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23rd International Pig Veterinary Society (IPVS) Congress

VOLUME II

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Volume 2

Poster presentations

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Preface

The 23rd International Pig Veterinary Society Congress is held in Cancun, Mexico…

We welcome you to the 23rd International Pig Veterinary Society Congress! This is the second grand opportunity for México's swine veterinarians to host IPVS … this time in beautiful Cancún! The meeting will open with the presentation of the Tom Alexander Memorial Lecture, "Accountabilities in the Age of Transboundary and Emerging Swine Diseases" by Dr. John Harding, Professor in the Department of Large Animal Clinical Sciences at the Western College of Veterinary Medicine in the University of Saskatchewan. After leading off on this very timely theme, we will hear 15 Lead Lectures (five each day) from leading experts from around the world. Finally, the program will be supported by 210 oral presentations, 53 "corners" (oral poster presentations - a first for an IPVS) and 689 posters. The Scientific Committee has had the pleasure of organizing IPVS 2014 and thank all those who have contributed to the success of the 23rd International Pig Veterinary Society Congress here in México where "Mi casa es tu casa".

Jesús Hernández and Jeffrey Zimmerman

Acknowledgements

A congress the size and scope of IPVS 2014 would not have been possible without the financial, professional and moral support of our Congress Sponsors.

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Locations of IPVS Congresses

1969	Cambridge, UK
1972	Hannover, Germany
1974	Lyon, France
1976	Ames, USA
1978	Zagreb, Yugoslavia
1980	Copenhagen, Denmark
1982	Mexico City, Mexico
1984	Ghent, Belgium
1986	Barcelona, Spain
1988	Rio de Janeiro, Brazil
1990	Lausanne, Switzerland
1992	The Hague, Netherlands
1994	Bangkok, Thailand
1996	Bologna, Italy
1998	Birmingham, UK
2000	Melbourne, Australia
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Control of respiratory disorders with Pulmotil® Premix (tilmicosin phosphate) in nursery pigs: Growth performance and economic evaluation

A Hidalgo, A Cox
Elanco Animal Health, UK and Ireland, hidalgo_alvaro@elanco.com

Introduction

Swine respiratory disease (SRD) has a great economic impact in pig production worldwide, affecting typically finishing pigs. SRD is often caused by interaction of two or more pathogens, including bacteria such as *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae* or *Pasteurella multocida*, and viruses as PRRSV, PCV2 or SIV (1). As a result, affected pigs usually present severe pneumonic lesions and impaired growth. This study aims to evaluate the use of Pulmotil® Premix in nursery pigs to minimize respiratory disorders and their associated cost throughout the finishing period.

Materials and Methods

This study was conducted in a 500-sow farrow to finish farm between May and September 2012. The herd has a recent history of respiratory disease in fattening pigs involving *M. hyopneumoniae*, *A. pleuropneumoniae*, and *P. multocida*. Pigs in one batch of production were ear tagged at weaning, weighted and randomly allocated to each experimental group. Pigs in the Pulmotil group (n= 142) were treated with tilmicosin phosphate (Pulmotil® Premix, Elanco AH) at 400 mg/kg feed for 2 weeks at the start of the nursery period. Pigs in the control group (n=137) did not received any medication at that time. Average weight at the beginning of the study (T0, 28 days old) was similar in both experimental groups. Pigs were individually weighed at the end of the nursery period (T1, 53 days old), at the start of the finishing period (T2, 81 days old) and before pigs were sent to slaughter (T3, 153 days old). Mortality was recorded by group. Average daily weight gain (ADWG) and weight coefficient of variation (CV), defined as the standard deviation divided by the mean, were calculated for both experimental groups. Data was recorded and analysed at a significance level of α=0.05 using JMP® version 9.0.3 (SAS Institute Inc., Cary, NC, USA).

Results

ADWG improved significantly in the Pulmotil group compared to the control group, with pigs gaining 22 g/d more during the nursery period and 27 g/d more during the growing period. From wean to finish, pigs in the Pulmotil group gained 20 g/d more than pigs in the control group (Table 1). Weight variation, as measured by CV, was consistently lower in the Pulmotil group when compared to the control group. At the end of the study, uniformity within the Pulmotil group was 2.4% higher than in the control group.

Table 1. ADWG at different stages by group.

	ADWG (g/d)		
	Pulmotil group	Control group	Difference
Nursery (28-53 d)	280	258	22*
Growing (53-81 d)	675	648	27*
Finishing (81-153d)	818	802	16
Total (28-153 d)	687	667	20*

*, Difference is statistically significant between groups

Table 2. Percentage (%) of light pigs at weighing times.

	Pulmotil	Control	Difference
T1 (<14 kg LW)	24.64	38.24	13.6*
T2 (<30 kg LW)	12.14	24.24	12.1*
T3 (<80 kg LW)	3.25	15.83	12.58*

*, Difference is statistically significant between groups

Mortality rate in the Pulmotil group was 1.40% whereas in the control group it increased up to 3.64%.

Conclusions and Discussion

Growth performance improved significantly in the Pulmotil group, with pigs gaining 20 g/d more than in the control group. In addition, the use of Pulmotil® Premix for respiratory disease produced heavier pigs throughout the finishing period, as evidenced by the higher percentage of light pigs in the control group at each weighing time (Table 2). Similar results following treatment of nursery pigs with Pulmotil® to control respiratory disease throughout the finishing period have been described before (2, 3, 4). Based on market conditions at the time of the study (1,70€/kg deadweight) and a 75% of carcass yield, pigs treated with Pulmotil® Premix had an extra revenue of 3.2 €, with a return of investment after treatment >5.

In summary, the use of Pulmotil® Premix at the start of the nursery period minimized respiratory disorders and related mortality. As a result, ADWG was significantly improved, group variability was reduced and fewer light pigs were produced.

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Chronic pleurisy score in slaughter pigs in relation to the ApxI-III toxins antibodies score

P Astrup[1]
[1]*Business Unit Swine, MSD Animal Health, Denmark,* peter.astrup@merck.com

Introduction

In Denmark, it is common practice to use lung lesion scores for pleurisy as an aid in the diagnosis of infections with *Actinobacillus pleuropneumoniae* (Ap) in pigs. The scores are usually done according to the system designated as USK (expanded health control) (1). For the purpose of determining the optimal timing of vaccination against Ap and for detecting the time point of infection with Ap, ApxI-II and III serology (2) in non-vaccinated animals is a frequently used management tool.

The objective of this study was to clarify a possible statistical relationship between chronic pleurisy in slaughter pigs measured by lung lesion scoring at the Laboratory for Pig Diseases in Denmark and the ApxI – III serology results in blood samples from the same groups of pigs and examined at R&D Service Lab, MSD Animal Health, The Netherlands.

Materials and Methods

A single batch of approximately 30 lungs each from a total of 123 farms was examined at slaughter. The lungs were scored for pleurisy expressed as the percentage of lungs with at least one chronic pleurisy lesion and they were scored separately for lesions in the cranio-ventral as well as caudo-dorsal parts. The caudo-dorsal lesions are used only for the analysis in this abstract.

Before slaughter and for each farm, the same group of 5 pigs was bled at approximately 16 and 20 weeks of age. The samples were examined and the results were expressed as log2 titer values for each of the toxins ApxI, II and III. For this study the serological results were scored according to a custom made system. See table 1.

Table 1. Definition of serological score values for level of one or two Apx toxin titers

All Apx titers below 10 log2	Score = 0
ApxII titers equal to or greater than 10 log2	Score = 1
Two Apx titers equal to or greater than 10 but below 12 log2	Score = 2
Two Apx titers equal to or greater than 12 log2	Score = 3

For each score value, the mean pleurisy percentage was calculated and analyzed by One-way ANOVA (Statgraphics Centurion XVI.II, Statpoint Technologies, Warrenton, VA 20186, USA).

Results

The scores are an ordinal variable but the results show an increasing average value of percent pleurisy with basic values averaging 24 % at score 0 ending at 84 % at score 3 (Figure 1). All means are significantly different ($P<0.05$).

Conclusions and Discussion

Under Danish conditions, the expression of ApxII alone may indicate an infection with Ap type 12 and the expression of more than one Apx type most probably indicate the infection with Ap type 2, 5 or 6.

Apx titers below 10 log2 may originate from maternally derived antibodies or cross reacting organisms. Low numbers of pleurisy must be expected from other agents than Ap. The increased pleurisy percentage at score 1 may imply the ability of Ap12 to elicit pleurisy at moderate levels. The apparent correlation of score 2 - 3 and pleurisy percentage may reflect different levels of severity of infection or differences in time of sampling and time of actual infection.

In conclusion, it is proven that the level of Apx toxin response may predict the average level of pleurisy score in a group of slaughter pigs.

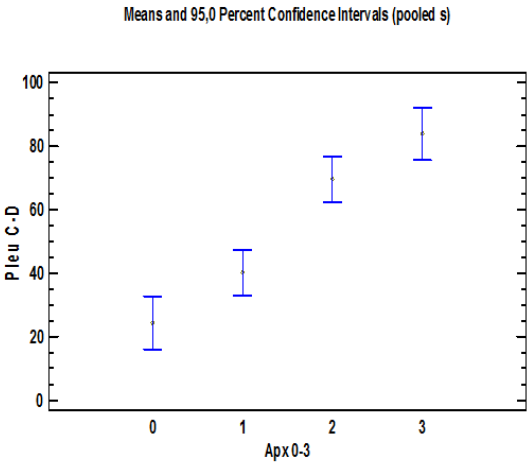

Figure 1.Mean percentage of lungs with at least one chronic pleurisy lesion (Pleu-CD) by Apx serological scores (Apx0-3). All means are significantly different, $p<0.05$.

Acknowledgments

Laboratory for Pig Diseases, Danish Agriculture and Food Council, Denmark.

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P.003

Efficacy of an alternative Tilmovet® treatment scheme in pigs

M Karanikolova[1], S Vesselova[1], V Nazarov[1], S Ivanova[1], S Petkov[2], W Depondt[2], A Kanora[2]
[1]*Department of R&D, Biovet JSC, Peshtera, Bulgaria,* [2]*Huvepharma NV, Belgium,* wouter.depondt@huvepharma.com

Introduction

Tilmicosin (TMS) is semi-synthetic broad-spectrum macrolide, currently approved for veterinary use in pigs, poultry and cattle. TMS maintains high concentrations in the lung tissue several days after the treatment has ended[1]. This is probably why practitioners often use an alternative treatment scheme: 5 days medication-2 days off medication (pause interval)-5 days medication. This seems mainly be driven in their aim to reduce the consumption of antibiotics within the specifications of the SPC. The current study aimed to evaluate the efficacy of this treatment scheme. For this reason the concentrations of TMS were measured at several timepoints during the treatment scheme and if the concentrations of TMS during the 2 day pause interval are sufficient to prevent occurrence of clinical symptoms and lesions after inoculation with *Actinobacillus pleuropneumoniae* (App) serotype 2.

Materials and Methods

Nine groups of 4 SPF pigs (Danube White), equal number of each sex (20.5-25.0 kg), 10-12 weeks of age, were used. All pigs were infected with App serotype 2. Group I didn't receive any medication (control group). Five days before the challenge, group II-IX received orally 16 mg TMS per kg bodyweight as Tilmovet®, following the scheme: 5-day treatment, 2-day pause and 5-day treatment. At each time point (see table 1) one group of the treated animals was euthanized and necropsied to assess concentrations of TMS using HPLC method for determination in plasma and lungs of pigs. Efficacy of the medication was evaluated by differences in pathological (App gross lesions) and microbiological (App reisolation) parameters between Group I (control) and group IX at the last time point (72 hours after the second treatment). The lungs were scored for App gross lesions from 0 to 5, depending on the percentage (%) of the lung surface affected with App gross lesions (0= no lesions; 1=up to 5%;2=6%-25%;3=26%-50%;4=51%-75%;5=76%-100%). Student-Fisher t-test was used to compare the results.

Results

No statistically differences of TMS concentrations in lung and plasma were found on day 5 of both treatments and 24 h and 48 h after both treatments. There was a statistically significant difference in TMS concentration in plasma and lungs on the 72nd hour after the second treatment (Table 1).

A statistically significant difference was remarked in App gross lesions between group I (control) and group IX. App could not be reisolated in group IX (Table 2).

Table 1

Days/ hours	Tilmicosin concentrations		
	Plasma, µg/mL	Lung, µg/g	Lung/Plasma ratio
Day 5 of the treatment	0.077[a]	2.302[a]	30
24 h after the treatment	0.110[a]	2.686[a]	24
48 h after the treatment	0.102[a]	2.865[a]	28
Day 1 of the second treatment	0.089[a]	2.344[a]	26
Day 5 of the second treatment	0.082[a]	2.680[a]	33
24 h after the treatment	0.103[a]	2.970[a]	29
48 h after the treatment	0.091[a]	2.183[a]	24
72 h after the treatment (= last time point)	0.060[b]	1.004[b]	17

a, b $p \leq 0.05$; LOQ<0.05 µg/mL

Table 2

Pathological, microbiological parameters			
Groups	Animals with lung lesion / total No.	App gross lesions	App reisolation
I	3/4	6.75[a]	2/4
IX	1/4	0.5[b]	0/4

a, b $p \leq 0.05$

Conclusions and Discussion

During the double 5 days treatment scheme with a 2 days pause interval, the lung and plasma concentrations stayed the same for 12 days. Only 72 hours after the last treatment the concentrations of TMS started to decrease in plasma and lung. Pigs inoculated with App in the 2 days pause interval, did not develop any clinical signs or lesions. This alternative treatment scheme can be considered as efficient in the treatment and control of respiratory disease.

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Clinical diagnosis of lung diseases in pigs by infrared thermography

A Menzel[1], C Siewert[2], D Hoeltig[1], H Seifert[2], I Hennig-Pauka[3]
[1]*Clinic for Swine and Small Ruminants, Forensic Medicine and Ambulatory Services, University of Veterinary Medicine Hannover, Germany,* [2]*Institute for General Radiology and Medical Physics, University of Veterinary Medicine Hannover, Germany,* [3]*Clinic for Swine, University of Veterinary Medicine, Vienna, Austria,* anne_menzelr@web.de

Introduction

Actinobacillus pleuropneumoniae (A.pp.) is a causative agent of respiratory tract diseases and has led to high economic losses worldwide. Further research on the disease itself but also new prophylactic and therapeutic approaches are needed. Infrared thermography (IRT) of the thorax might offer a new selection method of high specificity to select swine with basic lung alterations for further diagnostics as a cheap and non-invasive method.

Materials and Methods

Clinical, computed tomographical (CT) and IRT examination were performed on 50 *A.pp.*-infected and 10 control pigs prior to infection, 4 and 21 days after infection.
Clinical scores (ClS) were recorded daily according to the classification by Hoeltig et al. (2008). CT scores (CTS) were calculated as previously described by Brauer et al. (2012). Both scores allowed the assessment of disease severities during the trial and were compared with IRT findings.
IRT images were taken under controlled condition from both thorax sides after a 15 minutes cooling period. Absolute surface temperatures of the lung as well as temperature differences between lung and abdomen were compared between the three examination days.
During necropsies at the end of the trial lung lesion scores (LLS) were determined according to Hannan et al. (1982).

Results

On days 4 and 21 after infection, significant correlations were found between ClS, CTS and LLS and also on day 21 after infection between ClS and CTS. Influencing factors for the skin surface temperature of the thorax were ambient temperature, abdominal surface temperature and body temperature. On day 4 but not on day 21 after infection the right thoracal temperature was significantly higher and the difference between a thoracal region in the height of the left 10th vertebra and an abdominal region was significantly lower in infected pigs than in control pigs.

Conclusions and Discussion

An increase in body temperature above the normal is a fundamental physical component of inflammation besides swelling, pain and redness. Skin temperature is influenced by many different factors such as sweat evaporation, vascular perfusion, local tissue metabolism and ambient temperature (So, 1989).
Anyhow, infrared thermographical images captured under controlled conditions may be a helpful diagnostic tool to monitor the reaction of patient's physiology to different types of stress, such as inflammatory or thermal (Zaproudina, 2008).
Results demonstrate that IRT can help to detect inflammatory lung alterations in the pig lung. The acute stage of infection can be better detected by temperature pattern in IRT than chronical stages.
Ambient temperature plays is major interfering factor during the diagnostic of surface temperature.
However, IRT examination does not give information about etiology of respiratory disease.

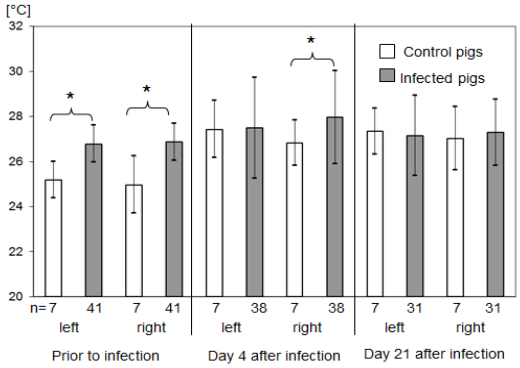

Figure 1. Absolute skin surface temperatures of the left and right thorax at different stages of infection. Asterics indicated significant differences between groups ($p \leq 0.05$). Selected pigs were examined in an ambient temperature range between 7-14°C during IRT.

Acknowledgments

This research was supported by the German Research Community (DFG, HE 6419/1-1).

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P.005

Montecarlo approaches to compare the treatment efficacy of pig respiratory disease with two pharmaceutical products containing florfenicol as active ingredient

C Vilalta[1], S Colomer[2], M Perelló[2], M Busquet[2], L Fraile[a]
[1] *Universitat de LLeida, Lleida, Spain* [2]*Laboratorios Hipra SA, Girona, Spain,* lorenzo.fraile@prodan.udl.cat

Introduction

Antimicrobial drugs have been classified as concentration-dependent or time-dependent. The concentration-dependent are those where increasing concentrations at the locus of infection improve bacterial kill. The time-dependent are those where exceeding the minimum inhibitory concentration (*MIC)* for a percentage of the inter-dosing interval (*T>MIC)* correlates with clinical efficacy. Florfenicol is an antimicrobial widely used in swine medicine that has been described as concentration or time-dependent relying on the bacterial species involved. The goal of this trial is to foresee the clinical efficacy of a pharmaceutical product (Selectan®) whose main active ingredient is florfenicol to treat *Actinobacillus pleuropneumoniae* (APP) and *Pasteurella multocida* (PM) infection in pigs in comparison with the reference product (Nuflor®).

Materials and Methods

A model was developed to predict the likelihood of attainment of the Pharmacokinetic (PK)/Pharmacodynamic (PD) parameters that determines florfenicol efficacy on APP and PM infections in pigs. For this analysis, Montecarlo simulations were performed using the pharmacokinetic data calculated for Selectan® and Nuflor® (Laboratorios HIPRA, registration dossier data) and the MICs for APP and PM published in the scientific literature (Lizarazo et al, 2006, Gutierrez-Martin, et al, 2006). Thus, a population of 100.000 pigs and strains was created to run the simulations. Area under the curve (AUC)/MIC over 50 and T>MIC 40% of the dose interval are the PK/PD parameters to be associated with antibacterial efficacy according to the literature for florfenicol. The likelihood of attainment of the Pharmacokinetic (PK)/Pharmacodynamic (PD) parameters that determines florfenicol efficacy on both microorganisms was calculated using CrystalBall Software (V. 11.1.2.0.00; Oracle Corporation, RedwoodShores, CA, USA) as previously described (Messenger, 2012).

Results

After running the model, the probability of clinical success, using the AUC/MIC>50 and T>MIC 40% of the dose interval as threshold values for Selectan® and Nuflor® are shown in table 1.

Table 1. Probability of clinical success for Selectan® and Nuflor® using AUC/MIC>50 and T>MIC 40% as threshold values

	PK/PD parameter	
Selectan®	**AUC/MIC>50**	**T>MIC40%**
APP	90	94.7
PM	87.8	94.2
Nuflor®		
APP	86.7	91
PM	74.9	81.5

Conclusions and Discussion

The ideal situation is to know the antimicrobial susceptibility of any microorganisms before applying antimicrobial treatments. However, this information is not available in many occasions under practical conditions. On the other hand, it is necessary to apply an antibiotic treatment with a high probability of clinical success. In this trial, it is described a rational approach to compare the clinical efficacy of two pharmaceutical products based on florfenicol. In this study, it is clear that Selectan® and Nuflor® are bioequivalents from the clinical point of view and both products would be efficacious in most cases to treat pig respiratory disease due to APP and PM. As demonstrated above, he foreseen clinical efficacy of Selectan® is better than for Nuflor® for both APP and PM treatment. A possible explanation could be the higher homogeneity observed in the pharmacokinetics of Selectan® versus the reference product at population level.

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P.006

In vitro activities of Tiamulin against European *A. pleuropneumoniae* field isolates

M Vallé[2], O Roy[3], X Fleurant[3], U Klein[1]
[1]*Novartis Animal Health Inc., Basel Switzerland,* [2]*AM Consultant, St Bénigne France,* [3]*CEBIPHAR, Fondettes France,* ulrich.klein@novartis.com

Introduction

Actinobacillus pleuropneumoniae (*App*) is the causative agent of severe fibrinous pleuropneumoniae, chronic pleuritis and pulmonary sequestration and abscessation, with generally high morbidity and mortality in pigs. *App* are commensals on mucous membranes of their host and commonly found in European pig farms. The aim of the study was to evaluate the antimicrobial susceptibility of *App* isolates against tiamulin based on MIC studies and kill curve determinations. The studies were conducted on field *App* strains isolated from clinical cases of respiratory infection from different European countries.

Materials and Methods

Bacterial strains: In total 220 *App* isolates from a European survey collection of clinical submissions (lung tissue or nasal swabs; BE, DK, FE, GE, IT, NL, PL, SP, UK) generated between 2002 to 2006 were tested. All isolates sampled before treatment originated from different pig farms distributed across the corresponding country.

Antimicrobial susceptibility tests: Tiamulin minimum inhibitory concentration (MIC) was determined according to broth microdilution CLSI standard method[1,2].

Statistics: Kruskal-Wallis test to compare the tiamulin MIC distribution among countries and Fisher's Exact test to compare rates of resistance among countries (significance if $p \leq 0.05$).

Kill curve determination: carried out on 4 fields *App* strains (Be, Fr, Ge, Nl; tiamulin MIC=8µg/mL) with an initial inoculum between 5.10^5 and 5.10^6 CFU/ml, in a supplemented cation adjusted Mueller-Hinton broth. Tiamulin concentrations tested: 0, 1/8, 1/4, 1/2, 1, 2, 4, 8 and 16xMIC during 24h.

Antimicrobial agent: Tiamulin hydrogen fumarate THF (Novartis AG, Basel, Switzerland).

Results

The tiamulin MIC results were previously published3,4. . The combined results5 show a tiamulin MIC range between 0.25 and 16 µg/mL with both MIC50 and MIC90 = 8 µg/mL (the main class of MIC distribution). According to the CLSI clinical breakpoints, all App strains were susceptible (100%). No significant difference in MIC distribution (Kruskal-Wallis test) and no significant difference of percentage of resistances (0%) (Fisher's exact test) were observed between the countries. Tiamulin showed a bactericidal time-dependant action (3 log10 reductions in viable cells, regardless of antimicrobial concentration) after THF exposure against all tested App strains at 24h at ≥2xMIC concentrations. This bactericidal activity has been observed within 6h for one strain, within 10h for one strain and within 24h for the two other strains. Moreover, tiamulin showed during the same time a bacteriostatic activity at sub-MIC concentrations (1/2, 1/4 and 1/8 MIC) within a time period of 6 to 24 hours for 3 tested strains. For the last strain tested the bacteriostatic activity was determined at the THF MIC and at lower concentrations an inhibition of growth was observed before the last sample time point (24h).

Conclusions and Discussion

Tiamulin MIC results of the pan-European survey reveal a very high susceptibility of *App* strains: no significant difference could be observed between countries during the period studied (2002-2006). Moreover, tiamulin *in vitro* curve killing determination against field *App* with a MIC = 8 µg/mL showed on all tested strains a bactericidal activity at a concentration ≥2xMIC and a bacteriostatic activity at least at 1/2xMIC and below for three strains of four. In theory MIC is by definition the first bacteriostatic concentration of tested antibiotic, but for tiamulin/*App* the bacteriostatic activity was observed at lower concentrations. The proven bactericidal & bacteriostatic action explain the high effect of tiamulin treatments (Denagard®) in cases of acute, sub-acute and chronic respiratory infections in pigs induced by *App*: fast and strong activity with an extended clinical effect. These new data must be correlated with pharmacokinetics in pig[6] and with other tiamulin *in vitro* MIC results[7].

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P.007

Antimicrobial resistance genes of *S. aureus* from pork

VT Tra An[1], DT Xuan Thiep[1], AC Fluit[2]
[1]*Department of Veterinary Biosciences, Faculty of Animal Science and Veterinary Medicine, Nong Lam University, Ho Chi Minh City, Vietnam* [2]*Department of Medical Microbiology, University Medical Centre Utrecht, G04.614, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands* an.vothitra@hcmuaf.edu.vn

Introduction

Staphylococcus aureus is a cause of many diseases in both humans and animals. Pork contaminated by staphylococci can be derived from the gastrointestinal tract, transport, facilities or personnel in slaughter houses and can cause food poisoning (Lin *et al*, 2009). If antibiotic-resistant *S. aureus* from pork infects humans through the food chain public health would be at risk because of the difficulties of antimicrobial therapy. This bacterium can be a reservoir for spread of resistance genes to the community and environment. Therefore, this study determined the presence of antimicrobial resistance genes among *S. aureus* isolated from pork meat in Ho Chi Minh City, Vietnam.

Materials and Methods

A total of 152 pork samples were collected in markets and supermarkets in Ho Chi Minh City, Vietnam. All of the *S. aureus* isolates have been cultured as described previously. *S. aureus* ATCC 25923 was used as quality control organism.

Antimicrobial susceptibility of the isolates to antimicrobial agents was determined according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).

Isolates which were resistant against oxacillin, ampicillin and/or penicillin, gentamicin and/or kanamycin, tetracycline, erythromycin and clindamycin were tested for *mecA*, *blaZ*, *aacA-aphD*, *tetM*, *msr*A and *linA*, respectively.

A rapid boiling lysis procedure was used to prepare template DNA from *S. aureus* isolates according to Queipo-Ortuno *et al* (2008). The primers used for PCR amplification have been described (Meshref and Omer, 2011; Milheiriço et al, 2011; Strommenger *et al*, 2003; Lina *et a*l, 1999). PCR reaction mixtures contained 12.5 µl Go *Taq*^{R} Green Master Mix 2X (Promega, USA), 2 µl oligonucleotide primers (25 pM) (IDT, USA), 2 µl DNA template and 8.5 µl free nuclease water, in a total of 25 µl for a PCR reaction (Promega, USA).

Representative resistance genes were sequenced and BLASTed. A high degree of similarity (> 90%) to corresponding genes in GenBank, would confirm the presence of the expected genes in PCR positive *S. aureus* isolates.

Results

The percentage of resistance genes was shown in Table 1.

Table 1. Resistance genes found among *S. aureus*

Gene	No. isolates tested	No. of positive	Percentage
mecA	6	0	0
blaZ	66	65	98.5
aacA-aphD	37	8	21.6
tetM	3	1	33.3
linA	9	4	44.4
msrA	10	0	0

Discussions

blaZ genes were detected with a high percentage among *S. aureus* isolates from pork (98.48%). This is nearly the same percentage that is reported in Italy (100%) (Desj et al, 2008). Although *mecA* was not found among 6 oxacillin-resistant isolates these isolates carried *blaZ* gene and were resistant against up to nine antibiotics. *aacA-aphD* encoded resistance to gentamicin, kanamycin, tobramycin and amikacin (Strommenger et al, 2003). While it was reported in 100% (Strommenger et al, 2003) and 28% (Nakaminam et al, 2008) of *S. aureus* in previous studies it was detected in only 21.6% of the tested isolates. One fourth of tetracycline-resistant *S. aureus* isolates contained *tetM* gene encoding for resistance to all tetracyclines, including minocycline (Trzcinski et al, 2000).

Acknowledgement

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P.008

The possible use of a *B. bronchiseptica aroA* deletion mutant expressing *P. multocida* toxin antigen as a candidate vaccine strain

Z Peng[1,2], W Liang[3,4], F Luo[1,2], R Hu[1,2], B Wu[1,2]

[1]*State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University,* [2]*College of Veterinary Medicine, Huazhong Agricultural University,* [3]*Key Laboratory of Animal Genetics, Breeding and Reproduction, Ministry of Education, Huazhong Agricultural University,* [4]*College of Animal Science, Huazhong Agricultural University, Wuhan, 430070, China,* pengzhong525@outlook.com

Introduction

The use of *Bordetella bronchiseptica aroA* deletion mutant as a live vector to deliver heterologous antigens has been reported (1, 2, 3), for the attenuated *B.bronchiseptica* can be administered directly to the respiratory tract, stimulating mucosal and systemic humoral and cellular immunity against *B.bronchiseptica* (1). In this study, a recombinant *B.bronchiseptica aroA* mutant (QH0814ΔaroA/tox-N) that can express the N-terminal fragment of *tox*A gene who encodes *Pasteurella multocida* toxin (PMT) has been constructed without antibiotic resistance markers. Mice experiment shows the mutant can induce the production of anti-*B.bronchiseptica* and anti-toxA-N, which implies the mutant could be used as a candidate vaccine strain to against infections caused by *B.bronchiseptica* and *P.multocida.*

Materials and Methods

Primers in this study were listed in Table 1. *B.bronchiseptica* QH0814, *P.multocida* HN06, *E.coli* X7213, plasmid pBluescript II SK(+), suicide plasmid pRE112 were used to construct the recombinant mutant as described previously (4). All strains were cultured in a suitable environment, as previously described (4).

Western-blot was worked to examine the expression of tox-N protein in the mutant.

Table 1 Primers used in this study.

Primers		
F1	TGGCGCCTGCCCTAT	Amplified *fla* gene (237bp), to identify *B.bronchiseptica*
F2	AGGCTCCCAAGAGAGAAA	
A1	GCGCGGTACCCGACGTGCTGGGCCATCGC (KpnI)	Amplified upstream flank of *aroA* gene (1388bp)
A2	TATAGTCGACCCGGAACGCGGTGCCGG（SalI）	
A3	ATATAAGCTTGATCCGGGTTGCGTCAGCAAG(HindIII）	Amplified downstream flank of *aroA* gene (1383bp)
A4	ATATGAGCTCACCAGCGAACCGGGCAGGAAC（SacI）	
A5	GCAATTGGCGCAAGGATTCC	Amplified *aroA* gene (1329bp)
A6	GGATGCGGAAATAGCCATGG	
A7	CGGCGATTACCGCCTGTCCG	Amplified the deleted *aroA* fragment (649bp)
A8	GGCGTCGGGAATCAGGTTGAAG	
T1	CGCGTCGACAATGAAAACAAAACATT（Sal I）	Amplified toxA-N fragment (933bp)
T2	GTGAAGCTTTCACGAGAAATGTTTG（Hind III）	
Cm1	TAAATACCTGTGACGGAAGAT	Amplified Cm resistant gene in pRE112 (924bp)
Cm2	TATCACTTATTCAGGCGTAGC	

60 4-week-old Balb/c mice were used to evaluate the virulence of QH0814ΔaroA/tox-N, compared with the parent strain, QH0814. LD_{50} of two strains were calculated on the basis of Korbor law.

Serum was collected from the mice in the 0, 14, 28, 35 day after the mice were challenged intranasally with the 1×10^6CFU mutant suspension to detect antibodies against *B.bronchiseptica* and toxA-N, using a direct-ELISA that by ourselves, as previously described (4).

Results

The mutant strain QH0814ΔaroA/tox-N was constructed successfully, since a 1241bp copy and the toxA-N fragment (933bp) could be amplified with primers A5/A6 and T1/T2, respectively, while the deleted *aroA* fragment (649bp) and the Cm resistant gene (924bp) could not be amplified with primers A7/A8 and Cm1/Cm2, using PCR (data not shown). Wstern blot demonstrated tox-N protein could be expressed in the mutant. LD_{50} of the strain QH0814 and QH0814ΔaroA/tox-N was 2.5×10^6 CFU and 1.8×10^7 CFU, showing virulence of QH0814ΔaroA/tox-N reduced almost 7 times, compared with QH0814. The immune efficacy test in mice revealed antibodies against *B.bronchiseptica* and toxA-N were induced in the serum by the mutant. OD_{630} of IgG titers against *B.bronchiseptica* were about 0.38, 0.79, 1.36 in the 14th, 28th, 35th day after vaccination (Fig 1a), while OD_{630} of IgG titers against toxA-N in these days were about 0.32, 0.63, 0.94 (Fig 1b), both of them showed a great increases, compared with the control groups.

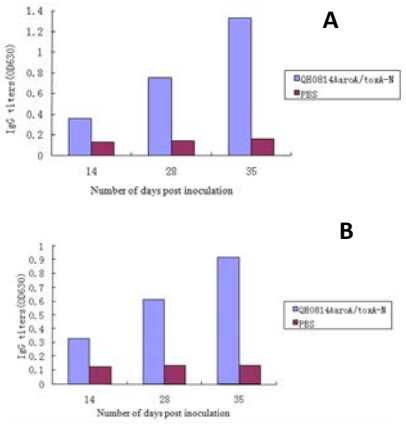

Figure 1. IgG titers in the serum of experimental mice induced by QH0814ΔaroA/tox-N after vaccination. (A) antibodies against B.bronchiseptica. (B) antibodies against toxA-N.

Conclusions and Discussion

In this study, we constructed a recombinant *B.bronchiseptica* mutant that could express the N-terminal fragment of *tox*A gene who encodes the PMT. Virulence of the mutant showed a great decrease compared with the parent strain. The mutant could stimulated the production of anti-*B.bronchiseptica* and anti-toxA-N, observably. What's more, the mutant did not contain any antibiotic resistance markers. All these implies that the mutant QH0814ΔaroA/tox-N may be suitable for the development of a bivalent vaccine that could prevent infections caused by *B.bronchiseptica* and *P. multocida.*

Acknowledgments

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P.009

Expression of the N-terminal fragment of *P. multocida* toxin antigen in an attenuated *B. bronchiseptica*

Z Peng[1,2], W Liang[3,4], F Luo[1,2], R Hu[1,2], B Wu[1,2]

[1]State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, [2]College of Veterinary Medicine, Huazhong Agricultural University, [3]Key Laboratory of Animal Genetics, Breeding and Reproduction, Ministry of Education, Huazhong Agricultural University, [4]College of Animal Science, Huazhong Agricultural University, Wuhan, 430070, China. pengzhong525@outlook.com

Introduction

The use of *Bordetella bronchiseptica aroA* deletion mutant (BbΔaroA) as a live vector to deliver heterologous antigens has been reported (1, 2, 3), for the attenuated *B.bronchiseptica* can be administered directly to the respiratory tract, stimulating mucosal and systemic humoral and cellular immunity against *B.bronchiseptica* (1). The purpose of this study was to determine whether a recombinant BbΔaroA that can express the N-terminal fragment of *tox*A gene (tox-N) who encodes *Pasteurella multocida* toxin (PMT), termed QH0814ΔaroA/tox-N, could stimulate the production of anti-*B.bronchiseptica* and anti-tox-N antibodies in mice.

Materials and Methods

B.bronchiseptica QH0814, *P.multocida* HN06, *E.coli* X7213, plasmid pBluescriptII SK(+), suicide plasmid pRE112 were used to construct the recombinant mutant, and all strains were cultured in a suitable environment, as previously described (4).

Western-blot was worked to examine the expression of tox-N protein in the mutant.

Mice experiments were worked for evaluating the virulence (based on LD_{50}, compared with the parent strain, QH0814) and the immune efficacy of the mutant. Serum was collected from the mice in the 0, 14, 28, 35 day after the mice were challenged intranasally with the mutant suspension at a dose of 1×10^6 CFU to detect antibodies against *B.bronchiseptica* and tox-N, using a direct-ELISA that by ourselves, as previously described (4).

Results

Wstern blot demonstrated tox-N protein could be expressed in the mutant. LD_{50} of the two strains and OD_{630} value of IgG titers induced by the mutant were listed in Table 1.

Table 1 Relevant data of the mice experiment.

Strains	QH0814	The mutant		
LD_{50}	2.5×10^6 CFU	1.8×10^7 CFU		
IgG titers induced by the mutant				
Dpi		14	28	35
OD_{630} (IgG against Bb)		0.38	0.79	1.36
OD_{630}(IgG against tox-N)		0.32	0.63	0.94

Conclusions and Discussion

In this study, we constructed a recombinant *B.bronchiseptica* mutant that could express the N-terminal fragment of *tox*A gene who encodes the PMT. Virulence of the mutant showed a great decrease compared with the parent strain. The mutant could stimulate the production of anti-*B.bronchiseptica* and anti-tox-N antibodies, observably. What's more, the mutant did not contain any antibiotic resistance markers. All these implies that the mutant QH0814ΔaroA/tox-N may be suitable for the development of a bivalent vaccine that could prevent infections caused by *B.bronchiseptica* and *P. multocida.*

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P.010

Efficacy of tulathromycin (DRAXXIN 25 mg/mL) for the treatment of swine respiratory disease associated with *B. bronchiseptica*

H Moyaert[1], A Palzer[2], L Noé[1], B Liu[1], MR Stegemann[1]
[1]*Zoetis VMRD,* [2]*Tierarztpraxis Scheidegg, Germany,* hilde.moyaert@zoetis.com

Introduction

The objective of this study was to evaluate the efficacy of tulathromycin (Draxxin 25 mg/mL) vs tildipirosin (Zuprevo®) for the treatment of naturally occurring Swine Respiratory Disease (SRD) associated with *Bordetella bronchiseptica*, a causative agent of bronchopneumonia in piglets and older animals (1,2).

Materials and Methods

At the age of 6 weeks, a total of 192 cross-breed slaughter piglets were enrolled from a commercial swine production unit in Germany with a known history of SRD associated with *B. bronchiseptica*. From birth until study completion no bacterial respiratory disease vaccines, except *Mycoplasma hyopneumoniae*-specific vaccines, were used. From 14 days preceding enrolment up to and including study completion on Day 21, study animals did not receive antibacterial compounds, except in-feed colistin for 7 days during an outbreak of *Escherichia coli* diarrhea from study Day 11 to Day 17. At study inclusion (Day 0), pigs had moderate (score 2) or severe (score 3) clinical signs of SRD (depression, dyspnoea, coughing and sneezing) in combination with pyrexia (rectal temperature ≥ 40.0°C). The nature of the disease was assessed by broncho-alveolar lavage (BAL) from the first 25 piglets enrolled, before treatment administration. Each animal was randomly allocated in a 1:1 ratio to either T01 (tildipirosin 4 mg/kg bodyweight (BW)) or T02 (tulathromycin 2.5 mg/kg BW) and treated once on Day 0. The examining veterinarian remained blinded to treatment throughout the study. On Days 1 to 14, inclusive, and on Day 21, clinical observations and rectal temperature measurements were performed. Animals were weighed on Day 0 and Day 14. The primary efficacy variable was the cure rate on Day 14 with the objective to demonstrate non-inferiority of tulathromycin compared to tildipirosin based on percentage of clinical cure (SRD score ≤ 1) on Day 14. A non-inferiority margin of 15% at a one-sided 0.025 level of significance with the individual animal as experimental unit was applied. Secondary efficacy variables were SRD-related mortality, prevalence and severity of clinical signs, rectal temperature, average daily weight gain (ADG, Day 0 – Day 14) and relapses on Day 21.

Results and Discussion

The target pathogen *B. bronchiseptica* has been isolated from 22 out of 25 BAL samples collected on Day 0, with an equal distribution among both treatment groups.
The primary objective of this study has been met, i.e. treatment with tulathromycin has shown to be non-inferior to treatment with tildipirosin based on percentage of clinical cure on Day 14 with 93.4% clinical cure in the tildipirosin-treated group (T01) vs 93.7% in the tulathromycin-treated animals (T02); Difference T02-T01 = 0.3% with 95% Confidence Interval -6.9% to 7.4%. Relapse rates on Day 21 were 2.4% (T01) vs 4.5% (T02), which was not significantly different between the treatment groups. There were no SRD-related mortalities or withdrawals during the study.
The percentage of animals with clinical signs of SRD on Days 3, 7, 14 and 21 was 57.7%, 9.3%, 6.6% and 2.3% in T01 vs 41.4%, 6.3%, 6.3% and 4.2% in T02, respectively. On Day 3, the observed difference between T01 and T02 (16.3%) was statistically significant, i.e. tulathromycin was superior to tildipirosin (P = 0.0181). Throughout the study, the mean rectal temperature varied between 39.8°C to 40.8°C in T01, and between 39.9°C to 40.9°C in T02, with no significant differences between the treatment groups, except on Day 8 where the mean rectal temperature in T02 was significantly lower compared to T01 (P = 0.0153). Overall, rectal temperature remained relatively high throughout the study, which can likely be attributed to stress due to frequent handling of the animals.
In both treatment groups, ADG was 0.26 kg/day.

Conclusion

Tulathromycin (Draxxin 25 mg/mL) has shown to be non-inferior to tildipirosin (Zuprevo®) for the treatment of SRD associated with *B. bronchiseptica* based on Day 14 clinical cure rate. There were no significant differences between treatment groups regarding rectal temperature (except on Day 8), Day 21 relapse rate and ADG. The clinical signs were decreasing more rapidly in the group of animals treated with tulathromycin.

Acknowledgments

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P.011

The effect of avilamycin on finishing swine administered a *B. hyodysenteriae* challenge

T Marsteller[1], M Knauer[2], M Pierdon[3], D Beckler[4]
[1]*Elanco Animal Health;* [2]*Department of Animal Science, North Carolina State University, Raleigh NC;* [3]*University of Pennsylvania, School of Veterinary Medicine;* [4]*GutBugs Inc, Fergus Falls, Minnesota*
marsteller_thomas_a@elanco.com

Introduction

Brachyspira hyodysenteriae is an enteric pathogen prevalent worldwide in most swine rearing geographies (1,2,3). The majority of the research work completed with *B. hyodysenteriae* is concerning the discovery of new serovars, disease diagnosis and measuring the disease impact on animal performance with little study on treatment options.

Avilamycin is an orthosomycin antibiotic used only in animals to control enteric disease. Therefore, the objective of the present study was to determine the effect of avilamycin in a swine dysentery challenge model.

Materials and Methods

One hundred pigs were allotted to twenty pens with 5 pigs per pen. On study day 7 the pigs weighed 32 kg and were randomly assigned to control or treatment feed (avilamycin 83 ppm). On study day 12 a challenge model utilizing a known pathogenic strain of *B. hyodysenteriae*, was grown in pure culture and a dose of 10^{10} cfu bacteria was administered. All challenged pigs were administered the bacterial inoculums orally twice, once on study day 12 and again on day 13. The challenge dose was split in half and given over the course of the 2 challenge days.

Pen feed, individual pig weights and clinical scores including: fecal score, animal appearance and animal behavior were evaluated on study days 7, 14, 21, and 28. All pigs were necropsied and intestinal lesions were evaluated on study day 29. Fresh and fixed tissues and fresh feces were collected at necropsy.

Results

This *B. hyodysenteriae* challenge did not cause any animal mortality during the experimental period. There were no differences in animal appearance or behavior during the study period. There was evidence of diarrhea as demonstrated by fecal score differences between negative control fed pigs versus the treated pigs during study days 14 and 28 (Table 1). Fresh fecal samples submitted to the University of Minnesota Diagnostic Laboratory to determine fecal PCR's for *B. hyodysenteriae* were all negative. Intestinal lesion scores were not different between treatments.

Conclusions and Discussion

B. hyodysenteriae challenge models have been very difficult to readily reproduce. This oral challenge model did produce changes in fecal scoring on study days 14 and 28, but Koch's postulates were not fullfilled due to the inability to find *B. hyodysenteriae* tissue lesions or organism after challenge. Further work will be needed to determine if this isolate will need other factors such as: dietary, animal age, quantity of infectious inoculum or growth phase of inoculum changes to clearly reproduce disease. Avilamycin treatment decreased fecal scores from this *B. hyodysenteriae* challenge and improved growth in this finisher study.

Table 1. Effect of treatment on weekly fecal score.

	TREATMENT GROUP		
DAY	CONTROL	AVILAMYCIN	SE
14	1.14[a]	1.00[b]	0.047
21	1.04	1.00	0.023
28	1.14[c]	1.02[d]	0.037

[a,b]Row Means with different superscripts differ (P<0.5).
[c,d]Row Means with different superscripts differ (P<0.1).

Table 2. Effect of treatment on performance (7-28 days).

	TREATMENT GROUP		
MEASURE	CONTROL	AVILAMYCIN	SE
ADG, kg	1.03[a]	1.19[b]	0.04
ADFI, kg	1.83[a]	2.10[b]	0.08
Feed:Gain	1.76	1.76	0.03

[a,b]Row Means with different superscripts differ (P<0.01).

These results justify additional research on *B. hyodysenteriae* disease modeling diagnostic responses and animal growth. These data support the continued evaluation of avilamycin for swine enteric disease.

Acknowledgments

North Carolina State University, Swine Evaluation Station, Clayton, NC 27520, USA.

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Sensitivity profiles of *B. hyodysenteriae* strains based on Pleuromutilin susceptibility testing in different European countries

U Klein[1]
[1]*Novartis Animal Health Inc., Basel Switzerland,* ulrich.klein@novartis.com

Introduction

Brachyspira hyodysenteriae is the causative agent of swine dysentery (SD), one of the most important gastrointestinal disorders among pigs and commonly found in European pig farms. The aim of the comparative survey was to evaluate the antimicrobial susceptibility of *Brachyspira hyodysenteriae* isolates against tiamulin and valnemulin based on thirteen MIC studies published between 2008 and 2014. The studies were conducted in different European countries representing large, medium and small scale pig production in Europe.

Materiasl and Methods

Bacterial strains: In total 1050 *Brachyspira hyodysenteriae* isolates from clinical submissions of pig faecal samples generated between 2000 to 2013 were tested in different countries (B, CH, D, DK, E, I, Pol, S, UK) and institutes. All isolates originated from different pig farms distributed across the corresponding country.
Antimicrobial susceptibility tests, antimicrobial agents: The methods used for susceptibility tests varied between the laboratories. Based on non-existence of CLSI standard method, laboratories used the internationally accepted methods (broth microdilution or agar dilution) for Brachyspira antimicrobial susceptibility testing. The antimicrobials tested were tiamulin and valnemulin (Novartis AG, Basel, Switzerland). The minimum inhibitory concentration (MIC) was determined as the lowest concentration of the antimicrobial agent that prevented visible growth. The interpretation of MIC values was based on criteria proposed by different authors [1,7,11].

Results

Country-specific differences in the sensitivity profiles of the Brachyspira strains were found. In the majority of the European countries (CH, D, DK, I, Pol, S) high susceptibilities to tiamulin and valnemulin were found. The MIC50 (0.063-1.0) and MIC90 (0.063-2.0) values for tiamulin and MIC50 (0.031-0.5) and MIC90 (0.031-4.0) values for valnemulin indicate minor differences between both antibiotics. Trends of an increase of the Pleuromutilin MICs over time periods of several years were not found in these countries.
Data from Belgium13 (tiamulin MIC50 0.25-8.0, MIC90 2.0-8.0; valnemulin MIC50 0.03-8.0, MIC90 0.5-8.0) show higher MICs. This is also the case for tiamulin in one study from Spain6 and from UK9.
In the overall Europe summary the MIC50 value for tiamulin was 0.76 µg/ml, the MIC90 for tiamulin was 3.7 µg/ml. No tiamulin resistance was found in studies in five European countries (CH, D, I, Pol, S). The mean tiamulin resistance rate in all the other European countries is about 16%.
In the overall Europe summary the MIC50 (0.73 µg/ml) and MIC90 (3.46 µg/ml) values for valnemulin were lower vs. those calculated for tiamulin. In the majority of the studies the resistance rate on valnemulin was not determined.

Conclusions and Discussion

The results of the comparative European survey reveal sensitivity to both Pleuromutilin antibiotics which are the main antibiotics used for treatment of SD in Europe. Country-specific differences in the sensitivity profiles of *Brachyspira hyodysenteriae* isolates have to be considered and highlight the importance of monitoring programs to detect changes in the susceptibility of those field isolates. The findings are in good correspondence to the clinical effect of both Pleuromutilin antibiotics seen in field cases of swine dysentery all over Europe.

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Control of swine dysentery with an inactivated autovaccine against *B. hyodysenteriae* in a finish pig farm of Spain

J Deza[1], G Pappaterra[2]
[1]*NIG (Nutrición, Inseminación Artificial, Genética), Córdoba, Spain.* [2]*Laboratorios Calier, Barcelona, Spain* gpappaterra@calier.es

Introduction

Swine Dysentery (SD), caused by *Brachyspira hyodysenteriae*, is responsible for severe economic losses to the pig industry (1). For this reason, is important to control and / or eradication measures to reduce the negative effects on porcine production. However, the changes in antimicrobial susceptibility, the absence or failures in biosecurity measures, management conditions and farm structure, can result in a control failure.

In Spain, there are positive experiences in the use of autogenous bacterin as part of the strategy to control of SD disease (2, 3).

The aim of this study was to evaluate the utility of an autovaccine against *B. hyodysenteriae* for the control of SD in a finish farm.

Materials and Methods

The study was performed in a growing - fattening farm of 1430 pigs, localized in the south-west of Spain. All piglets, of 6-7 kg of weight, have as origin the same sow farm, without clinical signs and diagnosis of SD. The pigs are sent to slaughter at 100 kg

In May 2012, severe mucohemorragic diarrhoea in fattening pigs (60-70 kg bw), for first time was observed. A treatment with tylosin in water and injectable route was implanted, but the clinic not was controlled. In July, similar diarrhoea in other areas was present. The animals were medicated with lincomycin 100 ppm in the feed, with improving cases, but not completely controlled. Immediately, faecal samples of pigs were collected for laboratory diagnosis. *B. hyodysenteriae* was identified and the strain was isolated. Medication was changed to tiamulin 100 ppm and chlortetracycline 300 ppm the first three weeks of consumption of feed in the growing, repeating 3 weeks later in fattening. Animals with diarrhoea, growth retardation and death was continued being observed.

In June 2013, using this strain, an autogenous vaccine (10^9 bacteria/dose) was prepared (Laboratorios Calier, S.A.; León, Spain). Piglets from 4 weeks-old were injected intramuscularly with two doses of the autogenous bacterin separated by an interval of 3 to 4 weeks. Medication with tiamulin 50 ppm and chlortetracycline 150 ppm until 3 weeks after the second vaccination was used. Medication was then removed.

Results

A decrease significant of SD clinical signs was observed in pigs after application of autogenous vaccine. The prevalence of clinical disease (typical SD diarrhoea) was higher than 35 % before vaccination and 0% after finishing the vaccination protocol. Mortality rate due to SD also showed a significant decrease in 7%. Improving the feed conversion index in more than one point and an important reduction feed costs and, also markedly, the drug costs was reduced as a consequence of the reduction, and even suspension, of previous treatments against B. hyodysenteriae (all data in Table 1)

Table 1. Data production before and after application of the autovaccine

	Pre vaccination	Post vaccination
Pigs with diarrhoea	35%	0%[a]
SD mortality	9%	2%[b]
Feed conversion index	3,62 kg	2,57
Cost medication./Anim sold	4,83 €	1,83 €
Cost feeding / Anim sold	105,11 €	74,18 €

(a, b) statistically significant difference ($p \leq 0,05$)

Conclusions and Discussion

These results allow us to conclude that an autogenous inactivated vaccine against B. hyodysenteriae is a useful tool in the control of this disease that can be used as a complementary strategy to antimicrobial therapy and significantly improving the farm productive parameter

Acknowledgments

To Rico Brothers by data provided

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P.014

Reducing fecal levels of *C. perfringens* in sows using dietary *B. subtilis* C-3102 (Calsporin®) direct fed microbial

J Schleifer[1], T Lohrmann[1], N Otomo[2], T Hamaoka[2], T Marubashi[2], M Kato[2], D Hooge[3]
[1]*Quality Technology International (QTI) Inc., Elgin, IL.* [2]*Calpis America, Inc., Peachtree City, GA.* [3]*Hooge Consulting Service Inc., Eagle Mountain, UT,* johns@qtitech.com

Introduction

The US swine industry continues to be plagued by enteric clostridial infections (1). *Clostridium perfringens* type A and type C cause infections in neonatal piglets. *C. perfringens* type A infections are difficult to control due to the commensal nature of this strain (3). The clostridia are commonly found in the feces of the sow which serves as the source of the neonatal pathogen . *C. perfringens* in the spore form is resistant to most cleaning and disinfection methods. This makes the spread of these bacteria difficult to control. Therefore reducing the fecal shedding of *C. perfringens* should have a positive effect toward reducing the incidence of disease in neonates. Previous reports have shown that feeding the DFM (direct fed microbial) *Bacillus subtilis* C-3102 (Calsporin®) to monogastric animals results in a reduction of *C. perfringens* in fecal samples. The purpose of these studies is to assess the effect on fecal *C. perfringens* levels from sows fed Calsporin®.

Materials and Methods

Two separate studies were performed on commercial operations of 5,000 and 1,500 sows respectively. Representative fecal samples were collected from each group prior to administration of the Calsporin®. Varying dosages of the Calsporin® were used in the trials. The dosages fed were at the highest level during gestation and lactation. Bacterial enumeration for *C. perfringens* was conducted on representative fecal samples and at specified time increments during the sow reproductive cycle.

Results

A greater than 2.0 log10 reduction in the levels of *C. perfringens* in fecal samples was observed during the lactation phase within 7 months after initiation of the dietary Calsporin® in the first trial. A similar reduction of clostridial levels was observed in the second trial with in a three month span of time. In the second trial, the swine herd was continually assessed for levels of fecal *C. perfringens*. A continued reduction of *C. perfringens* enumeration rates was observed as the sows entered the lactation cycle.

Conclusions and Discussion

A significant reduction in the levels of *C. perfringens* of sow fecal samples occurred with the use of dietary Calsporin®. The reductions in *C. perfringens* numbers occurred over a span of time of three to seven months. Fecal levels of *C. perfringens* continued to trend lower or remained at a lowered level with continued feeding of Calsporin®. The reduction in levels of *C. perfringens* in fecal samples should result in less pathogen exposure to the neonates. This reduction in fecal clostridial numbers may explain the reduction in pre-weaning mortality rates and increase in the number of pigs weaned observed in Trial 1. Improved weaning weights were observed in Trial 2.

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P.015

Detection of antibodies of the *C. novyi* type B toxoid in swine sera

SM Kim, JY Jung, BE Park, JH Jo, JH Han
College of Veterinary Medicine and Institute of Veterinary Science, Kangwon National University, Chuncheon, Gangwon, Republic of Korea, wowoops11@gmail.com

Introduction

Clostridium(*C.*) *novyi* is a gram-positive, obligate anaerobic, and endospore- forming bacteria. *C. novyi* is classified into 4 strains (A to D type) by the exotoxins, and the lethal and necrotizing alpha toxin is considered to be the principle toxin of the type B strain in pigs. This toxin causes necrosis by increasing permeability of the cell barrier, and disrupting intercellular junctions. So, *C. novyi* type B commonly causes infectious necrotic hepatitis, so called black diseases, and it has been observed in sheep and other animals. The infected liver uniformly infiltrated with many gas bubbles, so the liver presents a spongy appearance on the cut surface. Although the *C. novyi* infections are unusual in pigs, cases of sudden death in sows have been reported in Europe.This experiment detected the production of specific antibodies of *C. novyi* type B toxoid using ELISA in swine sera.

Materials and Methods

In this experiment, sera of 343 sows from 27 farms were used for ELISA(Hipra, Spain) detecting antibodies against *C. novyi* type B toxoid. First, the plate was coated with the *C. novyi* type B antigen diluted 1:100 with carbonate buffer and incubated all night at 4℃ in the humidity chamber. After blocking the plate, the diluted to 1:200 test sera including the positive and negative control sera were added to the plate. For the secondary antibody, the monoclonal antibody against porcine IgG conjugated with horseradish peroxidase was used. Then, the plate was added with the substrate dilution and stop solution and the plate was read at OD 405nm. The sera was evaluated by calculating the reference index percentage for each of them, and the sera was considered as positive if the value of the tested sera bigger than 10.
The tested sample was taken statistics by the positive rate of the total sows and farms.

Results

In this experiment, 129 sows of 21 farms have been revealed as a positive for *C. novyi* type B. So the positive rates of total sows and farms were 77.8% and 37.6% respectively (Table 1). And looking the range of the rate of farms positive for antibodies against *C. novyi* type B, there were most of farms at range 33~66% (Table 2).

Table1. Positive rate of antibodies against *C. novyi* type B infection by farms and sows

	Farms	Sows
	21/27*	**129/343***
Positive rate (%)	**77.8**	**37.6**

*No. of positive / Total

Table2. Distribution according to positive rate of antibodies against *C. novyi*

	Positeve rate			
	0	0<x≤33	33<x≤66	66<x≤100
Farms	**6/27***	**6/27**	**12/27**	**27/3**
%	**22.2**	**22.2**	**44.4**	**11.2**

*No. of positive / Total

Conclusions

Although the infection of the *C. novyi* type B is uncommon, these results can be a useful data for further investigation of *C. novyi* type B because there was few survey detecting the infection of *C. novyi* type B for now.

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Frequency of *E. coli* ETEC positive for K88 fimbriae (F4) in farms with post-weaning diarrhea in Brazil

M Moreno[1]; O Eckhardt[2], E Nadeau[3]; JS Ferreira Neto[4]; MR Felizardo[4]; VTM Gomes[4], AM Moreno[4]
[1]*Virbac do Brasil Ind. e Com. Ltda, Brazil,* [2]*Virbac SA, France,* [3]*Previtec Microbia Inc., Canada* [4]*Department of Preventive Veterinary Medicine and Animal Health, University of São Paulo, SP – Brazil*
marina.moreno@virbac.com.br

Introduction

In swine, the *Escherichia coli* species presents a variety of strains, mostly non-pathogenic and are a part of the normal population of bacteria in the digestive system. However, some subpopulations of *E. coli* may exhibit virulence factors leading to the emergence of diseases, including diarrhea[2]. The post weaning diarrhea caused by enterotoxigenic *E. coli* (ETEC) has occurred worldwide and is recognized as a disease of great impact on pig production, since it leads to a significant reduction performance and can cause increase of mortality[1]. The aim of this study was to evaluate which fimbriae were involved in the pathogenesis and which toxins produced by ETEC strains were involved in cases of post-weaning diarrhea in commercial farms in Brazil, with emphasis on the frequency of ETEC isolates with F4 fimbriae.

Materials and Methods

Between November 2011 and April 2013 were collected rectal swabs from 579 pigs from 46 farms located in different regions of Brazil. Swabs were collected from animals with clinical signs of diarrhea in the beginning of nursery at the mean age of 20 to 30 days. Fecal swabs with Stuart transport medium were sent to the Laboratory of Swine Health and Virology - FMVZ - USP under refrigeration. The cultivation was conducted in McConkey agar, blood agar base with 5% of defibrinated sheep blood and Luria Bertani (LB) broth incubated at 37°C for 18-24 hours. An aliquot of 200µl of LB broth was subjected to DNA extraction by boiling method. From positive animals (positive in LB broth), five colonies from agar plates were selected and confirmed as positive to virulence genes. *E. coli* strains and LB broth were investigated for genes encoding the toxins STa, STb, LT and VTe and F18 and K88 (F4) fimbriae[3]. The amplified fragments were stained with the dye Blue Green (LGC Biotechnology) and observed under ultraviolet illumination.

Results

In 43.48% of farms with problems of diarrhea, *E. coli* positive for F4 fimbriae was present, whereas *E. coli* positive for F18 fimbriae was present in 39.13% of the farms. Among the farms positive for *E. coli*, the association of F4 and F18 fimbriae was observed in 33% of the farms. With the toxins produced, the most frequent was the STb, present in 82.61% of cases, followed by STa in 80.43% and LT in 54.35% of cases. The VTE toxin was also investigated and was detected in 10.87% of cases. Considering the individuals in a total of 579 piglets with diarrhea in post-weaning, were observed the presence of *E. coli* positive for F4 fimbriae in 25.51% of the animals.

Conclusions and Discussion

This study in Brazil showed that ETEC-F4 is an important agent involved in the pathogenesis of post weaning diarrhea. However, the lack of clinical signs as consequence of the intensive use of antimicrobials in this phase of production makes difficult the correct diagnostic of this problem. But due to the possible impact on performance, specific prevention strategies should be implemented to improve livestock performance and better financial results in the production.

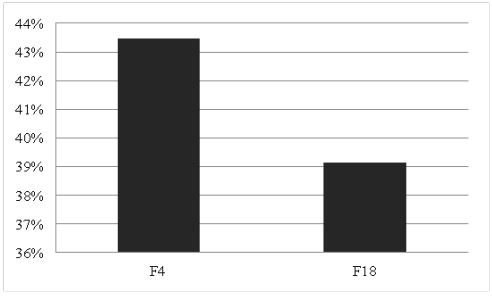

Figure 1. Percentage of farms with at least one sample positive for *E. coli* ETEC with F4 and F18 fimbriae.

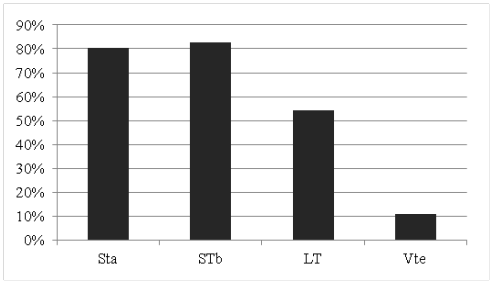

Figure 2. Frequency of farms with at least one sample of *E. coli* positive for the toxins STa, STb, LT and Vte.

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Prevalence of haemolytic *E. coli* in piglets in Australian commercial pig herds

L van Breda, M Ward, O Dhungyel
Faculty of Veterinary Science, University of Sydney, Australia, lechelle.vanbreda@sydney.edu.au

Introduction

Pathogenic *Escherichia coli* (*E. coli*) disease causes severe diarrhoea in weaner and suckling piglets. It is a cause for substantial concern as significant production losses are experienced, including reduced growth rates, high medication costs and high levels of mortality and morbidity [1]. Piglets are most susceptible to *E. coli* disease during weaning, when they are experiencing changes in diet, gut flora and movement to a new environment [2]. There are several pathogens responsible for causing diarrhoea in piglets, including enterotoxigenic strains of *E. coli* (ETEC) which generally appear haemolytic [3, 4]. The aim of this current study was to identify and determine the prevalence of *E. coli* disease in South-eastern Australian pig herds.

Materials and Methods

Faecal samples were collected off the floor from 17 farrow-to-finish farms over a 6-month period to determine beta-haemolytic *E. coli* prevelance. To determine pen- and herd-prevalence 50 samples from each farm, 5 per pen, 10 from pre-weaned and 40 from post-weaned piglets were collected. Selective culture for haemolytic *E. coli* was performed on Sheep blood agar and also by selective enrichment in Buffered-peptone water. DNA extractions were performed using the boiling technique and PCR were performed on 16S gene for *E. coli* isolate confirmation.

Results

A total of 870 faecal samples were collected, from which haemolytic *E. coli* was isolated from 368 (42%) of the isolates were, including 49 of 236 (21%) isolates from pre-weaned piglets and 319 of 624 (51%) isolates from post-weaned piglets. Prevalence level of haemolytic *E. coli* is shown in Table 1. The mean and median pen prevalence was 49% and 40%, respectively.

Table 1. Total number of faecal samples collected from Australian pig farms.

Prevalence of haemolytic *E. coli* (%)	Number of farms in each category	Diarrhoeal samples
High (100-71%)	3	45 (150)[1]
Medium (31-70%)	7	62 (350)
Low (0-30%)	7	25 (370)

[1] Total number of samples collected from each farm in parentheses.

Conclusions and Discussion

Beta-haemolytic *E.coli* strains were present at farms displaying both clinical and sub-clinical cases of diarrhoea. Survival of these strains in the environment could possibly lead to further spread of infection via the fecal-oral route. Further analysis is required to determine pathogensisity and virulence properties.

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Pharmacokinetics of marbofloxacin in porcine uterine tissue after a single intramuscular dose of 8mg/kg and PKPD integration

M Schneider[1], FEl Garch[1], PA Perrin[2], F Woehrlé[1]
[1]*Vétoquinol Global Development Centre, 70200 Lure, France,* [2]*Vétoquinol Research & Medical Department, 75009 Paris, France.* pierre-alexandre.perrin@vetoquinol.com

Introduction

Marbofloxacin was recently approved in sows for the treatment of *Escherichia coli* induced Metritis Mastitis Agalactiae (MMA) syndrome as a single intramuscular administration of Forcyl®, a 16% solution for injection, at a dose of 8mg/kg. An epidemiological survey performed from 2005 to 2009 in different European countries showed that about 50% of the bacterial pathogens involved in infectious MMA syndrome are *E. coli*, and their susceptibility remained stable (1). The geometric mean MIC_{90} value for the *E. coli* strains collected in the study (n = 234) was 0.32µg/mL.

Materials and Methods

For dose optimization, PK/PD integration was performed using the epidemiological survey results and the marbofloxacin concentration in the entire uterine, given the fluid associated with metritis is very difficult to collect for a pharmacokinetic study. The Mutant Prevention Concentration (MPC) was determined by a previously described method (2) for *E. coli* with various MICs. A single dose of Forcyl® (8mg/kg) was administered intramuscularly to 6 sows weighing 203 to 222kg, 1 to 3 days post partum, and to 18 gilts weighing 47 to 62kg. The sows represent the true target population but gilts were also included in order to perform the PKPD integration with more accurate values. The animals were sacrificed 1, 2, 4, 6, 10 and 24h after administration (1 sow and 3 gilts at each time point). Blood and the whole uterus were collected and the concentration of marbofloxacin was determined with HPLC methods.

Results

At all time points, the concentration of marbofloxacin in the uterine tissue was about 50% higher than in plasma (Figure 1). The C_{max} and the AUC values in the uterine tissue were 8.43µg/g and 121µg.h/g respectively in the sows and 9.35µg/g and 105.4µg.h/g respectively in the gilts. Thus the values obtained in gilts may be considered as a worst case.

The MPC value obtained for *E. coli* strains with MIC=0.5µg/ml representative of the MIC_{90} modal class, was 2µg/ml thus a ratio MPC/MIC of 4. Considering the MIC_{90} value, an estimated MPC_{90} of 1.29µg/ml was determined.

The ratio of uterine C_{max} values divided by the MIC_{90} value was 26.3 in sows and 29.2 in gilts. The ratio of uterine AUC values divided by the MIC_{90} value (AUIC) was 378h in sows and 329h in gilts. The ratio of uterine AUC values divided by the estimated MPC_{90} value was 94h in sows and 82h in gilts.

Conclusions and Discussion

Considering *E. coli* MIC_{90}, the commonly accepted surrogate markers of clinical efficacy for fluoroquinolones (a value of 10 for the ratio C_{max} / MIC and of 125h for the AUIC) (3, 4) were reached in plasma and in uterine tissues, meaning that clinical success of a single 8mg/kg dose of marbofloxacin for the treatment of *E. coli* metritis is expected.

More recently, a pharmacodynamic threshold at which resistant mutants are not likely to be selected in vivo, has been determined for fluoroquinolones and *E. coli* (a value higher than 20h for the ratio AUC/MPC at the infection site (5). Considering *E. coli* estimated MPC_{90}, this threshold was exceeded meaning that emergence of resistance in the target pathogens is not likely to occur with this dose regimen.

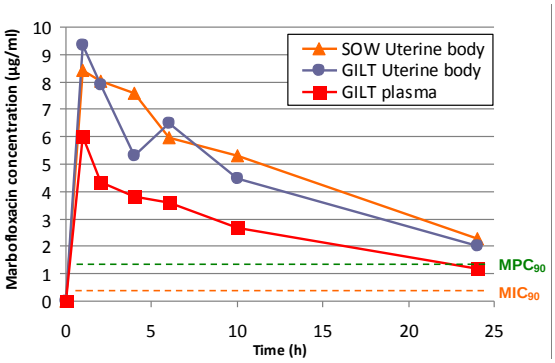

Figure 1. Mean marbofloxacin concentration in plasma (gilts) and uterine tissues (gilts and sows) relevant to *E. coli* MIC_{90} and estimated MPC_{90}.

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Frequency distribution of *H. parasuis* serotypes from clinically suspicious cases

E Banholzer[1], J Boehmer[2], A Koehrmann[1], D Goldstein[2], K Strutzberg-Minder[2]
[1]*Zoetis Deutschland GmbH, Berlin,* [2]*IVD GmbH Innovative Veterinary Diagnostic Laboratory, Hannover, Germany,* annika.koehrmann@zoetis.com

Introduction

Haemophilus parasuis (Hps) colonizes the upper respiratory tract of clinically healthy pigs as a commensal microorganism but it is also the etiological agent of Glässer's disease (fibrinous polyserositis and arthritis) and can cause pneumonia and meningitis in swine. Isolates were grouped on the basis of similar antigens into 15 serotypes (1), correlating to some degree with the grade of virulence (2) and legend of Fig.1.

Materials and Methods

458 *Hps* isolates from October 2009 to May 2013 were serotyped by indirect hemagglutination (IHA; 3). The isolates originated from autopsies of swine suspected of Glässer's disease or any other disorder caused by *Hps*. Species identity was confirmed by PCR of single colonies (IVD, 2009). The distribution of *Hps* serotypes was analyzed according to the origin of isolates.

Results

356 *Hps* (77.7%) were isolated from the respiratory tract (bronchoalveolar lavage fluid, bronchus and lungs) and 58 *Hps* from the thoracic and abdominal cavities (thoracic cavity, pleura, heart, pericardium and peritoneum). The remaining 44 *Hps* isolates were cultured from the central nervous system, joints, and unspecified organs or tissues.

Most *Hps* were isolated from weaned pigs (n = 339). 315 (68.8%) of all isolates originated from the respiratory tract of piglets. The most prevalent of all *Hps* isolates was serotype 4 (n = 84), followed by serotypes 2 and 13 (n = 60), 5 (n = 50), 1 (n = 48), 12 (n = 33), 15 (n = 32). Less prevalent (< 5%) were serotypes 3 (n = 10), 6 (n = 15), 7 (n = 13), 8 (n = 4), 9 (n = 12), 10 (n = 3), 11 (n = 10), 14 (n = 6). In all, only 17 of the isolates (3.8%) showed no reaction with any of the 15 serotype-specific antisera and were thus not typeable (n.t.).

Analysis of serotype distribution from isolates originating only from the respiratory tract showed a very similar profile (Fig.1A), while the distribution profile of *Hps* isolates from the thoracic and abdominal cavity was different (Fig.1B). The most prevalent serotype was 13 (n = 11), followed by 4 (n = 7), and 1 and 15 (both n = 6); frequencies of serotypes less prevalent both generally and in the respiratory tract (e.g. 7 and 9) were relatively high (8.6% and 6.9%), but the numbers of isolates were much lower.

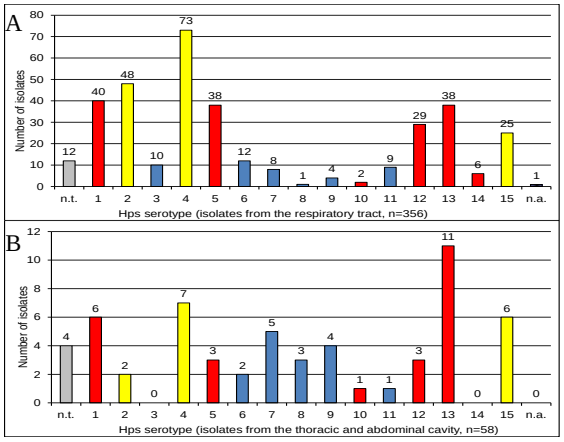

Figure 1. Frequency distribution of *Hps* serotypes isolated (A) from the respiratory tract and (B) from the thoracic and abdominal cavity of swine. *Red columns represent serotypes associated with death; yellow with a moderate to severe degree of virulence; blue, with no lesions (2); grey: n.t. (not typeable); black: n.a. (not analyzable). Please note different y-axis scales.*

Conclusions and Discussion

This is the first study since 1998 (5) to analyze the frequency distribution of *Hps* in a defined population. Additional information e.g. origin of isolate and age group of its porcine host made secondary epidemiological analysis possible. *Hps* was isolated predominantly from the bronchus or lungs of weaned pigs, and serotype 4 was the most prevalent. As no strong correlation has yet been shown between serotype and virulence, and molecular typing methods also failed to determine the virulence (4), serotyping is therefore still a useful tool for analyzing isolates on the basis of antigenicity. Analysis of the origin of *Hps* isolation revealed different serotype distribution profiles, and there may be different associations of different serotypes with different tissue types, but more isolates from these sources must be analyzed to establish such associations.

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Prevalence of faecal shedding of *L. intracellularis* in fattening pigs in the United Kingdom and Ireland

A Hidalgo, P Macdonald, A Cox
Elanco Animal Health, UK and Ireland, hidalgo_alvaro@elanco.com

Introduction

Lawsonia intracellularis is the aetiological agent of porcine proliferative enteropathy, commonly referred as ileitis. Three forms of the disease have been described: acute, chronic and subclinical ileitis (1). Subclinical ileitis is mainly characterized by impaired growth and worsened performance (1,2). This study aims to investigate the prevalence of faecal shedding of *Lawsonia intracellularis* in fattening pigs to gain knowledge in the epidemiology of ileitis.

Materials and Methods

Faecal samples were collected from 640 pigs between 6 and 19 weeks of age in 24 commercial pig farms across UK and Ireland in 2012. Ten pigs were sampled at random per group of age (n=64). A minimum of 2 and a maximum of 4 groups of age were sampled per farm. Samples were analysed using Lawsonia FIRSTtest (MicroCoat Biotechnologie GmbH), an ELISA-based test for detection of *L. intracellularis*. A cut-off value ≥ 3 positive samples within a group was used for positive batches, correlating with a prevalence of ≥ 40% pigs shedding *L. intracellularis* within group (3).

Results

L. intracellularis was detected in 308 out of 640 (48.13%) faecal samples. The prevalence of farms with at least one pig shedding *L. intracellularis* was 95.83%. In 49 out of 64 (76.56%) analysed batches, 3 or more samples tested positive within a batch. The prevalence of farms where at least 40% of the pigs within a batch were shedding *L. intracellularis* was 87.5% (21 out of 24). The distribution of positive groups by week of production is shown in Figure 1.

Conclusions and Discussion

This study shows that *L. intracellularis* is highly prevalent in the UK and Ireland, with 95.83% of the farms investigated presenting one or more pigs shedding bacteria at the time of sampling. Our results are in agreement with previous serological studies (4, 5). Prevalence of groups shedding *L. intracellularis* increased slightly from 6-8 to 9-11 week group, peaking at 12-14 weeks (93% of positive groups). After that, it decreased progressively, with 50 % of the groups above 17 weeks being positive. This distribution pattern suggests that for most of the farms transmission rate achieves a maximum during the growing period, being consistent with a grower infection pattern (6). On the other hand, the initial high prevalence for the 6-8 week group could indicate predominantly farms with a nursery infection pattern, as previously described (6). It is noteworthy that a considerable proportion of pigs were eliminating bacteria at later stages of production (>17 weeks). It is known that infected pigs can eliminate bacteria for long periods of time (7); however, recent infections at this stage cannot be excluded.

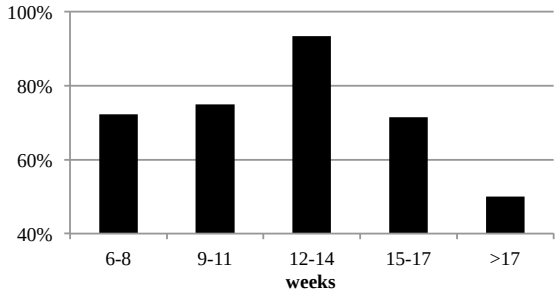

Figure 1. Distribution of positive groups by age.

Under experimental conditions, it has been shown that shedding of *L. intracellularis* in faeces precedes seroconversion (8). In field studies, a span of 2-8 weeks between the two events has been reported (9, 10), making it difficult to rely merely on serological diagnostic tools to determine the time of infection in a pig population. In this study, the use of Lawsonia FIRSTtest in cohorts of pigs enabled to identify when shedding started. Knowing the time of infection is essential to implement comprehensive control measures in farm and minimize the impact of ileitis. That is particularly important in cases of subclinical ileitis in which diarrhoea may not be present but average daily gain and feed conversion ratio are severely affected (2).
In summary, *L. intracellularis* is still highly prevalent in pig farms in UK and Ireland. The use of Lawsonia FIRSTtest allows the implementation of control measures based on the time of infection.

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Effect of oral vaccination against *L. intracellularis* in the fattening units through drinking water in a Spanish pig production company

V Rodriguez-Vega[1], S Figueras-Gourgues[1], I Hernández-Caravaca[1]
[1]*Boehringer-Ingelheim España. Spain*, victor_2.rodriguez@boehringer-ingelheim.com

Introduction

Lawsonia intracellularis (L.i.) is the causative agent of porcine proliferative enteropathy (PPE). PPE is a relevant economic enteric disease that causes diarrhea and reduces weight gain in growing pigs (1). The subclinical form has a negative impact on performance as well as farm economics. L.i. is endemic in most of the Spanish farms (2). The aim of this study was to evaluate the efficacy of Enterisol® Ileitis (Boehringer Ingelheim Vetmedica GmbH) in a commercial pig production system in Spain.

Materials and Methods

This study was conducted in a production system with 6 multi-site sow farms (total 2,500 sows) located in the eastern part of Spain. Pigs at fattening were suffering clinical PPE and L.i. infection was confirmed by ELISA (IgG). A total of 37,125 fattening pigs were included into the study (32,456 non-vaccinated and 4,969 vaccinated with the oral atenuant live vaccine Enterisol® Ileitis). From March 2013 all the piglets were orally vaccinated via drinking water after placement in the fattening unit using Thiosulfate Blue (Boehringer Ingelheim Vetmedica GmbH) as water stabilizer. All the animals were raised under similar conditions. To compare batches before and after vaccination, the parameters recorded were: average daily gain (ADG, g/d), feed conversion rate (FCR), FCR corrected (FCR_{18-100}), mortality rate (%) and medication costs (€). Data has been analyzed using two methods. To analyze whether the vaccination had modified the performance pattern, a Statistical Process Control (SPC) was carried out using R software (qcc package). To analyze the effect of the treatment an analysis of variance was performed using SAS (Cary, NC). The Return of Investment of vaccination was calculated using "BECAL calculator" provided by Boehringer Ingelheim, using an average price for feed of 300 €/ton and for the slaughtered pigs of 1.37 €/kg.

Results

The results are summarized in Table 1. Due to differences in initial and final weight, corrected FCR 18-100 was used. The average mortality was 19.47% lower in vaccinated group (4.59 % vs 5.7 %) and in addition FCR_{18-100} was reduced by 0.1 in the vaccinated group.

Table 1. Efficacy of Enterisol Ileitis® in fattening.

Variable	Non vaccinated	Vaccinated	P-value
Animals (batches)	32.456 (42)	4.969 (8)	
Animals per batch	772	621	
Start weight	20.3 ± 0.34	18.4 ±0.80	0.0333(*)
End weight	112.3 ± 0.52	110.9 ± 1.96	0.2949(NS)
FCR	2.67 ± 0.019	2.54 ± 0.045	0.0107 (*)
FCR 18-100	2.55 ± 0.020	2.45 ± 0.045	0.0528 (NS)
Mortality (%)	5.70 ± 0.34	4.59 ± 0.78	0.2003 (NS)
Med. cost (€/pig)	3.31 ± 0.55	3.50 ± 1.26	0.8879 (NS)

NS: non signifiant; * p<0.05

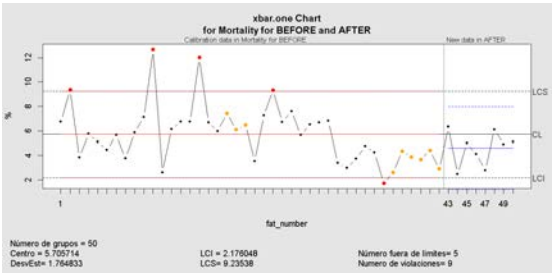

Figure 1. SPC (Statistical Process Control) graph showing the impact of the vaccination on mortality and FCR_{18-100}. The Upper and Lower Control limits (UCL and LCL) were calculated using 2 standard deviations.

Conclusions

In this field observation, FCR was significantly improved and mortality reduced in the batches vaccinated with Enterisol® Ileitis. An economic evaluation of the vaccination resulted in a ROI of 2.23:1.

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The influence on performance in fattening pigs of *L. intracellularis* and PCV2 infections as measured in feces by Q-PCR

GJR Groenland[1], VNAM Geurts[2]
[1]*De Heus Voeders b.v. Ede, The Netherlands, ggroenland@de-heus.nl,* [2] *MSD-AH, Boxmeer, The Netherlands,*
g.j.r.groenland@hotmail.com

Introduction

There often reports of disappointing growth rates in the absence of any clinical signs in fattening pigs, when from their genetic capacity, feed quality and feed intake, better performance would be expected. When there are no respiratory problems, intestinal disorders are most probably responsible. Two important pathogens of the intestine in the Netherlands are *Lawsonia intracellularis* (Li) and PCV2. The aim of this study was to investigate the influence of Li and PCV2 infections on performance by measuring the level of both pathogens in the feces by Q-PCR in field situations.

Materials and Methods

On the trial farm ('the Vlierbos' of de Heus) 656 fatt. pigs from three different farms were housed separately in nine units each containing eight pens. The piglets were vaccinated against *Mycoplasma hyopneumoniae* and PRRS, but not against Li or PCV2. The animals in each pen were weighed, their feed intake measured, and feces and blood samples taken at 10, 15, 20 and 25 weeks of age. Diarrhea was rarely observed. A pen's fecal sample consisted of a mixture of five teaspoonsful of the wettest feces in the pen[1]. The samples were analyzed for Li and PCV2 by Q-PCR at the GD-lab in Deventer. The detection level for PCV2 was 50,000 and for Li 300 DNA copies/ml. Growth (ADG) and feed intake (ADFI) were calculated for each growth period. Decreases and increases in DNA copies for both Li and PCV2 were compared between the 72 pens, and the significance of any differences was calculated using ANOVA. The possible influence of farm of origin, total Li and total PCV2 count (from 10 to 25 weeks) was determined by regression variance analysis.

Results

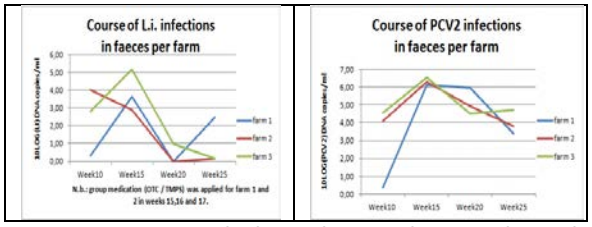

Figure 1. The course of infection for each of the three farms of origin. *Farms 2 and 3 already had Li and PCV2 infections at week 10. Medication of pigs on farms 1 and 2 at 15-17 weeks with OTC and TMPS resulted in a sharp decrease in Li in the feces. Pens on farm 1 which had had no infections at the start became infected later.*

In Table 2 ADG and ADFI is shown for pens with an increase/decrease for Li and PCV2 between 10-15 resp. 15-20 weeks.

Table 2. Results for ADG and ADFI

Li: 10-15 wk	n pens	ADG 10-15 wk	ADFI 10-15 wk	ADG 15-20 wk	ADFI 15-20 wk	ADG 20-25 wk	ADFI 20-25 wk	ADG total
decr.	17	748 [a]	1,443 [a]	849 [a]	1,901 [a]	944	2,718	860
incr.	48	669 [b]	1,271 [b]	755 [b]	1,710 [b]	917	2,541	799
diff.		-78	-0,173	-94	-0,191	-27	-0,177	-61
PCV2: 15-20	**n pens**	**ADG 10-15 wk**	**ADFI 10-15 wk**	**ADG 15-20 wk**	**ADFI 15-20 wk**	**ADG 20-25 wk**	**ADFI 20-25 wk**	**ADG total**
decr.	53	692	1,313	789	1,782	934	2,631	822
incr.	18	668	1,333	743	1,654	913	2,481	795
diff.		-24	0,019	-46	-0,128	-22	-0,150	-27

By regression analysis, the significance of the influence of farm, total Li and total PCV2 count (from 10 to 25 weeks) was P=0.000, 0.045 and 0.820 respectively.
Total count for each pen was determined by the highest Log_{10} value recorded during the fattening period.

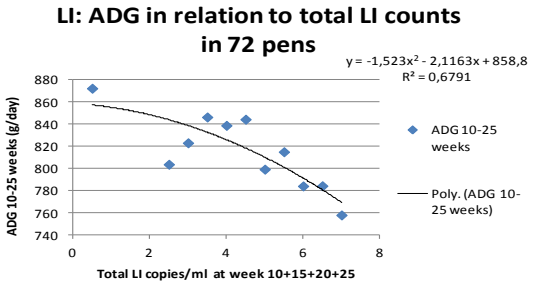

Figure 3. The relationship between total Li count and ADG from 10 to 15 weeks

Conclusions and Discussion

For Li, pens with an increase in DNA copies between 10 and 15 weeks, but also between 15 and 20 weeks had a significantly lower ADG and ADFI compared with pens with a decreased number of copies. For PCV2 there is the same trend but differences were not significant.
In this field trial pens with high total copies of Li had lower ADG, which has been shown by A. Collins after experimental challenge [2].
The 3 farms exhibited different contamination levels of Li and PCV2 in feces as measured by Q-PCR. The farm with the lowest Li and PCV2 counts at10 weeks of age experienced the greatest increase in pathogens during the first 5 weeks. Significant reductions in ADG and ADFI were evident in animals with a raised fecal level of Li. By regression analysis, farm origin which include breed, birthweight and infection status, had the most significant influence on ADG, followed by total Li count.
Even in the absence of clinical signs, growth rate and feed intake are likely to be reduced by Li infections. There is a trend that also PCV2 infections can exert a similar negative influence.

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P.023

Seasonal variation in *M. hyopneumoniae* prevalence in weaned piglets in Spain

P Sanchez[1], P Nuňez[1], A Saez[2], G Ramis[2]
[1] *Elanco Animal Health, Alcobendas, Spain,* [2] *University of Murcia, Murcia, Spain,*
sanchez_uribe_pedro_jose@elanco.com

It has been demonstrated by several research groups that infections with *Mycoplasma hyopneumoniae* (*M.hyo*) in Spanish pig herds may already occur starting from 21 days of age (*Sibila et al., 2007*; *Villarreal et al., 2010; Segalés et al., 2012*). The aim of the present study was to elucidate eventual differences in the detection rate of *M.hyo* and its quantities in Spanish piglets around weaning age between seasons and months. The study was conducted between February 2012 and June 2013 in a total of 43 Spanish pig herds. In each herd, at least 30 tracheo-bronchial swabs were collected (*Fablet et al., 2012*) from 3- to 4-weeks-old piglets and tested for the presence of *M.hyo* using a RT-PCR assay (*Marois et al., 2010*). 28 out of the 40 tested herds (70.0%) and 242 out of the 1256 tested piglets (19.3%) tested positive for *M.hyo*. The prevalence of *M.hyo* in spring (178/540 piglets; 33.0%) was significantly higher than the one in the 3 other seasons ($P<0.001$). Similarly, the prevalence in April (172/510 piglets; 33.7%) was significantly higher than the one in the other months of the year ($P<0.001$). The lowest prevalence was observed during July (7/120 piglets; 5.8%). Analysis of the *M.hyo* quantities in the tracheo-bronchial swabs revealed significant differences between winter and the other 3 seasons ($P=0.0045$) and between February and the other months of the year ($P=0.001$). In conclusion, the present study confirmed that 3- to 4-weeks-old piglets may already be infected with *M.hyo* and that the season and months of sampling determined the probability for being positive for *M.hyo* at 3 to 4 weeks of age. Indeed, samplings in spring and April were positively associated with the probability of being *M.hyo*-positive at 3 to 4 weeks of age, even if the *M.hyo* quantities were highest in winter and February.

Efficacy of a vaccination against *M. hyopneumoniae* at seven days of age under field conditions

P Sanchez[1], P Nuñez[1], G Labarque[2]
[1]*Elanco, Alcobendas, Spain,* [2]*Elanco, Neuilly sur Seine, France*
sanchez_uribe_pedro_jose@elanco.com

Introduction

Mycoplasma hyopneumoniae (*M.hyo*) is the primary pathogen of enzootic pneumonia, a chronic respiratory disease in pigs. Moreover, *M.hyo* is considered to be one of the primary agents involved in the porcine respiratory disease complex (PRDC). It has been demonstrated that infections with *M.hyo* in Spanish pig herds may already occur starting from 21 days of age (5,6,8). Stellamune® Once (Elanco Animal Health) has been shown to provide a protective immunity starting from 21 days of age following its administration at 7 days of age (7). The objective of this study was to confirm the efficacy of this vaccination schedule under field conditions by comparing its performance with another one-shot *M.hyo* vaccine administered at 7 days of age.

Materials and Methods

The study was conducted in an *M.hyo*-positive farrow-to-finish herd in Spain between November 2012 and May 2013. Before the beginning of the study, piglets were vaccinated against *M.hyo* with a one-shot vaccine at 21 days of age. Two consecutive batches were included. The piglets of each of the two batches were randomly divided into two treatment groups: one treatment group (n=481 pigs) was vaccinated with Stellamune® One (Elanco Animal Health) at 7 days of age and the other treatment group (n=475 pigs) was vaccinated with another one-shot *M.hyo* vaccine (designated vaccine A) at the same age. Both *M.hyo* vaccines were administered according to the manufacturer's instructions. Tracheo-bronchial swabs (1) were collected of 30 four-weeks-old piglets and tested for the presence of *M.hyo*, using a real-time polymerase chain reaction assay (4). The efficacy of both *M.hyo* vaccines was evaluated using clinical [the results of post-mortem lung examinations at the slaughterhouse] and growth performance parameters during the fattening period [average daily weight gain (ADWG), feed conversion ratio (FCR), carcass weight at slaughter]. Data were statistically analyzed using the JMP® statistical software version 9.0.3 (SAS Institute Inc., Cary, NC, USA).

Results

The tracheo-bronchial swabs of 2 out of the 30 piglets sampled at 4 weeks of age (6.7%) tested positive for *M.hyo*, indicting the existence of early infections with *M.hyo*. The results of the study are summarized in Table 1. No respiratory signs were observed during the study. There were no significant differences in the studied parameters between the two treatment groups, except for the prevalence of pleurisy at slaughter and the FCR between 150 and 170 days of age (P<0.05). Although not statistically different, the ADWG during the fattening period was numerically (5 g/pig/day) higher in the pigs vaccinated with Stellamune® One when compared to the pigs vaccinated with vaccine A.

Table 1. Clinical observations and growth performances in the fattening period following two different vaccination schedules against *M.hyo*.

Parameter	Stellamune® One (7 days)	Vaccine A (7 days)
% of pigs with lung lesions	73.4%^{A}	75.3%^{A}
Lung lesion score of all lungs	5.30^{A}	4.05^{A}
Lung lesion score of affected lungs	9.23^{A}	9.47^{A}
% of pigs with pleurisy	6.6%^{A}	16.5%^{B}
ADWG (g/pig/day)	675^{A}	670^{A}
FCR (150-170 days of age)	3.16^{A}	3.59^{B}
Carcass weight (kg)	84.67^{A}	84.50^{A}

^{A,B}: Superscripts indicate statistically significant differences between treatment groups (P<0.05).

Conclusions and Discussion

The herd where the study was conducted was exposed to a high *M.hyo* challenge, as evidenced by the high pneumonia rate observed at slaughter (74.3%). The detection of *M.hyo* in tracheo-bronchial swabs in some pigs at 4 weeks of age indicates that the *M.hyo* infection started already in the nursery or the early post-weaning period. The latter confirms the widespread prevalence of *M.hyo* infections at weaning age under Spanish field conditions (5,6,8). The use of Stellamune® One at 7 days of age reduced the consequences of the high infectious pressure with *M.hyo* in this herd, as evidenced by a lower pleurisy rate and a better zootechnical performance when compared to a vaccination with another one-shot *M.hyo* vaccine also administered at 7 days of age. The etiology of pleurisy in the present study was not investigated, but pleurisy has been shown to be associated with a high percentage of *Actinobacillus pleuropneumoniae* (APP)-positive pigs at herd level (2). Since previous studies have demonstrated that *M.hyo* may potentiate APP infections under experimental conditions (3), the present study suggests that an effective control of *M.hyo* may reduce the clinical consequences of an APP infection.

Acknowledgments

The authors are grateful to the herd owner and the herd veterinarian for their collaboration.

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P.025

Lung lesion scoring, a valuable tool to decide on *M. hyopneumoniae* vaccination

S Gram[1], P Sanchez,
Elanco Animal Health, [1]gram@elanco.com

Introduction

The SPF health Department in Denmark surveys 2797 production herds and 262 breeding herds. 70% of the production herds (1975 farms) and 28,6% (75 farms) of the breeding herds are positive for Mycoplasma Hyopneumoniae[1]. On these farms, the farmer and their veterinarian need to decide on the need for vaccination against M. Hyo and evaluate the return of investment (ROI). Several studies show that the percentage of the lungs being affected with M. Hyo will correlate to loss in daily weight gain (ADG) from 23-71 gram per 10% lung affected[2,3]. This poster will look at lung lesion scorings done at 121 farms in total: 78 farms that do not vaccinate, 25 farms that vaccinate with Stellamune One (Elanco) and 18 farms that vaccinate with Ingelvacc Mycoflex (Boehringer Ingelheim). Lung lesion scoring as a diagnostic mean to decide on the need for and benefit of vaccination will be investigated.

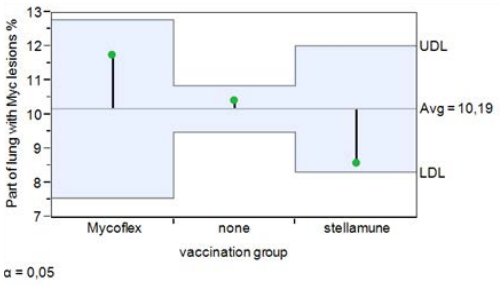
Materiasl and Methods

Lung Lesion Scoring is done routinely in Denmark. The veterinarian can request the slaughterhouse to take out 25 sets of lungs and have them send to the laboratory at the Pig Research Center for further evaluation. The lungs will be scored according the method developed by Gorm Christensen[4] which scores the lungs based on the percentage of the lung being affected. When examining the lung, all parts of the lungs are thoroughly palpated and inspected. The different types of pneumonia are divided into the groups: Uncomplicated catarrhal pneumonia, complicated catarrhal pneumonia, scars from older M. Hyo infections, and finally acute and chronic pleura-pneumonia. The volumes of the different pneumonic types are registered as percentage of the lung volume. Pleura are examined for fibrous or fibrinous pleurisy and the affected areas are registered as the percentage of the lung surface area. Furthermore it is registered whether the changes are located in the cranio-ventral or the dorsocaudal part of the lungs.
For each farm, the following information has been registered: vaccination, total number of lungs, number of lungs with M. hyo lesions, % part of lung with M. Hyo lesions, number of lungs with scars, % part of lung with scars, and number of lungs with at least one lesion due to M. Hyo. The data have been analyzed using JMP version 9.A. Tukey test was used to compare all means in each case.

Results

In the Stellamune One vaccinated group there was a significant lower *total number of lungs with M. Hyo lesions* compared to Mycoflex vaccinated pigs (P=0,0372) and to the non-vaccinated group (P=0,004). There was also a significant difference between Stellamune One vaccinated pig to non-vaccinated in number of *lungs with at least one lesion due to M Hyo* (P=0,0162).

	Non Vacc	Ingelvacc Mycoflex	Stellamune One
Total farms	78	14	25
Total number of lungs	1686	311	549
Lungs with M. Hyo lesions,	813[a]	152[a]	158[b]
Lungs with lesion, % of total	48,2	48,9	28,8
Extent of lesions per lung, %	11,8	11,8	8,6
Lungs with scars	392	60	88
Lungs with scars, % of total	23,3	19,3	16
Extent of scar lesions per lung, %	4,0	4,8	3,4
Lungs with at least ONE lesion due to M Hyo	1011[a]	191 [a,b]	223[b]
Lungs with at least ONE lesion due to M Hyo, % of total	60%	61,4%	40,6%



There is a tendency that Stellamune One vaccinated pigs have *smaller lesions* than Ingelvac Mycoflex vaccinated pigs (P=0.0852) and *lower number of lungs with at least one lesion* due to M Hyo (P=0.0663). However, there were not cases enough to show a significant difference (P<0,05).
There were no significant differences between the Ingelvac Mycoflex vaccinated pigs to the non-vaccinated pigs in any of the parameters that were tested.

Discussion

The lung lesion scoring is a macroscopic diagnostic tool that demands a great deal of experience from the pathologist performing it in order to ensure a consistent and uniform evaluation of the lungs. The laboratory at the Pig Research Center in Denmark perform around 300 lung lesion scorings each year (around 7500 individual lungs) and have a significant experience in doing so. Differential diagnosis to M. Hyo lesions would be PCV, PRRS, APP, Pasteurella Multocida, SIV, and Streptococcus spp infections.

Conclusion

Several studies have concluded that lung lesion scoring correlates to performance results[2,3]. This survey shows that lungs from pigs vaccinated with Stellamune One have significantly lower amount of lesions compared to lungs from non-vaccinated pigs and lungs from Ingelvac Mycoflex vaccinated pigs. Vaccination with Stellamune One should therefore be considered in order to reduce the impact of M. Hyo on pig performance. Lung lesion scoring is a valuable tool to evaluate the need for vaccination but not all vaccines will decrease the amount of lesions.

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An Australian field trial demonstrating equivalence of a novel "one shot" nasal spray live *M. hyopneumoniae* vaccine "Vaxsafe® MHP" and a "two shot" commercial vaccine

Y Abs EL-Osta, R Youil
Bioproperties Pty Ltd. 36 Charter St. Ringwood, VIC. 3134 Australia. Youssef.abs.el-osta@bioproperties.com.au

Introduction

Vaxsafe® MHP is a live attenuated vaccine intended for the protection against *Mycoplasma hyopneumoniae* (Mh) in pigs. A number of pen trials have demonstrated safety, efficacy and colonization of the vaccine in pigs. In this paper, the field efficacy of the vaccine is reported.

Materials and Methods

Efficacy of Vaxsafe® MHP was demonstrated in an "equivalence study" whereby Vaxsafe® MHP was compared to an inactivated commercial vaccine. For confidentiality purposes we refer to that vaccine as (Vaccine X). A commercial farm in Victoria Australia, with a history of Mh infection was selected for the study. The farm normally vaccinates with Vaccine X. Sows farrow once a month over three day period. Farrowed piglets from two consecutive months were involved in the study. Each month, half of the total number of litters was vaccinated with the Vaccine X (control group) and the other half with Vaxsafe® MHP taking into consideration equal distribution of parity between groups. A total of 495 piglets were involved in the study (246 pigs vaccinated with Vaccine X and 249 pigs with Vaxsafe® MHP. Vaccine X was delivered twice by injection into the neck muscle, the first at 3-5 days of age (doa) and the second at 3 weeks of age (woa). Vaxsafe® MHP was administered to piglets of 3-5 doa as a single dose delivered via nasal spray into one nostril. Pigs from both groups were comingled, maintained under normal farm management and received feed and water free from antibiotics. Pigs from both groups were weaned at 3 woa and at 20 woa all pigs were weighed and sent to slaughter at 21-24 woa. All pigs were monitored during the trial for clinical signs. At slaughter, the lungs were removed and examined for pneumonic lung lesions. The percentage of observed lesions in each lobe was recorded using the Hannan method with the maximum lung score of 55.

Results

There were no significant difference in the incidence and severity of lung lesions between the control group and the Vaxsafe® MHP group (Figs 1, A &B). Both vaccines showed significant level of protection from the development of lung lesions. Also, there was no significant difference in the daily weight gain (DWG) (0.61g for control group and 0.60g the group vaccinated with Vaxsafe® MHP).

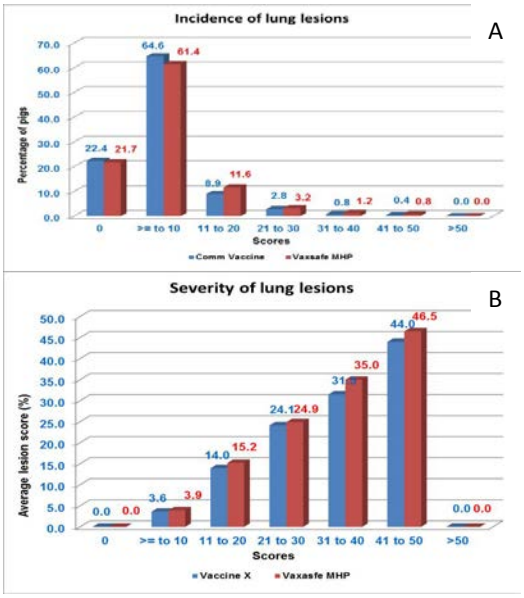

Figure 1. A: incidence and B: severity of lung lesions

Conclusions and Discussion

In Australia, it is hard to find an Mh infected farm that does not vaccinate against Mh. Withdrawal of the vaccination program would raise animal welfare concerns. Therefore, this study was designed as an "equivalence study" comparing the performance of Vaccine X to that of Vaxsafe® MHP. Vaccine X has a demonstrated field efficacy. When administered as a single shot it was shown to reduce the mean lung lesions by 4% when compared to non-vaccinated pigs and also aided in growth performance by reducing weight losses and improved average DWG. Therefore, this was a stringent comparative study between Vaccine X when given twice verses Vaxsafe® MHP that when given once.
In conclusion Vaxsafe® MHP given at 3-5 days of age by nasal spray performed equivocally to Vaccine X when delivered twice by injection at 3-5 doa and at 3 woa.

Acknowledgements

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Large scale field observation of the efficacy of MycoFLEX® in an integrator company

J Herrera[1], S Figueras Gourgues[2], V Rodriguez-Vega[2], I Hernandez Caravaca[2]
[1]Agroturia S.A., Spain [2] Boehringer Ingelheim España S.A., Spain, ivan_2.hernandez@boehringer-ingelheim.com

Introduction

There are a lot of production parameters that directly impact the pig production costs and with increased feed costs .The feed conversion ratio (FCR) is one of the most important parameter. Beside genetics, nutrition and pig density the health status of pigs is a key component influencing FCR. Improving the health status will therefore improve the production parameters of pigs.

Mycoplasma hyopneumoniae (M. hyo), the causative agent of swine enzootic pneumonia, is considered one of the most important agents of chronic diseases in pig herds, as the infection causes reduced performance1.

The objective of this large field study was to evaluate the efficacy of vaccination against Enzootic Pneumonia (Ingelvac MycoFLEX®), when it is used in combination with a vaccine against PCV2 (Ingelvac CircoFLEX®) to improve the parameters that influence directly the pig production costs in a big integrator in Spain.

Materials and Methods

This study was conducted in a 2200-sow, three sites commercial herd in Cuenca, Spain. The herd is positive for PRRS, M. hyo and PCV2 in a subclinical form. Pigs are weaned weekly at 3 weeks of age and slaughtered at 102 kg LW on average. Pigs were vaccinated with Ingelvac CircoFLEX® for more than a year. To evaluate the improvement of the production parameters after introduction of M. hyo vaccination the vaccination program was modified by the introduction of FLEXcombo® (Ingelvac CircoFLEX® mixed with Ingelvac MycoFLEX®). Two groups were evaluated: CircoFLEX® group: 24 batches finished from December 2011 to June 2012 (762 pigs per batch, 20305 pigs in total) were vaccinated at weaning (3 weeks of age) with Ingelvac CircoFLEX®. FLEXcombo® group: 25 batches finished from July 2012 to December 2012 (956 pigs per batch, 22958 pigs in total) vaccinated with FLEXcombo® at weaning (3 weeks of age). Facilities and management were identical for both groups. Parameters involved in production costs were taken into account. Mortality; FCR (18-100kg) corrected; and medication costs were evaluated during the fattening phase.

The FCR (18-100) corrected, eliminates the seasonal effect and shows only the difference by the intervention. It was calculated by using the differences in FCR during the two different periods in the whole company. This difference was deducted to the CircoFlex Group to eliminate this seasonal effect.

Data were processed by analysis of variance implemented using the GLM procedure of SAS. Mortality was analyzed using a chi-square test.

The ROI (return on investment) on the use of FLEXcombo® instead of CircoFLEX® alone was calculated by using BECAL (Boehringer-Ingelheim Economic Calculator). The feed price in this calculation was fixed to 300€/ton and the carcass price to 1.37€/kg live weight.

Results

The results are summarized in Table 1. Due to differences in start weight this parameter was used as a covariate.

Table 1. Efficacy of Ingelvac MycoFLEX® after mixing with Ingelvac CircoFLEX®

Variable	CircoFLEX	FLEXcombo	p
Animals per group	20305	22958	
FCR(18-100) corrected	2.55 ± 0.021	2.50 ± 0.020	0.1103 NS
Mortality (%)	5.21 ± 0.38	3.42 ± 0.38	0.0031**
Medication costs (€)	1.93 ± 0.24	2.52 ± 0.24	0.1108 NS

NS: non significant; * p<0.05; ** p<0.01

Figure 1. SPC chart of Mortality of the 49 batches.

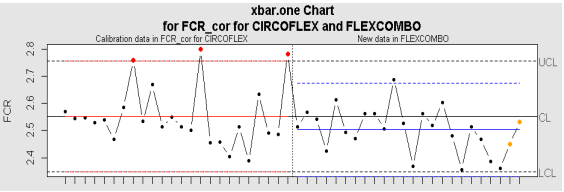

Figure 2. SPC chart of FCR of the 49 batches.

Conclusions and Discussion

In this field experience with more than 43,000 animals, it was demonstrated that mortality was significantly and FCR was efficiently reduced by the introduction of Ingelvac MycoFLEX®.

The return of investment of the intervention was calculated by BECAL to be 4.29:1.

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P.028

Eradication of *M. hyopneumoniae* from AI Stud

K Tarasiuk[1], M Starzewski[1,2]
[1]*PIC Polska, Wazow 8A, 01-986 Warsaw, Poland,* [2]*AI Stud, Skotniki, Poland,* kazimierz.tarasiuk@genusplc.com

Introduction

Mycoplasma hyopneumoniae (Mhp) is one of the most insidious swine pathogens having a very negative impact on pig farm economics. Partial depopulation has been shown to be very successful and well documented in Mhp eradication programs from pig farms (1,2,3). Although Mhp does not create any risk of transmission through the semen but in spite of that the decision was to eradicate the pathogen from the unit. The major reason of that was fact that the AI Stud was continuously replaced with Mhp (-) boars. To avoid any health problems after introduction of naïve animals into the Mhp positive environment we decided to eliminate this pathogen from the stud. In this study the eradication program of the disease was based on medication of all boars using first Draxxin and later Tiamutin.

Materials and Methods

The eradication procedure was applied in AI stud with 91 boars. The unit consists of three barns with 40, 23 and 28 individual pens respectively. The particular barns are connected to each other with a corridor. The stud was initially considered of high health status being free of PRRS, Mhp and other major infections. The first clinical signs (coughing) appeared in two barns in March, 2013. The Mhp infection was confirmed with serology (ELISA) as well as PCR (tracheobronchial swabs). Since the AI stud was intensively replaced with the new boars from Mhp-negative source farms, the decision was to eliminate that pathogen as soon as possible with the relevant treatment procedure.

Eradication protocol:

- Particular barns were vaccinated twice 2 weeks apart against Mhp.
- Every barn was vaccinated with 3 days difference between each other to avoid any problem with the semen quality.
- Two weeks after the second vaccination was over the first injection of Draxxin was given to every boar. All boars have estimated body weight using flank taping method for accurate dosing.
- Ten days after the first antibiotic injection, a second Draxxin injection was given to every single boar.
- In the meantime all the boar pens were cleaned and disinfected twice a week with 1% solution of Virkon S.
- Thirty days after the second medication with Draxxin, all boars were treated with Tiamutin premix in a dose 10mg/kg for 2 weeks.

The eradication process started in April 2013 and was finalized in August 2013.

The unit was clinically observed and examined on a daily basis by the herd veterinarian. Eleven sero-negative sentinels were introduced into the AI stud right after the medication with Tiamutin was finished. The sentinel pigs were allocated in the unit in a proportion of one sentinel pig per 8 local boars. Serological monitoring using ELISA (Idexx Herd Chek) covered only sentinel pigs and started one month after the treatment with Tiamutin was over.

Results

No clinical signs of Mhp were observed during the first 5 months after the treatment. Serological monitoring of sentinel pigs conducted for 5 consecutive months after introduction has shown to be negative for Mhp. Tracheobronchial swabs were taken monthly from 6 randomly selected boars and pooled by 3. Until now all the swabs were tested negative.

Conclusions and Discussion

Five months after the eradication program, all sentinel pigs remain serologically negative and there are no clinical signs of the disease (coughing) observed in boars. Additionally, all tracheobronchial swabs have been negative as well. No vaccine or anti-mycoplasmal antibiotics have been used since the treatment program was completed. Draxxin and Tiamutin treatments applied together showed to be very effective in elimination of Mhp from this AI stud as it was confirmed during the first 5 months after the treatment was completed. Further clinical observation as well as laboratory investigations will be continued in the future until the definite conclusion is to be made about the freedom of the AI stud from Mycoplasma hyopneumoniae.

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Use of serology to investigate the involvement of *M. hyopneumoniae* in the development of EP like lesions

R Neto
MSD AH UK, Ricardo.Neto@Merck.com

Introduction

National abattoir assessment has shown that there was an increase in the proportion of individual pigs affected by *Mycoplasma hyopneumoniae* (M hyo) like lesions since 2010 to mid 2012 in the UK[1].

Due to the costs involved in accurate slaughter house lung lesion assessment, it would be beneficial if serology would allow practitioners to assess which diseases may be responsible for respiratory disease, clinical signs commonly associated with it (cough) and lesions found at slaughter. The objective of this study was to determine if M hyo serology could be used with confidence to assess the involvement of M hyo in the development of Enzootic Pneumonia like lesions (EPL) at slaughter.

Materials and Methods

Consignments of pigs from a large pig producing company were assessed from 4th October to 2nd November 2013. Pigs were vaccinated for PCV2 and M hyo. Some of the pigs assessed were vaccinated against PRRS. To assess the EPL at slaughter, lesions were scored by assessment of antero-ventral pulmonary consolidation resulting in individual scores from 0 to 55[2]. Ten blood samples of each of the consignments assessed were collected at slaughter and the samples were tested for M hyo antibodies using the IDEXX M. hyo Ab Test. The data was analyzed using proprietary statistical software.

Results

Thirty three consignments of pigs were assessed at slaughter, resulting in the assessment of 2930 pigs respiratory tracts, 330 blood samples were tested

The total average individual EPL was 5.47 and the average batch EPL 5.39. A summary of the batch results may be seen in Table 1.

	Total	ELISA positive	EPL > 2	EPL > 4
N° consignments	33	30	22	14

Table 1. Analysis of consignments EPL and Mhyo ELISA

The mean EPL was significantly higher in consignments with high M hyo a.b. titres.

A significant positive correlation was found between mean EPL and M hyo a.b. titres (0.443 p = 0.011). Using the obtained regression formula, it was possible to predict the expected EPL (for animals with non-zero values) if the M hyo ELISA titres are known in consignments with EPL > 4 (Figure 1).

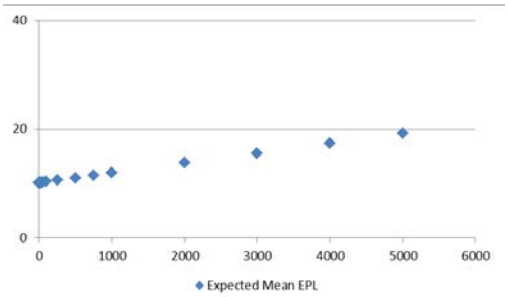

Figure 1. Expected mean EPL (for animals with non-zero values) for known mean M hyo ELISA.

Conclusions and Discussion

Assessment of EPL at slaughter and blood sample analysis collected at slaughter allowed to establish a significant positive correlation between mean a.b. in pigs at slaughter and EPL. The regression formula allowed to predict mean EPL scores when we know the M hyo a.b. titres. This cannot however predict EPL scores less than 10 and may only be useful in distinguishing higher EP scores when the mean M hyo titre is greater than 500.This may allow practitioners to investigate the involvement of *Mycoplasma hyopneumoniae* in respiratory disease in finishing pigs associated with high EP like lesions at slaughter relying on clinical disease, lung score lesions and blood sampling at the finishing stage.

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P.031

Complete genome sequence of *P. multocida* serotype A strain HB03, isolated from swine

W Liang[1,2], Z Peng[1], WJ Liu[1], ZF Xu[1], HC Chen[1], B Wu[1*]
[1]*State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University,* [2]*Key Laboratory of Animal Genetics, Breeding and Reproduction, Ministry of Education, Huazhong Agricultural University, Wuhan, 430070, China.* wanleung828@hotmail.com

Introduction

Pasteurella multocida is a bacterial pathogen that can infect swine, bovine, buffalo, sheep, avian, rabbit and even human. Many key virulence factors, such as capsule, lipopolysaccharide, fimbriae and adhesions, toxins and iron regulated and iron acquisition proteins have been identified (1). But the pathogenic mechanism has not been well explained. Now we have finished the complete genome sequencing, assembly and annotation of *Pasteurella multocida* strain HB03. It is a well supplement for the data base of *Pasteurella multocida* genome.

Materials and Methods

The strain was isolated from the lung of a swine in Hubei province of China in 2010. Whole-genome shotgun sequencing was performed by using Illumina Solexa platform. Glimmer[2] and GeneMarks[3] were used to predict the protein-coding sequencing. The CDS was annotated with gene coordinates taken from *Pasteurella multocida* strain Pm70 and 36950. Paralogous gene families were defined by using a cutoff E value of 10-5 with at least 40% query coverage and 40% identity. tRNAs and rRNAs were identified using tRNAscan-SE[4] and RNAmmer[5], respectively.

Results

The genome of *Pasteurella multocida* serotype A strain HB03 (GenBank: CP003328) contains a signal circular chromosome of 2,307,684 bp. The average GC content is 40.4%. The HB03 genome encodes 2,118 CDSs, accounting for about 89% of the genome. The entire chromosome contains 53 tRNA genes, 6 complete rRNA operons (16S-23S-5S) and an additional 5S rRNA. In addition, 25 pseudogenes and a IS element were predicted.

Genomic analysis showed that HB03 contains 7 pair of two-component system, but there isn't any report about its function. HB03 encodes the complete genes of Flp operon, which is very important for the colonization and reproduction. Comparative genomic analysis showed that HB03 has two large specific regions with 37,342 bp and 18,757 bp in length, respectively. The 37,342 bp region contains phage related genes and the 18,757 bp region encoding tetracycline resistance related proteins.

Conclusions and Discussion

The acquirement of the whole genome sequence of *Pasteurella multocida* strain Pm70, 36950, 3480 and HN06 have done a great job of finding new virulence factors. Our job perfects the whole genome database and tries to define the relationship between serotypes and pathogenic difference. Based on the BSR method, 1,812 genes of HB03 were highly conserved among the 5 genomes, and 79 proteins were showed great genetic diversity between HB03 and other strains, respectively. More *Pasteurella multocida* genome information is a pressing need so that we can take full advantage of them to declare the mechanism of *Pasteurella multocida* infection.

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Identification and research of novel surface immunogenic proteins of *P. multocida* HN06

DZ Zeng[1,2], HJ Kong[1,2], Z Peng[1,2], W Liang[1,2], J Fan[1,2], RM Hu[1,2], B Wu[1,2]
[1]*College of Veterinary Medicine, Huazhong Agricultural University,* [2]*National Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan, Hubei 430070, PR China,* dongzhuzeng@live.com

Introduction

Immuneprotmics technology was used to screening diagnostic markers and drug target. In the life sciences or medical applications, The further research about proteins to help develop new vaccines and drugs(1,5,6). PGAM are key enzyme of glycolysis process, indirectly inducing the production of pyruvate, the loss of the function of PGAM in humans can cause disease, the increasing of PGAM can induce tumor cell growth(2,3,4). In bacteria, few studies reported about PGAM, the research is expected to diagnosis and prevention of *Pasteurella multocida* for preparing subunit vaccine.

Materials and Methods

Pasteurella multocida HN06 generated *toxA* cultured in Tryptic Soy Broth(TSB) medium plus 10%bovine sera.
Convalescent sera and negative sera was collected from after six pigs which are free of *Pasteurella multocida* immunized.
Using Triton-114 to prepare for surface proteins, 2-DE (GE Healthcare, USA), the Immobiline DryStrip^{TM} IPGstrips of 13cm(pH 3-10). Western blotting was immunoblotting analysis.
After in-gel protein digestion, MALDI-TOF-MS was identified proteins.
To express proteins in E.coli, gene sequences of interest was amplified by PCR. The recombinant protein was purified from the supernatant by Ni-NTA agarose (Qiagen GmbH, Germany) column on an AKATA FPLC(GE healthcare, USA).
Western blotting researches the immune activity of the recombinant protein, determination of OD_{600} value study the effect of PGAM on *PM*

Results

Four novel immunogenic proteins identified was shown in Table 1. Through Western blotting indicated the immune activity of the recombinant PGAM (Figure 1), can promote the growth of HN06 (Figure 2).

Table 1. MALDI-TOF-MS identified four novel immunogenic proteins.

Gene	Score	Protein
TorA	75	Trimethylamine-N-oxide
Tsf	167	Elongation factor Ts
GpmA	111	Phosphoglyceromutase
Prx	218	Peroxiredoxin

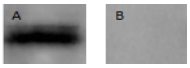

Figure 1. Immunoblotting analysis: convalescent sera(A); negative sera(B)

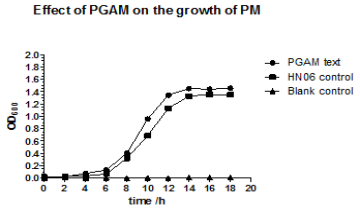

Figure 2. 100ug/ml PGAM in medium in PGAM text, determination of OD_{600} per two hours per group

Conclusions and Discussion

A conventional approach to identify immunogenic proteins. Four novel immunogenic proteins was found at the first time: 1, Trimethylamine-N-oxide; 2, Elongation factor Ts; 3, Phosphoglyceromutase;4, Peroxiredoxin.
According to the latest research reports, the function of PGAM may be of potential value. The loss of function of PGAM can cause animal disease.
The study on PGAM found the immune activity of the recombinant PGAM, can promote the growth of *PM* HN06. Probably, PGAM are key enzyme of glycolysis, increasing pyruvate production, promoting TCA circulation, improving the metabolism of bacterial growth.

Acknowledgments

Nationl Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, professor Wu and professor Chen, China

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Assessment of tools for monitoring infection by *P. multocida* in pigs

M Moreno[1], JS Ferreira Neto[1]; MC Dutra[1]; MR Felizardo[1]; TSP Ferreira[1], AM Moreno[1]
[1]*Department of Preventive Veterinary Medicine and Animal Health, University of São Paulo, SP - Brazil*
marina.moreno@usp.br

Introduction

Pasteurella multocida is an important pathogen for pigs, causing progressive atrophic rhinitis, pneumonia, pleurisy, and septicemia. For implementation of strategies to control and prevent infections, it is necessary to know about the profile of agent spread in natural conditions and in different types of production system. Given the lack of standardized serological techniques for this purpose, this study aims to evaluate the influence of different methods and sites of sample collection in the detection of animals with *P. multocida* by polymerase chain reaction (PCR) and ELISA.

Materials and Methods

Blood samples and swabs from the nasal cavity and tonsil were collected from of 90 animals in slaughter age. Examined pigs were divided in two groups originated from two different properties. The animals were examined before slaughter and after slaughter before scalding. Samples were collected at a slaughterhouse, located in Carapicuíba, São Paulo – Brazil. Extraction of bacterial DNA from nasal and tonsil swabs was performed according to the method previous described[1]. Detection of *P. multocida* was conducted by PCR using primers previous described[2] for identification (kmt gene). Antibodies against P. multocida were detected in sera using ELISA kit "*Pasteurella multocida* Antibody Test Kit - Flock chek" (IDEXX Laboratories - Westbrook, Maine, USA, lote: 09251JD-JD703). For use this test the antibody conjugated to peroxidase from original kit was replaced by a swine anti-IgG conjugate from the kit M. hyopneumoniae Antibody Test Kit IDEXX Laboratories - Westbrook, Maine, USA- lote: 06733DD116). The positive control of porcine origin was obtained from the serum of two animals with approximately 90 days of age with pneumonia caused by *Pasteurella multocida* examined in Swine Health and Virology Laboratory at University of São Paulo - USP and confirmed by bacterial isolation and PCR .

Results

Among the 90 animals evaluated, 14 (15.5%) were positive for *Pasteurella multocida* by polymerase chain reaction (PCR), all of which were nasal swabs. There are no positive animals among the tonsil swabs. In the ELISA, 25 (27.7%) were positive, and of these, three (3.3%) were positive in both the polymerase chain reaction and the ELISA and the remaining 22 (24.4%) was positive by ELISA and negative by polymerase chain reaction.

Conclusions and Discussion

Using comparison of proportion analysis, was observed a significant differences in the methodology of monitoring in different tests considering p-value <0.0001 and a confidence interval of 95%. Concluding, the frequency of positives was significantly higher in ELISA than by the polymerase chain reaction.

Table 1. Number and frequency of positive animals to *Pasteurella multocida* by ELISA and polymerase chain reaction according to the site examined (nasal cavity and tonsil)

Test	Positive Number (%)	Negative Number (%)
ELISA	25 (27,77%)	65 (72,23%)
PCR nasal swab	14 (15,55%)	76 (84,45%)
PCR tonsil swab	0	90 (100%)

The difference found between the sites of collect, nasal swabs and tonsil was expected, as previouslly described[3]. The presence, maintenance and multiplication of the agent in the nasal cavity may be favored by abundant mucus covering this site. The fact that the present study did not detect any positive in tonsil swabs may be related to poor elimination of the agent at the time of collection or reduced adherence capacity of strains of *P. multocida* and maintaining on the surface of tonsil tissue. The swabs of tonsil can be used succefully for detection of other respiratory agents in diagnostic practice, as *Mycoplasma hyopneumoniae*, *Mycoplasma hyorhinis* and *Actinobacillus pleuropneumoniae*, since collection is not invasive and is easy to perform. Moreover, the ELISA test seems promising for the indirect diagnosis of infection by *P. multocida* in porcine and suggest that further studies involving gold standard method consistent and higher number of samples should be conduced for better understanding of the patogeny and epidemiology o *P. multocida*.

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Diagnostic investigation of swine salmonellosis in Korean farms

KY Park[1], JS Heo[1], HI Kim[2], SH Shin[2]
[1]*Bayer Animal Health, Bayer Korea,* [2]*Optipharm Inc., Osong, Choongbuk, Korea*
jaesung.heo@bayer.com

Introduction

Salmonella infection in rearing pig is typically endemic and largely asymptomatic (1). In Korea this situation is unknown, but considering the potential risk of zoonosis, the objective of this study was to evaluate the prevalence of this disease in pigs at different production stages, weaners, growers and finishers from different farms in the country through bacterial analysis from diarrheic animals.

Materials and Methods

To investigate the prevalence of swine salmonellosis in Korea, samples from diarrheic animals were sent to two diagnostic laboratories for isolation and serotyping. Bacterial isolates were identified biochemically and serotyping was performed using standard agglutination test with O and H antisera.

Salmonellosis cases were diagnosed by the clinical laboratory of AH Bayer Korea during the period of 1992-2010 and Optipharm Solution Lab between 2010 - 2012.

199 salmonellosis cases were divided by occurrence time of the year to compare seasonal difference.

142 cases of salmonellosis were classified by the ages of diseased animals in three groups: weaners(70d<), growers(70-120d) and finishers(>120d).

Results

Swine salmonellosis was identified consistently during all the seasons of the year.

The frequencies of salmonellosis were higher in finishers than weaners before 2003 but after 2004, occurrence was predominant in weaners(Figure 1).

Most(96.5%) of the salmonella isolates belonged to *Salmonella enterica* subsp. *enterica* serotype Typhimurium (*S.* Typhimurium) except for a few ones which were *Salmonella enterica* subsp. *enterica* serotype Derby(*S.* Derby) and group C(Table 2).

Conclusions and Discussion

The frequency of swine salmonellosis showed little difference in seasonality in Korea as reported in Denmark (2) and other countries in the northern hemisphere.

Swine salmonellosis was more prevalent in finishers than in weaners before 2003 but was more prevalent in weaners after 2004. Postweaning multisystemic wasting syndrome (PMWS) associated with PCV-2 reached its peak around 2004 in Korea, immune depressed weaners seemed to be infected with *Salmonella* at a high rate.

Table 1. Swine salmonellosis cases diagnosed each season in Korea (1992-2012)

Season	Winter Dec-Feb	Spring Mar-May	Summer Jun-Aug	Fall Sep-Nov
No.of cases	44	62	45	48

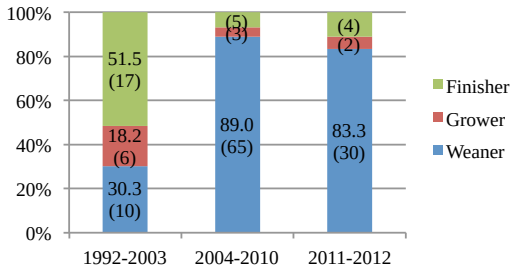

Figure 1. Swine salmonellosis cases diagnosed each phase of production in Korea (1992-2012)

Table 2. The serotypes of Salmonella isolated from diarrheic pigs in Korea (1992-2012).

Serotype /Specimen	Intestine, Feces	Lung	Liver	Total (%)
ST	134	1	2	137(96.5)
Non-ST	4	1	-	5 (3.5)
Total	138	2	2	142 (100)

* ST : *S.* Typhimurium, Non-ST: *S.* Derby and group C

PCV-2 vaccine has been used since 2008 in Korea and salmonellosis in weaners assumed to be decreased slightly.

Pallares FJ et al. (3) reported that the main causes of bacterial septicemia in cases of PMWS were *Streptococcus suis* and *Salmonella sp*. As a result of this study the dominant serotype of *Salmonella* isolates was *S.* Typhimurium. This suggests that isolates derived from immune depressed diarrheic animals.

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Evaluation of phage cocktails for control of *Salmonella*

BY Jung[1], KC Lee[1], HY Park[1], SC Jung[1], JS Son[2], WI Kim[3]
[1]*Bacteriology Division, Animal and Plant Quarantine Agency, Anyang,* [2]*iNtRON Biotechnology Inc., Seongnam,* [3]*Chonbuk National University, Jeonju, Republic of Korea* jungby@korea.kr

Introduction

Salmonellae are widely distributed throughout the world, gaining entry to almost all aspects of human food chain. Pork has been reported to be associated with as much as 15% of human cases of salmonellosis (1). It was also estimated that 70% of carcass contamination resulted from infected pigs (2). Despite the multiple treatments to eliminate *Salmonella* spp. in pig farms, it still remains a considerable problem due to limiting effective treatments. The use of specific bacteriophages could be a promising strategy for controlling *Salmonella* colonization in pigs. Therefore, the aim of this study was to evaluate the phage cocktails which were the mixture of *Salmonella* specific lytic phages.

Materials and Methods

Fecal and sewage samples were collected from pig farms for phage isolation. The presence of phages was investigated by a phage enrichment technique using *Salmonella* Typhimurium ATCC 14028 and confirmed by spotting assay. Double layer plaque assay was performed to obtain pure phage isolates. Transmission electron microscopy (TEM) was also performed for investigation of phage morphology (3).

For the estimation of lytic capability, reference strains representing 34 serotypes and 190 *Salmonella* isolates representing 16 serotypes were tested using spotting assay.

Results

Four phage cocktails were prepared with mixture of individual phages: cocktail A (SEP-1, SGP-1, STP-1, SS3P-1, EK9P-1; $\geq 10^9$ pfu/ml), cocktail B (SEP-1, SGP-1, STP-1, SS3P-1, EK9P-1; $\geq 10^{11}$ pfu/ml), cocktail C (SEP-1, SGP-1, STP-1, SS3P-1, STP-2, SCHP-1, SAP-1, SAP-2; $\geq 10^9$ pfu/ml), and cocktail D (STP-2, SCHP-1, SAP-1, SAP-2, EF4P-1, EK8P-1, EK9P-1, CP-3, CP-5; $\geq 10^9$ pfu/ml), respectively. Morphological analysis by TEM revealed that the phages were divided into three types of families; *Siphoviridae*, *Myoviridae* and *Podoviridae*.

All the reference strains were clearly lysed in cocktails B and C. However, only 8 (23.5%) serotypes of reference strains showed complete lysis in cocktails A and D.

In the present study, the numbers of completely lysed isolates were different with the type of individual phage of cocktail and titer of each phage. Among 190 *Salmonella* isolates, 177 (93.2%) and 176 (92.6%) isolates were clearly lysed with cocktails B and C, respectively. However, 96 (50.5%) and 86 (45.3%) isolates were lysed with cocktails A and D, respectively.

All the tested Typhimurium (n = 93), the most prevalent serotype in pig farms in Korea, were completely lysed in cocktails B and C. Serotypes London (n = 11), Schwarzengrund (n = 8) and Derby (n = 6) were also clearly lysed in cocktails B and C. On the other hand, no lysis was observed in serotype Rissen (n = 58) with cocktails A and D, but 47 (81.0%) and 46 (79.3%) isolates were shown with complete lysis patterns in cocktails B and C, respectively.

Conclusions and Discussion

In the present study, phage cocktails B and C showed lytic capability against broad spectrum of *Salmonella* serotypes. Cocktails A and B had the same composition of individual phages, except phage titer. Cocktail B was more efficient compared to cocktail A. This result demonstrated that the phage titer was important to improve the antibacterial effect of phages.

In comparison of cocktails C (mixture of 8 individual phages) and D (mixture of 9 individual phages), cocktail C was much more efficient than D. For this reason, we consider that accurate selection of phages are required for the better efficiency of phage cocktails.

We proposed the usage of phage cocktail as a way to control of *Salmonella* infection in pig farms.

Acknowledgments

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Field experience of mixture of Ingelvac Mycoflex with Ingelvac Circoflex on a 500 sow level farm in Malaysia

C-K Yong [1], K-Y Kam[1]
[1]*Boehringer Ingelheim (Malaysia) Sdn Bhd*
chiun-khang.yong@boehringer-ingelheim.com

Introduction

Porcine respiratory disease complex (PRDC) is a major concern in modern swine production. It can be caused by multiple infectious agents, in combinations of primary and secondary respiratory pahtogens[1]. Mycoplasma Hyopneumoniae and porcine circovirus type 2 are two pathogens of those commonly associated with PRDC[1,2,3]. Finishing pigs at 14 to 20 weeks of age are most likely to be severely affected[2]. The affected herd may suffer increased mortality. The objective of this study was to evaluate the efficacy to control mortality due to PRDC when Mypravac suis vaccine (Hipra) and Ingelvac CircoFLEX were used (April to Oct. 2011) and when a mixture of Ingelvac MycoFLEX with Ingelvac CircoFLEX as a FLEXcombo was used in the farm (Nov. 2011 to Aug. 2012).

Materiasl and Methods

The study was performed in a 500 sow, farrow to finish farm located in a high disease pressure area within Peninsular Malaysia. The farm was having severe respiratory disease problem for years. Before Nov 2011, respiratory signs such as coughing, dyspnea and increased mortality were noticed when pigs were reaching 3 months of age. Swine enzootic pneumonia with secondary bacterial complications was found during the postmortem to diagnose the cause of death. Previously the producer was using 2 dose of Mypravac Suis vaccine and 1 dose of Ingelvac CircoFLEX according to the recommended regimen from the product's labels. However since Nov 2011, the producer replaced the 2 doses Mypravac Suis vaccine with Ingelvac MycoFLEX and mixed with Ingelvac CircoFLEX, given as a FLEXcombo the pigs at 3 weeks of age.

Mortality rates were recorded when the vaccinated pig entered the grower-finisher stage. Statistical Process Control charts (Statistic aver 8.0) and student's t-Test were used for analysis

Results

The average mortality from the grower and finishers was 3.35% when 2 doses Mypravac suis and 1 dose CircoFLEX were used. It was reduced to 2.10% after implementation of MycoFLEX and CircoFLEX, 1 dose (2 ml) at 3 weeks of age (Figure 1). There was a significant difference on mortality of grower-finisher pigs between the two groups (Table 1).

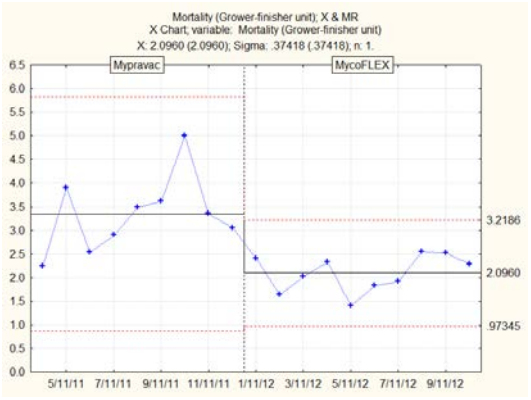

Figure 1. Comparison of Mortality in grower-finisher unit before and after the mixture of Ingelvac MycoFLEX with Ingelvac CircoFLEX

Table 1. Monthly mortality rate mean (Mean ± SD) grower-finisher before and after the mixture of Ingelvac MycoFLEX with Ingelvac CircoFLEX

Group	Mortality rate (%)
2 doses Mypravac suis and 1 dose CircoFLEX (5449 pigs)	3.3467 ± 0.8157 *
Mixuture of Ingelvac MycoFLEX with Ingelvac CircoFLEX (6074 pigs)	2.0960 ± 0.3891 *

*Statistically significant between the groups ($p<0.05$)

Conclusions and Discussion

This study demonstrated that a single dose; 2ml mixture of Ingelvac MycoFLEX with Ingelvac CircoFLEX at 3 weeks of age was effective to significantly reduce the mortality in the grower-finisher unit by 37.37%, from average mortality rate of 3.35% to 2.10% in a pig farm with high disease pressure. Besides the mortality rates, coughing signs were also reduced in farm.

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PRRSV control using Ingelvac® PRRS MLV in a Korean farm infected with European type PRRSV

HJ Chae[1]
[1] *Boehringer Ingelheim Vetmedica Korea Ltd*
Heejin.chae@boehringer-ingelheim.com

Introduction

The swine industry in South Korea is suffering from respiratory diseases caused by PRRSV as a primary pathogen. Although most of the farms have the North American isolate (Type II), recent studies have shown some farms infected with European PRRSV (Type I).
The objective of this study was to evaluate the efficacy of a PRRSV Type II based vaccine (Ingelvac® PRRS MLV) to control PRRSV in a Korean farm affected with European PRRSV (Type I).

Materials and Methods

This field case study was conducted in a 200 sow farrow to finish farm. Weaning age is 27 days of age and stay in a nursery until 90 days of age and then, pigs are transferred to growing and finishing barns. Several clinical signs include "greasy pig", diarrhea, weakness in the pre-weaning phase while coughing, thumping were observed in nursery house. Poor growing performance, with high mortality was observed around 2~10 weeks of age. Based on diagnostic results, the farm decided to implement mass vaccination in the breeding herd twice (at 4 weeks interval between 1st and 2nd mass vaccination) in 1st week of February and March followed by quartely mass vaccinations (every three months).
Before and after MLV PRRS vaccine implmentation, blood samples were taken for examination by ELISA (IDEXX ELISA X3) and RT-PCR. f*Five blood samples were taken in pigs from 15, 40, 70, 100, 130 days of age. Serological result and mortality were compared before and after the mass vaccination.

Results

Before mass vaccination, PRRSV was first detected in 15 day old pigs and then again 20 days later. (Table 1). After mass vaccination, no viruses were detected at any ages.

Table 1. Result of PCR test before and after mass vaccination of Ingelvac PRRS MLV

	Before vac.		After vac.
	1st blood samples	2nd blood samples	blood samples
15days	+(EU strain)	-	-
40days	-	+(EU strain)	-
70days	-	-	-
100days	-	-	-
130days	-	-	-

Serologically, before mass vaccination maternal antibody was low (Figure 1), however after mass vaccination, the S/P ratio of 15 day old pigs increased. This coincides the reduction in mortality in both the pre-weaning and nursery stage after mass vaccination (Figure 2).

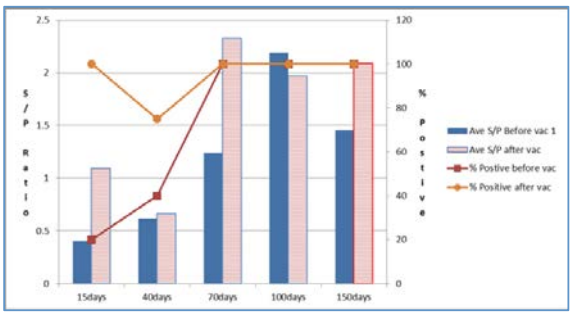

Figure 1. Average S/P ratio and % Positive of ELISA test before and after mass vaccination of Ingelvac PRRS MLV

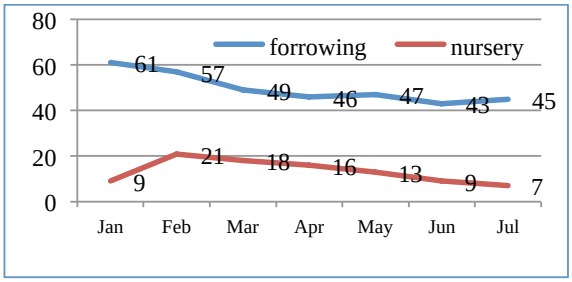

Figure 2. Number of dead pigs in farrowing and nursery house

Conclusion

Based on the diagnostic results, the breeding herd was unstable, measured by PCR + results at farrowing house and nursery pigs. After the implementation of breeding herd mass vaccination, PRRS stabilization was achieved and an overall improvement in productivity was observed. This PRRS stabilization had an impact in PRRSV dynamic in pigs moving the exposure later in nursery pigs (table 1) providing a better scenario for piglet vaccination. .
This field case provides strong evidence of controling PRRSV in farms infected with European type under Korean farms conditions.

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Field observation of the efficacy of Flexcombo in finishing performance in Thailand

W Thongmak[1] ,T Yong Sripanyarit [2], S Kongtes[3] , N Duangwhae[3]
1 Live-infomatics company 2 BestAgro group company 3Boehringer Ingelheim (Thai) Co;Ltd
nathaya.duangwhae@boehringer-ingelheim.com

Introduction

Diseases associated with Porcine Circovirus Type 2 (PCV2) and *M. hyopneumoniae*(M hyo, enzootic pneumonia) infections are a major concern in the swine industry. M hyo has also been considered as one of the major co-factors in the development of PCVAD.[1,2] The objective of these studies was to evaluate the efficacy of both Porcine Circovirus Type 2 and M hyo vaccines when the monovalent licensed vaccines for the two agents are mixed and administered in a single combined injection in a PRRS stabilized farm.

Materials and Methods

The retrospective field observation was conducted on a 3 site production farm with 2,350 sows in Thailand. The sow herd is stabilized for PRRS by mass vaccination with Ingelvac PRRS MLV. Piglets are weaned at 26 days of age and routinely vaccinated with Ingelvac PRRS MLV at 2 weeks and Ingelvac Mhyo and Ingelvac CircoFLEX at 4 weeks of age, respectively, Pigs are shipped to contract fattening farms at the age of about 8 weeks. 14 consecutive batches with 7004 pigs were evaluated in this study. 2920 pigs from 7 batches were separately vaccinated with Ingelvac CircoFLEX and Ingelvac M hyo and 4084 pigs from 7 following batches were vaccinated with FLEXcombo (Ingelvac CircoFLEX + Ingelvac MycoFLEX, licensed to be mixed and administered in a single combined injection).. Separate as well as FLEXcombo vaccinations were applied at 4 weeks of age. All animals were kept under the same management program and feed formulation. The monitored parameters were average daily weight gain (ADWG), Feed conversion ratio (FCR) and total losses. Parameters were evaluated using standard statistical process control (SPC) performed by Statistica version 8.1.The differences between the groups were evaluated by students T-test.

Results

The results of production parameters are shown in Table 1.

The overall growth performance and FCR showed significant differences between both groups. The chart of growth parameters such as the ADG is shown in figure2

Table 1. Evaluation of fattening pigs batches with two different vaccinations schemes.

	Separate	FLEXcombo	p-value
Prod. Batches (N)	7	7	n/a
Avg. Weight In (Kg)	20.4	19.50	n/a
Avg. Weight Gain (Kg)	97.85	95.44	0.333
ADGW (g/d)	643	683	0.045
FCR	2.66	2.54	0.022
%Total loss	4.30	6.03	0.158

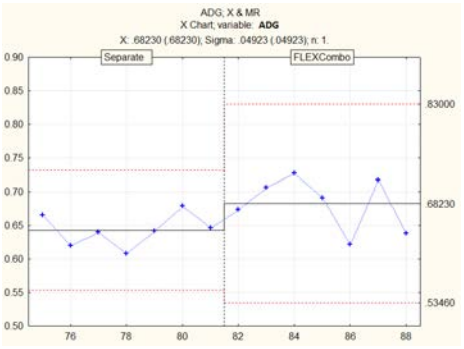

Figure1. SPC I-Charts for Average daily weight gain and FCR in finishing period

Conclusions and Discussion

The mixture of Ingelvac MycoFLEX and Ingelvac CircoFLEX delivered in a single 2 ml injection was safe and efficacious as the conventional separate vaccination scheme with Ingelvac M.hyo and Ingelvac CircoFLEX . This is in line with previous studies demonstrating the efficacy of FLEXcombo (1,2). This mixing license not only provides the protection for both pathogens, but also reduces the number of injections, pigs stress and labor requirements.

References

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Reduction in PDNS cases after PCV2 vaccination on a Dutch fattening farm

R de Groot[1], NWertenbroek[2]
[1]*Veterinary praxis Boxmeer, Boxmeer, The Netherlands;* [2] *Boehringer Ingelheim Vetmedica, Alkmaar, The Netherlands,*
info@dapboxmeer.nl

Introduction

PCVD has a significant impact on the technical performance on pig farms around the world. Since 2006 different vaccines have been on the market against this financially devastating disease, and have shown to be effective against many aspects of this disease (1). Not many reports have described the impact of PCV2 vaccination on the number of PDNS cases (2). The objective of this study was to evaluate the effect of a single dose PCV2 vaccination on the technical results and number of PDNS cases under field conditions.

Materials and Methods

Production data of a 350 sow farm with 2000 fattening places was retrospectively reviewed for the period September 2011 until August 2013. The fattening unit had a history of suboptimal growth, despite feeding adjustments, and cases of PDNS. Other clinical signs included an in increased number of runts and pigs growing apart. Diagnostic blood samples taken from 15 pigs showed high levels of PCV2 virus load. The blood samples were negative for PRRS and Mycoplasma. In May 2012 the farm started vaccinating with Ingelvac CircoFLEX® (1 ml) at 10 weeks of age, at the introduction on the fattening unit. Continuous flow data of the fatteners was used for evaluation. 12 months before vaccination (approx. 6000 pigs) were compared to 12 months in which vaccinated pigs were present on the farm (approx. 6000 pigs). The farmer recorded individual data about the PDNS cases. The parameters average daily weight gain (ADWG), feed conversion (based on kg), mortality and PDNS cases were monitored for the fatteners on monthly bases.

Results

The number of PDNS cases dropped from 11 to 0 cases for the CircoFLEX vaccinated pigs (table 1). This was very remarkable for the pig owner. Also the growth and mortality (Figure 1) improved strongly. Since the pig owner had a low antibiotic use in the period before, the reduction after the implementation of the 1 shot PCV2 vaccine was small.

Table 1: Technical results of fattening pigs before and after implementation of CircoFLEX®

Parameter	Before	CircoFLEX	difference
Period	Sept 11 Aug 12	Sept 12 Aug 13	
Growth / day (gram)*	769	812	*+43*
Feedconversion *	2,58	2,49	*-0,09*
Mortality (%)	2,13	1,11	*-1,02*
PDNS cases	11	0	*-100%*

*Corrected data for 25-112 kg

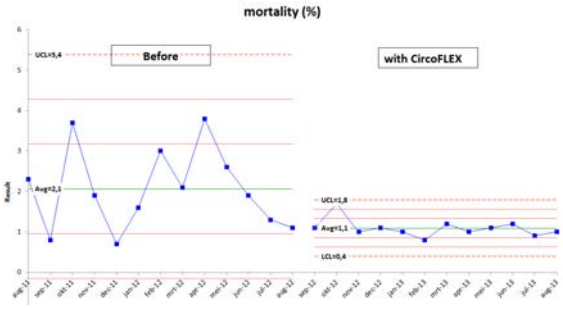

Figure 1. Mortality in time, Before and with CircoFLEX

Conclusions and Discussion

This retrospective analysis of a Dutch pig farm demonstrates that the use of a 1 dose PCV2 vaccine improved the technical performance and reduced the number of PDNS cases even when vaccinating at entry to fattening. Based on an average (2012) feed price of 0.28 €/kg and a (slaughtered) carcass price of €1,55 an improved gross margin of 3,79€ per CircoFLEX vaccinated pig was established (ex vaccine).These results suggest that there are situations where PCV2 vaccination decreases the number of PDNS cases and improves the production and economic performance. Similar findings are confirmed in other reports (2,3,4,5).

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Field observation: Success full control of an ileitis outbreak in a Dutch sowherd by oral vaccination

A Schuttert[1], M Steenaert[2]
[1]*De Oosthof samenwerkende dierenartsen, The Netherlands,* [2]*Boehringer Ingelheim B.V., Alkmaar,The Netherlands,*
arjan.schuttert@deoosthof.nl

Introduction

Ileitis is caused by the bacterium *Lawsonia intracellularis* (Li), found all over the word in pig production. The general assumption is that for commercial swine operations it is very hard to become and stay Li free for a prolonged time period. Due to enteric mucosal changes, ileitis in growing pigs causes lower feeding efficiency resulting in a higher feed conversion rate and/ or decreased daily gain. In older pigs, like breeding animals, the main clinical signs observed are severe intestinal bleeding which is causes by enteritis. This form is known as Proliferative Hemorrhagic Enteropathy (PHE).(2)
The present paper describes a field observation in which a PHE outbreak in a sow herd was controlled by oral vaccination.

Materiasl and Methods

A high health sow farm (free of PRRS/App/M.hyo) of 500 sows regularly introduced replacement breeding gilts at the age of 6 months coming from a PRRS/App/M.hyo SPF breeding herd. The sow farm was started 5 years before and historically the gilts were tested serologically negative for Li.
In April 2012, 2 out of 24 gilts of one batch showed 5 weeks after arrival clinical signs of PHE while they were housed under quarantine. The entire batch of gilts was treated with tylosine (oral, 10 mg/ kg BW, 10 days). One week later, 2 days after these gilts from the affected batch were placed into the mating room, some older parity sows in the mating room showed clinical symptoms of PHE. Oral treatment with tylosine 10 mg/kg BW for 8 days was started in all the sows and piglets on the farm. Severely diseased animals were treated by injection (tiamulin 8 mg/ kg BW, 3 days). Diagnosis was confirmed by autopsy of a sow at the GD Animal Health Service (Deventer, the Netherlands). After the medication all sows and breeding gilts present on the farm were vaccinated individually by administrating the vaccine (Enterisol® Ileitis, Boehringer Ingelheim) in the trough, each dose diluted in 20 ml of pasteurized fatfree milk.
To ensure vaccine efficacy no antibiotics were administered to the vaccinated animals for 7 day around vaccination – from 3 days before to 3 days after vaccination (3). The onset of immunity (OOI) after EI vaccination is 3 weeks. In this OOI period the use of oral tylosine was continued to prevent Li infection, by medicating all sows on the farm weekly three times for 2 consecutive days (tylosine, oral, 5 mg/kg BW) starting 5 days after vaccination.
After the initial mass vaccination of all breeding animals a routine vaccination program for replacement gilts was implemented (1) where gilts are vaccinated via trough on the day of arrival at the sow farm, without the following tylosine treatment. Replacement breeding gilts now stay in quarantine for at least 3 weeks after vaccination.

Results

On the farm 12 sows (2,4%) died during the outbreak of PHE. The last sow showing PHE signs was observed at day 7 after starting the oral treatment of the whole herd with tylosine. A total number of 50 affected sows (10%) were treated with injectable tiamulin. Since the initial treatment and vaccination no more clinical cases were reported.
Lawsonia intracellularis is still present in the sowherd, proven by seropositive samples taken in sows 4 and 12 months after the initial outbreak.

Table 1. Number of positive samples / sample size (Lawsonia antibody Elisa)

Date	Positive samples
August 13, 2012	5/ 5
April 15, 2013	5/ 6

Conclusion and Discussion

Introducing gilts from quarantine to an apparently naive herd too early after clinical signs of ileitis was probably the cause for the outbreak of ileitis in the sow herd.
On this sow farm the combination of curative antibiotics and preventive Enterisol® Ileitis oral vaccination showed to be an effective program
of controlling an ileitis outbreak.. Preventive Enterisol® Ileitis oral vaccination of replacement gilts is a sustainable tool to prevent sow herds from PHE outbreaks.

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Reduction of antibiotic use and improvement of production results in a Dutch farrow-to-finish farm after implementation of oral Ileitis vaccination

J Kwinten[1], N Wertenbroek[2], M Steenaert[2]
[1] *VGTZ, Oisterwijk, The Netherlands,* [2] *Boehringer Ingelheim B.V., Alkmaar, The Netherlands,*
jkwinten@dierenartsenoisterwijk.nl

Introduction

The antibiotic use in the food producing animals is of a growing concern for consumers, human health care, politicians and retail. The antibiotic use in the Netherlands was considered to be one of the highest in the EU (1,2) and has therefore come under greater governmental attention the last years. The Dutch government issued the goal of a 50% reduction on the use of antibiotics by 2013 compared to 2009 (3).Vaccination is one of the tools to prevent diseases and therefore can be a useful tool in the reduction of the use of antibiotics. Furthermore vaccines can improve the production results, resulting in a better economical payoff for the primary producer (4,5). The objective of this study was to evaluate the effect of ileitis vaccination on the antibiotic use and the production results in a fattening unit under field conditions.

Materials and methods

The study was done on a 540 sow farm with 1700 finishing places. The finishing unit had a history of acute mortality in the heavy pigs close to slaughter and of suboptimal production results. Ileitis was diagnosed as the primary cause by the clinical signs in combination with the results of blood and fecal samples and autopsies. In the past antibiotics were used to control ileitis. This resulted in an improvement of uniformity and average daily gain but the acute form of ileitis was not prevented. In June 2012 the farm started oral ileitis vaccination (Enterisol®Ileitis, Boehringer Ingelheim). Vaccination was performed one week after entry in the finishing unit at the age of 10 weeks, according to the manufactures instructions of the vaccine in the drinking water. A batch of 600 control pigs was followed by a batch of 600 Enterisol vaccinated pigs. Close out production results were registered for the batch without ileitis vaccination and the batch with ileitis vaccination. Parameters monitored were: average daily gain (ADG), mortality, feeding conversion ratio (FCR) and antibiotic use. For evaluation and comparison of the antibiotic use, the standardized method of Defined Daily Dosages of antibiotics used per animal year was applied (DDD) (2,6,7). The DDD at this farm was already relatively low compared to the Dutch average. The low antibiotic use resulted in higher mortality and lower performance compared to a period with higher antibiotic that was used to control ileitis. Ileitis diagnoses was confirmed again and vaccination was started.

Results

The vaccinated pigs had better production parameters compared to the non-vaccinated pigs (see table 1) The average age of mortality was lower in the vaccinated group (55 vs. 131 days of age), which added to the economic result. The improvement of production results was combined with a reduction of antibiotic use (0.2 vs. 2.4 DDD), a reduction of 92%.

Table 1: production parameters for control and Enterisol vaccinated pigs.

	Control	Enterisol	difference
ADG (g/day)	799	846	47
FCR	2.64	2.48	0.16
Mortality(%)	5.5	2.2	3.3

Discussion

This study demonstrates that the use of Enterisol®Ileitis vaccination in the beginning of the finishing period can improve production results as well as reduce antibiotic usage in the finishing period. Based on an average (2012) feed price of € 0,28/kg and a (slaughtered) carcass price of € 1,55/kg an improved gross margin of € 9,00 per vaccinated pig was established (cost for vaccine excluded). The results on this farm are in line with the results in other reports (8,9,10). Together this results in a more sustainable pig production, including better economic performance.

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P.042

Two commercial one-shot Mhyo vaccins show comparable production results in a large Dutch fattening farm

M Schuttert[1], M Steenaert[2], N Wertenbroek[2]
[1]*De Varkenspraktijk, Someren, The Netherlands,* [2]*Boehringer Ingelheim B.V. Alkmaar, The Netherlands,*
m.schuttert@devarkenspraktijk.nl

Introduction

In the Netherlands fattening farms generally operate as continuous flow systems, having the pigs managed all in - all out per room. Respiratory disease is common in these systems (1) and *Mycoplasma hyopneumoniae* (Mhyo) vaccination is used in more than 50% of the Dutch piglets (2). Despite of what the official registrations of the various products state, sometimes the efficacy of different competitive products is argued by farmers. The objective of this field study was to evaluate two commercial mycoplasma vaccines on a Dutch fattening farm on production parameters under field circumstances.

Materials and Methods

The 7000 head farm consisted of 3 different closed barns with automatic ventilation. At slaughterhouse check an unexpected high percentage of lung lesions were recorded, with minimal corresponding clinical signs at the farm. Laboratory results confirmed Mhyo-infection, which was preceded by infections with App, Influenza and PRRSV. During the study every week on average 350 piglets arrived with a weight variation of 19,5 to 23,5 kg on average per batch. All piglets came from the same sow herd and were PCV2 vaccinated (CircoFLEX®) at 3 weeks of age. Every week a batch was housed in 2 or 3 different rooms and vaccinated within 3 days after arrival with either MycoFLEX® or Stellamune One®, alternating the brand of vaccine per batch. In total 3 batches were vaccinated MycoFLEX® and 3 batches were vaccinated Stellamune One®. During the study 5 bloodsamples per room were collected within 4 weeks before slaughter to monitor the Mhyo infection by serology (Herdcheck Mycoplasma hyopneumoniae ELISA,IDEXX Laboratories, Westbrook, Maine). The primarily production parameters for evaluation were the Average Daily Gain (ADG) and mortality, secondary parameter was lung lesion score at slaughter.
Lung lesions were registered at the slaughterhouse by dedicated slaughterhouse staff, reported as the total percentage of registered lungs with any kind of pneumonic lesions.

Results

Mhyo infection during the study was confirmed by serology performed in all batches.
See table 1 for the production parameters of the two groups.

Table 1. Production parameters of the pigs in fattening unit for 2 mycoplasma vaccines.

	MycoFLEX	Stellamune One
no of pigs	976	1088
% boars	53	57
ADG (gr/day)	805	794
mortality %	3,6	3,3
% lungs with lesions	61	56

Conclusion and Discussion

As shown in table 1 the production parameters of both groups were comparable.
The percentage of boars per group has to be comparable, for boars generally have better performance results (3) which could influence the study results.
When lung lesions at the slaughterhouse are scored one has to realize that the lesions can be the result of anything that has happened in the pig's life. The percentage of lung lesions is in general not only influenced by Mhyo-infections, but also by other infections such as PRRSV and PCV2 (4, 5), SIV (6) and by non-infectious causes (7). This has to be taken in consideration when using lung lesions as a parameter for evaluation.
The low number of batches in this field study did not allow statistical evaluation.
The results suggest that the efficacy of both vaccines is comparable.
The used vaccines have different registrations regarding the administration of the vaccine concerning age, mixability and safety. With comparable technical performance these characteristics are of importance for the pig farmer in the choice of a Mhyo vaccine.

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P.043

Retrospective field evaluation of efficacy of separated injection of Ingelvac® PRRS MLV and FLEXcombo® vaccines compared to a single injection of 3FLEX® vaccine in Thailand

W Thongmak[1] , T Yongsripanyarit [2], S Kongtes[3] , N Duangwhae[3]
1 Live-infomatics company 2 BestAgro group company 3Boehringer Ingelheim (Thai) Co;Ltd
nathaya.duangwhae@boehringer-ingelheim.com

Introduction

PRDC is a major problem in swine industry worldwide. The three pathogens which play major role are PRRSV, PCV2 and *Mycoplasma hyopneumoniae*. Vaccination against these 3 pathogens is a common program in Thailand. The conventional separated vaccination program could represent some disadvantages like labor effort, piglets stress and vaccination program design and/or flexibility. The objective of this retrospective study was to confirm the efficacy of 3FLEX® vaccine (vaccine with PCV2, Mhyo & PRRS in a single injection, Boehringer Ingelheim, St Joseph Missouri USA) compared to separated injections of Ingelvac® PRRS MLV and FLEXcombo® (vaccine with PCV2 and Mhyo in a single injection, Boehringer Ingelheim, St Joseph Missouri, USA) in fattening period under Thai field conditions.

Materiasl and Methods

The study was conducted in a multiple site farm with 2,350 sows. Sow herd was PRRS stable through mass vaccination with Ingelvac PRRS MLV four times a year. Piglets are weaned at 26 days old and housed in a nursery site until 7-8 weeks old. The vaccination program to control PRDC in piglets was Ingelvac PRRS MLV at 14 days and FLEXcombo® at 4 weeks of age. A new vaccination program was implemented with a single injection with 3FLEX® at 14 days of age. A before and after analysis was done having 7 batches vaccinated with Ingelvac® PRRS MLV and FLEXcombo® were compared to 37 batches vaccinated with 3FLEX® vaccine. The performance parameters used in this observation were: Total loss, ADG and FCR using standard statistical process control (SPC) method performed by Statistica version 8.1. and Student T-test.

Results

The results of production performance are shown in Table 1. In both groups, the performance of fattening pigs showed no significant differences as before and after. Furthermore there were no adverse reactions observed in 3FLEX® vaccination groups. A chart of key parameters such as % Total loss and ADG is shown in figure 2.

Table 1. Evaluation of fattening pigs batches with two different vaccination schemes.

	FLEXcombo	3FLEX	p-value	
Prod. Batches (N)	7	37	N/A	
Avg. Weight In (Kg)	19.50		16.19	N/A
Avg. Weight Gain (Kg)	95.44	95.01	0.401	
FCR	2.54	2.50	0.296	
ADGW (g/d)	682	665	0.159	
%total loss	6.03	6.37	0.674	

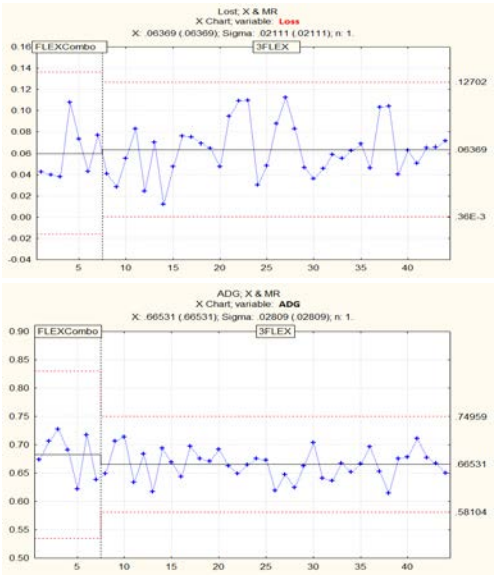

Figure 2. SPC chart of Percentage Loss and ADG comparing 2 periods

Discussion

A 3FLEX® vaccination scheme can reduce labor effort and piglet stress with no negative effects in fattening pig performance, which fits to modern management in todays pig production industry.

Field observation of the efficacy of Flexcombo in finishing performance in Thailand

W Thongmak[1],T Yong Sripanyarit[2], S Kongtes[3], N Duangwhae[3]
1 Live-infomatics company 2 BestAgro group company 3Boehringer Ingelheim (Thai) Co;Ltd
nathaya.duangwhae@boehringer-ingelheim.com

Introduction and objective

Diseases associated with Porcine Circovirus Type 2 (PCV2) and *M. hyopneumoniae*(M hyo, enzootic pneumonia) infections are a major concern in the swine industry. M hyo has also been considered as one of the major co-factors in the development of PCVAD.[1,2] The objective of these studies was to evaluate the efficacy of both Porcine Circovirus Type 2 and M hyo vaccines when the monovalent licensed vaccines for the two agents are mixed and administered in a single combined injection in a PRRS stabilized farm.

Material and Method

The retrospective field observation was conducted on a 3 site production farm with 2,350 sows in Thailand. The sow herd is stabilized for PRRS by mass vaccination with Ingelvac PRRS MLV. Piglets are weaned at 26 days of age and routinely vaccinated with Ingelvac PRRS MLV at 2 weeks and Ingelvac Mhyo and Ingelvac CircoFLEX at 4 weeks of age, respectively, Pigs are shipped to contract fattening farms at the age of about 8 weeks. 14 consecutive batches with 7004 pigs were evaluated in this study. 2920 pigs from 7 batches were separately vaccinated with Ingelvac CircoFLEX and Ingelvac M hyo and 4084 pigs from 7 following batches were vaccinated with FLEXcombo (Ingelvac CircoFLEX + Ingelvac MycoFLEX, licensed to be mixed and administered in a single combined injection).. Separate as well as FLEXcombo vaccinations were applied at 4 weeks of age. All animals were kept under the same management program and feed formulation. The monitored parameters were average daily weight gain (ADWG), Feed conversion ratio (FCR) and total losses. Parameters were evaluated using standard statistical process control (SPC) performed by Statistica version 8.1.The differences between the groups were evaluated by students T-test.

Result

The results of production parameters are shown in Table 1.

The overall growth performance and FCR showed significant differences between both groups. The chart of growth parameters such as the ADG is shown in figure2

Table 1. Evaluation of fattening pigs batches with two different vaccinations schemes.

	Separate	FLEXcombo	p-value
Prod. Batches (N)	7	7	n/a
Avg. Weight In (Kg)	20.4	19.50	n/a
Avg. Weight Gain (Kg)	97.85	95.44	0.333
ADGW (g/d)	643	683	0.045
FCR	2.66	2.54	0.022
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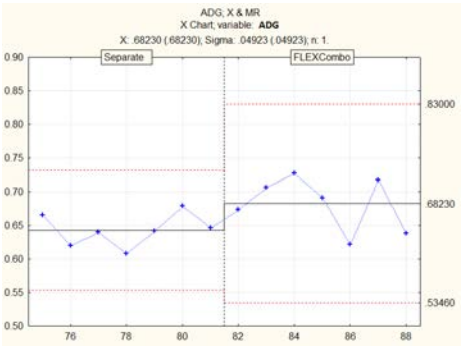

Figure1. SPC I-Charts for Average daily weight gain and FCR in finishing period

Conclusions and Discussion

The mixture of Ingelvac MycoFLEX and Ingelvac CircoFLEX delivered in a single 2 ml injection was safe and efficacious as the conventional separate vaccination scheme with Ingelvac M.hyo and Ingelvac CircoFLEX . This is in line with previous studies demonstrating the efficacy of FLEXcombo (1,2). This mixing license not only provides the protection for both pathogens, but also reduces the number of injections, pigs stress and labor requirements.

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Long term observation on efficacy of 3FLEX vaccination scheme in breeder herd in Thailand

S Kongtes[1] , J Channarong[1] , N Duangwhae[1] , J Permsub[2]
[1]*Boehringer Ingelheim (Thai) Co;Ltd* [2]*MG.Pharma Co;Ltd,* Sopon.kongtes@boehringer-ingelheim.com

Introduction

Aside from PRRSV, PCV2 also causes reproductive system problems in sows. Furthermore *Mycoplasma hyopneumoniae* (Mhyo) coinfections with these 2 viruses are the three major pathogens that play a role in PRDC. Sow herd mass vaccination against PRRS with MLV is an effective tool to control the disease. Breeder herd vaccination with PCV2 vaccine is also recommended by practitioners to minimize the reproductive negative impact of this disease[1]. The objective of this retrospective study is to observe the efficacy and safety of 3-way vaccination scheme against PRRS, PCV2 and Mhyo (3FLEX) compared to PRRS MLV mass vaccination program alone in a breeding herd under Thai field condition as shown in previous report[2].

Materials and Methods

This retrospective study was conducted in a single site farm with 1,900 sows. Sow herd had beenmass vaccinated with Ingelvac PRRS MLV 4 times a year since 2006. In 2012 this farm imported new genetic replacement females to increase herd size and vaccinated sow herd with 3FLEX 4 times a year. Monthly basis reproductive performance of year 2011 was compared to year 2012 and 2013 (Jan-July) performance. The parameters evaluated in this observation were farrowing rate, Stillbirth, born alive and weaned pigs per litter. Data was evaluated by using standard statistical process control (SPC) performed by Statistica version 8.0 and ANOVA performed by SPSS version 13.

Results

The result of production parameters is shown in Table 1 In both periods, the breeding herd performance werenot significantly different except born alive and weaned pigs per litter which were influenced by new genetics and stillbirth caused by management practices in year 2012. The chart of Pigs weaned per sow, is shown in figure 2. The values went out of control in October 2011 due to a PED outbreak in the herd.

Table 1. Production performance in breeding herd from year 2011 - July 2013

	Ingelvac PRRS MLV only (2011)	3FLEX (2012-2013)
	Mean	Mean
Average no. of sows	**1,784**	**1,898**
Farrowing rate (%)	**82.41**	**82.38**
Still Birth(%)	**8.74**	**10.08**
Born Alive	**11.26**	**12.09**
Wean/litter	**10.91**	**11.44**

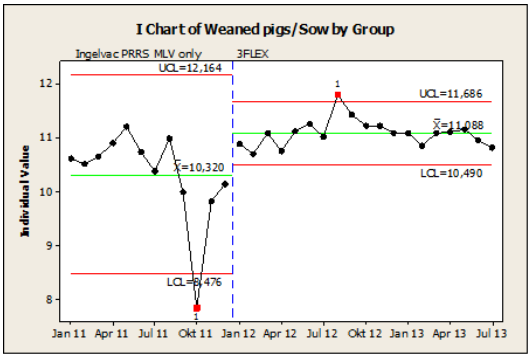

Figure 2. SPC chart of Pigs Weaned per Sow by month

Conclusions and Discussion

In this study 3FLEX vaccination scheme in breeding herd to control PRRS, PCV2 and Mycoplasma have no negative effects onbreeding performance. Furthermore, it reduced labor effort and pig stress which fits to modern management in todays pig production industry. The genetic improvement starting 2012 may have contributed to the advantage of the 3FLEX group

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Additional *Mycoplasma* vaccination improves Dutch pig herd performance

D Struik[1], M Steenaert[2], N Wertenbroek[2]
[1]*Dierenkliniek Noord Nederland, The Netherlands,* [2]*Boehringer Ingelheim Vetmedica, Alkmaar, The Netherlands,*
danielstruik@live.nl

Introduction

Infections with *Mycoplasma.hyopneumoniae* (M. hyo) are still a major concern in pig production, being one of the pathogens in Porcine Respiratory Disease Complex (PRDC) (1). The objective of this study was to evaluate the effect of an additional Mycoplasma vaccination (Ingelvac MycoFLEX) under field conditions at a M hyo positive farm. The Mycoplasma vaccination was applied in a licensed mixture together with a PCV2 vaccine (Ingelvac CircoFLEX), which was already used for some years in this farm.

Materials and Methods

Production data of a 2 site production farm with 10.000 fattening places were retrospectively reviewed for the period June 2011 till December 2012. The fattening unit had a history of recurrent coughing beginning at 16-18 weeks of age and at slaughter sub-optimal uniformity. Diagnose was based on serology and additional slaughter checks of the lungs. In December 2011 vaccination with MycoFLEX was started in the piglet producing farm. The piglets were already vaccinated with CircoFLEX for some years and MycoFLEX was applied in the mixture together with CircoFLEX following the label instructions. The mixture (FLEXcombo) was applied at the age of 3 weeks. The pigs were moved to the fattening unit at 10 weeks of age. Continuous flow data of the fatteners was used for evaluation. 11 months before the additional vaccination with MycoFLEX were compared to 7 months in which only FLEXcombo vaccinated pigs were present on the farm. Due to another change in management system the data was limited until December 2012 for evaluation. The economical relevant production parameters were used for evaluation.

Results

The general health improved, with less coughing in the pigs and less runts and losses. All major production parameters improved (see table 1) in the timeframe with the added Mycoplasma vaccination. Also the carcass quality improved. The amount of antibiotics used for respiratory pathogens was reduced in the FLEXcombo group with half the amount of oxytetracycline and no doxycycline in this period.

Table 1: Performance data of fattening pigs before and after implementation of MycoFLEX® in the FLEXcombo.

Parameter	CircoFLEX	FLEXcombo	difference
Period	June 11 Apr 12	June 12 Dec 12	
Growth / day (gram)*	755	775	*+20*
Feedconversion *	2,8	2,71	*-0,09*
Mortality (%)	2,6	1,9	*-0,8*
mm muscle	61,95	66,22	*+4,27*
mm fat	16,41	15,89	*-0,53*

*Corrected data for 25-112 kg

Conclusions and Discussion

This retrospective analysis of a Dutch pig farm confirms that the Mycoplasma vaccine applied in a 1 dose mixture of a PCV2 and Mycoplasma vaccine improved not only the clinical symptoms but also the technical performance of the pigs. This is in line with other reports (2,3,4). Based on an average (2012) feed price of 0.28 €/kg and a (slaughtered) carcass price of €1,55/kg an improved gross margin of € 2,91 per MycoFLEX vaccinated pig was established (ex vaccine). On top of this the extra earnings of the improved carcass quality should be added.

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Attempted eradication of *A. pleuropneumoniae* by Pulmotil® premix and its economic evaluation in a three-site pig production farm in Japan

H Ishikawa[1], S Ishizeki[1]
[1]*Summit Veterinary Services, Japan*, ishikawa@svs-jp.com

Introduction

Actinobacillus pleuropneumoniae (App) is a causative pathogen for pig pneumonia, emerged as one of the most significant diseases with heavy economic loss in the swine industry. Previous studies reported that App could be eradicated in two or three site pig production systems with appropriate medication or vaccination for both sows and piglets (1). In this study, we report the attempted App eradication by Pulmotil® premix (Tilmicosin phosphate, Elanco Animal Health) in the 2nd and the 3rd sites in a three-site pig production farm in Japan.

Materials and Methods

This trial was conducted in a three-site pig production farm with 1,600 sows. The 1st site (breeding farm) was App serotype 2 positive. Piglets were weaned at 3 weeks of age or earlier, then moved to the 2nd site and finally to the 3rd site at approximately 12 weeks. Partial all-in all-out was practiced in the 2nd and the 3rd sites. High mortality and poor growth due to pneumonia caused by App and PRRS were significant problems in the 3rd site.
Pulmotil® medicated feed was included at 400 ppm of Tilmicosin phosphate and fed to sows from 1 week before farrowing to weaning before the trial. Pulmotil® medicated feed at 400 ppm was also fed to fattening pigs from 1 week prior (79d) to the transfer to the 3rd site, ended at 120d. The medication continued from Jun. 2012 to the end of Oct. 2012, a period of 5 months.
App and *Mycoplasma hyopneumoniae* combined vaccine was administrated to sows 1 month prior to farrowing and *Mycoplasma hyoneumonie* and *Porcine Circovirus Type 2* vaccines were also administrated to piglets at weaning, respectively.
Sera for antibody tests were randomly collected from 5 pigs at 30d, 60d, 90d, 120d, 150d and 180d in May 2012 prior to Pulmotil® medication and the same herds were monitored from 30d to 180d during Pulmotil® medication. CF reaction tests were conducted for antibodies for App serotypes 1, 2 and 5. Clinical observations for pigs were also performed monthly.

Results

CF antibody values were positive at 120d (1/5, x32) prior to Pulmotil® medication and also positive at 150d (3/5). In contrast, pigs were negative for App until 165d and changed to positive at 172d (4/5) during Pulmotil® medication (Figure 1).
Coughing from pigs was not observed from 21d to 140d, but emerged after 140d.
App serotype 2 was isolated from lung at 160d in Sep. 2012 and at 140d in Nov. 2012, respectively.

Conclusions and Discussion

Serological / bacteriological tests and clinical observations suggested that Pulmotil® medication sustained App negative status in the 2nd site, but failed to complete eradication in the 3rd site.
However, Pulmotil® medication brought about additional 3 pigs marketed per sow per year, 3.49 points improvement in mortality and 40g improvement in ADG (Table 1). Furthermore, those improvements continue after medication stopped.
The economic evaluation of Pulmotil® medication in this trial resulted in a profit of 12.4mil JPY annually, due to the improvements in mortality and ADG.

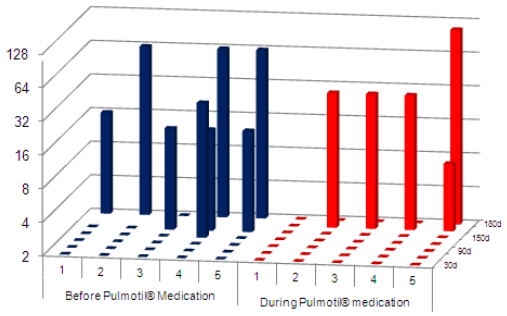

Figure 1. App CF test value trends

Table 1. Productivity trends, 2010-2012

	2010	2011	2012
Pigs marketed (heads/sow/year)	22.22	22.63	25.58
Mortality after weaning (%)	10.97	10.35	6.86
ADG (g/day, days marketed – born)	494.6	530.8	571.1

These results suggest that Pulmotil® medication enabled App eradication in the 2nd site and delayed App infection period, resulting in improvements in mortality, ADG and pig numbers per sow. However, App eradication may have not been completely achieved in the 3rd site due to partial all-in all-out per unit practices.

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Field efficacy evaluation of two PRRS modified live vaccines against highly pathogenic PRRSV

Dedicated to the memory of Xianjin Yang

J Chen[1], S Sun[2], T Tan[2], L Zhu[2], G Chen[2]
1 Dongguan Entry-Exit Inspection and Quarantine Bureau, Dongguan523071, China
2 Boehringer Ingelheim Int'l Trading (Shanghai) Co. Ltd, Beijing100004, China
tao.tan@boehringer-ingelheim.com

Introduction

Highly Pathogenic Porcine Reproductive and Respiratory Syndrome Virus (HP-PRRSV) is a widespread pathogen in China, causing big loss in pig farms since 2006[1]. Chinese farmers have been trying their best to control this fatal disease and PRRS modified live vaccine (MLV) has been their main choice as a primary tool. The objective of this field study was to compare the efficacy of two MLV vaccines (Ingelvac® PRRS MLV and an attenuated live PRRS vaccine (manufactured by a local company) in a farm in south China.

Materials and Methods

The study was implemented in a 2000-sow farm located in Guangdong province in China with a continuous flow system. Breeding herd was routinely vaccinated against Classical Swine Fever Virus (CSFv), Foot and Mouth Disease Virus (FMDv) and Pseudorabies Virus (PRv), three times a year (labeled dose); PPv and JEv were vaccinated in the sow herd in a seasonal fashion. The vaccination scheme for piglets was the following: CSF at 35 and 75 days of age (local manufacturing); PCV2 (Ingelvac CircoFLEX®) at 21 days of age; FMD at 70 and 100 days of age.

In 2011, a local PRRS live vaccine was introduced into this farm, sow herd was vaccinated 3 times a year while piglets were vaccinated at 35 days of age. However, since March 2012, fattening pigs showed PRRS-like respiratory symptoms followed by nursery pigs. The main symptoms were dyspnea, anorexia, radish skin, high fever. Morbidity in nursery was around 45% and 50% of them were dead. Blood and tissue samples were collected and sent to a diagnostic lab for ELISA and PCR test. PCR result showed HP-PRRSV as the main pathogen. In April 2012, a comparative study was set to evaluate the efficacy of two PRRS MLV's. 776 piglets at 3 weeks of age were randomly allocated into 3 groups. The piglets in group 1 were injected with Ingelvac® PRRS MLV, 1 dose per pig; the piglets in group 2 were injected with a local PRRS MLV, 1 dose per pig; the piglets in group 3 were treated with normal saline, 2ml per pig.

Pigs from each treatment group were raised in different barns on the same site under the same management and housing conditions. Mortality and morbidity were recorded as primary parameters and analyzed by Chi-Square test (Minitab 16.2.3, State College PA USA).

Results

Morbidity and mortality in pigs vaccinated with Ingelvac® PRRS MLV were significantly reduced in nursery and fattening facing HP-PRRS infection, compared to the local MLV and control group; on the other hand, there was no statistically significant difference in these parameters between the local PRRS MLV and the control group.

Table 1 Morbidity and mortality in the 3 different groups.

Group	Group 1 BI PRRS MLV	Group 2 Local MLV	Control Non-Vx
Number of pigs	259	259	258
Sick pigs	19	71	68
Dead pigs	14	34	37
Morbidity（%）	7.34[a]	27.41[b]	26.36[b]
Mortality（%）	5.02[a]	13.13[b]	14.34[b]

Different letters indicate that values differ significantly at P<0.05 (Chi-Square).

Discussion

In this study, Ingelvac® PRRS MLV showed a significantly better efficacy (P<0.05) against HP PRRS in pigs facing an exposure in growing phase, indicating a good level of cross protection against HP-PRRS as documented in a previous studies[2,3]. This farm began to use Ingelvac® PRRS MLV as a routine vaccination program and the PRRS status has been stable since then.

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Management of pig manure in anaerobic lagoons with added biological substrates

R Braun[1], M Muñoz[1], S Pattacini[1], G Scoles[1], M Bellozas Reinhard[1]
[1] *Department of Agronomy, National University of La Pampa, Km 334 35th Street, Santa Rosa, La Pampa, Argentina,* braun@agro.unlpam.edu.ar

Introduction

The pig manure in the stabilization lagoons, are used by anaerobic and facultative bacteria to produce volatile organic acids. These organic acids, anaerobic bacterium, used to convert CH_4 and CO_2 optional. This stage of depuration, is essential for the subsequent removal of organic matter (OM) and contribute to the lower biological oxygen demand (BOD_5) and chemical oxygen demand (COD) of the medium by the action of aerobic bacterium and redox reactions. The methane bacterium is very sensitive to pH < 7. If, the digestion not exist in the environment a sufficient number of methane-forming bacterium occurs an accumulation of acids and pH decreases. At pH values below 6.8 the activity begins to decline methane activity. When this occurs organic acids that can have unpleasant odors and compounds such as hydrogen sulfide (H_2S), mercaptans or skatole giving odor and indicate malfunction in the lagoons are released (1,2,3). If these lagoon operate with small retention time, the hydrolytic acidogenic phase may develop, but the formation of methane, which is slow, and such odors are produced and little removal of MO is obtained (2,3). In this experience pH, T°, redox potential, concentration of sulfides, BOD_5, COD, *Escherichia coli* and coliforms NMP (Number method most likely) was estimated in stabilization lagoons using a preparation of bacterium for commercial use as biological activator enzyme action.

Materials and Methods

The experiment was conducted during September and October 2012 in six anaerobic stabilization lagoons of 250 m^3 each average that began filling in the months of January and February of the same year. Three of them (Treatment 1 = T1) on first September were introduced 285 g of a concentrate of 1.5 billion / g of microorganisms without excipients, the three other (Treatment 2 = T2) was untreated. At 30 and 60 days of aggregate external biological substrate 10 samples per lagoon each treatment were collected and evaluated on the organic substrate pH, T°, redox potential, concentration of sulfides, BOD_5, COD, *Escherichia coli* and coliforms NMP. The analysis of the data corresponded to a Student test (df ∞).

Results

The results are shown in Table 1.

Table 1. Mean variables ± 1 standard error

Indicators contamination	30 days of initiation		30 days of initiation	
	T1	T2	T1	T2
T° (°C)	24	18	32	27
	(± 0,3) ***a***	*(± 0,2)* ***b***	*(± 0,2)****a***	*(± 0,4)****b***
pH	6,9	6,0 *(±0,04)****b***	7,0	6,3
	*(±0,02)****a***		*(±0,01)****a***	*(± 0,05)****b***
Redox Potential	-0,49	-0,42	-0,52	-0,54
(volts)	*(± 0,001)* ***a***	*(±0,003)* ***b***	*(±0,002)* ***a***	*(±0,003)* ***b***
Sulfides(mg/L)	123	206	0,61	146
	(± 10) ***a***	*(± 17)* ***b***	*(±0,01)* ***a***	*(±) 11* ***b***
BOD_5 (mg/L)	4547	10903	717	7762
	(±114) ***a***	*(±203)* ***b***	*(± 21)* ***a***	*(± 186)* ***b***
COD (mg/L)	7561	15977	2107	5681
	(±177) ***a***	*(±243)* ***b***	*(±97)* ***a***	*(±134)* ***b***
Escherichia coli	866	1412	171	433
(Col/100 ml)	*(± 49)* ***a***	*(±75)* ***b***	*(±11)* ***a***	*(±38)* ***b***
Coliforms NMP	4765	9670	551	4530
(Col/100 ml)	*(±102)* ***a***	*(±246)* ***b***	*(± 31)* ***a***	*(±189)* ***b***

Values with same letter in the row are not statistically different (P<0.05).Distribution of critical values "t" from equal variance and one - direction test.

Conclusions and Discussion

The results are significantly advantageous in T1 because the variables indicate that stabilizing the OM to end products CO_2 and CH_4. The bacterium grow best methane the higher the Tº (T1). The redox stable in T1 values correspond to a reducing environment, rich in hydrogen gas, and therefore, suitable for the growth of strictly anaerobic microorganisms, which indicate decreased elimination of H_2S, <BOD_5 <COD < *Escherichia coli* and total coliforms; and a further reduction of COD and BOD_5 medium. An > pH in the presence of T1 benefited these sulfide-oxidizing bacterium preventing the formation of odors associated with the release of H_2S.

Addition of biological activators of enzymatic action to increase the anaerobic depuration, increases the elimination of small lagoon OM retention times.

Acknowledgments

Department of Chemistry, Faculty of Exact and Natural Sciences, UNLPam, Argentina.

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The prevalence of milk spots in pigs at slaughter in Ireland

A Hidalgo[1], A Cox[1], P Kirwan[2]
[1]*Elanco Animal Health, UK & Ireland,* [2]*Pat Kirwan & Associates, Dublin, Ireland,* hidalgo_alvaro@elanco.com

Introduction

Ascaris suum infestation is the most important parasitism of pigs worldwide, having a negative effect on performance and associated high economic costs (1). As a part of *A. suum* hepatotracheal migration route, L_3-larvae travel through the liver damaging it. Consequently, whitish healing foci occur in the liver that are referred to as "milk spot". Such lesions disappear within 4 weeks (1). The presence of milk spot has been used to monitor national and herd prevalence of ascariasis before (2, 3), being indicative of recent migration of *A. suum*. However, current information on milk spot prevalence in Ireland is scarce. This study aims to investigate the prevalence of liver milk spot in pigs at slaughter as an indicator of *A. suum* infestation in pig herds in Ireland.

Materials and Methods

A total of 12,597 finishing pigs sent to slaughter to a dedicated pig abattoir in Ireland during the last weeks of March 2012 and 2013 were included in this study. A detailed description of the number of batches inspected is presented in Table 1.

Table 1. Description of pigs and batches studied by year.

	2012	2013	Total
Pigs assessed	7,043	5,554	12,597
Batches	63	52	115
Pigs/batch	111.8	106.8	109.5
Herds	45	40	85

Liver milk spots (presence or absence) and hepatic scarring (presence or absence) were assessed individually in the processing line. Data was recorded and stored electronically. Statistical analysis was conducted using JMP® version 9.0.3 (SAS Institute Inc., Cary, NC, USA).

Results

The prevalence of milk spot and hepatic scarring positive herds by year is presented in Table 2.

The average percentage of milk spot positive pigs within a batch was 11.5% for the combined period, 13% and 9.6% in 2012 and 2013 respectively. No statistically significant differences were detected between years when within batch prevalence was compared (p=0.334). Similarly, within batch average prevalence of hepatic scarring was 3.9% in the 2012-2013 period (2.9% in 2012 and 5.2% in 2013 [p=0.008]). Within batch milk spot prevalence in 2012 and 2013 is depicted in Figure 1.

Figure 1. Distribution of within batch prevalence of milk spot positive herds.

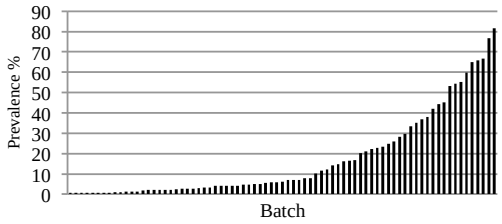

Table 2. Prevalence of positive herds.

Prevalence (%)	2012	2013	Total
Milk spot	84	62.5	74.4
Hepatic scarring	86.9	72.5	82.2

Conclusions and Discussion

This study confirms a high prevalence of *A. suum* in Irish pig farms. Since milk spots heals within 4 weeks, being just indicative of recent migration of *Ascaris suum*, true prevalence figures of this parasite is likely to exceed levels detected in this abattoir survey. With at least 3 out of 4 farms being positive for *Ascaris suum* infestation, our results are in agreement with a previous report analyzing data from 2003 to 2005 (3). In addition, the number of herds investigated per year in this study was considered to be representative of the commercial Irish pig farms, representing approximately 15% of them (4).

A. suum infestation can impact dramatically on pig performance, worsening average daily gain and feed conversion ratio up to 9% and 15% respectively in case of heavy infestation (5). In this study, the prevalence within a batch exceeded 10% in 42.8% (33 out of 77) of milk spot positive batches, suggesting the need of more effective control measure to reduce the impact of this parasitosis. Together with the need of reviewing managing practices, a strategic deworming program with flubendazole based on the prepatent period of *A. suum* has been succesful in reducing milk spot prevalence before (6,7).

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P.051

The development of a pig model to test the role of exogenous lipases in fat absorption when fed human infant milk formula

SG Pierzynowski[1], K Goncharova[2], T Kovalenko[2], I Osadchenko[2], G Ushakova[3], G Skibo[2], A-Ch Olsson[4], J Svendsen[4]

[1]*Dept of Biol., U of Lund, Sweden & Inst of Rural Health, Lublin, Poland,* [2]*Dept of Cytology, Key State Lab, Bogomoletz Inst of Physiol, Kyiv, Ukraine,* [3]*Dept of Biochem and Biophys, DNU, Dnepropetrovsk, Ukraine,* [4]*Swedish Univ of Agricul Sciences, Dept of Biosys and Techn (BT), Alnarp, hyocon@gmail.com*

Introduction

The exocrine pancreas is fully functional in the newborn pig. However, the neonatal human must obtain pancreatic lipases from mother's milk. Thus, the human infants fed milk formula cannot properly digest dietary fat, which is necessary, e.g., for the proper growth of the neonatal brain. It has been shown that many aspects of postnatal development are similar between the human and the piglet, with the exception of the exocrine pancreas. A model of exocrine pancreatic insufficiency in the young pig (EPI pigs) has been developed and is well established; these animals do not grow and have very poor fat and protein absorption (1,2,3,4). Thus the possibility of using this animal model for studying lipid metabolism and absorption in human pre- and full-term infants was investigated in the following study.

The aim was to determine the effect of feeding a standard neonatal formula, treated and not treated with microbial lipases to EPI pigs and relate the observations to those observed in infants fed non-treated similar formulae.

Materials and Methods

Animal material, maintenance systems and pancreatic duct ligation surgery, recovery and adaptation procedures have been reported (2). A partially hydrolyzed infant starter formula milk NAN Pro 1 Gold (Nestle) was used. Based on avg. body weight, the EPI pigs were fed approx. 400-500 g formula powder daily in 4 feeds. Microbial lipase was used for the digestion of the long chain polyunsaturated fatty acids (LCPUFA) in the formula. 13 male EPI pigs (aged 8±2 wks and weighing ca 13±2 kg at start), received formula during the 2 wks of pre-treatment period. For the 1 wk treatment period, they were divided into 2 groups. The control group (n=4) was fed formula only. The experimental group was fed milk prehydrolyzed with microbial lipase (n=9). Blood samples were collected daily before feeding.

The animals were sacrificed at the end of the study and post-mortem examined. Histological examination of the gastrointestinal tract was carried out using standard methods. Standard methods were used to analyze the levels of total fat, free fatty acids, triglycerides (TG) and non-esterified fatty acids (NEFA). The Lipemic Index (LI) and coefficient of fat absorption (CFA) were calculated. Statistical analyses using Statistica 7 (StatSoft, USA) were carried out.

Results

The lipase group ate less than the control and no EPI group gained weight. The lipase treated group had lower stool weights and significantly ($p<0.01$) lower total fat content than the control group (53.5% vs 30%). The coefficient of fat absorption (CFA) in the EPI pigs increased up to 87% ($p<0.05$) with lipase treatment, whereas that of the control pigs remained ca. 66-67%. The lipase treated group showed a significant increase in the LI, and significant changes in post prandial serum NEFA. Neither the mucosal thickness of the small intestine nor the number of goblet cells in the mucosa of the EPI pigs were increased by predigestion of the formula with lipase, in contrast to observations made on intact pigs fed formula.

Conclusions and Discussion

Formula intake is optimal for term and preterm neonates when breast feeding is impossible, and if pasteurization is required for donor breast milk. Infants fed formula supplemented with taurine, a bile acid, showed a higher CFA than those fed untreated formula (5). A similar increase in the CFA of the EPI pigs was found when they were fed lipase treated formula. The results of the present study indicated that the response of the EPI pigs was similar to those of infants with neonatal physiological pancreatic insufficiency.

Thus there are indications that the EPI pig can be used as an animal model of the infant of 3-6 months of age to test lipid metabolism and absorption of pre-hydrolyzed TG-fats.

Acknowledgments

SGPlus – Sweden

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Exocrine pancreatic insufficient pigs as an animal model to study brain structure and function during pancreatic insufficiency in humans: Effect of pancreatic-like enzyme replacement therapy

SG Pierzynowski[1], T Kovalenko[2], I Osadchenko[2], K Goncharova[2], G Ushakova[3], G Skibo[2], J. Botermans[4], J Svendsen[4]

[1]*Dept of Biol., U of Lund, Sweden & Inst. of Rural Health, Lublin, Poland,* [2]*Dept of Cytology, Key State Lab, Bogomoletz Inst of Physiol, Kyiv, Ukraine,* [3]*Dept of Biochem and Biophys, DNU, Dnepropetrovsk, Ukraine,* [4]*Swedish Univ of Agricult Sciences, Dept of Biosyst and Technol (BT), Alnarp,* hyocon@gmail.com

Introduction

Acute and chronic pancreatic insufficiency in humans is often associated with marked neurological alterations related to cognitive and sensory motor function. However, no animal model permitting brain studies under these conditions is available (1). A model of exocrine pancreatic insufficiency in the young pig (EPI pigs) has been developed and is well established; these animals do not grow and have very poor fat and protein absorption (2,3,4,5). - The aim was to study changes in neurospecific protein distribution, hippocampal morphology and behaviour of EPI pigs, and to study if replacement therapy with pancreatic-like enzymes of microbial origin (PLEM) can alleviate these changes.

Materials and Methods

Twelve castrated male piglets 6±2 wks of age and 11.3±2 kg at surgery (pancreatic duct ligation [2]) were randomized into 3 groups: Control (n=4) intact pigs; EPI pigs (n=4), and PLEM pigs (n=4) where EPI pigs received pancreatic-like enzymes of microbial origin in their diet (5). The pigs were fed a cereal based feed for young growing pigs (Lantmannen, Sweden) enriched with ca. 15% extra fat twice daily for three weeks. The treated pigs received 4 PLEM capsules at each meal. Each capsule contained 57.4 mg enzyme mix: 30,000 lipase U, 20,000 protease U, 3,000 amylase U, using commercially available enzymes.

Using standard methods, animal behaviour and activity were evaluated on 2 consecutive days at the end of the study. The pigs were then sacrificed and the brain removed and sampled for biochemical analyses and the evaluation of morphology. Statistical analyses using Statistica 7 (StatSoft, USA) were carried out.

Results

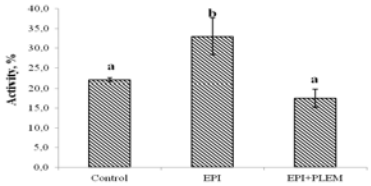

Figure 1. Changes in pig activity (% obs. time, n=12)

The EPI group showed significantly ($p \leq 0.01$) more activity than the control (intact) **(**Fig. 1**).** and the PLEM pigs, respectively. A significant reduction in the levels of glial filament protein (GFAP) and calcium binding protein, S100b, was found in the hippocampus (the CA1 area) of EPI pigs and slightly higher levels of soluble GFAP. PLEM treated EPI pigs showed levels of these and other biochemical parameters (e.g., neural cell adhesion molecule [NCAM]) similar to that of the control pigs. The amount of hippocampal neurons were significantly ($p \leq 0.05$) lower in EPI pigs than the controls, and also lower than those receiving PLEM (Fig. 2).

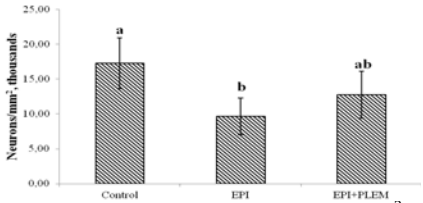

Figure 2. No. neurons per 1 mm^2 in the CA1 hippocampal area in the pigs (n=12)

Conclusions and Discussion

The present study demonstrates that EPI may result in the reduction of the number of pyramidal neurons in pigs' hippocampus, reduction in the NCAM level, and a pathological increase in animal activity. These negative effects in pigs can be significantly reduced by pancreatic-like enzymatic replacement therapy. Thus this animal model may be relevant with respect to studying the clinical use of pancreatic enzymes under EPI conditions in humans.

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P.053

Improving reproductive performance of weaned primiparous sows with low body condition score by using Altrenogest

N Am-in[1], P Pearodwong[1], P Tummaruk[1]
Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand, nutthee.a@chula.ac.th

Introduction

Sows weaned with poor body condition score do not usually resume estrus within 7 days after weaning. The reason is mainly due to negative energy balance during lactation period[1]. It has been demonstrated that split-weaning and the use of altrenogest treatment are able to resume anabolic state in weaned sows. It was found that both LH secretion and the number of medium size follicles (diameter >3 mm) at weaning were higher in the treatment group compared with control group[2]. Additionally, it was found that an increase in progesterone level increase litter size in sows[2]. It is hypothesized that application of progesterone treatment at weaning in sows may enhance the body condition score, number of large follicle and subsequent litter size. Therefore, the objective of the present study was to investigate the effect of altrenogest treatment for 8 days after weaning on WSI interval, number of large follicles, farrowing rate and litter size in primiparous sows.

Materials and Methods

The study was conducted in a commercial swine herd in Thailand from January 2012 to November 2013. Weaned primiparous (n=40) sows with poor body condition score (BCS <3) and losing backfat thickness > 3 mm during lactation were assigned into 2 groups: treated with

- Treatment group: 20 primiparous sows were weaned at day 24 and orally dosed with altrenogest for 8 days (20 mg/day ; Virbagest®, Virbac, France)
- Control group: 20 primiparous sows were weaned at day 24

The sows received fence line boar contact from 3 days after weaning or at the end of altrenogest treatment to facilitate estrus detection. During estrus, number of dominant follicle (>7 mm) were recorded using ultrasonography. Sows were artificially inseminated with 3×10^9 motile sperm at 12 and 36 h after estrus. Numbers of dominant follicle and total born piglets were compared between groups by Student's t-test. Pregnancy rate was analyzed by Fisher's exact test.

Results

Five sows of control group were culled because they did not present estrous sign within 14 days after weaning. The sows of the treatment group gained 2.8±0.8 mm, and the control group can gain 2.1±0.3 mm of backfat thickness from weaning to estrus. Sows in treatment group had a higher number of dominant follicles, a higher farrowing rate and larger litter size than control group (Table 1.) WSI of treatment group was longer than control group because we plus 8 days of feeding altrenogest. The sows of treatment group returned to estrus within 3.5±1.1 days after withdrawing altrenogest.

Table 1. Comparison of WSI, number of dominant follicle, farrowing rate and litter size between control and treatment group (mean±SD.)

Parameter	Control	Treatment
WSI (days)	8.6±2.2 (n=15)	11.6±1.2 (n=20)
Number of dominant follicles	15.3±2.2[a] (n=15)	20.4±1.9[b] (n=20)
Farrowing rate (%)	66[a] (n=15)	95[b] (n=20)
Total born (piglets)	8.1±1.2[a] (n=10)	10.6±1.0[b] (n=19)

[a,b]Rows with different superscripts differ $P \leq 0.05$.

Conclusions and Discussion

It is known that low feed intake during lactation results in excessive loss of body condition. These sows may have depressed fertility as indicated by longer WSI, low number of dominant follicles, low pregnancy rates, increased embryo mortality and decreased litter size. In the present study, altrenogest treatment can suppress estrus and allow the sows to have an anabolic status. After withdrawing the altrenogest, those sows have a good body condition, normal number of dominant follicles and exhibit a good standing estrus. Additionally, the sows in treatment group presented a better farrowing rate and total born piglets vthan the control group. This is in agreed with our previous study in multiparous sows[3]. In conclusion, the treatment of altrenogest for 8 days after weaning improved number of dominant follicles, farrowing rate and total born piglets in primiparous sows with poor body condition score.

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Virbac Thailand Co., Ltd., Pakkret, Nonthaburi 11120 Thailand

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P.054

Association between *P. carinii* and bacterial lung pathogens in pigs with pneumonia

C Weissenbacher-Lang[1], B Kureljusic[1], N Nedorost[1], D Stixenberger[1], D Binanti[2], M Viehmann[3], H Weissenböck[1]
[1]*Institute of Pathology and Forensic Veterinary Medicine, University of Veterinary Medicine Vienna, Vienna, Austria,*[2]*Department of Veterinary Science and Public Health, Faculty of Veterinary Medicine, Veterinary University Hospital – Pathology Unit, Universit à degli Studi di Milano, Lodi, Italy,*[3]*Clinic for Swine, University of Veterinary Medicine Vienna, Vienna, Austria,* christiane.weissenbacher-lang@vetmeduni.ac.at

Introduction

Several respiratory diseases in swine with unknown etiology seem to be multifactorial in origin or break out due to immune suppressive concomitant circumstances. In the case of *Pneumocystis carinii* (P.c.) it remains unclear whether other respiratory agents could be positively influenced by the presence of this fungus. Synergistic effects with PCV2 or PRRSV have been discussed, but solid data on the association of P.c. with bacterial pathogens are sparcely available (1). The aim of the present study was the evaluation of a possible association between P.c. and *Bordetella bronchiseptica* (B.b.), *Mycoplasma hyopneumoniae* (M.h.), and *Pasteurella multocida* (P.m.).

Materials and Methods

A total of 219 formalin-fixed paraffin-embedded lung tissue samples of pigs with respiratory symptoms were analysed. The detection of P.c. was carried out by *in-situ* hybridization, B.b., M.h., and P.m. were detected using immunohistochemistry. H&E staining was used for assessment of histological lung lesions.

Results

P.c. could be detected in 60% of the samples. IHC gave a positive result for B.b. in 6%, for M.h. in 17%, and for P.m. in 24%. Co-infections with two, three or four pathogens were present in 22% of the cases. None of the associations was significant. Co-infections with P.c. and P.m. were most commonly seen (5.9%), followed by triple infections with P.c., M.h. and P.m. (4.1%) as well as double infections with M.h. and P.m. (3.7%). All other possible combinations were observed in lower numbers between one and three cases. Quantities and distribution patterns of pathogens are presented in table 1. Different combinations of distribution patterns could be seen. Histologically, the lungs infected with P.c. mainly showed an interstitial pneumonia. In contrast, the B.b., P.m. and M.h. positive lungs were associated with fibrinous or fibrinous-haemorrhagic bronchopneumonia or catarrhal-purulent bronchopneumonia. A single infection with P.c. could be detected in 43%, with B.b. in 0.5%, with M.h. in 4%, and with P.m. in 6%. 25% of the samples were negative for the presence of the investigated pathogens.

Table 1. Quantities/distribution patterns of pathogens

	Localization	(+)	+	++	+++
P.c.	Alveoli	5	62	57	7
B.b.	Infiltrate in airways	0	1	1	3
	Infiltrate in alveoli	0	0	1	4
	Necrotic tissue	0	0	2	7
M.h.	Brush border of airways	8	1	1	2
	Infiltrate in airways	9	9	10	3
	Infiltrate in alveoli	3	6	4	2
	Necrotic tissue	0	0	0	3
P.m.	Infiltrate in airways	8	18	9	9
	Infiltrate in alveoli	8	8	6	6
	Necrotic tissue	1	0	2	9
	Septicaemia	0	0	0	1

(+): minimal, +: few, ++: moderate, +++: multiple organisms or bacteria

Conclusions and Discussion

P.c. attaches to the pneumocytes and gradually fills the alveolar lumina. For this process, the lung tissue needs to be more or less intact. Severe lesions of the lung tissue, as they are caused by the three bacteria under investigation, do not offer the optimal basis for *Pneumocystis* development anymore. P.c. mainly occurred as single infection, whereas the three bacteria especially could be observed in the cases infected with more than one pathogen. The progeny of this fungus is triggered under immunosuppressive circumstances, which can be caused in pigs easily due to a variety of reasons. As P.c. showed a high prevalence and was also occurring as co-factor in multifactorially caused infections, its impact has to be further elucidated. P.c. could act as a precursor for other respiratory diseases by causing premature damages in the lung tissue through oxygen deficiency, but it could also facilitate infection of other pathogens by supporting immune suppression. Furthermore, associations to other bacterial or viral diseases have to be illuminated.

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Epidemiology of NNPDS in four Danish herds

H Kongsted[1,3], N Toft, [2], JP Nielsen[3]
[1]*Pig Research Centre, Danish Agriculture & Food Council,* [2]*National Veterinary Institute,Technical University of Denmark,* [3] *Centre for Herd-oriented Education, Research and Development, University of Copenhagen,* hko@lf.dk

Introduction

New Neonatal Porcine Diarrhoea Syndrome (NNPDS) is a newly emerged syndrome, characterized by diarrhoea within the first week of life, which is un-responsive to antibiotics and not associated with known pathogens (1). Our study focused on describing the course of clinical symptoms within the first five days of life in piglets from four affected herds. Furthermore, we evaluated potential sow- and piglet-level risk factors for development of NNPDS. We defined NNPDS as diarrhoea during the second to fifth day of life.

Materials and Methods

A total of 941 piglets from 86 litters in four herds diagnosed with NNPDS were included in the study. Consistency of faeces was evaluated on a daily basis by use of rectal swabs.

The sow factors evaluated included parity, litter size, stillborn piglets and disease (a combination of different disease-parameters) on the day of parturition. The piglet factors included birth weight and gender as well as liquid consistency of faeces, hollow flanks and rough hair coat on the day of birth. Risk factors were evaluated by generalized linear mixed models using the lme4 package in R (2). Litter was inserted as random effect. Herd was inserted as fixed effect.

Results

Liquid consistency of faeces on the day of birth was highly prevalent in all herds (18-40% piglets within herds experienced this). In Herd 1, 2, 3 and 4, a total of 70%, 42%, 41% and 27% of piglets developed NNPDS. Many piglets (46%) with liquid consistency of faeces at birth did not develop NNPDS. First parity litters were most severely affected in terms of both number of piglets affected and duration of symptoms. In most cases, symptoms lasted for 1-2 days. Herd of origin and parity of sow were the most important factors for developing NNPDS, whereas birth weight and faecal consistency on the day of birth had minor associations (Table 1).

Table 1. Risk factor analysis for NNPDS development.
[#]: The random variation by litter is presented as the Intra Class Correlation Coefficient.

Risk factor	Coefficient	SE	OR_{PA} [$]	P-value
Intercept	-1.63			
Herd				$3.1*10^{-10}$
Herd 4	0			
Herd 2	0.49	0.36	1.4	
Herd 3	1.11	0.37	2.7	
Herd 1	2.68	0.38	12.8	
Parity				$3.5*10^{-7}$
2nd-7th	0			
1st	1.54	0.28	4.1	
Birth weight				0.003
Per 100g increase	-0.09	0.03	0.8	
Liquid faeces day one				0.006
No	0			
Yes	0.54	0.19	1.5	
ICC[#] litter	21%			

Conclusions and Discussion

The pattern of diarrhoea differed much between herds suffering from NNPDS, but an association with first parity litters was seen across herds. This association may be explained by an insufficiency of colostral antibodies, thus suggesting an infectious etiology of the syndrome. Overall, herd of origin and parity of sows were the most important factors associated with the development of NNPDS. Birth weight and faecal consistency at birth were risk factors of minor importance.

Acknowledgements

The study was supported by the Danish Ministry of Food, Agriculture and Fisheries. We wish to thank herd-owners and staff-persons for their help and co-operation during the field-work.

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P.056

The use of pressure mat gait analysis in pigs: Vertical impulse asymmetry as an objective indicator for lameness

E Meijer[1], FJ van der Staay[1], M Oosterlinck[2], A van Nes[1], W Back[2,3]
[1]*Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, The Netherlands,*
[2]*Department of Surgery and Anaesthesiology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium,*
[3]*Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands,*
e.meijer1@uu.nl

Introduction

Lameness is a common problem in modern swine husbandry [1]. This condition causes severe welfare problems in the affected pigs [2, 3], and accounts for considerable economic losses in the porcine industry [3]. To reduce the incidence of lameness, evidence-based prevention and treatment advices need to be provided by veterinarians, for which methods to objectively quantify lameness are instrumental.

Traditionally, however, lameness is quantified by using visual scoring methods. Although these are fast and inexpensive, they are subjective, especially in hands of untrained observers and in mild lamenesses [4–6]. Kinetic gait analysis has been used extensively in human, equine and canine gait research, and recently some promising results have been obtained in pigs as well [7]. In this study, a pressure mat was used to establish left- versus right limb asymmetry indices, which were compared with the visual lameness scores. An optimal cutoff point was determined that allowed objectively distinguishing lame from sound piglets.

Materials and Methods

Ten (6 males and 4 females) clinically lame (Topigs 20 x Tempo) pigs aged between 4 and 10 weeks were selected by a consulting veterinarian at a commercial breeding farm. The pigs were lame at only one limb and did not show any symptoms of other diseases. An age-matched group of 10 pigs from the same breed and source served as sound controls. All pigs were trained to walk over a runway (4.83 x 0.40 m) that contained a Footscan® 3D Gait Scientific 2.00 x 0.40 m pressure mat (RsScan International, Olen, Belgium) with an active sensor surface of 1.95 m x 0.32 m containing 16384 sensors. Vertical impulse (VI) was recorded in three runs and the mean Asymmetry Index (ASI) values were calculated for the whole group of the fore and hind limbs, and across the medial body plane (left fore plus left hind versus right fore plus right hind). Visual scoring was performed according to the protocol by Main et al. [4]. After locomotor data collection the piglets were euthanized, and the limbs were subjected to a pathological examination.

Spearman's rank correlation coefficient was used to determine correlations between visual scores and ASI; an independent-sample *t*-test was used to statistically compare the collected data from the lame to those of the sound control pigs. Receiver-operated curve analysis was performed to assess the suitability of the ASI of VI for diagnosing lameness ($P<0.05$).

Results

Visual scoring of lameness was highly positively correlated with VI ASI. Lame pigs had significantly higher ASI of VI compared to sound pigs ($P<0.05$). Using the average ASI, a clear cutoff point, yielding 100% sensitivity and specificity, could be determined using the ROC analysis.

Conclusions and Discussion

Pressure mat analysis of pig gait symmetry data allowed to objectively distinguish clinically lame from sound pigs. The ASI correlated well with the severity of lameness determined by visual scoring. Thus, pressure mat analysis of gait may be a useful tool to objectively quantify lameness in pigs. Further research, assessing the sensitivity of VI ASI to detect very subtle lameness (that cannot be detected by visual inspection) is underway, and the potential of this approach for both research and practical applications of pressure mat analysis in lameness detection will be evaluated.

Acknowledgments

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The effect of Virkon S disinfection on the reduction of losses caused by PFTS

L Czanderlova[1], S Odehnalova[1], M Zizlavsky[1]
[1]*Sevaron Counselling, Brno, Czech Republic,* linda@sevaron.cz

Introduction

Porcine periweaning failure to thrive syndrome (PFTS) is a clinical condition characterized by anorexia, lethargy, and progressive debilitation of pigs occurring within 1 to 3 weeks after weaning. Pigs are apparently healthy and in good body condition at the start of the syndrome. In clinically affected pigs the weight loss and poor body condition continue over next 1 to 3 weeks, the case fatality rate is usually high (more than 50 to 100%). The syndrome can result in mortality rates of 5 to more than 20%. The etiology, pathophysiology, and pathogenesis of PFTS have not been determined, although several infectious agents have been identified in affected pigs. Histopathologic lesions of chronic active rhinitis, superficial gastritis, atrophic enteritis, superficial colitis, and thymic atrophy are observed in most PFTS-affected pigs. The basis for a presumptive diagnosis of PFTS includes the age of onset, the presence of typical clinical signs, the presence of collective histopathologic lesions, and, importantly, the ruling out of other known swine diseases (1).

Materials and Methods

The farm where this syndrome was seen was a 300 sows commercial production system. This farm is positive for PCV2, *Mycoplasma hyopneumoniae*, *Haemophilus parasuis (serotype 5 and 14)*, *Actinobacillus pleuropneumoniae* (serotype 8), *Streptococcus suis and PED*. Pigs were vaccinated against *Actinobacillus pleuropneumoniae* and swine erysipelas. Sows were vaccinated against PCV2, *Actinobacillus pleuropneumoniae*, *Escherichia coli*, TGE and PED (autogenous vaccine), PRRSV, PPV and swine erysipelas. Amoxicillin was used for periweaning medications (10 days treatment). Piglets were placed in a nursery facility, where they spent 5 weeks, then they were transferred to a facility for growing pigs.

The outbreak of PFTS started in the beginning of the year 2013 and significantly increased the mortality on this farm. Weaned piglets in good condition, with an average weight of 7.3 kg began to show the first signs of failure to thrive and wasting approximately 7 days after weaning, even at the time of amoxicillin medication. In the following days the health condition deteriorated and affected piglets died or were killed. Comprehensive laboratory tests did not reveal infection with any specific pathogen in dead piglets. Virological tests for rotavirus, coronavirus, PCV2, PRRS and SIV were negative. Parasitological examination of faeces and intestinal contents was negative. Cultivation examination found only *Pasteurella multocida* and *Streptococcus suis* with good antibiotic sensitivity (including amoxicillin) in the lung tissue of several pieces. Of intestinal pathogens, *Salmonella* sp., *Brachyspira* sp. and *E. coli* with virulence factors were excluded repeatedly. The quality of feed was also analyzed, including the determination of DON and ZEA mycotoxins, again with negative results. The health status did not improve even when the medication was changed to another antibiotic (florfenicol). On the contrary, losses increased (Table 1). Due to the presence of typical clinical symptoms and the exclusion of other diseases, PFTS was diagnosed. A disinfection programme was recommended, which included regular decontamination of empty sections with 1% Virkon S and regular decontamination of air by fogging 0.5% Virkon S in the presence of animals, always twice weekly (Monday and Thursday). Immediately after the start of this disinfection programme on 15/04/2013 there was a significant loss reduction in weaned piglets (Table 1).

Results

Almost immediately after the start of this disinfection programme on 15/04/2013 there was a significant loss reduction in weaned piglets (Table 1). The onset of effect was very rapid, while 21 piglets died in the first half of April, only one piglet died in the second half of April. The health situation in the herd has remained stable with a long-term and consistent disinfection of the environment including regular fogging.

Table 1. Mortality and ADG in weaned piglets before and after the start of Virkon S disinfection

Month	Mortality (%)	ADG (g)
January	3.39	249
February	5.07	245
March	9.88	167
April	6.20[1]	300
May	2.24	271
June	1.29	278
July	0.26	296

[1]Virkon S disinfection started on 15/04

Conclusions and Discussion

The results of this field evaluation have confirmed a significant decrease in mortality and the total loss and improvement of performance parameters in the group of weaned piglets treated with Virkon S disinfection.

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Collaborative information system for PRRS management: From farm to cell phones

L Urizar[1], C Klopfenstein[1], V Dufour[1], R Doré[1]; J Rivest[1]
[1] *Centre de développement du porc du Québec inc. (CDPQ),* lurizar@cdpq.ca

Introduction

The availability of an efficient PRRSV monitoring information system for a large scale project remains a major issue. The purpose of this paper is to present the system developed by CDPQ to support Quebec's province-wide PRRS monitoring effort (3000 sites).

Materials and Methods

Conceptual framework

The system has three main components: 1) the database system, 2) a data collection and management tool[1], and 3) an information delivery system adapted to clients (producers, veterinarians, laboratories and the University of Montréal's research department).

Each information system partner (producers, veterinarians, laboratories and researchers) has its own database that communicates and shares specific data with the other databases (see figure 1). This structure, which does not have a central database, guarantees high quality data in the system at the lowest cost (no intermediary).

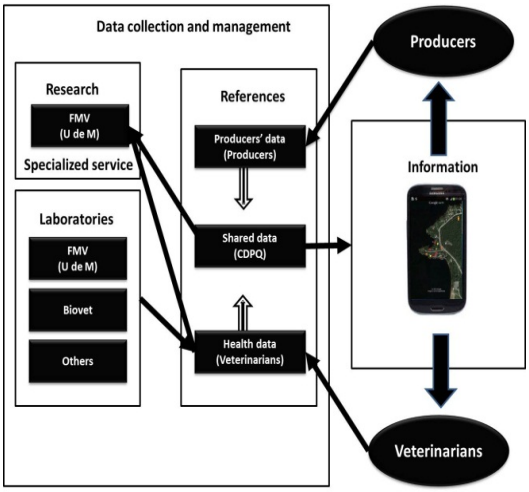

Figure 1. Operational framework of the information system

The data collection and management tool gathers the data and inputs it into the information system. CDPQ, an independent third party, manages this tool. This data management tool is built on Microsoft SQL server and comprises two data collection tools (data importation facilities and web-based data validation and import tools).

All the data obtained from various sources is then used to produce information for clients (producers, veterinarians and other consultants involved in swine health management). Information is transferred to specific clients through Dropbox linked to their cellphone, tablet or pc.

Technology

The technology to support this system is based on freeware. Moreover the technology is compatible with most platforms (Android, IOS and Windows).

Ownership and control

This system allows the producer (owner of the pigs) to have full control over the entire system. At any given time, a producer can display basic health-related information on a cellular phone, and see who has access to the information on his sites.

Shared information

The information currently shared comprises: statistical reports about PRRS status and virus strains in the area; and geographical maps which indicate the location of the production sites, the type of production and the health status of the sites. Different levels of confidentiality are offered, depending on what producers want.

Results

This monitoring system facilitates analysis of the PRRS status profile of a group of farms or in a region. It can also be used to monitor other types of disease (Porcine Epidemic Diarrhea, Influenza, etc.).

The system lets the producer, herd veterinarian and technical staff share strategic and standardized information between themselves, as well as within the community projects framework.

It also allows the optimization of the efficacy of the work being done by the teams on the field.

Finally, it provides information to researchers involved in disease control projects.

Cost of the system

The anticipated cost of running this information system should be less than CAD 100 per client per year.

Conclusions and Discussion

We are entering a new era where smartphone technology and apps will be widely used.

CDPQ believes this low cost collaborative information system for monitoring PRRS provides a sustainable template for disease control initiatives in the province and elsewhere.

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1. Portail et Zoom santé du CDPQ, copyright 2013

P.060

Susceptibility of *B. suis* biovar 2 to antibiotics of current use in pigs

L Dieste-Pérez[1], PM Muñoz[1], MJ De Miguel [1], JM Blasco [1], L Fraile[2]
[1] Unidad de Sanidad Animal, CITA, *Zaragoza, Spain* [2]*Animal Production Department, University of Lleida, Spain,*
lorenzo.fraile@prodan.udl.cat

Introduction

Antibiotic treatment of *Brucella* infections is used successfully in humans. Doxycycline- aminoglycoside combinations are considered the most effective treatment for human brucellosis, whereas the oral association of doxycycline and rifampicin is a good practical alternative (Ariza et al., 2007). However, treatment of brucellosis in animals is not regularly performed due to their high costs and the existence of compulsory testing and slaughtering programs. Swine brucellosis caused by *Brucella suis* biovar 2 is an emerging disease in Europe, and represents a common infection in pigs reared in outdoor breeding systems, likely as a spill-over from brucellosis of wild boar and hares, which are considered the wild reservoir of this infection (Muñoz et al., 2010). Due to its very low zoonotic involvement, and the epidemiological particularities of this infection (Olsen et al, 2012), the antibiotic treatment could be a suitable alternative to culling infected herds. However, there is a paucity of data on antibiotic treatment of swine brucellosis. The first step to establish an adequate antibiotherapy against this infection would be the selection of the most suitable antibiotics and the assesment of their efficay *in vitro*.

The purpose of this study was to determine the *in vitro* susceptibility of *Brucella suis* biovar 2 to the most suitable antimicrobial candidates.

Materials and Methods

The 3 *Brucella suis* (*B. suis*) reference strains for biovar 1, 2 and 3 (1330, Thomsen and 686, respectively) and 33 *B. suis* biovar 2 field strains representing the main haplotypes (Muñoz *et al.* 2010), and isolated from natural brucellosis cases in swine and wildboar occurred in Europe between 1990 and 2013 were used. Antimicrobial susceptibility (MIC) was assessed using a broth microdilution method. Briefly, each bacterial suspension was inoculated on a 96-well microplate loaded with 2-fold dilutions (from 0.01 to 8 µg/mL) of each antibiotic in Mueller-Hinton broth. The final bacterial concentration in each well was around 1 x 10^4 CFU/mL. Microplates were incubated for 7 days at 37ºC with 10% CO_2 and without CO_2 atmosphere. Controls for strains growth and sterility were included. The MIC_{50} and MIC_{90} values were defined as the lowest antimicrobial concentrations inhibiting bacterial growth in the 50% and 90% of strains, respectively, in the specific incubating conditions. The antibiotic tested, including two new generation macrolides (tulathromycin and tildipirosin), are shown in Table 1.

Results

The MIC_{90} for all *B. suis* strains were comprised between 0.01 to 0.25 µg/mL or 0.01 to 0.12 µg/mL depending whether they were incubated with or without CO_2, respectively (Table 1). Tulathromycin, tildipirosin and dihydrostreptomycin MICs were higher when incubating with CO_2. By contrast rifampicin MICs were lower. No differences were observed between the different haplotypes of *B. suis* biovar 2 neither with the *B. suis* biovar 2 (Thomsen) and biovar 3 (686) reference strains. Curiously, *B. suis* biovar 1 (1330) reference strain showed higher MICs for tildipirosin (8 µg/mL and 0.25 µg/mL, with and without CO2, respectively).

Table 1. MIC results of the 36 *B. suis* strains tested.

	MIC range obtained after incubation with / without CO_2	
Antibiotic	**MIC_{50}**	**MIC_{90}**
Oxytetracycline	0.01/0.01	0.01/0.01
Doxycycline hyclate	0.01/0.01	0.01/0.01
Dihydrostreptomycin	0.06/0.01	0.25/0.03
Rifampicin	0.03/0.06	0.06/0.12
Tulathromycin	0.12/0.01	0.12/0.01
Tildipirosin	0.03/0.01	0.06/0.01

Conclusions and Discussion

The MICs of all the antimicrobials tested were very low against all haplotypes of *B. suis* biovar 2 described in Europe, paving their potencial use independently of the haplotype present. Additional research should be conducted to determine their pharmacokinetics and efficacy *in vivo* to establish the proper treatment in swine. Experiments in animals are in progress.

Acknowledgments. Aragón Government PhD grant (L. Dieste-Pérez) and INIA project RTA2011-00103-00-00.

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P.061

Comparative efficacy of two vaccines against Atrophic Rhinitis in pigs under field conditions in Germany

P Latell[1], O Niemann[2]
Veterinary practice Latell[1], Köthen, Germany; HIPRA Deutschland GmbH[2], Düsseldorf, Germany, olaf.niemann@hipra.com

Introduction

RHINISENG® is a vaccine to protect against progressive (PAR) and nonprogressive atrophic rhinitis (NPAR), indicated for pregnant sows and gilts to passively protect their offspring[1]. This study evaluated the clinical efficacy of RHINISENG® compared to the control scheme in force in an endemically infected farrow-to-finish herd in the East of Germany.

Materials and Methods

The study was carried out on a sample from a 1200-sow farrow- to-finish herd. At the time of the study, the farm reported good reproductive parameters. However, clinical signs of PAR occur regularly in the nursery and fattening units, and growing pigs were suffering from porcine respiratory disease complex (PRDC). The farm was endemically infected with PMT as evidenced by previous laboratorial examinations. In consequence, a vaccination schedule in sows, consisting of two shots of a commercial vaccine (Vac B) at 6 and 3 weeks before farrowing was in force. Because of the clinical evidence of PAR in piglets, they were also being injected with Terramycin LA on day 1, 7 and 14 of age. Other vaccines used comprised *E. coli* + *Clostridium perfringens*, and porcine parvovirus + swine erysipelas in sows. Piglets were also vaccinated with *Mycoplasma hyopneumoniae* and PCV2 vaccines.

Because the studied herd was being managed under a 1-week production rhythm at the time of the trial, experimental groups of animals (average of 60 sows and 850 piglets per group) were consecutively designated G1-G4, and treated following the same rhythm in order not to disturb the production flow, but ensuring that no changes were implemented other than the vaccination and antimicrobial treatments as follows: G1: Vaccination with RHINISENG® (vaccine A) 6 and 3 weeks before farrowing[2]. Piglets remained untreated; G2: Vaccination with RHINISENG® (6 and 3 weeks before farrowing). Piglets were injected with Terramycin LA at a dose of 200 mg/kg body weight (deep intramuscularly) three times (D1, D7, and D14 of age); G3: Vaccination with vaccine B using the same injection schedule, and following the vaccine label instructions. Piglets were treated the same as G2; and G4: Vaccination with vaccine B the same as G3. Piglets remained untreated. At the end of the nursery period (11 weeks of age), 8 piglets with clinical signs for AR in each group were selected for necropsy. Evaluation of vaccine efficacy was based on the proportion of individuals showing gross lesions of pneumonia and rhinitis.

Results

Groups with Rhinseng® vaccination showed less gross lesions than the groups vaccinated with the vaccine B

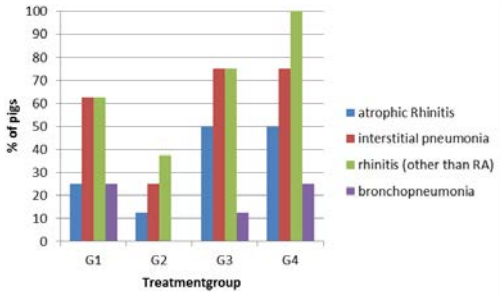

Figure 1. Especially the numbers of pigs with typical gross lesions of AR were lower. Only in the group G2 pigs without gross lesions could be found (3/8).

Conclusion and Discussion

The vaccination with Rhiniseng® with and without antibiotic treatment shows a higher efficacy in controlling AR on the farm. In the follow the health status in the nursery and fattening improved significantly.

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Presence of astrovirus in healthy piglets and in piglets with signs of neonatal diarrhoea of unknown origin

P Wallgren, K Ullman, C Baule, L Liu, M Juremalm
National Veterinary Institute, SVA, Uppsala, Sweden. Per.Wallgren@sva.se

Introduction

Despite proper vaccination routines of sows the incidence of neonatal diarrhoeas has increased during the last decade in several countries, indicating other sources to neonatal diarrhoeas than *E. coli* and *Cl. perfringens*. The phenomenon has been called New Neonatal Porcine Diarrhoea (NNPD) and both viral and bacterial agents have been suggested to have caused the disease. Astrovirus has previously been demonstrated in diarrheic neonatals from many species including mink [1] and humans [2]. In the present study, the relevance of porcine astrovirus (PoAstV) as cause of neonatal diarrhoeas of undiagnosed origin in pigs was evaluated.

Materials and Methods

Step I. A broad range semi nested RT-PCR for detection of astrovirus [3] was used and positive samples were confirmed as PoAstV by sequencing.

Faeces from 17 piglets with neonatal diarrhoea were analysed. They emanated from three herds previously scrutinized with respect to *E. coli* but without presence of pathogenic *E coli* [4].

In addition, faeces from six piglets with diarrhoea and six healthy piglets in a herd clinically affected by NNPD were analysed (**Case I**).

As control, faeces from ten piglets in each of three herds free from neonatal diarrhoeas were analysed (Control A, B and C).

Step II. Presence of PoAstV was detected by an in house developed real-time RT-PCR targeting genotypes 2 and 3.

Faecal samples from diarrhoeic and healthy piglets in four herds clinically affected with NNPD were analysed (**Case II, III, IV and V**).

As a control, faecal samples from piglets in a healthy herd that had been closed (only introducing semen) for more than 20 years were analysed (Control D).

Results

Step I. In **case I**, PoAstV was demonstrated in both diarrhoeic and healthy piglets. However, PoAstV was only demonstrated in one out of 17 piglets with neonatal diarrhoea not associated with *E. coli*, and PoAstV was also demonstrated in all three apparently healthy herds scrutinized (Table 1). **Step II.** PoAstV was demonstrated in all piglets with neonatal diarrhoea and from none of the healthy piglets in **case II**. In **case III**, PAV was detected in both healthy and diarrheic pigs, whereas PoAstV not was detected at all in **case IV** and **case V**. Neither was PoAstV demonstrated in the healthy herd that had been closed for more than 20 years (Table 2).

Table 1. Results from step I of the study

Herds	Status Herd	Status piglets	n	PoAstV-positive
3 herds [4]	NNPD	diarrhoea	17	1
Case I	NNPD	diarrhoea	6	3
		Healthy	6	2
Control A	Healthy	Healthy	10	1
Control B	Healthy	Healthy	10	3
Control C	Healthy	Healthy	10	1

Table 2. Results from step II of the study

Herds	Status Herd	Status piglets	n	PoAstV-positive
Case II	NNPD	Diarrhoea	6	6
		Healthy	6	0
Case III	NNPD	Diarrhoea	5	3
		Healthy	5	5
Case IV	NNPD	Diarrhoea	6	0
		Healthy	4	0
Case V	NNPD	Diarrhoea	5	0
		Healthy	1	0
Control D	Healthy	Healthy	12	0

Conclusions and Discussion

The result from **case II** clearly indicated that PoAstV may induce diarrhoea in neonatal piglets and also was the causative of the NNPD in that herd. Although less clear, the results obtained in **case I** and **case III** does not rule PoAstV out from being causative to the NNPD in those herds. However, failure to demonstrate PoAstV in **case IV** and **case V** clearly suggest other causes to the NNPD in those herds.

In conclusion, PoAastV was confirmed to be able to induce diarrhoea in neonatal piglets[5], and occasionally also to do so. However, already in this pilot study PoAastV was also conclude to not be the sole cause of NNPD. PoAstV was also detected in herds free from NNPD

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P.063

Estimating the impact of live virus inoculation for PRRS control in a production system

A Oropeza, J Kolb, M White, R Philips
Boehringer Ingelheim Vetmedica Inc. Saint Joseph, MO, arturo.oropeza@boehringer-ingelheim.com

Introduction

The cost to the swine industry associated with the presence of PRRSV infection continues to be a significant problem faced by swine producers. This is a case study evaluating the production impact of the use of Live Virus Inoculation (LVI) as a method to generate homologous immunity for the control of PRRS.

Materials and Methods

In the spring of 2011, a collaborative PRRS control project was initiated involving 26,000 sows distributed in 12 different sow farms, two gilt development units, one boar exposure site and two nurseries. The primary interventions consisted of herd closure for 210 days, application of live resident wild-type PRRSV twice, four weeks apart, to all sows and gilts. Production parameters were compared using a before and after approach. Production data from eight weeks prior to and following the LVI intervention was compared. A limited number of nursery closeouts were available in the 8 week period prior to the intervention so 7 additional closeouts representing a 20 week period prior to the intervention were included. . The impact on the breeding herd was assessed by calculating pigs not weaned from sows due to PRRS induced abortions. Post-weaning effects were assessed by tracking the pigs born from LVI exposed sows through both the nursery and grow-finish stage of production. A total of 46,168 pigs were followed through the nursery phase and 36,835 pigs were followed through the finishing phase. Pigs lost during the nursery period were calculated as the difference in mortalities before and after the intervention. Impact on nursery and finisher performance was calculated using the difference in pounds gained between the before and after period. No seasonal adjustments were made as the objective was to assess the immediate effects of the LVI intervention on production. Calculations were made accounting for all growing pigs in the production groups as well as the impact to breeding and weaning groups. In addition, SPC charts were used in assessing the effects of LVI as a process change.

Results

Table 1 summarizes the effects of the LVI intervention. Additionally, the LVI intervention increased cull rates for females, increased non-productive days, and increased culls in the finisher (Not quantified or data not shown).

Table 1. Summary of LVI effects

Parameter	Difference	Long term financial impact
Pigs not weaned due to abortions	-3312	Opportunity loss, under-utilization of buildings
Nursery pig losses	-835	Opportunity loss, under-utilization of buildings
Reduction in Nursery exit weight (lbs/pig)	-2.92	Impact on finisher performance
Reduction in Finisher exit weight (lbs/pig)	-4.33	Impact on revenue

Conclusions and Discussion

The use of LVI can have a measurable biologic impact as a PRRS control intervention. Linhares et al. reported farms implementing LVI as an exposure protocol lost 2665 weaned pigs and estimated opportunity costs of $177,809.[1] This case study showed the intervention of LVI for breeding herd stabilization to negatively impact the number of pigs weaned, nursery mortality, nursery and finishing ADG for a period of time following the intervention. A reduction in pigs weaned due to abortions has significant economic consequences due to both opportunity losses and potential under-utilization of buildings. Losses occurring in the nursery period represent additional opportunity losses, further under-utilization of facilities, and a loss of the investments already placed into the pig, such as feed, vaccines, and antibiotics. Losses in nursery performance were not compensated for in the finisher period, and instead continued in the finisher period. When developing a PRRS stabilization protocol, factors such as “time to stability” (TTS), “time to base-line performance” (TTBP), and expectations for the impact of the intervention need to be considered.

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P.064

Clinical and serologic investigations of foot-and-mouth disease outbreak in the Kinmen Island with animals under compulsory vaccination in 2012

SP Chen, YF Sun, MC Lee, PC Yang
Division of animal Medicine, Animal Technology Institute Taiwan, Chunan, Miaoli, Taiwan pchen342@gmail.com

Introduction

In Taiwan, NSP has been annually used for the routine surveillance to monitor virus circulation. Every year, about 50000 samples including Kinmen Island were collected from different auction markets and farms which were tests with two ELISA kits, one for screening test and another one for confirmatory test (Chen et al., 2011). The prevalent rate was about 0.02% each year. Since the low level of prevalence and the quick turn-over of pig growth, Taiwan had developed a new strategy for the control of foot and mouth disease (FMD). After FMD was recurred in 2009, the prophylactic vaccination measure with one vaccination in fattening pigs was implemented and twice for cattle, sheep, goats and deer per year. This is to report the quick control and eradicate FMD outbreak of O SEA on pig farms in Kinmen Island with compulsory prophylactic vaccination after the implement of move restriction, depopulation of infected farms and emergency revaccination.

Materials and Methods

1 Sampling method

In Kinmen, fattening pigs and cattle were sent directly to slaughterhouses by trucks. Pigs and cattle are blood sampled before slaughtering. The pigs and cattle are systematically sampled with 1 to 5 serum samples being taken from each farm. Before the blood samples are taken the staff check the pigs first and any pigs with abnormal hoof lesions or abnormality in gaits have blood collected, otherwise the pigs for testing are randomly selected.

2 Samples collected

Sera were collected from slaughterhouse every month throughout the year. The numbers collected were proportional to the pig farm sizes throughout Kinmen Island. 2,200 and 264 serum samples were collected from pigs and cattle respectively in the slaughterhouse in 2012.

3 The NSP ELISA kits used in the serosurveillance

Two commercially available ELISAs for detection of antibodies directed against FMDV non-structural proteins (NSP ELISAs) were used. The UBI FMDV NS ELISA Swine was obtained from United Biomedical Inc., Nauppauge, The Ceditest and the UBI ELISA kits were used as the screening test and confirmatory test, respectively.

4 Follow-up procedure in case of positive NSP test results

Pigs or cattle giving positive reactions were traced back to the farms of origin where a thorough investigation was conducted using relevant clinical, epidemiological, and serological examinations. They visited the farm to examine hoofs and snouts of fattening pigs and cattle of all age groups for evidence of current clinical lesions or healing lesions that resemble FMD. Further blood sampling was performed with 14 samples collected from the fattening pigs or cattle, preferably from those batches to be sold soon. The sampling protocol was designed to detect at least one positive reactor, given 20% herd prevalence, with 95% confidence.

Results

Samples tested with two NSP ELISA kits used:

A total of 2,464 serum samples collected from pigs and cattle in the slaughterhouse were submitted in 2012. There were two positive reactors in the 2012 surveillance with the screening test (Ceditest). No positive reactors were found after reconfirmation test.

Follow-up procedure in case of positive NSP test results:

No follow-up procedure were carried in 2012 surveillance because of none of the farms was classified as a suspected farm.

Conclusions and Discussion

Following the culling of 540 animals in four farms of this outbreak, the Taiwanese government adopted emergency revaccination, movement restriction as stipulated control policy with a vaccination-to-live strategy for effectively controlling the epidemic to minimize economic losses. The different epidemiological pattern of limited cases has been found in Kinmen Island FMD outbreak with O SEA virus, which was causing wide spread of disease in Korea in 2010-2011. However, in this outbreak the only clinical lesions were mainly found in sows and fattening pigs which is different from those found in cattle in 1999 in Kinmen Island and the losses in Korea in 2010, but similar to the one found in Taiwan in 1997. In this investigation in 2012, we have shown that the FMD virus was not circulated in the susceptible animals in Kinmen Island with compulsory vaccination program.

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Pig; a potential channel for transmission of norovirus, rotavirus, and astrovirus

AR Kim[1], MK Choi[1], HC Chung[1], VG Nguyen[1], EO Kim[1],
HJ Yang[1], JA Kim[1], BK Park
[1]*Virology Laboratory, College of Veterinary Science, Seoul National University, Korea* parkx026@snu.ac.kr

Introduction

Norovirus, Rotavirus, and Astrovirus induce gastroenteric diseases in various animals as well as human. Especially, they are known for infecting young children and occurring severe symptoms such as anorexia, vomiting, and diarrhea (2). Porcine strains of the viruses have a variety of serotypes and genotypes, and among them, some strains are closely related to human strains. Besides, Pigs are one of the animals mentioned that these viruses can infect and transmit into human. Therefore, this study tries to find some possibilities that pigs serve as a potential agent for transmitting viruses, which have high danger for zoonosis (1, 3).

Materials and Methods

Total 121 stool samples of pigs were collected. All these samples were conducted for extraction of RNAs in feces following to TRIzol LS method. Next, these extracted RNA samples were reverse-transcribed using M-MLV polymerase. With cDNA samples, specific primer sets were used for detecting noroviruses, rotaviruses, and astroviruses. The prevalence of each virus was calculated. After acquiring sequences, phylogenetic analysis was performed with sequences found in this study and those registered in GenBank on MEGA 5.

Results

The results (Table 1) indicated the presence of NoVs, RVAs, and AstroVs in porcine fecal samples. Each detection rate of the viruses was 0, 12, and 9 percent. Based on the result, simultaneous infection of RVAs and AstroVs was found showing 5% of positive rate. On phylogenetic analysis, sequences of RVAs were arranged into G5, and G9.

Table 1. Porcine NoV, RVA, and AstroV detection rates

	NoV	RVA	AstroV
Positive	0 (0%)	15 (12%)	11 (9%)

Conclusions and Discussion

The detection rates of porcine RVAs and AstroVs were low, which is considered as it was. On the result of NoVs detection, it was a little suspected whether primer sets used worked well though we utilized 3 sets of primer targeting the regions of RdRp, Capsid, and ORF1-ORF2 junction. Figure 1 showed that all RVAs we detected were the most prevalent strains in pigs. However, the positive rate of G9, which is closely related to human strains, was not overlooked because it seemed high than other studies.

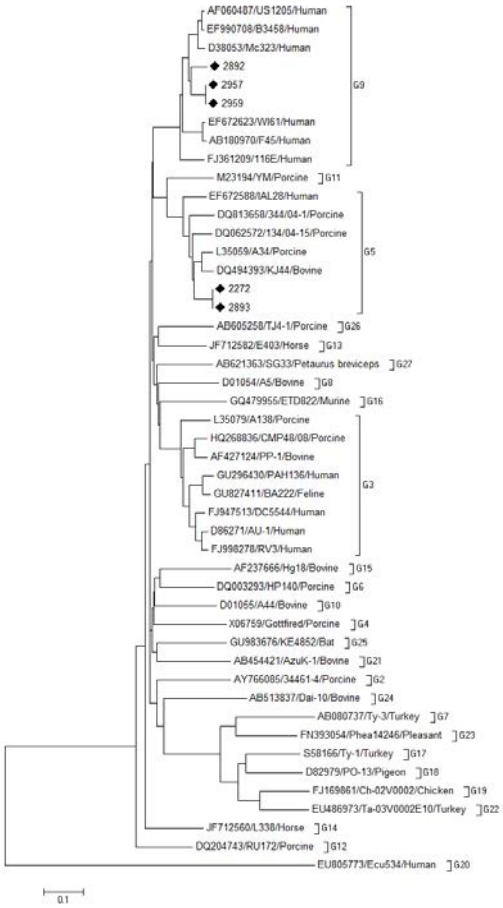

Figure 1. Genotype analysis of the porcine RVAs based on partial VP7 protein.

Acknowledgements

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P.066

Helicobacter infection in piglets: Immunohistochemical analysis in mucosal samples collected using gastroscopy

RL Silveira[1], AC M Cruz[1], VAN Degani[1], JA Câmara Filho[1], [2] FAGC Weber, ECQ Carvalho[3]
[1]*MMO, MZO, NAL, Fluminense Federal University,* [2]*Unigranrio University,*
[3]*LMPA, North Fluminense State University.* renatosilveira@vm.uff.br

Introduction

Gastroesophageal ulcer (GEU) diagnosis is fundamental for the treatment and recovery of the affected animal stock. GEU is a condition affecting animals, resulting in depletion of animal stock and subsequent economic losses. *Helicobacter* spp. have been associated with GEU. Modern endoscopes are important for detecting the stage of the breeding process at which the ulceration occurs. However, few studies regarding early detection of GEU have been conducted. Therefore, we aimed to identify whether GEU lesions were related to *Helicobacter* spp. infection, using gastroscopy as a diagnostic technique for macroscopic and histopathological analyses.

Materials and Methods

Twenty piglets (both male and female) with a mean age of 65 days were included (weight, 22–26 kg). We used a Karl Storz Gastrovideoscope (model 1380NKS). Samples from nonglandular and glandular (cardia, fundus, and pylorus) regions were collected for the ultra-rapid urease test and for histopathological and immunohistochemical (IHC) evaluations.

Results and Discussion

Eleven animals (55%) showed macroscopic lesions in the nonglandular region during endoscopy, and 15 animals (75%) showed parakeratosis on histological analysis. Lesions in at least 1 glandular region were observed in 18 animals (90%). The lesions were bigger in the cardiac region, followed by those in the antrum and the fundus. With regard to the ultra-rapid urease test, 7 animals (35%) were negative in all 4 regions and 13 (65%) were positive in at least 1 region. On IHC, 10 animals (50%) were negative in all 4 regions and 10 (50%) were positive in at least 1 region.

Conclusions

Although pre-ulcerative findings were not correlated with *Helicobacter* spp. infection in the present study, early diagnosis of GEU is vital for its treatment. The positive IHC findings for *Helicobacter* spp in regions without ulcerative lesions suggest its saprophytic and opportunistic nature.

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Relationship of *Helicobacter* spp. to gastroesophageal pre-ulcerative lesions in minipigs: Immunohistochemistry and ultra-rapid urease test analysis

RL Silveira[1], ACM Cruz[1], CRR Almeida[2], RM Medina[3], EJ Abílio[3], ECQ Carvalho[3]
[1] MMO, MZO, NAL, Fluminense Federal University, [2] LMPA, North Fluminense State University, [3]LMPA, North Fluminense State University, renatosilveira@vm.uff.br

Introduction

Studies of gastric ulcers in pigs have become of great interest in recent years. This has occurred due to its similarity to the human disease, the discovery of *Helicobacter* spp., and the relationship to reduced weight gain and therefore economic loss. This study aimed to obtain and evaluate the features of subclinical gastric lesions naturally occurring in minipigs, and to determine their relationship to the presence of *Helicobacter* spp.

Materials and Methods

In total, 40 piglets, with an average weight and age of 39.0 kg and 17 months, respectively, were euthanized and necropsied, and their stomachs collected for evaluation.

Aglandular region integrity was macroscopically evaluated, and ulcerative lesions were graded from zero to five.

Anatomical samples from aglandular (quad esophageal) and glandular (the cardium, the fundus, and the pylorus) regions were collected for rapid urease testing and histopathological and immunohistochemical evaluations.

Results and Discussion

Macroscopic lesions were observed in aglandular sections in 29 animals and microscopically showed parakeratosis.

In 36 animals, there was a change in at least one of the three regions (the cardium, the fundus, and pylorus), and four animals showed no change in any region.

Glandular lesions were larger at the cardium, followed by the antrum, and the fundus.

For the urease rapid test, 22 animals were negative in all regions, and 18 were positive in at least one, while none were positive in any region. In the antrum, eight were positive and 32 were negative. In the aglandular region, six were positive and 34 were negative. In the cardium, 13 were positive and 27 were negative. In the fundus, one animal was positive and 39 were negative. Differences were statistically significant relating to the bacteria.

In relation to immunohistochemistry, eight animals were negative in all regions and 32 were positive in at least one, but only one animal was positive in all regions. In the antrum, 10 were positive and 30 were negative. In the aglandular region, four were positive and 36 were negative. In the cardium, 23 were positive and 17 were negative. In the fundus, three were positive and 37 were negative.

We found significant statistical differences in bacteria present in the aglandular and cardium regions, while in the other regions, the bacteria was evident on immunostaining but was not associated with the affected region.

Conclusions

Pre-ulcerative findings were closely related to Helicobacter spp. in minipigs. Bacterial immunostaining not associated with gastric lesions in certain regions demonstrates its saprophytic and opportunistic character.

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Inactive vaccine to control PEDV outbreak, a Chinese observation

L Li[1], Y Huang[2]

[1]*Hangzhou Beta Veterinary Diagnostic Laboratory, 588 Feijiatang Rd, Hangzhou, China;* [2] *Institute of Preventive Veterinary Medicine Zhejiang University, Hangzhou, China,* dragonli97@aliyun.com

Introduction

Porcine epidemic diarrhea virus (PEDV), porcine transmissible gastroenteritis virus (TGEV) and porcine rotavirus (PROV) are three major viral agents to cause suckling pig diarrhea. Before 2011, these pathogens had been in endemic circulation in Chinese farms for more than 20 years. The new outbreak of sever newborn piglets diarrhea started from early spring of 2011 in China. PEDV still occupied the predominant pathogen, confirmed by RT-PCR. It causes average 60-100% mortality in the herd infected within 7 days old.

Materials and Methods

The onsite PEDV circulations were observed and concluded from more than 300 farms with PEDV outbreak. The autogenous inactivated PEDV vaccine was made from isolate HZ-2012-36. The vaccination trial was conducted in a 6000-sow one-site farm, during PEDV outbreak. Mortality data was collected from field with double-blind design. The groups received/not feedback were layout in the same farrowing unit in two rows. The positive control data was collected during the outbreak.

Results

PEDV usually first infects fatting/gilt pigs in a negative farms. Than the accumulated virus attaches gestation to make sows carry virus, when move them into farrowing crates. Sucking pigs are infected from subclinical sows afterwards, and then getting the outbreak point in very young piglets. This circulation is modeled in fig 1.

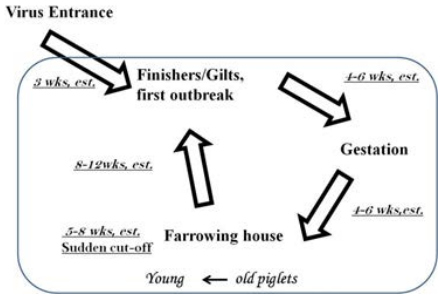

Figure 1. The onsite PEDV circulation model concluded from field outbreak cases.

Feedback was selected, by us also, as the first choice to shut down PEDV outbreak. And it has been very effectively. However, most of those farms fell in re-outbreak in 8-16 weeks after feedback. Targeting this phenomena, we made an assumption that PEDV mucosal boosting program, as shown in fig 2.

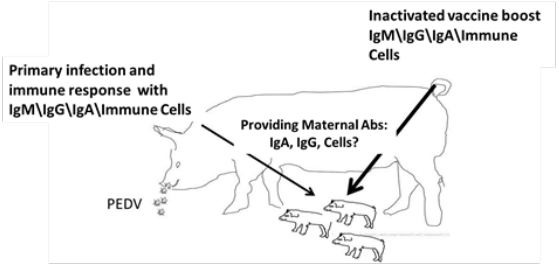

Figure 2. Maternal immune boosting model with PEDV inactivated vaccine

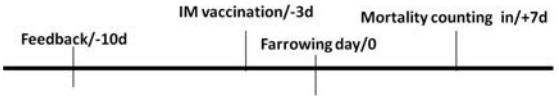

Figure 3. A rational design using feedback and vaccination combination

Following this assumption, we further made a vaccination trial as show in fig 3 and table 1. The results turned out that this boosting program worked and the protection period lasted much longer (>16wks) that feedback alone.

Table1. Clinical protection of feedback and vaccination combination in controlling PEDV outbreak

Trials	Feedback (w/o)	Inactivated Vaccine (w/o)		Litters	Total born in alive	Mortality in 7days	
						Each Group	In average
1	W	HZ-2012-36	W	34	352	7.4%	14.7%
2	W		W/O	34	349	19.5%	
3	W/O	W/O		102	1052	60.8%	60.8%

Construction and immunogenecity analysis of recombinant attenuated *S. choleraesuis* strains expressing protective antigens of PEDV

L Xu, F Chen, A Guo, H Chen, Q He
State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan, China, he628@mail.hzau.edu.cn

Introduction

Porcine epidemic diarrhea (PED) is an economically important enteric pathogen for swine, especially in Southeast Asia countries. The disease is characterized by severe enteritis, vomiting, watery diarrhea and weight loss in adult pigs and is lethal in piglets (1). Since October of 2010, more severe PED began becoming epidemic in the China. In 2011, variant PEDV strains had been conformed almost in the whole China (2,3). The effect of immunization relies mainly on the mucosal immunity (SIgA), not on the IgG level. Previously, we have constructed the new vaccines against PED with attenuated Salmonella as live vector. In this study, we here explore the antigen characteristic of two recombinant *Salmonella Choleraesuises* strains which respectively express partial S gene of PEDV (COE and SD gene) in mice and pig model

Materials and Methods

The two fragments cover main antigen epitopes of PEDV, i.e., SD domain and COE, were amplified through RT-PCR using CV777 strain as template. The amplicons were confirmed via sizes and sequence analyses. Then, the amplified products were singular or fusion inserted into asd+ expressing plasmid, pYA3493, followed by electro-transformation with attenuated *Salmonella choleraesuises* to yield two recombinant *S.cholerasuises* expressing the target genes, designated as C501-COE and C501-SD. Seventy six-week old SPF mice were randomly divided into seven groups, in which the mice were vaccinated intramuscularly or orally with $10^{8.0}$CFU, the mice administrated with the parental *attenuated Salmonella* C500 and TBS were served as control. Clinically oral administration with a dose 3×10^{9} CFU of the mixture of two recombinant strains were carried out to evaluate the safety of these two strains. The potential vaccines containing two recombinant strains were also clinically applied in diarrhea-effected pig farms to primarily determine the immunogenecity.

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Results

The two recombinant genes were successfully constructed and the targeted SD and COE were expressed on the surface of the *Salmonella*. The recombinant *Salmonella* strains had the same biochemical characteristics as parental strain C500 in phenotypic characteristics and hereditary stability. The mice model showed that the secretory IgA titers of orally immunized C501-COE and C501-SD groups were l:1280 and l:2048, respectively. While the secretory IgA titers of the intramuscular injection groups were l:640 and l:1024, respectively, lower that the oral administrated groups. The intramuscular injection groups could induce IgG titers with l:4096 and l:2560 higher than orally immunized groups, 1:2048 and 1:1280, respectively. The intramuscular injection groups could induce more IFN-γ than the orally immunized groups. The primary clinical applications of the recombinant *Salmonella* strain in porcine showed these strains were safe as no side-effect including fever and inappetence was observed. The clinical test in PED-affected farms implied the decrease mortality rates from 80% before utilization to 20%-50% after treatment and the rate of diarrhea had fallen from100% to 18%-30%.

Conclusions and Discussion

The S gene sequence is a distinguishing feature of PEDV strains' virulence and evolution. The residue (499-638aa) on the S protein of PEDV is an important neutralizing epitope region (4). The epitopes S1D5 (residues 744-759) and S1D6 (residues 756-771) are two linear epitopes of the PEDV S protein (5). So these two domians can be used to study vaccines. In our experiment, these two proteins were succesful expressed, and the recombinat *Salmonella* strains showed good genetic stability, good safety and good antigen characteristic. The two different inoculation ways of these two recombinant strains could not only induce mucosal immunity and humoral immunity response, but also cellular immune response as well. The level of IgG was higher in intramuscular injection groups. While the secretory IgA level was higher in the orally adminstration groups. What's more, the *Salmonella strain* itself can work as adjuvant. All of the above data indicated these two recombinant as potential vaccines strains.

Acknowledgments

China Agriculture Research System (CARS-36). We also thank Dr. Roy Curtiss III, University of Washington, USA, for his gift of the plasmid pYA3493 and host bacterial X6097.

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Experimental inoculation with PEDV in sparrows and mice

JH Lee[1], DS Song[2], HK. Jeong[3], JH Shon [3], BK Park[1], HS Joo[4]
[1]Seoul National University, [2]Korea Research Institute of Bioscience and Biotechnology, [3]Hansoo Pig Res Institute, S. Korea, [4]College of Veterinary Medicine, University of Minnesota, St. Paul, U.S.A. hoony9913@hotmail.com

Introduction

Porcine epidemic diarrhea virus (PEDV) causes significant economic loss due to high mortality in pigs less than 1 week of age with vomiting and severe diarrhea. Transmission of the virus between swine farms commonly occurs by introduction of the carrier pigs. The virus also can be transmitted by mechanical carriers such as contaminated vehicles. Several wild animals are suspected to carry PEDV and transmit the virus between and within swine farms. Among the wild animals, sparrows and mice are commonly found around swine farms and are likely carry the virus. Sparrow has been speculated to transmit transmissible gastroenteritis virus that causes very similar clinical signs in pigs. The objective of this experiment is to investigate if PEDV can replicate in intestinal tract of sparrows and mice following experimental inoculation with a wild type PEDV.

Materials and Methods

PEDV used was a pig intestinal homogenate that had been inoculated with a field sample from a pig showing typical clinical signs of PEDV infection. The virus was confirmed by RT-PCR using a PEDV specific primer and characterized genetically in a previous study (1, 2). Wild sparrows that were caught from fields by using a net and laboratory mice were purchased from commercial sources. Twenty sparrows and 20 mice were used in this experiment. The sparrows were housed in a 90 x 50 x 40 cm cage, and the laboratory mice were housed in a 40 x 20 x 20cm cage with sodas bedding. At day post-inoculation (dpi) 0, 4 sparrows and 4 mice were sacrificed without an inoculation and used as controls. Sixteen sparrows and 16 mice were inoculated orally with approximately 0.5 ml of PEDV using pipet tips. The inoculated sparrows were subsequently allowed to drink water that had been mixed with 20 ml of the virus for overnight. The mice were also allowed similarly to drink water containing 20 ml of the virus. Four sparrows and 4 mice each were killed 1, 2, 3, and 4 dpi, and small intestines were collected for RT-PCR and IHC. Small intestines from the sparrows and mice were collected and divided into 2 parts. One part was stored at -20C for real time RT-PCR (rRT-PCR) and another part was in 0.5% formalin phosphate buffered saline for immunohistochemistry (IHC). For the rRT-PCR, cT values of 35 or higher were considered as negative. The rRT-PCR and IHC were carried out as described previously (3, 4)

Results

Two pools of 2 sera each from sparrows and mice were negative by PEDV serum neutralization test. The inoculated sparrows and mice did not show any abnormal clinical sign during the experimental period. All intestinal samples collected every day from 20 sparrows were negative by PEDV rRT-PCR (Table 1). All samples collected from mice were also negative. Two intestinal samples each from sparrows and mice collected at 1, 2, 3, and 4 dpi were examined by IHC, and no infected cells were found in the samples from both sparrows and mice.

Table 1. Viral RNA detection using rRT-PCR after inoculation of a wild type PEDV in sparrows and mice

	Days post-inoculation				
Intestine	0	1	2	3	4
Sparrows	neg	neg	neg	neg	neg
Mice	neg	neg	neg	neg	neg

***** On each day, 4 sparrows and 4 mice were tested.

Conclusions and Discussion

No evidence of PEDV replication in small intestinal cells of sparrow and mice was found in this experiment. These results suggest that both sparrow and laboratory mice may not to act as biological carrier for PEDV. However, it will be necessary to test further with different PEDV strains to conclude inability of the replication in intestinal cells of sparrows and mice.

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Dong Bang Co., Ltd, Suwon, S. Korea 443-390

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Isolation and genome characteristic of the epidemic PEDV variant strain CH/YNKM-8/2013

F Chen, S Wan, Q He
State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan, China,
he628@mail.hzau.edu.cn

Introduction

The PEDV, an important pathogen for global swine industry, especially for Southeast Asia countries, is the causative agent of porcine epidemic diarrhea (PED), which is characterized by severe enteritis, vomiting, watery diarrhea and weight loss in adult pigs and lethal in piglets(1,2). From late 2010, the variant PEDV strains were believed to responsible for the outbreaks of viral diarrhea in many parts of China which caused massive economic losses in the swine industry(3). There was rarely reported of PEDV in Europe countries and USA in the recent decade. However, in the 2013, the similar PEDV variant strains and similar clinical cases were reported in USA(4). So the study of genome genetically characterize of the Chinese isolated variant PEDV strain (CH/YNKM-8/2013) and the wild strain CH/FJZZ-9/2012 had an important global meaning, which would lay the foundation of better elucidation of the epidemic of the variant PEDV strains.

Materials and Methods

In Sep. 2012, wild-type PEDV strain CH/YNKM-8/2013 and CH/FJZZ-9/2012 was obtained from fecal sample of a 3-day-old piglet on a commercial swine farm in Yunnan Province (southwest of China) and Fujian Province (Southeast of China). The mortality and morbidity in suckling piglets of these piglet herds were high (100% and 90%, respectively). And only the wild-type PEDV strain- CH/YNKM-8/2013 was successfully passaged in Vero cells. After passage 30 times, 48 h after inoculation, the virus can produce an obvious cytopathic effect on Vero cells characterized by cell fusion and syncytium formation, and the cell-adapted isolate, CH/YNKM-8/2013, was obtained. As described previously, 13 pairs of oligonucleotide primers were designed to amplify the different regions of the CH/YNKM-8/2013 and CH/FJZZ-9/2012 genomes which encompass the entire genome. The 5' and 3' ends of the genome were amplified by rapid amplification of cDNA ends (RACE) methods. The PCR products were cloned into pMD-18T vector (Takara) and sequenced with an ABI3730XL genome sequencer. All the data were assembled and processed by the MEGA (version 5.10). The genome similarity plot of the PEDV isolates was done by Simplot (version 3.5.1).

Results

An obvious cytopathic effect (CPE), which characterized by cell fusion, syncytia formation and eventual cell detachment was observed for CH/YNKM-8/2013 from the passage 20(P20). In order to determine whether the isolated virus can be stabile propagated and maintained in cell cultures, the virus isolate was further serially passed in Vero cells and now up to 45 passages (P0-P45). Prominent CPE was usually observed within 48 hours post inoculation during the propagation process. The IFA, Electron microscopy (EM) and M gene based Real-time PCR menthod were also verified the existence of this variant PEDV strain. The complete genome sequences of CH/YNKM-8/2013 and CH/FJZZ-9/2012 are 28,038 nucleotides (nt) in length. The genome organization is 5'UTR-ORF1a/1b-S-ORF3-E-M-N-3'UTR. The sizes of them were 292 nt, 20,345 nt, 4,161 nt, 675 nt, 231 nt, 681 nt, and 1,326 nt and 334 nt, respectively. And the nucleotide sequences identity of these two strains was 98.9%. As well as I known, the complete genome sequence of 33 PEDV strains were reported. The nucleotide sequences identity of these strains range from 96.2%-99.7%. The nucleotide sequences identity of these genes were 93.6%-100%, 97.2%-99.8%, 93.0%-99.7%, 94.8%-100%, 95.2%-100%, 97.0%-100%, 95.5%-99.8%, respectively. Phylogenetic analysis of the complete genome of these genome shows that CH/YNKM-8/2013, CH/FJZZ-9/2012, USA/Colorado/2013 and other recent isolated variant strains belong to the same group, but they are distant from the vaccine strain CV777 and Korean vaccine strain DR13. The present study indicates that the PEDV variant strain isolated in our study shows similarity mutations characteristic with those isolated in south China and the recently reported USA strains.

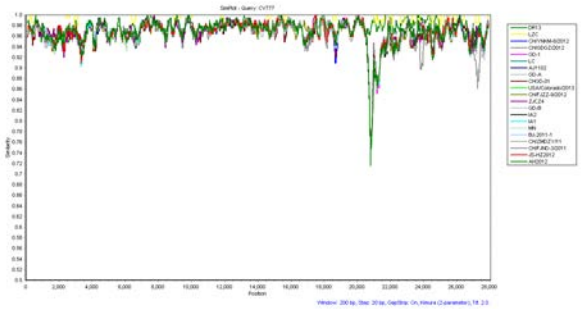

Figure 1 Similarity plot of the genome of PEDV isolates. The similarity plot was constructed using the two-parameter (Kimura) distance model with a sliding window of 200 bp and step size of 20 bp.

Conclusions and Discussion

From late 2010, the variant PEDV strains were believed to responsible for the outbreaks of viral diarrhea in many parts of China which caused massive economic losses in the swine industry. But the biology mechanism behind that is unrevealed. As well as I known, the compare of complete genome sequence of 33 PEDV strains were reported in these study. Just as what had showed in the Fig.1, the variant strains showed great diversity with the vaccine strains CV777 and Korean vaccine strain DR13. The length of most USA strains are 28380bp, but the length of Chinese strains are more abundant, from 27930-28047bp. The great differences regions, especially those in the S gene can be the important virulent difference research regions. With the collecting genome dates, the genome difference research of these strains can be meaningful. Knowledge of its sequence will not only facilitate future investigations on the genetic variations of PEDV field strains in China, but also contribute to prevent and control PEDV infection in future.

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Isolation and characterization of US PED viruses

Q Chen, G Li, J Thomas, W Stensland, A Pillatzki, P Gauger, K Schwartz, D Madson, K-J Yoon, G Stevenson, E Burrough, K Harmon, R Main, J Zhang

College of Veterinary Medicine, Iowa State University, Ames, IA; jqzhang@iastate.edu

Introduction

Porcine epidemic diarrhea virus (PEDV), a member of the family *Coronaviridae*, can cause acute diarrhea in all ages, and high mortality in piglets and result in significant economic losses. PEDV was detected for the first time in US swine in April 2013.[1] It was critical to obtain US PEDV isolates that can grow efficiently in cell culture for studying the pathogenesis, developing diagnostic assays, and for vaccine development. An additional objective was to determine which gene(s) of PEDV is most suitable for studying the genetic relatedness of the virus.

Materials and Methods

Fifty-two fecal and 22 intestinal tissue samples that tested positive by a PEDV specific real-time RT-PCR were selected for virus isolation attempts on Vero cells as previously described[2] with modifications. The resulting PEDV isolates were serially passed in cell culture. Virus growth in cells was examined by PEDV-specific real-time RT-PCR, immunofluorescence assay, and electron microscopy. The infectious titers of the viruses were determined by end-point dilution assay. The full-length genome sequences of the isolates at selected passages were determined using next generation sequencing technology. Sequences were compared to 8 additional US PEDV strains and 23 non-US strains whose whole genome sequences were available in GenBank.

Results

Five PEDVs were successfully isolated in cell culture. They were designated as USA/IN/2013/19338E (from piglet small intestine collected in Indiana on May 16, 2013), USA/IA/2013/22038 (from piglet small intestine collected in Iowa on June 6, 2013), USA/NC/2013/35140 (from piglet feces collected in North Carolina on September 12, 2013), USA/IA/2013/49379 (from piglet feces collected in Iowa on December 9, 2013), and USA/NC/2013/49469 (from piglet feces collected in North Carolina on December 10, 2013). All PEDV isolates induced distinct cytopathic effect, characterized by syncytia formation and eventual cell detachment, at either passage 0 or passage 1. Virus growth was confirmed by an immunofluorescence assay using a PEDV specific monoclonal antibody and a real-time PEDV RT-PCR. The isolates 19338E, 22038, 35140, 49379, and 49469 have been serially propagated in cell culture for 70, 70, 35, 5, and 5 passages. Viruses were observed to grow faster upon further passaging. The infectious titers of virus isolates reached approximately 10^5 $TCID_{50}$/ml at passages 5-10 and could increase to 10^6-10^7 $TCID_{50}$/ml after passages 25. When the entire genome sequences of 5 PEDV isolates were compared to other US and non-US PEDVs, all 13 US PEDV strains were genetically closely related to each other (99.7-100% nucleotide identity). The US PEDVs had 96.3% to 99.6% nucleotide identity to the 23 non-US PEDVs at the entire genome level with the closest genetic similarity to some PEDVs reported in China during 2011-2012.

Conclusions and Discussion

The PEDV isolates appeared to be adapted to Vero cells as successively passed in cell culture, producing more progeny viruses. Sequence analyses of three PEDV isolates (19338E, 22038, and 35140) that were sequenced at different cell passages indicated that the viruses were genetically stable during the first 10 passages in cell culture.

Phylogenetic analyses based on the entire genome sequences or individual gene sequences of US and non-US PEDV indicated that the S1 portion is appropriate for sequencing as a routine diagnostic service to demonstrate the genetic relatedness of different PED viruses.

Availability of the US PEDV isolates collected from different states at different time points provides an important tool for PEDV pathogenesis study, virological and serological assay development, molecular epidemiology, and vaccine development.

Acknowledgements

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Incomplete effective protection provided by classical PRV maternal antibodies to piglets challenged with new PRV variant strains in China

T Yu, X Ku, S Li, F Chen, H Hu, Q He

Animal infectious disease laboratory, Veterinary Medicine College, Huazhong Agricultural University, Wuhan, Hubei Province, China, 430070, He628@mail.hzau.edu.cn

Introduction

Pseudorabies virus (PRV), also called Aujeszky disease virus(ADV), is a member of the family Herpesviridae and genus Varicellovirus(1). This pathogen can infect livestock and wild animals and increase these morbidity and mortality, resulting in significant economic losses for the livestock industry finally. In China, the PRV live attenuated vaccine vaccinated with the vast majority of farms and pseudorabies was well controlled(2). However, In 2012, pseudorabies has occurred on many large pig farms in vaccinated swine herds. The positive rate of PRV gE Antibody showed more than 50%(3). To determine the virulence of the newly isolated PRV strains among PRV maternal antibodies piglets, we identified the protection of the newly PRV.

Materials and Methods

The PRV HNXX strain was isolated from the farms with clinical cases of pseudorabies' disease, and it was purified and cultured in PK-15 cells.

Ten 21-days piglets were confirmed to have high level PRV gB Antibody enzyme-linked immunosorbent assay(ELISA) kit (IDEXX Laboratories, Westbrook, ME, USA), were obtained from the large pig farm where the classical live vaccine were used. All piglets were confirmed to be free of PRV wild-type strain infection by using gE PCR detection and using a gE Antibody.

The ten piglets were randomly divided into two groups, Group A: Five piglets were in the test group. They were injected intramuscularly with a 1mL inoculum containing $1\times10^{7.0}$ $TCID_{50}$ of PRV strain HNXX. Group B: The other piglets were used as a control and injected intramuscularly with 1 mL of PK-15 cell culture supernatant. Clinical symptoms were checked daily, and rectal temperatures were recorded daily until postinoculation day 28.

Results

The piglet serums were collected before PRV inoculated. Mean PRV gB and gE ELISA S/P ratios are shown in Table1.

The fever symptoms(rectal temperature≥40.0°C) developed in PRV-inoculated piglets 2–5 days after challenge in group A, and the rectal temperatures returned to normal 7 days(Figure 1). Loss of appetite was observed in the 5 piglets at 3 to 6 days after PRV HNXX strain challenge. Itching Symptoms appeared at the injection site 4 days after challenge and remained itching until the 21 days(Figure 2). The control piglets remained normal until the end of the experiment.

Conclusions and Discussion

The piglets weren't vaccinated with attenuated or inactivated PRV vaccines, and comfirmed to be free of PRV wild-type strain infection. The mean gB ELISA S/P ratios were showed high level, and gE ELISA S/P ratios was negative. These data support the piglets contain high level maternal antibodies. The piglets still showed fever symptoms and a long time itching Symptoms after PRV HNXX strain challenge. The results indicated that the piglets contain PRV maternal antibodies had not been given completely effective protection against newly isolated PRV strains in China.

Table 1. Mean PRV gB and gE ELISA S/P ratios before challenge PRV

Group	gB S/P ratio*					gE S/P ratio*				
	1	2	3	4	5	6	7	8	9	10
A	0.29	0.29	0.25	0.27	0.27	0.25	0.27	0.24	0.35	0.32
B	0.86	0.96	1.06	1.12	1.00	0.90	0.93	0.99	1.02	1.21

*Serum with an S/N ratio of < 0.60 was considered positive.

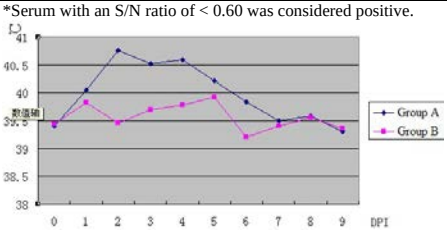

Figure 1. Rectal temperatures of PRV maternal antibodies piglets inoculated with pseudorabies virus strain HNXX

Figure 2. Itching Symptoms appeared at the injection site after challenge with pseudorabies virus strain HNXX in Group A.

Acknowledgments

This work was supported by China Agricultural Research System (CARS-36).

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P.076

H1N1 porcine influenza virus isolation in technified and untechnified farms in central Mexico

W González[1], E Lucio[1], M Escorcia[2], J Munguía[1]
[1] *Investigación Aplicada S.A. de C.V.*, [2] *Universidad Nacional Autónoma de México*, wgonzalez@grupoidisa.com

Introduction

The presence of porcine influenza virus subtype H1N1 has been reported in Mexico since 1982 with an outbreak in a farm of the state of Puebla [(1)]; subtype H3N2 has been reported in swine sera since 1979[(2)]. Porcine influenza has changed from a seasonal disease caused by a stable viral genotype to a respiratory disease that is constantly present throughout the year by means of multiple genotypes that are continuously changing [(3)]. The aim of this study was to isolate and characterize porcine influenza virus from nasal swabs collected from both technified and untechnified farms.

Materials and Methods

48 nasal swabs were collected from backyard farms from one county in Puebla and 5 from Oaxaca, from May to July 2010. On the other hand, 1,513 swabs from swine with respiratory disease signs were received from 17 farms of the states of Jalisco and Guanajuato, from January to August 2012. The latter samples were worked in the laboratory in 226 pools grouped by age and physiological status.

Samples were transported in 5 mL of minimal essential medium (MEM) with antibiotic and tamponaded with sodium bicarbonate at 0.5%, supplemented with 5 mg/mL of bovine serum albumin fraction V, samples were stored at -70°C until processing began.

Viral isolation was performed as per the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (OIE, 2012), the viral harvesting was done 48 hours postinoculation and the fluids were processed by means of hemagglutination using rooster red blood cells.

Viral RNA extraction was performed with viral fluids positive to hemagglutination using a commercial kit. RNA was amplified by means of RT-PCR for the gene responsible for codifying the hemagglutinin protein of porcine influenza virus. The reaction was performed with a commercial kit of a Perkin Elmer Cetus thermocycler model 480, following the manufacturer specifications at 58.5°C for H1 alignment and 48 °C for H3. Each reaction was analyzed with agarose gel electrophoresis at 1.8% using ethidium bromide.

The material obtained with RT-PCR was purified with the commercial kit QIA *quick gel extraction*. The sequencing took place in the Polymorphisms Identification and Sequencing Unit of the National Genomic Medicine Institute (IN.ME.GEN.), with a DNA analyzer, Automatic Sequencer 3730XL of *Applied Biosystems*. The sequence analysis was done with the *Sequencer 4.7 DNA Sequence Analysis Software*. Sequences were aligned and compared in order to determine virus homology percentages using the software DNASTAR.

Results

From the 48 samples collected from backyard swine only one was confirmed as positive. After sequencing it was determined to be of the H1N1 classical subtype.

From the 226 *pools* from technified farms, 5 were positive by means of viral isolation and RT- PCR. Sequencing also confirmed results as H1N1 classical subtype.

From the viral sequences obtained in this study, only four were added to the *Gen Bank* (KF 015226, KF 015225, KF 015224, KF 015223).

Conclusions and Discussion

It is important for swine production to consider the role of backyard animals in the circulation of the different viruses because normally it is a sector that lacks attention.

A study performed with 147 nasal swabs from backyard swine in a rural community of Veracruz, Mexico had 17 viral isolations from which 10 were confirmed as H1N1 and seven were H3N2[(4)], while other study performed in several states from lung samples obtained from slaughterhouses had 791 positive samples out of 1,246 analyzed samples, which represents a prevalence of 63.48%[(5)]. This study had substantially less positive samples probably due to the fact that the nasal swabs were obtained several days after the infection with porcine influenza virus, and this virus is located in nasal secretions 1 to 7 days after contact with the agent.

It is important to generate epidemiological information of this kind since the circulating influenza viruses change over time, and homology among the vaccinal strains and field viruses must be assured in order to afford an optimal response to immunization.

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Phylogenetic analysis of the HA gene of swine H3N2 strains isolated from 2012 to 2013 in Mexico

S Ramírez[2], A Flores[1], A Massa[1], R Raya[1], M Macías[1], A Franco[1], V Orozco[1], C Armenta[1]

[1]*Lapisa S.A. de C.V.; Carretera La Piedad-Guadalajara Km. 5.5, Col. Camelinas, C.P. 59375, La Piedad, Michoacán; México. www.lapisa.com,* [2]*Private practice,* mvzsusana@hotmail.com

Introduction

The hemagglutinin (HA) is the gene that determines the cellular receptors able to bind to the influenza virus, therefore the range of species that could be infected.[1] Between isolates, different genetic and antigenic groups exist. A phylogenetic analysis was performed on the HA gene of H3N2 strains in Mexico to identify genetic clusters circulating in the swine population.

Materials and Methods

The information of 90 of the total number of strains obtained from 2012 to 2013 was analyzed. The strains were isolated in a standard cell line[2] from nasal swabs and lung samples collected from animals exhibiting influenza-like illness in commercial farms from 7 states of Mexico. The sequences of the HA1 domain of these strains were determined, and reference sequences from genetic clusters identified previously[3,4,5] obtained from GenBank®. Phylogenetic relationships were estimated from nucleotide sequences by the method of Maximum Likelihood with a bootstrap resampling support.

Results

The nucleotide phylogram (Figure 1) indicates that the majority of the strains analyzed (80) are closer related to the strain A/Swine/Ontario/33853/2005 from Cluster IV[5] than the other reference strains categorized previously.[5] Two separate lineages (A and B) were detected.

Conclusions and Discussion

None of the sequences analyzed related with reference strains from clusters I to III[5], or to the double-reassortant reference strain (A/Swine/North Carolina/35922/98). Results indicate that in Mexico, triple-reassortant viruses are probably predominant in pigs. Cluster IV H3N2 viruses have been endemic in North American pig farms for more than 7 years.[5] Using the Blast® tool to search for close sequences, no related swine viruses were found for lineages A and B in the nucleotide database, and both clusters presented highest similarity with strains isolated from humans. This suggests that strains may belong to genetic groups not reported previously in swine, and further research should be carried on the HA and other genes to identify possible recombination or other details that lead to this placement in the phylogenetic tree. Although most strains are clearly related to Cluster IV, different antigenic groups are likely to exist, and other methods can be applied for further SIV characterization.

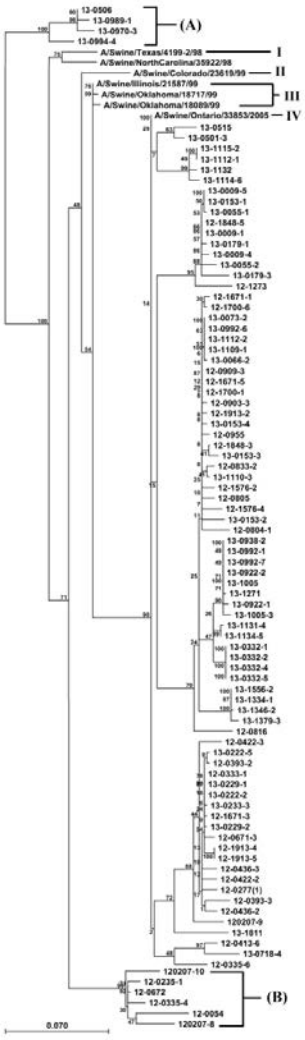

Figure 1. Porcine influenza nucleotide phylogram of the HA1 domain of Mexican (N=90) and reference strains (I, II, III, IV and A/Swine/North Carolina/35922/98) by the ML method with bootstrap resampling (200 replications).

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Complete genome characterization of influenza A virus in a breeding herd following a year of monthly surveillance in a breeding herd using next generation sequencing

A Diaz[1], M Torremorell[1] M Culhane[1], S Sreevatsan[1]
[1]*Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul MN, USA.*, diazj002@umn.edu, torr003@umn.edu

Introduction

Swine influenza A viruses (IAV) are worldwide distributed (1). IAV can persist for prolonged periods of time in populations and there are animal subpopulations that serve as a vehicle for virus maintenance. Newborn piglets and replacement animals are swine subpopulations considered important for introducing and maintaining IAV in swine herds. However the viral genetic and antigenic differences responsible for virus maintenance within these sub-populations are not known. The objective of this study was to characterize the complete genome of influenza viruses isolated from various animal subpopulations in a breeding herd throughout a 12 month period in order to identify mechanisms of virus introduction and maintenance in swine populations.

Materials and Methods

A 3,250 head breeding herd with history of endemic IAV was conveniently selected on November 2011 and sampled monthly throughout a year. Three-week old suckling piglets, gilts introduced > 4 weeks (gilts) and gilts recently introduced < 4 weeks (new gilts) were sampled. Thirty individual nasal swabs were collected from each subpopulation and tested for IAV by RT-PCR targeting the IAV matrix gene. Positive samples were used for IAV isolation in MDCK cells. The complete IAV genome from each isolate was amplified using previously described protocols (2), and sequenced using MiSeq Illumina platform. Viruses were sub-typed based on their hemagglutinin (HA) and neuraminidase (NA) configuration. Sequences where aligned by gene segment using ClustalW and the pairwise percentage of identity between sequences used to estimate the number of clusters within gene segments.

Results

A total of 360, 359 and 268 nasal swabs were collected from piglets, gilts and new gilts respectively. 110 (11%) of all swabs tested positive for IAV by RT-PCR (20 from piglets, 18 from gilts and 72 from new gilts). While piglets and gilts tested positive only once (month 1 and 4 respectively) new gilts tested positive 5 out of 12 times (months 4, 5, 6, 9, and 10). Seventy one viruses were isolated from positive samples (20 from piglets, 12 from gilts, and 39 from new gilts). H1N1, H1N2, and H3N2 subtypes were isolated from piglets but only H1N1 and H3N2 subtypes were isolated from gilts and new gilts. Additionally more than one subtype were co-circulating at once in a given population. Four different HA clusters were identified with an average of 99.7% and 62.15% identity alignment distance within and between clusters respectively. At the HA level (segment 4), sequences clustered within the same group at different points in time and different subpopulations indicating that the same virus (or very closely related ones) can persist over time at the farm level within and between subpopulations. All other gene segments, except segment 3, clustered in 4 groups as segment 4. Segment 3 clustered in 5 different groups indicating a reassorment event between different IAV.

Conclusions and Discussion

Multiple IAV strains and subtypes were circulating within animals subpopulations within the same farm throughout one year period. The viral diversity observed during this time was significant which makes the control of IAV within swine populations difficult. Moreover, this diversity is not limited to those gene segments that translate the main antigens of the virus HA and NA. There was no IAV at the same time in different subpopulations indicating that viruses that persist at the herd level may move between subpopulations over time. The direction of such transmission events still needs to be investigated.

Acknowledgments

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Influenza A virus (IAV) detection using oral fluid and nasal swab specimens from IAV-inoculated pigs

CK Goodell,[1] A Kittawornrat,[2] Y Panyasing,[2] C Olsen,[2] F Zhou,[2] T Overbay,[4] R Rauh,[5] WM Nelson,[5] CO'Connell,[6] A Burrell,[6] C Wang,[2,3] KJ Yoon,[2] RG Main,[2] J Zimmerman[2]

[1]IDEXX, Westbrook, ME, [2]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, Iowa; [3]Department of Statistics, Iowa State University, Ames, Iowa; [4]Abaxis, Inc., Union City, CA; [5]Tetracore, Inc., Rockville, MD; [6]Life Technologies Corp., Carlsbad, CA. christa-goodell@idexx.com

Introduction

Surveillance using nasal swab (NS) specimens is a labor intensive, yet relatively insensitive, method for detecting influenza A virus (IAV) in commercial swine populations (1). Alternatively, oral fluid (OF) samples are easily collected and have been shown to be a sensitive surveillance sample (2,3,4). The objective of this study was to compare the detection of IAV in individual pig nasal swabs and pen-based oral fluid samples collected over time from experimentally inoculated swine.

Materials and Methods

The study involved 82 piglets housed under BSL-2 conditions. A subset (n=28) was vaccinated twice with a multivalent IAV vaccine (FluSure XP™, Zoetis). Thereafter, pigs were either intratracheally inoculated with A/Swine/OH/511445/ 2007 γ H1N1, or A/Swine/Illinois/02907/2009 Cluster IV H3N2, or served as negative controls. Individual NS were collected on day post inoculation (DPI) 0-6, 8, 10, 12, 14, and 16. Pen-based OF samples were collected DPI 0-16. Samples were tested by rRT-PCR at 2 laboratories, virus isolation (VI), and for IAV antigen using a point-of-care (POC) kit (VetScan®, Abaxis Inc.).

Results

No significant difference noted in detection rates between IAV subtypes (H1N1 vs. H3N2).

False positive rRT-PCRs were reported in both OF (n = 2 of 28) and NS (n = 5 of 96) samples. One false positive (of 95) VI was reported in a NS sample. No false positives were observed with the POC test.

For OF, the sensitivity of the POC test improved if the assay was read at 30 minutes, rather than 15, and was equivalent to NS ($P = 0.74$).

To compare sampling methods at the pen level, a pen was classified NS positive if ≥ 1 pig in the pen was NS rRT-PCR, VI, or POC positive. Using this convention the following observations were made in pens of unvaccinated but inoculated pigs: (1) OF and NS rRT-PCR results were statistically equivalent through DPI 8, but more OF than NS were positive, thereafter. (2) There was no significant difference in the duration of detection by VI between OF and NS through DPI 6, but a higher proportion of NS samples were positive.

In pens of vaccinated inoculated pigs, (1) rRT-PCR positive NS and OF specimens were detected through DPI 14, although vaccination significantly reduced detection by NS after DPI 6. (2) Detection of IAV in OF and NS was significantly reduced by vaccination for both VI and POC assays.

Conclusions and Discussion

IAV rRT-PCR testing of pen-based OF was equal or better than detection using NS at the pen level. Oral fluid is a valid and useful sample type for the detection of IAV by rRT-PCR in both unvaccinated and vaccinated pigs for at least 14 DPI. Pen-based OF is a valid and useful sample type for VI in unvaccinated pigs for at least 6 DPI. In vaccinated pigs, if a virus isolate is desired, NS is the sample of choice. The POC test could be a useful pen-side test for the detection of IAV antigen in unvaccinated pigs during acute infection using either OF or NS but a 30 minute incubation is recommended for OF.

Acknowledgements

This study was supported by Pork Checkoff® funds through the National Pork Board (#09-193) and Zoetis™. Virus isolates were provided by Dr. Amy Vincent (USDA, NADC, Ames, IA) and Dr. Marie Gramer (University of Minnesota, St. Paul, MN). VetScan® AIV Rapid Test kits were provided by Abaxis, Inc. FluSure XP™ was provided by Zoetis™. rRT-PCR testing was provided by Tetracore Inc. and Life Technologies Corp.

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Detection of PorPV in circulating leukocytes in persistently infected pigs from a natural outbreak

R Lara-Romero[1], S Cuevas-Romero[3], JF Rivera-Benítez[2], V Quintero[1], S Mendoza[1], H Ramírez-Mendoza[2], E Hernández[1], J Vázquez[3], D Córdova[3]
[1]FES-C. UNAM, México.[2]DMI, FMVZ, UNAM, México.[3]CENID-MA, INIFAP, México. cuevas.julieta@inifap.gob.mx

Introduction

An outbreak of an unknown disease occurred in La Piedad, Mexico in 1980, in pig farms. This disease was characterized by encephalitis and corneal opacity in newborn piglets, also characterized by a high mortality rate. From this outbreak a hemagglutinating virus was isolated. The virus was eventually classified as a Paramixoviridae, Genus Rubulavirus. (*Rubulavirus porcino*, PorPV) (3). The clinical signs are dependent upon the age of the affected animals, with a nervous and respiratory picture in young animals and mainly reproductive problems in adults. For these reasons it is considered the fourth most important disease for the Mexican pig industry. The PorPV can establish persistent infections in adult pigs (1, 3). This disease is endemic in the central states of Mexico (3, 4). Our aim in conducting this study is to determine the presence of viral RNA in naturally convalescent pigs from an outbreak, since the persistent infection had been previously demonstrated in experimentally infected pigs. The technique chosen for this study was the real time RT-PCR from the circulating leukocytes (1).

Materials and Methods

The study was conducted at the CENID-MA INIFAP México and at the FMVZ, UNAM, México. We selected three farms, two that had undergone an outbreak (Zumpango and Guanajuato), confirmed by viral isolation and one without having undergone a previous infection, as confirmed by serology. (Toluca). We also included 8 SPF pigs as negative controls. The blood collected from all animals at the start of the experiment was centrifuged to separate serum for serology and White cells for RNA extraction. The RNA was quantified by real time RT-PCR, the antibodies were quantified by ELISA. The oligonucleotides used for the real time RT-PCR amplified a 71 BP fragment (4). The standard curve was obtained by ten-fold dilution of standard RNA (Figure 1).

Results

In the Zumpango farm (n=18) in the state of Mexico, 88.89% of the sampled saws sampled were positive with an estimated concentration of 47.67 ng/µl of viral RNA. From the farm located in Pénjamo Guanajuato, we sampled saws from 0 to 6 parturitions (n=30), where 96.67% were positive with 2.45 ng/µl of viral RNA and sementals (n=23), with a 69.57% boars positive with a concentration of 1.31 ng/µl viral RNA. In the farm located in Toluca, (n=21), Estado de México, an 80.95% of the animals were positive at 0.35ng/µl of viral ARN for the P gene. The ELISA test showed that in the three farms studies significant amounts of IgG for HN protein of the RVP.

Dilución	RNA ng/µl	Valor Ct
1	100	23.47
2	10	26.92
3	1	31.18
4	0.1	34.76

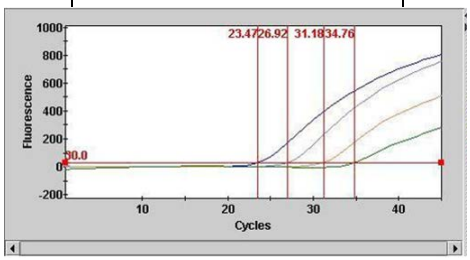

Figure1. Dilutions, RNA concentrations and Ct values of the standard

Conclusions and Discussion

The use of the Real time RT-PCR in leucocytes of peripheral blood resulted in a rapid method (90 min), quantitative and higly sensitive method for the detection of persistently infected with the Blue eye virus, since it allows the detection of minimal amounts of viral RNA, which represent an important method for the epidemiology of the Blue Eye Disease in Mexico

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POCKIT™ system, a point-of-need PCR detection platform, for rapid and sensitive diagnosis of PCV2

ST-Y Chung, Y-C Lin, C-H Ho, P-H Chou, Y-L Tsai,
P-YA Lee, H-TT Wang, H-FG Chang
GeneReach USA, Lexington MA, USA, peiyu329@genereachbiotech.com

Introduction

Porcine circovirus type 2 (PCV2), a single stranded circular DNA virus, is the essential cause of post-weaning multisystemic wasting syndrome. A simple and sensitive diagnostic method is needed for PCV2 detection to help on-site monitoring of virus infection. Insulated isothermal polymerase chain reaction (iiPCR) is a specific and sensitive nucleic acid detection method that requires a simple heating source (1, 2, 3). The POCKIT™ system (Figure 1) is designed specifically to facilitate iiPCR and process signals from fluorescent probe hydrolysis. This field-deployable device allows on-site application of iiPCR to facilitate point-of-need diagnosis of diseases. Simple readouts of the results are generated within one hour from nucleic acid samples. In this study, an iiPCR assay was developed for detection of PCV2 DNA.

Materials and Methods

Primers and probe were designed to target a highly conserved area in *ORF1* gene. Analytical sensitivity was evaluated using a standard plasmid containing the target region. Each dilution was done in 20 repeats. Sensitivity of the assay was compared to a reference real-time PCR (qPCR) assay (4) using serial dilutions of a clinical sample. Specificity was tested using a panel containing other circoviruses and porcine viral pathogens. Finally, accuracy was evaluated by comparative analysis of 40 clinical samples by PCV2 iiPCR and reference qPCR assays in parallel.

Results

The limit of detection 95% of the assay was about 18 copies of the standard plasmid DNA (Table 1). Comparative tests using serial dilutions of a clinical sample showed that PCV2 iiPCR reached sensitivity similar to that of reference qPCR assay. Exclusivity study, which tested PCV1, classical swine fever virus, porcine reproductive and respiratory syndrome virus, and chicken infectious anemia virus, demonstrated excellent target specificity (Table 2). Finally, specificity and sensitivity of iiPCR were comparable to those of the reference method.

Conclusions and Discussion

The PCV2 iiPCR could detect PCV2 sensitively and specifically. This simple PCV2 iiPCR assay shall facilitate timely on-site disease management of PCV2.

Figure 1 POCKIT™ Nucleic Acid Analyzer is a portable PCR amplification and detection system based on iiPCR technology.

Table 1. Analytical sensitivity analysis of PCV2 iiPCR assay using serial dilutions of standard plasmid

Standard (copies/rxn)	%
10^3	100%
10^2	100%
10^1	85%
10^0	25%
NTC	0%

Table 2. Exclusivity analysis of PCV2 iiPCR

Target pathogen	PCV2 iiPCR
PCV1	Negative
Classical swine fever virus	Negative
Porcine reproductive and respiratory syndrome virus	Negative
Chicken infectious anemia virus	Negative

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Experimental *in vivo* comparison of the virulence of a 2013 U.S. variant strain of PCV2b (mPCV2b) to well characterized U.S. PCV2a and PCV2b strains provides limited evidence of differences in virulence

T Opriessnig[1,2], P Gerber[1], C Xiao[1], P Halbur[1] S Matzinger[3], XJ Meng[3]
[1]*The Roslin Institute, University of Edinburgh, Midlothian, UK* [2]*Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA,USA* [3]*Department of Biomedical Sciences and Pathobiology, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA,* Tanja.Opriessnig@roslin.ed.ac.uk

Introduction

In 2012, a mutant porcine circovirus type 2b (mPCV2b) strain was identified in U.S. cases of porcine circovirus associated disease (PCVAD) (1). This strain, also known as mPCV2b, has several unique mutations throughout the capsid and its open reading frame (ORF) 2 length is 234 amino acids compared to 233 amino acids in traditional PCV2a and PCV2b strains. A similar strain was recently identified in China (2,3). When compared to traditional Chinese PCV2a and PCV2b isolates using a Chinese conventional pig model, enhanced virulence of mPCV2b was confirmed (3). The objective of this study was to conduct a head-to-head comparison of the virulence of mPCV2b with PCV2a and PCV2b isolates in cesarean-derived, colostrum-deprived (CDCD) pigs.

Materials and Methods

Twenty-nine CDCD pigs were purchased at 2 weeks of age and randomly assigned to one of 4 groups. At 3 weeks of age, the pigs were infected with saline, PCV2a, PCV2b, or mPCV2b. In brief, infections clones were constructed, sequenced, and after sequence confirmation, subsequently used to transfect PK-15 cells to generate the infectious virus stocks utilized to infect the pigs. All pigs were necropsied 21 days after experimental infection. Serum samples were collected weekly and tested for presence of PCV2 DNA by quantitative real-time PCR (4) and for presence of anti-PCV2 antibodies by ELISA (5). Amount of PCV2 antigen in tissues was determined by immunohistochemistry (IHC) (6).

Results

Clinical signs were not observed during the study; however, at necropsy a portion of the mPCV2b infected pigs showed mild to severe icterus and emaciation. Amount of PCV2 DNA was significantly higher in pigs infected with mPCV2b compared to PCV2b in serum samples at 7 days post PCV2 infection (dpi) and fecal swabs at dpi 14. All pigs were negative for anti-PCV2 IgG antibodies at inoculation and the majority of the pigs remained negative throughout the study. One of eight mPCV2b pigs seroconverted to PCV2 by 21 dpi and 3/7 PCV2a pigs and 1/7 PCV2b pigs were in the suspect range (ELISA S/P ratios between 0.1 to 0.2). Severe systemic microscopic lesions consistent with PCVAD (lymphoid depletion of follicles, replacement of follicles by macrophages, abundant PCV2 antigen in tissues) were seen in selected pigs in all groups (3/7 PCV2a pigs, 4/7 PCV2b pigs, 4/8 mPCV2b pigs) except the negative controls.

Conclusions and Discussion

The results indicate that in this model, all PCV2 isolates were capable of inducing clinical disease and moderate-to-severe microscopic lesions consistent with PCVAD and remarkable differences in virulence were not apparent. These findings are in contrast to work from China (3) suggesting that the mPCV2b was more virulent. In contrast to the Chinese model, we utilized infectious clones to better assure the purity of our inocula. We also utilized a CDCD pig model rather than a conventional pig model to better control for concurrent infections which could enhance expression of disease. Additional comparisons of these U.S. strains in larger numbers of vaccinated and unvaccinated conventional pigs should be done to further document our findings in comparative virulence and understand vaccine efficacy against the mPCV2b strain. Interestingly, pigs infected with mPCV2 had the highest viral load at 7 dpi which was correlated with higher fecal shedding during the subsequent week. Increased viremia and shedding could contribute to improved dissemination and pig-to-pig transmission of a virus in a pig population and this may explain the increasingly frequency of association of mPCV2 with PCVAD in pork production systems in the USA.

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Using placental umbilical cord serum to determine PCV2 statuses of breeding herds

T Fangman[1], D Baumert[2], TTe Grotenhuis[3], B Payne[1]
[1]Boehringer Ingelheim Vetmedica, Inc, St. Joseph, MO, USA, [2]Cargill Pork, Marshall, MO, USA
[3]College of Veterinary Medicine, Iowa State University, Ames, IA, thomas.fangman@boehringer-ingelheim.com

Introduction

This study was developed when periodic testing of comingled weaned pigs demonstrated PCV2 at 7 weeks of age. The occurrence of PCVAD was sporadic within each of the nursery sites and traced back to a specific flow. The primary objective was to determine sow herd PCV2 status and prevalence within six different sites using serum from placental umbilical cords.

Materials and Methods

This was a multiple test, cross-sectional research study of six breeding herd sites. Each sow unit was sampled three times during the summer, with sampling occurring in 30 day intervals. Each site completed a survey during the initial visit. Thirty placenta samples were randomly collected from each site. Placenta was the experimental unit used to determine PCV2 status of a litter. Analysis of qPCR levels from placental umbilical cord serum samples was used to classify breeding herd prevalence of PCV2. Serum was collected from a minimum of four umbilical cords per placenta and pooled for analysis. Performance data was analyzed by ANOVA using JMP 9.0 (SAS). Mean separation was conducted using either Tukey-Kramer HSD or Wilcoxon rank sums. Differences were considered significant at P<0.05.

Results

All sites were positive for PCV2 (Figure 1). Percent positives between sites ranged from 27-100% during the entire testing period. PCV2 qPCR values of the sites ranged from 2 to 9 logs. The prevalence of PCV2 varied dramatically within sites over the 3 periods. Three sites had a percentage of qPCR positive placenta blood was > 82% over the 3 periods and 1 site was constantly <40% positive. The greatest variation was found in Farm E which ranged from 43-93% positive. Table 1 shows the difference in the mean production parameters between the 6 breeding herds during the 90 day period of placenta blood collection. Total born, born alive, stillborns and mummies were significantly different between the sites (P<0.0001). Site C and D had the highest live born at 13.3 and 13.2 pigs, while sites A and F had the lowest live born at 12.6 and 12.8 pigs, respectively. Stillborns were higher at site B, C, D, and F compared to sites A and E. Conception rates were similar between the sites (P=0.405).

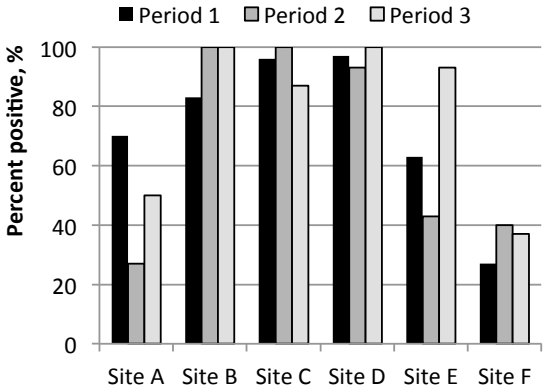

Figure 1. PCV2 qPCR results by site over time

Table 1. Breeding herd performance by site

Site	Total Born	Live Born	Still-born %	Mummies, %	Conception Rate, %
A	13.0^{c}	12.6^{c}	2.3^{b}	0.6^{d}	98.5
B	14.1^{a}	13.1^{ab}	4.1^{a}	3.2^{b}	98.8
C	14.1^{a}	13.3^{a}	3.7^{a}	2.0^{c}	99.2
D	14.3^{a}	13.2^{a}	3.4^{a}	3.9^{a}	98.7
E	13.4^{b}	12.9^{bc}	2.0^{b}	2.0^{c}	97.6
F	13.5^{b}	12.8^{c}	3.3^{a}	2.0^{c}	98.7

Columns with different superscripts are different, P<0.05

Conclusions and Discussion

This placenta blood sampling method may be utilized to suggest the prevalence of PCV2 in piglets at birth. This evaluation would suggest that placenta blood should be collected multiple times in order to understand the true prevalence and the variation. More sampling periods may be indicated depending on the goals of the investigation. Efforts were made to minimize environmental contamination, however, that can not be ruled out as a potential factor to explain the large variation within farm. Differences were observed in breeding performance between the 6 sites. However, sites with the lowest total and live born were not sites with the highest number of PCV2 positive placentas. Additional work is required in this area to determine if PCV2 at birth is detrimental to piglet birth weights or growth.

P.084

Evaluation of the prevalence of PCV2 viremia in Canadian breeding herds and piglets

B Tully[1,2], S Drapeau[2], A De Grau[2], Z Poljak[3],
[1]*Swine Health Professionals Ltd, Steinbach, MB, Canada,* [2]*Merck Animal Health, Kirkland, QC, Canada,* [3]*University of Guelph, Guelph, ON, Canada,* btully@shpswine.com

Introduction

Porcine Circovirus type 2 (PCV2) is a causative agent in PCV2- Associated Reproductive Failure (6). A possible source of sub-clinical infection in growing pigs is vertical transmission from sow to piglet, either in utero or through colostrum and milk to suckling piglets (4,6) leading to piglets already being born infected with virus and or having antibodies to PCV2 prior to ingesting colostrum (3,5). Since the advent of commercially available PCV2 vaccines in Canada over the past 6 years, clinical PCVAD has dramatically decreased in growing herds, however there is more interest in vaccination of breeding sows to impact PCVAD in the breeding herd or in downstream growing pig populations (8). Several studies have investigated the benefit to sow herd performance by vaccinating the sow herd, including; reduced wean-service interval, reduced sow mortality, reduced pre-weaning mortality, increased pigs weaned/sow, increased weaning weights, and increased farrowing rates (7,8).

A study was designed to determine the prevalence of PCV-2 viremia in replacement gilts and pre-suckle and weaning age piglets across Canada.

Materials and Methods

Sow herds with more than 500 sows were included in the study by simple random sampling using participating clinics as primary sampling units and provinces as strata. Serum samples were collected from 4 piglets/litter totaling 10 litters/farm before suckling colostrum (PS) and then again when the same piglets were weaned (W) on 57 farms across Canada. Thirty-five replacement gilts (G) were blood sampled within 30 days of entering the breeding herd. Sera were tested by PCV2 RT qPCR using primers and techniques previously described in the literature (9) in pools of 4. A positive pool was subsequently retested as individual sera. Individual sera from negative pools were considered negative. Samples with a CT value <=36 were considered positive and >36 considered negative.

Prevalence was evaluated at the farm level (1 or more positive piglet or gilts resulted in positive farm), litter-level (1 or more positive piglets resulted in positive litter) and individual (gilt) level. Prevalence and 95% confidence intervals were estimated by using the exact test based on binomial distribution; and second by using generalized estimating equations-based logistic regression with exchangeable correlation structure to account for within-herd clustering, when such approach was feasible.

Results

PCV-2 prevalence for replacement gilts, pre-suckle and weaning litters and farms are shown in Table.

Table 1. Prevalence (%) of PCV2 infection in piglets, litters and farms in Canada.

	Estimated Prevalence (%)	95 % CI
Pre-suckle (PS)	0.46	(0.22, 0.65)
Wean (W)	0.19	(0.05, 0.49)
Gilt (G)	3.47	(1.86, 6.38)
Litter (PS)	1.59	(0.71, 3.53)
Litter (W)	0.71	(0.28, 1.82)
Farm (PS)	10.53	(3.96, 21.52)
Farm (W)	7.02	(1.95, 17.00)
Farm (G)	42.11	(29.14, 55.92)

Conclusions and Discussion

PCV2 prevalence was determined to be much lower than previously reported in the USA. (1,2,8) Further investigation into the dynamic of PCV2 infection in replacement gilts and breeding sows over time would help practitioners make decisions about PCV2 vaccination strategies in breeding herd.

Acknowledgments

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PRRS in Vietnam and its diagnosis

NH Nguyen [1], THV Vuong [2], TH Vo [2]
[1] *Faculty of Animal Science and Veterinary Medicine,* [2] *HanViet Veterinary Diagnosis Laboratory, Nong Lam University in Ho Chi Minh city, Vietnam.* nguyenngochai@hcmuaf.edu.vn

Introduction

Porcine reproductive and respiratory syndrome (PRRS) caused by a small, enveloped RNA virus belonging to the *Arteriviridae* family, order *Nidovirales*, has become a serious challenge to the global pig industry. The efficacy of PRRS vaccine is very often discussed by the problem of homogenous or heterogenous PRRSV strains and genotypes. It is also important to note that the modified-live vaccine virus can persist in pigs and be disseminated through the herd (Martina Velasova et al., 2012). The study is realized to know which genotype of PRRSV infecting in Vietnam and the risk of virus persistence in the herds applied MLV vaccines. The results would be considered in taking the measures of PRRS control.

Materials and Methods

Forty two serum samples were taken from the pigs in PRRS outbreak, and 10 samples in the unapparent pig herds of PRRS that were either PRRS vaccinated or non-vaccinated.

Primers used in nested RT-PCR to differenciate North American (NA) and European (EU) genotypes were with outer primers[1] of F1: ATGGCC AGCCAGTCAATC and R1: TCGCCCTAATTG AT AGGTG, and inner primers[4] were F2: AGTCCAGAG GCAAGGGACCG and R2: TCAATCAGTGCCATT CACCAC, and F3: ATGATAAAGTCCCAGCGC CAG and R3: CTGTATGAGCAACCGGCAGCAT. And to determine chinese clade the using designed primers[5] were F: CGACGAGCTTAAAGACCAGATGG and R: CATCACAAGCCTCACGCATGA. The PCR products size expected for the primers F1 and R1 (PRRSV, NA and EU), F2 and R2 (PRRSV, NA), F3 and R3 (PRRSV, EU), and F and R for chinese clade were 433 bp, 337 bp, and 241 bp, 757 bp, respectively. ELISA kit (IDEXX) was used for determine the antibody titer in serum.

Results

Most of the PRRS cases in Vietnam caused by the PRRSV strains which belong to chinese clade, and there was two cases that was positive with both European (EU) and North American PRRS genotypes (NA) (table 1).

Despite the titer of antibodies against PRRSV by ELISA test there was the PRRSV infection in the pig herds.

Conclusions and Discussion

The presence of PRRSV chinese clade of NA PRRSV genotype was demonstrated in the study of Nguyen Ngoc Hai and Vo Khanh Hung, 2012. A multitype of PRRS genotype infection in the pig herds makes the difficulties not only in diagnosis, but also in vaccine application to control the PRRS. The PRRSV antibodies titer determined by ELISA was not a good indicator for PRRS protection. There was no correlation between ELISA antibodies against PRRSV and virus existence. The antibodies seronegative did not mean absence of virus infection in the herds, and the high titer of antibodies did not mean the protection or being free of virus infection in pig herd. The RT-PCR is very sensitive method in PRRSV diagnosis, but in the case of an unapparent pig herds, the virus could not be detected with conventional RT-PCR. Nested RT-PCR should be apllied for diagnosis PRRSV infection in monitoring PRRS.

Table 1. Genotypes of PRRSV in PRRS oubreaks

Source	Total	EU	NA	
			Classical clade	Chinese clade
HCM city, 2009	19	0	4	15
HCM city, 2010	12	2*	4*	8
Bac giang, 2010	4	0	0	4
Ha noi, 2010	2	0	0	2
Long an, 2013	5	0	0	5

(*): two samples was positive with both European and North American PRRS genotype

Conclusion

In despite of PRRS vaccination or non-vaccination, when herd monitoring is desired, a combination of nested RT-PCR and serology testing will be more valuable in determination of virus infection herd status.

Acknowledgements

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Phylogenetic analysis of ORF7 sequences of PRRSV

A Sotomayor [1], VH Anaya [2], ME Trujillo [1], E Sciutto [2], R Alonso [3], JI Sánchez [1]
[1] *Pig Medicine and Husbandry Department, FMVZ, UNAM;* [2] *Immunology Department, Biomedical Research Institute, UNAM,* [3]*Genetics and biostatistics Department, FMVZ, UNAM.* mvzaliciasotomayor@gmail.com

Introduction

Since PRRSV was first described in 1987, it has been demonstrated that it has great variability due to its high mutation rate. The genetic diversity among European and American strains is almost 60%, and it is high even among strains of the same genotype. The virus genome contains nine open reading frames (ORFs).(1,2,3) ORF7 is one of the most studied ORFs, it encodes for the nucleocapsid and is the most conserved gene. ORF7 is useful for the identification of strains and to improve diagnosis (4,5).

In this study we determine the position of some ORF7 sequences from PRRSV obtained in 2012 in various states of Mexico in respect to sequences reported in GenBank from.

Materials and Methods

Sampling (nasal swabs and lungs) was performed in the field in areas with PRRS prevalence in Mexico. Diagnosis was performed by RT-PCR using the extraction (Gibco Life Technologies 1996) and specific primers with the OneStep RT-PCR kit from QIAGEN®. (6) The PCR products were purified with the QIAmp® RNA/DNA Viral purification kit according to manufacturer's recommendations. The amplicons were sequenced at the Institute of Cellular Physiology, UNAM.

The ORF7 amino acid sequences reported in GeneBank where automatically obtained and verified to avoid duplication with an ad hoc script. Sequences were aligned with ClustalX, and edited using Sea View v.4. The best substitution model for inference of phylogenetic trees was determined using ProtTest 3.2. Phylogenies were reconstructed using PhyML; parameters were adjusted according to the substitution pattern FLU. Phylogenies were visualized and analyzed using FigTree v. 1.3.1, midpoint rooting is used to make the simplest visualization. (7, 8, 9, 10)

Results

Figure 1 shows that the American PRRS ORF7 sequences clusters with Asian, although they form a separate branch. American sequences are divided into two branches. In one we observed gi4680477 a sequence with three changes at amino acid level. The other branch of this clade includes all sequences obtained in field CSI_75-Fwd, Fwd-7V_75, CEI_75Fwd and the reference sequence (gbU87392.3); as well as two sequences that are separated from the group and are derived from an American strain introduced into Europe during a vaccination campaign with alive American virus.

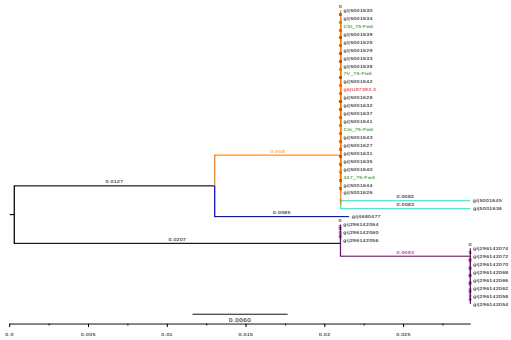

Figure 1. Phylogenetic analysis of ORF7 sequences of PRRSV.

Conclusions and Discussion

The high similarity between the sequences of ORF7 form different locations suggests that there are important selection pressures maintaining a relatively invariable sequence. The evolutionary conservation of the ORF 7 could be an interesting feature for diagnostic or vaccination.

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P.087

Genetic mutation of PRRSV under swIFN-β immune pressure and phosphorylation of interferon regulatory factor-3 suppressed by structural protein-5

H Dongsheng,C Rui-ai,N Xiaoyun,W Yanli, X Zhixuan, S Danping, Z Xianhao, P Zhangfu
MOA Key Lab. of Biotech. & Bioproducts Development for Animal Epidemic Prevention, South China Agricultural University, 510642, China, dhe@scau.edu.cn

Introduction

Our previous studied showed that in Marc-145 moderate swIFN-b promotes genetic mutation of porcine reproductive and respiratory syndrome virus (PRRSV) A1 strain. Cellular immunity plays an important role in the body against pathogens. The type I IFN system is a central feature of the antiviral innate immune response, which is the first defense against viral infection. IFN-α and IFN-β were more efficient in antiviral effect. IRF3 is one of the key factors in inducing the expression of IFN-β. When stimulated, IRF3 undergoes phosphorylation and nuclear translocation to induce the expression of swIFN-I. PRRSV propagation would be completely inhibited by high swIFN concentration, but our previous studied showed that moderate swIFN-b promotes genetic mutation of porcine reproductive and respiratory syndrome virus in Marc-145.

Materials and Methods

A highly pathogenic PRRSV GD strain was continuously propagated in Marc-145 cell cultures with or without swIFN-β to develop PRRSV GDβfn and PRRSV GDfn up to 50 passages. At every 10 passages, their biologics, sequence determination and capacity of induced host swIFN-β mRNA level and the effect of GP5 on IRF3 were analyzed.

Results

The ORF3 and ORF5 sequencing analysis indicated that under swIFN-β immune pressure molecular variation of PRRSV was accelerated in some genes (NS/S>2.5). swIFN-β mRNA level in cells with swIFN-β was lower than cells without swIFN-β, and both were far less than swIFN-β mRNA level in cells with Poly(I: C). Effect of GP5 on IRF3 was analyzed by SDS-PAGE and western-blot. Analysis results proved that GP5 protein could prevent IRF3 phosphorylation.

Conclusions and Discussion

In this study in another PRRSV GD strain, we confirm not only that swIFN promote viral mutation in GP5 but GP5 inhibits IRF3 from phosphorylation to escape from swIFN-β. Our results indicated that the mutative frequency of PRRSV passaged under swIFN-beta immune pressure was significantly faster than that without swIFN-beta. The immune-pressure of swIFN-beta accelerated genetic variation on PRRSV ORF5 and ORF3 and GP5 protein could prevent IRF3 phosphorylation in which may help PRRSV escape from innate immune of host cells.

Acknowledgments

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Effect of Tilmovet® on PRRSV viral loads

CN Lin[1,2], WH Lin[1], CH-Ching Wu[3], W Depondt[3], A Kanora[3], KL Li[1], MT Chiou[1]

[1]*Department of Veterinary Medicine, National Pingtung University of Science and Technology, Taiwan*

[2]*Veterinary Hospital, National Pingtung University of Science and Technology, Taiwan*

[3]*Huvepharma NV,* wouter.depondt@huvepharma.com

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is an economically important disease of pigs defined by severe respiratory disorders in piglets and widespread abortions in gestating sows and gilts, caused by the PRRSV (PRRSV). The antibody-dependent enhancement phenomenon (ADE) of the virus (1,2,3) and the high heterogeneity among PRRSV isolates is likely to be the main obstacle to effective control of PRRSV infection (4,5). Tilmicosin, the active of Tilmovet®, can inhibit replication of PRRSV in pulmonary alveolar macrophages in vitro (6). The aim of this study was to evaluate if tilmicosin is also effective in controlling the replication of PRRSV in vivo under field conditions.

Materials and Methods

Forty four-week-old weaned piglets were randomly chosen from a PRRSV-contaminated farrow-to-finish herd. The piglets were equally divided into two groups and housed in the same pen but separated into individual spaces. Tilmicosin (Tilmovet® 20% premix, 2 kg/ton) was administered to the 4-week-old piglets for 3 weeks (treated group). The control group did not receive any (untreated) treatment. Blood samples were collected from each animal every 2 weeks. The PRRSV antibody levels were determined by ELISA (HerdCheck PRRS 2XR) and the PRRSV viral load was quantified by ZNA probe-based real-time PCR. Student's *t*-test was used to compare the viral loads. Mortality rate and daily weight gain were recorded to evaluate clinical signs.

Results

The PRRSV load was significantly higher (P=0.0003) in the untreated group (ranging from 1.96 to 5.19 $\log_{10}$ PRRSV genomes /µl, median 3.17 $\log_{10}$) compared to treated group (ranging from 1.25 to 3.73 $\log_{10}$ PRRSV genomes/µl, median 2.50 $\log_{10}$). S/P ratios became positive (>0.4) in both groups as from 8 weeks of age. The treated animals exhibited a drop in mortality rate of 20% (5% versus 25%) and an increase of average daily weight gain of 0.3 kg (0.45kg versus 0.48 kg) during the period of the study.

Table 1 PRRSV load in serum collected from the untreated and treated groups

	Group		
	Untreated	Treated	***P* value**
Number of tested samples	93	96	
Number of positive pigs (%)	31 (33.3)	30 (31.3)	**0.759**
Mean ± SD [a]	3.38 ± 1.01**	2.58 ± 0.60	**0.0003**
Median [a]	3.17	2.50	
Range [a]	1.96 to 5.19	1.25 to 3.73	

[a] $\log_{10}$ copy number per microliter in serum
**** Statistical significance (P< 0.01)**

Conclusions and Discussion

This study showed that tilmicosin can reduce the PRRSV load in pigs under field conditions and reduce the clinical signs of PRRSV infection. This would be in agreement with previous studies, showing that reducing the PRRSV load in serum, prevents the development of clinical signs after PRRSV infection (7).

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Case study of a gilt development acclimatization protocol in a PRRS control program and impact on breeding herd stability in a U.S. production system

A Oropeza, J Kolb, R Philips, M White
Boehringer Ingelheim Vetmedica Inc., Saint Joseph, MO, arturo.oropeza@boehringer-ingelheim.com

Introduction

An important component to a successful system-based PRRS control program is replacement gilt development and acclimatization. This case study examines a gilt development, acclimatization protocol of a PRRS control program and its impact on PRRS stability in recipient breeding herds.

Materials and Methods

In the spring of 2011, a collaborative PRRS control project was initiated, involving 26,000 sows distributed in 12 different sow farms, gilt development units (GDU) and nurseries. The primary interventions consisted of herd closure for 210 days and application of live wild-type (wt) resident virus twice, four weeks apart, to all sows and gilts in sow farms. Sow farms were mass vaccinated with two doses of Ingelvac PRRS® MLV 30 days apart, beginning 30 days after the second application of resident wt virus. Ingelvac PRRS® MLV was then applied quarterly to all sows in the system. The gilt acclimatization protocol consisted of exposure to two wt PRRSVes resident to these sow farms, three weeks apart, followed by 2 doses of Ingelvac PRRS MLV. The acclimatization protocol was completed 40 days before gilts were moved to the GDU. The GDU operated as a continuous flow unit. The timing between exposure to PRRS wt viruses and entry into the GDU ranged from 70 to 98 days post exposure to wt virus. To evaluate PRRSV circulation, a monthly diagnostic protocol consisting of 20-60 piglet serum samples pooled into sets of 5 from ready to wean pigs, six oral fluid samples at weeks 10 and 18 in the gilt grow-out farms, and 6-18 oral fluid samples at the GDU. PRRS PCR was conducted on both pooled serum samples (5:1) and oral fluids and samples with CT values ≤33 were sequenced.

Results

After consistently implementing the above protocol for over 2 years, PRRS resident wt viruses continue to be detected at both the GDU (7/24 samples) and in the recipient parity 1 sow farms (31/81 samples, Table 1). Homology analysis suggested that the viruses found at P1 sow farms and the GDU were closely related genetically, and often temporally.

Conclusions and Discussion

The goal of developing both immune and non-infectious replacement gilts for entry into P1 farms with this protocol was not achieved. The presence of the resident wt virus in both the GDU and P1 farms suggests that gilts were not stable and transmitting wt virus to the P1 farms. Insufficient acclimatization time potentially allowed endemic circulation and evolution of resident wt PRRSV. At least 97 days was required for a field virus exposed and vaccinated population to stop shedding to sentinel pigs.[1] The acclimatization time ranged from 70-98 days in this protocol. Rather than create an immune, non-infectious replacement gilt, the protocol provided multiple points for ongoing PRRSV circulation and evolution of the resident field viruses. Ideally, replacement gilts will not be moved to the GDU until 120 days post-exposure to the resident wt virus.

Table 1. Diagnostic summary for P1 farms and GDU

		Parity 1 Farms		
Date	**GDU**	**Farm 1**	**Farm 2**	**Farm 3**
2011				
March	**NT**	**WT +/ Unk**	**WT +/ Unk**	**WT -**
April	**WT -**	**WT +/ Unk**	**WT +/ Unk**	**WT -**
May	**WT -**	**WT -**	**WT -**	
June	**WT -**	**WT -**	**WT -**	**WT -**
July	**WT -**	**WT -**	**WT -**	**WT -**
August	**WT -**	**WT -**	**WT -**	**WT -**
September	**WT -**	**WT +/NA T1**	**WT -**	**WT -**
October	**WT +/1-8-4**	**WT -**	**WT -**	**WT -**
November	**WT +/1-8-4**	**WT -**	**WT -**	**WT -**
December	**WT -**	**WT -**	**WT -**	**WT -**
2012				
January	**WT +/1-8-4**	**WT -**	**WT -**	**WT +/1-8-4**
February	**WT -**	**WT +/1-8-4**	**WT +/1-8-4, 1-19-2**	**WT +/NA T1**
March	**WT -**	**WT +/1-8-4**	**WT +/1-8-2**	**WT +/1-8-4**
April	**WT -**	**WT +/1-8-4**	**WT +/1-8-4**	**WT -**
May	**WT -**	**WT +/1-8-4**	**WT +/1-8-4, 1-8-2**	**WT +/1-8-4**
June	**WT -**	**WT +/1-8-4**	**WT +/Unk**	**WT -**
July	**WT -**	**WT -**	**WT -**	**WT -**
August	**WT -**	**WT -**	**WT -**	**WT +/1-8-4**
October	**WT -**	**WT +/1-8-4**	**WT -**	**WT -**
November	**WT +/1-8-2**	**WT -**	**WT -**	**WT +/1-8-4**
December	**WT +/NA T1**	**WT -**	**WT -**	**WT -**
2013				
January	**WT +/1-8-4**	**WT +/1-8-2, NA T1**	**WT -**	**WT -**
February	**WT +/ Unk**	**WT -**	**WT +/1-8-2, NA T1**	**WT -**
March	**Not tested**	**WT +/1-8-4, NA T1**	**WT +/NA T1**	**WT -**
April	**Not tested**	**WT +/1-8-4, NA T1**	**WT +/NA T1**	**WT +/1-8-4**
May	**WT -**	**WT -**	**WT -**	**WT -**
June	**Not tested**	**WT +/ NA T1**	**WT +/1-8-4**	**WT -**

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A summary of three large scale systems-based PRRS control projects

A Oropeza, J Kolb, R Philips, M White[1]
Boehringer Ingelheim Vetmedica Inc, Saint Joseph, MO, arturo.oropeza@boehringer-ingelheim.com

Introduction

The swine industry continues to experience significant losses due to PRRSV infections of sow farms and growing pigs.[1] As a result, productivity suffers in all phases of production. This is a summary of three collaborative large-scale, long term PRRS control projects applying a systems-based methodology. These projects occurred in three different geographical locations; all of which had previously implemented different methods to control PRRS with varying levels of success. The objective of these projects was to improve PRRS stability and improve overall growing pig performance through the strategic use of modified-live vaccine (MLV) compared to 15 to 24 months of prior production data.

Materials and Methods

Three breeding herd (BH) populations of 30,000, 70,000 and 24,000 sows and respective growing pig flows were involved in these PRRS control projects. All BH populations were infected with diverse heterologous PRRSV isolates and had experienced severe reductions in production. The primary interventions consisted of herd closures ranging from 147 to 210 days, mass vaccinations of BH populations with Ingelvac PRRS® MLV twice 30 days apart and re-vaccination quarterly. Replacement gilts were vaccinated with two doses of Ingelvac PRRS® MLV prior to introduction to BH's. The PRRS control protocol implemented for growing pigs included vaccination of all pigs with Ingelvac PRRS® MLV at weaning. System A used LVI on gilts one time at the start of the project followed by Ingelvac PRRS® MLV, System B did not use any LVI in any population. And System C used live virus inoculation (LVI) on gilts and sows at the initiation of project followed by Ingelvac PRRS® MLV, and continued to use LVI on gilts during the project. PRRSV circulation was monitored monthly in weaned pig and growing pig populations. To evaluate PRRSV circulation, a monthly diagnostic protocol for BH's assessed ready to wean (RTW) piglets. The growing pig populations were monitored monthly using serum samples and oral fluids. Production data for ADG, and mortality by phase of production was analyzed using SPC technology.

Results

All three projects demonstrated significant improvements in ADG and mortality, following the interventions for PRRS control in the growing pig phase of production. Results are summarized in Table 1.

Table 1. Summary of production changes 24 months post-implementation of the PRRS control protocol

	System			
	A	A-FP	B	C
ADG, % change				
Nursery	+23%	-	+7%	0.0%
Finisher	+6%	+5%	+7%	+2%
WTF	-	-	-	+6%
Mortality, % change				
Nursery	-63%	-	-23%	-38%
Finisher	-33%	-32%	-35%	-30%
WTF	-	-	-	-45%

FP= Feeder Pig Flow

Conclusions and Discussion

Managing and controlling PRRSV in large systems is a complex and challenging task. Consistent implementation of a methodology that utilized herd closure and modified-live vaccine for the control of PRRSV infections in both the BH and weanling pigs was effective in improving pig performance and reducing mortality. Reductions in mortality in the nursery period (38-63%), finisher period (30-35%), and WTF period (45%) were realized. Improvements in ADG in the nursery phase (7-23%), finisher phase (2-7%), and WTF (6%) were consistent and repeatable across the three systems. These three systems-based PRRS control projects demonstrate that the implementation of a methodology that utilizes modified-live vaccine for the control of wt-PRRSV infections can mitigate the consequences of infection on health and performance.

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To our customer for the trust and highly collaborative behavior in making these improvements possible

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Field efficacy study in weaned pigs with an inactivated PCV2 and *M. hyopneumoniae* vaccines, and a modified live PRRS vaccine administered as a trivalent mixture (3FLEX) in a 400 sow farrow-to-finish site

AC Bulay, III[1], CA Tiongson[2], RL Marquez [2], CU Maala[1]
[1]*Boehringer Ingelheim (Phil.) Inc.;* [2]*Swine Veterinary Partitionier*, andy.bulay@boehringer-ingelheim.com

Introduction

The clinical presentation of co-infections with various pathogens makes pig farming in Asia even more complex and challenging. Considering this scenario, well designed prophylactic programs to immunize pigs against most of pathogens present in a farm is imperative. Finding the right balance between multiple disease pathogen exposure and protective immunization with minimal stress often is difficult. 3FLEX™ is the trade name associated with the mixture of Ingelvac CircoFLEX®, Ingelvac MycoFLEX®, and Ingelvac® PRRS MLV swine vaccines (BoehringerIngelheim Vetmedica, Inc., St Joseph, Missouri) and it has been proven to be safe and efficacious[1].

The present 6-month study was conducted to evaluate the efficacy of the trivalent vaccine mixture 3FLEX under Philippine farm conditions wherein multiple forms of stress negatively impact growth efficiency parameters.

Materials and Methods

The trial was conducted in a 400-sow farrow-to-finish site located East of Manila and has been previously using FLEXCombo + PRRS MLV simultaneously administered on different neck sites. One hundred healthy pigs within the same batch, aged 21 days (±3d) on Day 0 were tagged, blocked by age and body weight and randomly assigned to treatment groups (Table 1). The farm tested positive serologically for PRRSV, *M. hyopneumoniae*, and PCV2 using commercial ELISA. Testing was conducted just prior to the start of the study, July, 2012.

Table 1. Vaccination program per group of pigs aged approximately 21 days (±3d).

Group	N	Treatment
Yellow	50	Trivalent vaccine mixture (3FLEX)
Blue	50	FLEXcombo + PRRS MLV

The 3FLEX (Yellow Group) was created by mixing equal volumes of the Ingelvac MycoFLEX and Ingelvac CircoFLEX products (each labeled as 1 ml/dose) and this mixture was used to rehydrate Ingelvac PRRS MLV vaccine cake. The mixture was then administered intramuscularly as a 2 ml/dose vaccine injected on the right side of the neck area. For the Blue group, 2ml of the FLEXcombo mixture was injected IM on the left side while 2ml of the Ingelvac PRRS MLV was given separately on the right portion of the neck area. To measure performance differences among the treatment groups, ADG and live body weights at the end of nursery and finishing, respectively, were recorded and statistically analyzed using Student's T-test. Other parameters like mortality and cull rate also were analyzed.

Results

In general, growing performance was numerically better in pigs vaccinated with 3FLEX (Yellow group) but was not statistically significant. (Table 2).

Table 2. Effect of Vaccine on Pig Performance

Parameter	FLEXcombo Ingelvac PRRS MLV	3FLEX	*P*-value	Diff.
Avg. Birth Weight, kg	1.37	1.40	0.5253	0.03
Avg. End Nursery Weight, kg	**44.15**	**45.21**	0.3797	**+1.06**
End Nursery Age, day	85	85		0
Nursery ADG, kg	**0.519**	**0.532**	0.3937	**+0.013**
Mortality, %	2	4		-2
Avg. End Finisher Weight, kg	**86.60**	**88.75**	0.2587	**+2.15**
Avg. End Finisher Age, day	157	151	0.1851	**<6**
Total Pigs culled, %	8.16	2.08		**+6.08**
Finisher ADG, kg	**0.519**	**0.539**	0.1194	**+0.020**

Conclusions and Discussion

Pigs vaccinated with 3FLEX did not show a statistical difference compared with the vaccinated group using separate vaccines concurrently administered (FLEXcombo + PRRS MLV) confirming under the conditions of this farm, the lack of any potential negative impact due to mixing of the three components as one injection, providing the convenience of a single shot and potentially leading to better performance due to reduced handling and injection stress.

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Isolation and genotyping of PRRSV on pig farms from Peru

M Ramírez, H Rivera, J More, A Manchego, KL Chiok
Laboratory of Veterinary Microbiology and Parasitology, Faculty of Veterinary Medicine, San Marcos University, Lima-Perú, mramirezv@unmsm.edu.pe

Introduction

The Porcine Reproductive and Respiratory Syndrome (PRRS) is a disease caused by a virus of the same name (PRRSV) and causing respiratory and reproductive problems such as premature deliveries, abortions, stillbirths and mummified fetuses, causing economic losses estimated at 560 million annually in U.S. dollars (Neuman *et al*, 2005; Beura *et al*, 2010). In Peru the PRRS was detected serologically in fattening pigs from some well managed pig farms from Lima province (Alegria *et al*.,1998). However, no virus isolation or genotyping of PRRSV strains were carried out. The aim of this study was to isolate and genotyping PRRSV strains from serologically positive pig farms from Peru.

Materials and Methods

Blood samples were collected from weaned pigs from positive pig farms in Lima (A=44, B=20; C=16) and Arequipa (D=32, E=92) provinces. Seropositive pig farms to PRRSV were identified by ELISA test. The serum samples (n=204) were processed in 51 pools of 4 samples each for virus isolation using Porcine Alveolar Macrophage (PAM) cell line. The genome of the virus isolated was identified by Reverse Transcription-nested Polimerase Chain Reaction (RT-nPCR). The complementary DNA (cDNA) of genotype 1 and 2 of PRRSV vaccine strains were used as positive controls and cDNA of equine viral arteritis virus, classical swine fever virus and PAM cells as negative controls in the RT-nPCR.

Three blind passages in PAM cell line with each of the 51 pool were done before searching PRRSV antigen by Immunofluorescence test (IF). All samples were processed by a RT-nPCR reported by Gilbert *et al*., (1996), with slight variations.

Results

The 19.6% (10/51) of samples were positive to viral antigen by IF. Eight out of ten positive samples were confirmed as PRRSV using RT-nPCR test and 1 of the 41 negative samples was positive to PRRSV by RT-nPCR. The 17.6% (9/51) of isolated were positives to PRRSV using primers that recognize the common genomic sequence to genotype 1 and 2 and all belonged to genotype 1. Besides, 77.8 (7/9) and 22.2% (2/9) of the samples were identified as genotype 1 from pig farms in Arequipa and Lima, respectively.

Conclusions and Discussion

These results indicate that all the PRRSV strains confirmed by RT-nPCR test belonged to genotype 1. This is the first evidence of genotype 1-European of PRRSV in pig farms in Peru. The absence of genotype 2 strains of PRRSV by RT-nPCR could be due to the origin of the samples, the use of the PAM cell line that is more sensitive for the isolation of strains of genotype 1 and others factors not yet identified. The pig industry in the world has developed in part, with the use of imported semen from countries where genotype 2 was widely reported (Zimmerman *et al*., 1997), in our case, that may have been the way how the virus entered to the country by the 80 and 90 decades when the PRRSV was not on the list of diseases to be considered sanitary barriers. Currently the disease is endemic globally (Shi *et al*., 2010). Therefore, the present study does not rule out the presence of genotype 2 PRRSV in Peruvian swine population.

Acknowledgements

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PRRSV elimination from a recently infected boar stud through partial depopulation

C Corzo[1], D Dagieu[2], A Juarez[2], JA Ugalde[3], E Vera[3], R Mendoza[3], C Rodriguez[3], J Gonzalez[3], J Becerril[4]
[1]*PIC, Hendersonville, TN, USA,* [2]*PIC, Querétaro, México,* [3]*AGLPQ, Querétaro, México,*
[4]*Consultant, La Piedad, México,* cesar.corzo@genusplc.com

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is the most important swine disease from an economic standpoint (1). The virus can be disseminated though different means including semen (2), therefore, for a successful system-wide PRRSV prevention and control program boar studs are required to be PRRSV free. Biosecurity measures to maintain boar studs free of this virus have been successful in preventing the introduction of PRRSV; however, PRRSV continues to infect studs leading to complete depopulation of these valuable animals. The objective of this abstract is to present a case describing the elimination of PRRSV from a recently infected boar stud through partial depopulation saving >80% of the boar stud population.

Materials and Methods

The boar stud located in Mexico had a total population of 143 boars housed in five separate barns. Each barn contained between 19 to 29 boars individually housed in pens. The semen processing lab is located equidistant from all barns in the center of the premises.

In April 2013, as part of the PRRSV routine surveillance, 20 boars were blood sampled and serum sent to the local diagnostic laboratory for PRRSV testing through RT-PCR in pools of five. One of four pools tested positive. The five samples that comprised the pool were individually tested and only one yielded a positive result. For confirmation purposes the serum was re-tested in the same laboratory confirming the original result. Immediately the boar stud was closed for semen production and all sow farms receiving semen from this stud were asked to discard all semen stored and recently received.

In order to understand how widespread the virus was in the boar stud population it was decided to bleed all boars and run both RT-PCR and individual ELISA tests. Additionally, the boar that had initially tested positive was individually tested again. All RT-PCR tests yielded a negative result except the one from the index case. Two boars tested ELISA and IFA positive, one of them being the index case. The two positive cases were housed in the same barn. These two individuals were housed together with 25 boars more.

At this point it was decided to attempt saving as many boars as possible; therefore, the barn in which both positive boars were located was immediately depopulated, cleaned and disinfected. Tissues from both positive boars were sent to the University of Minnesota Veterinary Diagnostic Laboratory with the aim of obtaining a sequence. Partial sequences were obtained from both boars.

An intensive monitoring program began a week after partial depopulation. All boars were weekly tested by both RT-PCR (pools of five) and ELISA for the following three weeks.

Results and Discussion

All samples (RT-PCR and ELISA) from all the three weeks worth of sampling post partial depopulation yielded negative results confirming the remainder of the stud population to be PRRSV free.

The fact that two boars were already seropositive meant that the virus had been circulating for at least a week; however, it is difficult to explain why only one boar was RT-PCR positive.

Conflicting results were seen when all boars were tested through RT-PCR and all pools yielded negative results except the individually tested index case. This could be the result of imperfect sensitivity or that the amount of serum viral particles in the positive animal was descending.

At this point it is not well understood why the virus did not easily spread to other barns; however, compartmentalization may have played a role in containing the virus as well as increasing the chances of successful elimination.

Partial depopulation was effective in eliminating PRRSV from a recently infected boar stud population. This strategy was succesful due to the fact that decisions were made in the very early stages of the outbreak.

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Elimination of modified-live PRRSV from three breeding herds located in low pig-density areas

JP Cano[1], C Odland[2], P Yeske[3], R Philips[1]
Boehringer Ingelheim Vetmedica Inc, St. Joseph, MO[1], Pipestone Veterinary Clinic, Pipestone, MN[2], Swine Veterinary Center, St. Peter, MN[3]

Introduction

Herd closure programs consistently stabilize breeding herds infected with porcine reproductive and respiratory syndrome virus (PRRSV).[1] Modified-live virus (MLV) vaccine has demonstrated to be a valuable tool to achieve stability with sooner recovery of production and less total loss than wild-type virus (WTV) inoculation as the method to homogenize herd immunity.[2] Furthermore, the use of MLV vaccine is indicated in swine populations located in high pig-density areas because outbreaks in PRRS-free herds are more costly[3] and take longer to get stabilized than outbreaks in herds with previous immunity.[2] Once the breeding herd is stabilized, if the risk of infection is negligible, the elimination of both WTV and MLV is justified. The objective of this report is to summarize feasible procedures to eliminate MLV.

Materials and Methods

MLV vaccine elimination was attempted from three breed-to-wean herds with internal gilt development units (GDU) located in low pig-density areas in the upper Midwest of the US (Table 1). When the projects started, WTV or clinical signs associated with PRRS had not been detected in the herds for three years or longer. Ingelvac PRRS® MLV or Ingelvac PRRS® ATP (Boehringer Ingelheim Vetmedica, Inc, St Joseph, MO) had been routinely administered to sows and/or gilts for the last years. In herd A, the entire breeding herd was vaccinated twice 30 days apart after loading the GDU and closing the site for replacements. At 98 days post-vaccination (DPV), negative gilts were introduced to the same air space as vaccinated gilts. In herd B, the GDU was divided in three air spaces. Gilts in rooms II and III were mass vaccinated after room I was emptied. At 28 DPV, access to a cleaned and disinfected room I was blocked from the GDU and gilts in rooms II and III were revaccinated. At 42 DPV negative gilts were introduced to room I, and room II was emptied, cleaned, disinfected and isolated from room III in order to receive negative gilts. At 84 DPV room III was emptied, cleaned, disinfected and isolated from the rest of the farm in order to receive negative gilts. At 126 DPV the first group of non-vaccinated gilts was introduced to the breeding-gestation barn. In herd C, the last group of vaccinated gilts was moved to the breeding-gestation barn 49 DPV. The GDU was cleaned, disinfected and isolated from the rest of the farm in order to receive negative gilts at 56 DPV. Those gilts were exposed to the rest of the population at 91 DPV. PCR was performed on 60 serum samples from due to be weaned piglets in pools of five to monitor shedding during the program. A sample of 30 non-vaccinated gilts was periodically tested by PCR and ELISA to determine exposure in breeding-gestation area.

Table 1. Breeding herds attempting MLV elimination

Herd	# sows	Frequency of gilt introduction	# air spaces in GDU
A	5400	Every 3 weeks	2
B	1300	Every 6 weeks	3
C	1300	Every 6 weeks	1

Results

All serum samples from due to be weaned piglets tested negative during the elimination procedures in all herds. Non-vaccinated gilts tested PCR and ELISA negative in all herds for at least one year following the elimination program. For these three breed-to-wean herds, the use of internal biosecurity measures to avoid direct contact between vaccinated and non-vaccinated gilts for at least 91 days was enough to eliminate MLV (Table 2).

Table 2. Specific times and interventions by herd

Herd	Gilt flow interruption [a]	Neg gilt exposure [b]	Implemented interventions
A	91 days	98 days	Mass vaccination, herd closure, biosecurity
B	42 days	126 days	Mass gilt vaccination, internal biosecurity
C	56 days	91 days	Gilt vaccination, internal biosecurity

[a] Time between introduction of the last vaccinated and the first non-vaccinated gilts
[b] Time from last vaccination to exposure of non-vaccinated gilts to the rest of the breeding herd

Conclusions and Discussion

A larger number of MLV elimination cases need to be analyzed to determine whether the results of this small group of herds are repeatable. However, previously reported data showing minimal transmission of PRRS MLV,[4] probably caused by cell culture adaptation, may support the shorter time needed for stabilization in these herds when compared to populations infected with WTV.[5]

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Collection of oral fluid from individually-housed sows: Baseline parameters

B Pepin[1], R Main[1], A Ramirez[1], F Liu[2], J Zimmerman[1]
[1]*Department of Veterinary Diagnostic and Production Animal Medicine,* [2]*Department of Statistics, Iowa State University, Ames, IA,* jjzimm@iastate.edu

Introduction

Testing oral fluid samples by antibody- or PCR-based assays is an effective method to surveil for infectious agents in swine populations. Oral fluids are commonly collected from growing pigs, but boars can also be trained for oral fluid collection (1,2). Our premise was that the collection of oral fluids from individual sows would facilitate the surveillance of breeding herds for infectious diseases and reduce the need to collect blood samples. However, there is no published data on the collection of oral fluid samples from individually-housed sows. Likewise, there is little data on the repeatability of test results on successive oral fluid samples collected from individual sows. This study initiated an exploration into these issues.

Materials and Methods

The study involved 513 individually-housed, mixed-parity, gestating sows from which oral fluids had never been collected. Three parameters were of interest: (1) the relationship between sow age (parity) and oral fluid collection, (2) the effect of re-sampling ("training") on collection, and (3) the repeatability of diagnostic test results on two successive oral fluid samples from the same animal. The study was carried out by attempting oral fluid collection on two successive days from each animal, i.e., hanging a 1.59 cm (5/8 in) diameter 100% cotton rope at the front of each crate for 30-45 minutes and then manually extracting the sample from the rope. A volume ≥1.0 ml was considered a valid collection. After sampling was completed, paired oral fluid samples (days 1 and 2) from 48 randomly-selected animals were completely randomized, submitted for testing by PRRSV RT-PCR (TetraCore, Inc., Rockville, MD), and PRRSV antibodies (IDEXX Laboratories, Inc., Westbrook, ME).

Results

Parity was significantly associated with oral fluid collection ($p < 0.01$), where lower collection success was observed in higher parities. The total number of animals from which an oral fluid sample was collected was significantly higher on Day 2 vs. Day 1 ($p < 0.001$).

All samples (n = 96 from 48 animals) were PRRSV RT-PCR negative and PRRSV antibody ELISA positive. Analysis of the ELISA S/P ratios revealed a strong correlation between day 1 and day 2 (Pearson's correlation coefficient = 0.82) and no significant difference between days (Student's t-test).

Table 1. Actual and predicted oral fluid collection success (%) by sow parity

	Oral fluid collection (% successful)		Predicted oral fluid collection (% successful)[1]	
Parity[2]	Day 1[3]	Day 2[3]	Day 1	Day 2
0	14.6%	36.6%	29.5%	61.8%
1	34.8%	67.4%	25.1%	57.2%
2	25.5%	50.0%	21.3%	52.4%
3	33.8%	56.3%	17.8%	47.5%
4	16.7%	47.2%	14.9%	42.8%
5+	15.1%	33.6%	12.3%	38.1%

[1]Predicted expectation of oral fluid collection success based on a logistic regression model: (logit(p) = α + β1x1 + β2x2 + β3 x1x2), where p = probability of successful oral fluid collection; α = intercept; β1 = regression coefficient for day; β2 = regression coefficient for parity; and β3 = regression coefficient for interaction of parity and day

[2]Parity was associated with sampling success ($p < 0.01$)

[3]Day 2 collection rate was higher than day 1 ($p < 0.001$)

Conclusions and Discussion

This was a small study, but the findings suggest it may be possible to collect oral fluids from individual sows in commercial herds for surveillance purposes and that paired samples from the same individuals will produce highly repeatable diagnostic results. On the farm, incorporating the collection of oral fluid samples into routine quarantine-acclimatization procedures could be used to train sows for future oral fluid collection.

Acknowledgments

Our thanks to Dr. Craig Rowles and Dr. John Hicks for arranging access to breeding herd units.

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Outbreak and fade out of a genotype 2 PRRSV in three German SPF-herds: Role of vaccination and herd closure

P van Lith[1], G Bronsvoort[1], T Cruijsen[2], V Geurts[2]
[1]Tierarztpraxis Blumberg, Luckower Damm 1a, 16306 Casekow, OT Blumberg, Germany, [2]MSD-AH, Intervet Nederland BV, Boxmeer, The Netherlands, peter.t.schneider@gmail.com

Introduction

PRRS-negative farms can be created by depopulation and repopulation with negative piglets and gilts from PRRS-free farms. Following strict hygienic procedures, these farms can remain PRRS-free in non-pig-dense areas like the eastern part of Germany. In the summer of 2011, three large German multiplying pig farms became infected with a US-PRRSV (Type 2), possibly via infected sperm. All three farms experienced production problems like weak- and little piglets at farrowing with high mortality of pre- and post-weaning piglets. A Type 2 PRRSV was found via PCR in weak born piglets and had a sequence homology of 95-96% with VR2332.

The farms decided to implement a control / eradication strategy in line with Vogelmayer and van Groenland.[1,2] Porcilis® PRRS was the vaccine of choice based on evidence of cross-protection of EU-MLV vaccines against Type 2 PRRS [3] and reduced transmission of Porcilis®PRRS compared to the US MLV vaccine (Ingelvac PRRS-MLV).[4] Furthermore, to reduce the risk of transmission between pigs by needle injection, a needle-less vaccination system was used (IDAL®, intra dermal applicator of liquids, MSD Animal Health).

Materiasl and Methods

Herd

Farm A (Thuringia) is a multiplying farm with 1200 sows, no nursery and SPF-rearing gilts arrive at 150 days.

Farm B (Brandenburg) is a multiplying farm with 2000 sows, with a nursery on the same site and own rearing gilts until the moment of the PRRSV infection.

Farm C (Saxony-Anhalt) is a multiplying farm with 5000 sows, with a nursery on the same site and SPF-rearing gilts arrive at different ages every 3-4 months.

Farm management and vaccination protocol:

On farm A and B the strategy was divided into three stages: (1) 2 x a herd vaccination (4 wk interval) of sows with Porcilis PRRS (IDAL) and structural removal of all piglets older than 3 wks (inclusive non pregnant rearing gilts). (2) Stop import new gilts for at least 120 days followed by (3), input of PRRS free gilts which are sentinels for PRRS monitoring. Farm C did not opt to close the herd. All sows and rearing gilts were vaccinated 2x with an interval of 4 wks. Rearing gilts were vaccinated again after 4 wks (3th vaccination) and sows were vaccinated in the 2nd wk after farrowing and in the 9th wk of gestation. Since March 2013 sows are vaccinated once per cycle, in the 2nd wk after farrowing: rearing gilts are still vaccinated 3x after arrival.

Monitoring

Every 12 wks, 20 weak born piglets and their mothers were tested for PRRSV by PCR. Incoming gilts in farm A and B were tested serologically (PRRS Elisa Idexx) 4-8 wks after arrival with an interval of also 12 wks.

Results

Farm	First PRRS diagnosis	pigs PRRSv (-)	sentinel gilts remain (-)	PRRSv present after control./eradication
A	Aug. 2011	Dec. 2011	Jan. 2012	No
B	Sept. 2011	Jan. 2012	Jan. 2012	No
C	Sept. 2011	Jan. 2012	Not Done	No

Conclusion and Discussion

In 2 farms, Porcilis®PRRS herd vaccination combined with herd closure was successful. In farm C, where herd closure was not applied, this herd vaccination followed by continuous vaccination of the sows and rearing gilts was also successful in reducing genotype 2 PRRSV.

In farm A and B, the infection faded out together with the absence of susceptible pigs, resulting in the production of PRRSV-negative pigs.

In our study, herd closure ensured that during 120 days virus-carriers cannot infect susceptible pigs and might get free from the carrier status [5].

Whether eradication is achieved is not sure because not all sows and piglets were tested by PCR. Eradication is achieved if all incoming SPF gilts remain serologically negative after removal of all sows that were present at the time of infection.

Since cross protection against PRRSV Type 2 strains is demonstrated via EU-MLV vaccination[3], and following conclusions from vaccine strain prevalence studies as well as control and eradication publications, Porcilis®PRRS is a logical choice [1,2,4]. In this study, further spread of Type 2 PRRSV infection was prevented by this vaccination in combination (on two farms) with removal of susceptible pigs. Therefore we conclude that the EU-MLV vaccine likely contributed to cross-protection against Type 2 PRRS strains.

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HP-PRRSV challenge in pigs vaccinated with Porcilis® PRRS

KM Lager[1], SL Brockmeier[1], KS Faaberg[1], LC Miller[1], CL Loving[1], HC Yang[2], R Jolie[3]
Dr. K. M. Lager, National Animal Disease Center, USDA-ARS, Ames, Iowa 50011-1250, USA,
kelly.lager@ars.usda.gov, [1] National Animal Disease Center, USDA-ARS, Ames, Iowa, USA. [2]China Agricultural University Beijing China [3]*Merck Animal Health, Summit, NJ, USA.*

Introduction

In 2006, epidemics of swine disease causing high mortality were recognized in China and soon thereafter in many Southeast Asian countries. The epidemics were shown to be caused by, or associated with porcine reproductive and respiratory syndrome virus (PRRSV). These virus isolates are classified as Type 2 PRRSV with a unique molecular signature and became known as highly pathogenic PRRSV (HP-PRRSV). From the beginning, there were questions about control of HP-PRRSV through the use of current vaccine technology. To date, several studies have been completed demonstrating some efficacy of commercially available attenuated vaccines derived from Type 1[1] and 2[2,3] PRRSV isolates. A study evaluating the efficacy of Porcilis® PRRS vaccine, a Type 1 attenuated PRRSV, against HP-PRRSV challenge is described.

Materials and Methods

Conventionally-raised PRRSV-free pigs at 3 weeks-of-age were randomly allotted to one of three groups: Challenge control (n=15), Vaccinated (n=15), and Control (n=14). At 4 weeks-of-age, Vaccinated pigs received one injection of Porcilis® PRRS vaccine according to label. At 34 days post vaccination, or 0 days-post-challenge (dpc), Challenge and Vaccinated pigs were housed in separate pens within the same isolation room with snout-to-snout contact. Both groups received an intranasal challenge with 1 x 10^4 $CCID_{50}$ of the JXwn06 HP-PRRSV isolate.[4] Planned bleeding dates were 0, 2, 4, 7, 10, 14, and 21 dpc at which time necropsies were scheduled. Pig weights were recorded at -1, 7, 14, and 21 dpc. All sera and the bronchoalveolar lavage fluid (BALF) collected at necropsy were tested for infectious virus in MARC-145 cells. Aerobic bacterial isolation was conducted on BALF samples and on serosal surface in thorax and abdomen. Serum was tested for antibody by IDEXX PRRS X3 ELISA. There is a 61.5% nucleotide identity between the JXwn06 and Porcilis PRRS vaccine viruses at the genomic level.

Results

Control pigs remained normal and free of PRRSV throughout the experiment. At 0 dpc, all 15 vaccinated pigs were PRRSV antibody positive, and all non-vaccinated pigs were antibody negative. By 3 dpc, pigs in both challenge groups were becoming sick. Severe disease developed in each challenge group leading to death or euthanasia for 12/15 and 6/15 pigs from the Challenge and Vaccinated pigs, respectively. Virus was isolated from the serum of each challenged pig, Vaccinated pigs had less virus load than Challenged pigs. No difference in reduced weight gain and fever were found between Challenge and Vaccinated pigs. *B. bronchiseptica* was isolated from 8/14 control-pig BALF samples. Isolation of bacterial species other than *B. bronchiseptica* was more prominent in Challenged pigs 12/15 (with more bacterial species represented) as compared to Vaccinated pigs 4/14 (BALF not collected from 1 dead pig). Also, there was an increased systemic isolation (serosa) of bacteria from Challenge (6/15) vs. Vaccinated (0/14) pigs, which included species not typically found at that site (*P. multocida* and *S. aureus*).

Discussion

Since the discovery of PRRSV in 1991 many advances have been made in understanding the biology and ecology of the virus. However, it is still difficult to control the virus even though it has been 20 years since PRRSV vaccines became available. Results from this study are similar to what has been reported for Type 1 and Type 2 attenuated PRRSV vaccines against challenge with HP-PRRSV, i.e., the vaccines reduced clinical disease, mortality, and virus load, but did not induce sterilizing immunity. This was not unexpected since all attenuated PRRSV vaccine studies have similar results when the challenge virus is different or heterologous to the virus used to derive the vaccine. Differences found between homologous and heterologous challenge of PRRSV immunity indicate the remarkable capacity of the virus to affect the swine host and thwart a sterilizing immune response. In addition, although an immunosuppressive effect from viral infection was seen in both PRRSV groups resulting in an increase in secondary bacterial infections, vaccination mitigated this effect. Despite the lack of sterilizing immunity induced by PRRSV vaccines, their use can reduce clinical disease within the herd and perhaps transmission as well, making vaccination an important tool in the control of PRRS.

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PRRSV monitoring based on oral fluid antibody: A pilot study in a commercial farm in Sonora, Mexico

C Gomez[1], C Goodell[1], C Díaz Rayo[2], A Bedoy[2] , M Serrano[3], S Zimmerman[1]
[1]*IDEXX Laboratories Inc., Westbrook, Diagnosticos Integrales en Patologia Animal-ITSON,Mexico,* [3]*IASA,Mexico,*
carlos-gomez@idexx.com

Introduction

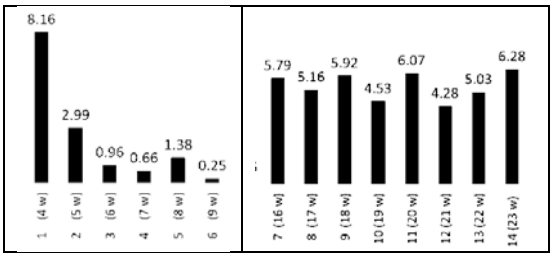
PRRS is one of the most costly diseases swine producers confront. Because the virus is endemic to most swine-producing regions, PRRS control is fundamental to herd health and productivity. However, s*ince there is no perfect control strategy*, effective PRRS control must be based on (and continually adjusted to) the actual situation on the farm. The long-term aim of this study (currently in progress) is to evaluate antibody-based PRRSV monitoring on a large farm using oral fluid samples. Herein we present the cross-sectional (single point in time) results from the first sampling of nursery and finishing barns.

Materials and Methods

This study was conducted in a 950-sow farm in Sonora, México. The farm had experienced a PRRS outbreak 6 months prior to the start of the study. Vaccination at 12 week intervals (Ingelvac PRRS® MLV, Boehringer Ingelheim; InmunoPRRS, Investigacion Aplicada SA de CV) had stabilized PRRSV in the breeding herd.
Oral fluids were collected by suspending a cotton rope in each pen for ~30 minutes. Samples were tested using a commercial PRRSV oral fluid antibody ELISA (IDEXX Laboratories, Inc., Westbrook, ME, USA). Samples with S/P ≥ 0.4 were classified as positive.

Results

PRRS oral fluid antibody ELISA S/P ratios by barn (pig age in weeks) are given in the Figure and Tables:



	Oral fluid ELISA S/P by BARN (age in weeks)					
PEN	1 (4 w)	2 (5 w)	3 (6 w)	4 (7 w)	5 (8 w)	6 (9 w)
1	8.39	3.91	0.35	0.54	1.60	0.14
2	8.45	1.78	1.62	0.42	1.58	0.44
3	7.92	4.33	0.39	1.06	2.55	0.34
4	7.81	2.79	1.59	0.40	0.30	0.13
5	8.23	2.13	0.84	0.89	0.86	0.19
Mean	**8.16**	**2.99**	**0.96**	**0.66**	**1.38**	**0.25**

	Oral fluid ELISA S/P by BARN (age in weeks)							
PEN	7 16 w	8 17 w	9 18 w	10 19 w	11 20 w	12 21w	13 22 w	14 23 w
1	7.16	5.24	3.48	1.67	5.78	7.82	8.49	7.15
2	4.37	5.30	6.37	5.82	6.21	3.64	3.30	5.15
3	5.84	4.95	7.92	6.10	6.20	3.63	1.03	6.56
Mean	**5.79**	**5.16**	**5.92**	**4.53**	**6.06**	**5.03**	**4.28**	**6.28**

Conclusions and Discussion

In this study, testing of oral fluid samples with the PRRS oral fluid antibody ELISA provided extensive information at low testing cost. A previous study (1) in 12 laboratories found that the PRRS oral fluid antibody ELISA was highly repeatable (laboratories were able to reproduce their own results) and reproducible (laboratories' results agreed with each other). The test has a wide "amplitude" and S/Ps of 12 are not uncommon. This greater range in S/Ps provides more information and facilitates comparisons within and between barns.

In this study, S/Ps ranged from 0.13 (barn 6, pen 4) to 8.49 (barn 13, pen 1). The data showed a steady decline of maternal antibody through barn 6 (9-week-old pigs), with PRRSV circulation, thereafter. This information can guide important management decisions, e.g., when / where to use vaccination or which samples to submit for PRRS RT-PCR or sequencing. For example, the samples most likely to be useful in molecular testing would be from barns in which seroconversion was occurring.

The current dataset represents a single instant in the past, but *PRRSV continues to move and change on the farm.* Every herd's PRRS control program must account for this though a process of continual evaluation and adjustment. Monitoring based on oral fluid collection at biweekly intervals fits this need by creating a dynamic picture of PRRSV circulation on the farm. This allows improvements in PRRSV control to develop rationally and efficiently through the use of relevant herd data.

Acknowledgments

Francisco Javier Madrid

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Impact of PRRSV aerosols on air filtration efficiency as a function of particle size

C Alonso[1], M Torremorell[1], BA Olson[2]
[1]*Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN* [2]*Mechanical Engineering Department, University of Minnesota, Minneapolis, MN* alons015@umn.edu

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is considered one of the most devastating viruses for the US swine industry. Air filtration systems in large sow farms have demonstrated to significantly reduce, but not eliminate, the risk of introduction of PRRSV contaminated aerosols (1, 2). Air filtration works by removing particles from the air entering into the animal facilities. This removal efficiency is dependent on particle size. MERV 14 and MERV 16 filters are commonly used in sow farms and they provide 75% and 95% minimum removal efficiency respectively in capturing particles from 0.3 to 1 microns in diameter, and >95% for particles of 1 to 10 micron size range. However, with these filters, a significant proportion of particles (25% of 0.3-1 micron particles in the case of MERV 14 filters) will penetrate the filters and will not be removed from incoming air. Understanding the relationship between airborne particle sizes and animal viruses in bioaerosols is important to advance the application of filtration technology and the risk of failure in filtered farms. Therefore, the objective of the present study was to assess the efficacy of commercially available MERV 14 and MERV 16 filters at eliminating PRRSV from aerosols of selected 'respirable' particle sizes following ASHRAE Standard 52.2-2012 methods.

Materials and Methods

Aerosols were generated into an ASHRAE 52.2 filter test facility using a high output aerosol generator tagged with fluorescent dye (Figure 1) (3). Aerosols were sampled, as a function of particle size, using an Optical Particle Counter (flow rate of 1.0 l/min, particle size range from 0.3 to 10 µm) and Andersen Cascade Impactor (flow rate of 60 l/min, particle size ranges from 0.28-6.2 µm) located both upstream and downstream from the filter. Infectious PRRSV aerosols were generated using 1, 10 and 20% KCl solutions and a modified live virus vaccine (10^7 viral RNA copies/ml). PRRSV RT-PCR was used to assess the presence of viral genetic material in the samples. PCR reactions were run for each sample size range collected on each of the 8 stages of both Andersen Impactor samplers.

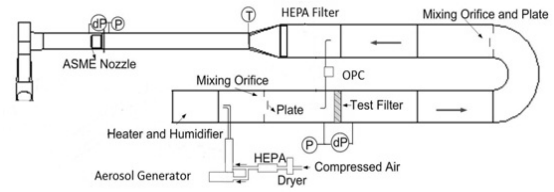

Figure 1. Diagram of ASHRAE 52.2 test facility

Results

Under the conditions of this study, efficiency of PRRSV removal was similar to removing KCl particles following ASHRAE 52.2 Standard (Figure 2). Using a MERV 14 filter, 30% of PRRSV aerosolized particles with a 0.35 µm mean particle diameter were able to penetrate the filter compared to 5% after testing the MERV 16 filter type. Efficiency of particle removal was similar across the different methods (OPC and PCR) results for both types of filters.

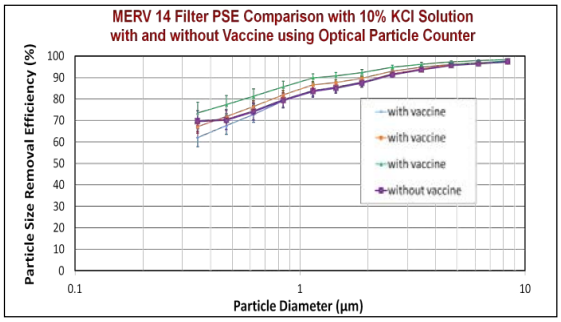

Figure 2. MERV 14 Particle Size Removal Efficiency. *(Particle Size Removal Efficiency (PSE), % = [1-downstream/upstream particle concentration] x 100) results by OPC comparing 3 replicates of virus challenge against a negative control (10% KCl solution).*

Conclusions and Discussion

The results presented in this study indicate that filter efficiency for removing PRRSV from aerosols was similar to filter efficiency for particles of similar size. PRRSV associated to small size particles were able to penetrate the filter, and thus potentially infect farms. This study emphasizes the need for understanding the role of particle size in PRRSV aerosol transmission and control to prevent the spread of airborne viruses. However, further research is needed to determine the particle sizes that are associated with PRRSV aerosols under field conditions.

Acknowledgments

The authors woudl like to thank the Swine Disease Erradication Center at the University of Minnesota for the funding of the project.

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Seroprevalence of PRRS in breeding herds from pig farms of Zulia State

W Mejia[1], T Padrino[1], D Zapata[1], A Aranguren[2], G Portillo[2], A Quintero[1], EJ Kwiecien[3], JC Negrete[1]
[1] *Laboratorio de Patología Porcina,* [2]*Laboratorio de Genética Molecular,* [3]*Doctorado en Ciencias Veterinarias. Facultad de Ciencias Veterinarias, La Universidad del Zulia* willian.mejia@fcv.luz.edu.ve

Introduction

PRRS is a viral disease characterized by two overlapping clinical presentations, reproductive impairment or failure in breeding animals, and respiratory disease in pigs of any age. The first official description of the disease in Venezuela was in 1998 (3). Serological studies have shown a widespread disease and have reported a seroprevalence in a range between 44% and 90% (2). The aim of this study was to determine the prevalence of PRRSV (PRRSV) in swine farms in Zulia state.

Materials and Methods

A study was performed in order to estimate the serological PRRSV prevalence of pig herds in Zulia State. The following criteria were assumed; a total census swine herds is 49 technified farms and a population of 102.049 pigs in 2008, a priori prevalence of 50%, a precision ± 5% and confidence level 95%, the number of swine herds was calculated to be 44 (Win episcope 2.0); however, were examined 45. In each selected pig farm at least 19 samples of sera were colleted (according to prevalence estimate of 15% ± 5% precision, and confidence level 95%) (Win epicospe 2.0). Samples were aseptically taken from external jugular vein in older pigs (Sows and Boars) with sterile 18G x 1½" and 7 mL BD vacutainer vacuum tube. Serology was performed with a commercial enzyme linked immunoasorbent assay (ELISA) kit, IDEXX PRRS X3 Ab Test. The plates were read at 650 nm using an ELISA plate reader controlled by commercial software (the xCHECK software (USA) version 3.3, of IDEXX). The presence or absence of antibody to PRRSV is determined by calculating the S/P ratio for each sample, considering positive for PRRSV antibodies, S/P ratio values grater or equal to 0.40, and negative for PRRSV antibodies, S/P ratio values less than to 0.40.

Results

A total of 1182 serum samples were tested by ELISA, of these 1078 (91.20%) were negative and 104 positive sera (8.80% [I.C.95%: 7.27 - 10.59]) was obtained. The number of positive samples were detected in 13 farms (28.89% [I.C.95%:16.84 - 44.52]) of the 45 evaluated. The prevalence of seropositive herds ranged from 2.86% to 100%. The interaction between sex was not statistically significant ($p \geq 0.05$). (Table 1).

Table 1. ELISA PRRSV general results from swin herds from Zulia state technified farms

Municipalities	Nº of Farms	Nº of Samples	Sex		Samples Positives of PRRS				Prevalence (%)			
			M	F	Farms	Samples	Sex		Farms	Samples	Sex	
							M	F			M	F
Jesús Enrique Lossada	17	392	61	331	6	31	6	25	32.29	7.91	9.84	7.55
San Francisco	9	331	37	294	3	29	7	22	33.33	8.76	18.92	7.48
Lagunillas	2	30	0	30	1	1	0	1	50	3.33	0.00	3.33
Cabimas	12	29	8	21	0	0	0	0	0	0	0	0
Santa Rita	1	34	0	34	0	0	0	0	0	0	0	0
Valmore Rodríguez	1	20	5	15	0	0	0	0	0	0	0	0
Mara	5	81	2	79	0	0	0	0	0	0	0	0
Machiques	1	12	1	11	0	0	0	0	0	0	0	0
Miranda	1	20	9	11	1	8	2	6	100	40.00	22.22	54.55
Cañada de Urdaneta	7	233	2	231	2	35	0	35	28.57	15.02	0.00	15.15
Total	45	1182	125	1057	13	104	15	89	28.89	8.80	12.00[a]	8.42[a]

(a) Superscripts indicate no statistically significant differences within main effect ($p \geq 0.05$)

Conclusions and Discussion

In our study a prevalence of 28.88% was found, this result contrasts with previous studies. This lower prevalence found could be due to several factors involved. Firstly to climatic factors. The PRRSV is very sensitive to the external environment. Transmission of PRRSV decreased when weather conditions were associated with a high intensity of sunlight a low wind speed, pressure, and low relative humidity (1). The above weather conditions are very common in the different municipalities of Zulia state and which would not allow the virus to remain for long outside the host and reducing the transmission of the disease and also prevent that the virus can travel long distances. Whence, the climatic factors may affect the prevalence of PRRS (4). Secondly, the low population density of pigs, as well as on the number of pig farms in Zulia, is another factor that could be involved in the low prevalence found.

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Genetic evolution of PRRSV in pigs vaccinated with modified live PRRSV vaccines of type I in comparison to type II in Thailand

S Boonyawatana[1*], G Temeeyasen[2], T Tripipat[2], D Nilubol[2]

[1]*Intervet (Thailand) Ltd. suraphan.boonyawatana@merck.com and* [2]*Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand,* suraphan.boonyawatana@merck.com

Introduction

Two distinct genotypes of PRRSV, Type I (European) and Type II (North American), have been recognized and the co-existence of both genotypes has been increasingly evident in several countries, including Thailand, Korea and China[1-3]. In Thailand, modified live vaccines (MLV) of both genotypes are commercially available, questions have been raised as to which should be used to control PRRSV and how PRRSV evolution would occur. The objectives of the study were to investigate the genetic evolution of ORF5 gene following MLV vaccination in previously infected pigs.

Materials and Methods

Experimental design

Two hundred 20 kg PRRSV-positive pigs were separated into three groups: Controls (n=30), and Type I, and Type II MLV-vaccinated groups (both n=85). Each group was housed in a separate pen in the same building with a stocking density of 1.5 m^2/pig. Pigs in vaccinated groups were vaccinated intramuscularly at 10 days after arrival with either MLV of Type I or II genotype in accordance with manufacturer's instructions.

Three pigs from each group were randomly selected, identified and blood sampled on 0, 7, 14, 21, 28 and 77 days post- vaccination (DPV). Sera were separated and assayed for the presence of virus by PCR. ORF5 genes were sequenced using the previously described method[3].

Results

The phylogenetic analysis demonstrated that both types of PRRSV were isolated from the study. All Type I and II isolates were further divided into 2 and 4 clusters, respectively. Type I MLV belongs to the cluster 1 of Type I isolates along with other Type I Thai isolates. The cluster 2 of Type II PRRSV was highly pathogenic (HP) PRRSV. Type II MLV belongs to the cluster 3 of Type II isolates and the cluster 4 isolates were reported to be the progeny virus of Type II MLV[4].

Prior to and following MLV vaccination, pigs in all 3 groups were co-infected with both Type I and II PRRSV and pigs in all 3 groups were infected with HP-PRRSV.

The results of ORF5 genes demonstrated that Type I and II PRRSV were consistently detected in all 3 groups following vaccination, regardless of MLV type. Type I isolates in Type I MLV vaccinated group were not genetically identical to Type I MLV, unlike in Type II MLV vaccinated group, in which isolates identical to MLV type were isolated from pigs 7 days DPV (Table 1).

Figure 1. Phylogenetic analyses based on PRRSV ORF5 genes corresponding to type I (left) and type II (right)

Table 1 The cluster of PRRS genotype from 3 pigs each per DPV sampled

Group	Type	D0	D7	D14	D21	D28	D77
Type I vaccinate	1	C1	C1	C1	C1	C1	
	2	C4	C4	C2,C4	C2,C4	C4	
Type II vaccinate	1	C1	C1	C1	C1		C1
	2	C2, C4	C2,C3, C4	C2		C2,C4	C2,C4
Control	1		C1		C1	C1	
	2	C4	C4,C4	C2,C4	C4	C2,C4	C2

Conclusions and Discussion

The results of the study suggested that vaccination with Type I MLV did not influence the diversity of PRRSV in the herd once the endemic virus belonged to the similar cluster as MLV. This is unlike Type II MLV, which influences PRRSV strain development in the herd as previously reported[4].

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Potential environmental contamination of PRRSV from livehaul vehicles

A Hintz[1], J Pittman[2]
[1]*University of Wisconsin School of Veterinary Medicine,* [2]*Murphy-Brown LLC,* ahintz2@wisc.edu

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is the most economically devastating disease in swine, costing the United States pork industry $664 million per year[1]. Both aerosol transmission[2,4] and transmission from contaminated transport vehicles[3] have been implicated in the transmission of PRRSV. However, no studies exist exploring the possibility of aerosol transmission of PRRSV from livehaul vehicles transporting confirmed PRRSV positive and shedding pigs. PRRSV infected pigs are routinely transported through high swine-traffic areas, but it is unknown what risk these animals pose. This study looked at the potential environmental contamination of PRRSV from livehaul trucks moving infected pigs under both controlled and field conditions.

Materials and Methods

Part 1: A trail vehicle was parked 20 feet behind a lead truck. One-hundred doses of Boehringer-Ingelheim Ingelvac® PRRS MLV were reconstituted and aerosolized from the truck toward the trail vehicle. Immediately after spraying, nine sites on the trail vehicle were swabbed using Swiffer® cloths soaked in 25 mL of minimal essential medium (MEM). The sites included the roof, windshield, hood, bumper, grille, left and right side mirrors and left and right front tires. The MEM was collected and PRRSV polymerase chain reaction (PCR) testing was run on the samples. The trail vehicle was washed and disinfected post-trial. Part 2: A trail vehicle drove 20 feet behind a lead truck at 15 to 20 miles per hour. 100 doses of Ingelvac® were aerosolized with a spray bottle from the lead truck while the two vehicles were in motion. Samples were collected and processed in the same manner as part 1. The trail vehicle was washed and disinfected post-trial. Part 3: Part three of the study involved following livehaul vehicles transporting PRRSV positive weaned and feeder pigs from their origin to their destination. These pigs were identified as PRRSV positive by routine monitoring of serum or oral fluid samples by PCR. The livehaul vehicles were followed for 15 minutes to 2 hours depending on the distance between sites. The trail vehicle followed up to 100 feet behind the livehaul vehicle at speeds of up to 60 miles per hour. Throughout each run, a cyclonic air collector was attached to the roof of the trail vehicle and was filled with 10 mL of Physiological Buffered Saline (PBS). Once each run was complete, three MEM samples were taken from the trail vehicle (bumper, hood, grille) and the PBS was collected. Each sample was tested for PRRSV by PCR. A total of 21 runs were completed and the trail vehicle was washed and disinfected after each run.

Results

Parts 1 and 2: In part 1, 9 of the 9 samples collected tested positive for PRRSV by PCR and in part 2, 8 of the 9 samples collected tested positive for PRRSV by PCR. Part 3: 0 of the 63 Swiffer® MEM samples tested positive for PRRSV by PCR and 1 of the 21 PBS cyclonic air collector samples tested positive for PRRSV.

Conclusions and Discussion

Under controlled conditions PRRSV was aerosolized from a lead vehicle and detected on a trail vehicle. However, the same results were not replicated under field conditions at this time. Future studies should look at the seasonal effect on PRRSV shedding, the effect of different age swine being transported on the amount of PRRSV aerosolized, and the possibility of the aerosolized PRRSV infecting naive swine carried on transport vehicles. Studies also can look at other viruses potentially contaminating livehaul routes through aerosolization, like porcine epidemic diarrhea virus, porcine circovirus type 2, and other viruses that may lead to epidemics. These results could change pig movements through high-traffic areas. If groups of pigs are shedding high levels of PRRSV, they pose a risk to naïve animals via aerosolization. Livehaul routes may be altered to move naïve groups of pigs through lower-traffic areas and minimize the chance of contact with aerosolized and perhaps infective PRRSV.

Acknowledgments

Boehringer Ingelheim Vetmedica, St. Joseph Missouri 64506
Murphy Brown LLC, Warsaw North Carolina 28398

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PEDV introduction into a United States sow farm

M Ackerman[1], J Stevens[2], J Waddell[3], G Cline[3]
[1]*Swine Veterinary Services, Greensburg IN,* [2]*Osgood, IN,* [3]*Boehringer Ingelheim Vetmedica Inc, St Joseph, MO,*
pigvet@aol.com

Introduction

Porcine Epidemic Diarrhea (PED) virus is a newly recognized pathogen in the United States. PEDV, a member of the *Coronaviridae* family is similar yet distinctly different from Transmissible Gastroenteritis (TGE)[1]. PED has been reported in Europe and Asia, but until now not in North or South America[2]. Diarrhea, vomiting and dehydration are commonly observed clinical signs with up to 100% mortality occurring in the youngest animals[3]. The objective of this abstract is to report on the second officially confirmed case of PED in the United States.

Materials and Methods

A 6000 head farrow to wean sow farm in the eastern corn-belt of the United States observed vomiting and diarrhea in sows in one of 8 gestation rooms. Fifty percent of all pigs in farrowing greater than 10 days of age were also exhibiting diarrhea. Diagnostic samples of ileum, jejunum, cecum and colon (fresh and fixed) from 2 pigs were submitted to the ISU-VDL for confirmation of TGE May 6th, 2012. Control strategies for TGE included immediate herd closure to new entries; harvesting intestines of recently deceased neonatal piglets which had succumbed to diarrhea; and multi-day oral exposure to all adult animals to the macerated neonatal intestines. Two days later TGE PCR and IHC were reported as negative. The samples were then submitted to NVSL and tested positive for PEDV.

Results

Reproductive performance remained normal on the farm throughout. The adjusted percent survivability (corrected for baseline pre-weaning mortality) of piglets in farrowing groups for the first 6 weeks post-intervention is shown in Figure 1. Survivability was calculated as: ((100 –(farrowing plus mortality through d7) minus the farms baseline mortality). A 100% survivability value indicates the weekly pre-weaning mortality was equivalent to the farms previous baseline level. Clinical signs of diarrhea was also observed in the nursery but resolved within 48 hours of weaning. As seen with TGE, the severity of duration and magnitude of clinical signs of PEDV observed decreased with increasing age. In 12 week or older pigs the pigs remained active, with normal water intake and slightly decreased feed consumption for three days.

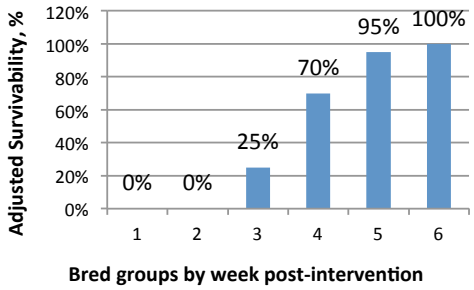

Figure 1. Percent adjusted survivability for 6 weeks following PEDV intervention.

Conclusions and Discussion

This abstract describes the first onset of clinical PEDV in the United States.

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Risk factors associated with swine flu depends on the subtype

C Tufiño-Loza[1], E Rojas-Anaya[1], E Loza-Rubio[1], MJJ Martínez[2], VF Diosdado[1], LAC Martínez[1], ME Manjárrez[3], GC Cabello[3], LD Córdova[1], FA García[1]

[1]CENID-Microbiología, INIFAP, Mexico; [2]FMVZ-UNAM, [3]INER, edith_ra23@hotmail.com

Introduction

Swine influenza is caused by type A influenza virus. Pigs can be infected by both avian and human influenza virus; therefore the influenza virus infection in pigs is considered an important public health concern (1,2,3,4). Therefore, the aim of this study was determine risk factors associated with the spread of swine influenza virus from mexican pigs in different states of the country (SIV).

Materials and Methods

Serum samples from 2048 pigs of 256 farms were analyzed for detection of antibodies against SIV subtypes H1N1, H3N2, and H1N1/2009 pandemic using IH test. A non-probabilistic transversal study was made in the states of Guanajuato, Jalisco, Michoacán, Querétaro and San Luis Potosí, using questionnaires in a backyard, semi-intensive and intensive farms. To evaluate the potential risk factors associated with SIV, were analyzed using X^2 test and logistic regression model.

Results

For the five states sampled, 45.7% were to the H1N1 subtype endemic, 20.4% for subtype H3N2 and 15.9% to subtype A/H1N1/2009 was observed. Statistically significant difference among the five states sampled (P <0.05). With these results, the risk factors associated with exposure to influenza were analyzed. The result of this analysis is shown in Tables 1-3 for each subtype. In Table 1, it is notable that the analysis revealed that the origin of the animal (own or purchased) resulted as a protection factor (PF) and no risk. In multivariate analysis factors with a P <0.1 in the bivariate analysis were used. The risk factors that were found significant (P <0.05), but with an OR <1, represent protective factors. These factors can be considered definitive in the study, since the latter analysis eliminated confounding variables that may have been skewing the true association between risk factors and exposure to subtype or presentation of the disease.

Conclusions and Discussion

The results showed that the risk factors are dependent on the virus subtype, since for each subtype were different.In conclusion, this study determined that there is a greater distribution of subtype H1N1 in the sampled states regarding the H3N2 subtype, indicating an active viral circulation. Knowing the different risk factors for each subtype is possible to establish the most appropriate prevention programs as well as improve biosecurity measures because most of the resulting risk factors arerelated to this; the above in order to reduce the occurrence and spreading of the disease in herds.

Table 1. Risk factors for exposure to viral subtype H3N2 endemic.

Category	OR	C.I 95%	P Value
Road	2.6414	1.1954-5.8363	0.0163
Food waste	3.6674	1.7149-7.8428	0.0008
Origin	*0.2906*	*0.0959-0.8809*	*0.0290 FP*
Female	2.1551	0.87295.3205	0.0959

Table 2. Risk factors for exposure to viral subtype H1N1 pandemic.

Category	OR	C.I 95%	P Value
Breeding-fattening pig farm	1.7608	1.0013-3.0963	0.0495
Weaning	21.8319	5.7201-83.3256	0.0000
Wetlands	2.0131	1.1214-3.6137	0.0191
Querétaro	176.9590	67.2425-465.6948	0.0000

Table 3. Risk factors for exposure to influenza H1N1 virus..

Variable	OR	I.C. 95%	P Value
Backyard	5.0209	1.2397-20.3353	0.0238
Guanajuato	5.2900	1.5976-17.5163	0.0064

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CONACYT-SALUD/IMSS/ISSSTE-127005, INIFAP.

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PorPV causes a respiratory disease in pigs after experimental infection

JF Rivera-Benitez[1], S Cuevas-Romero[2], J Reyes-Leyva[3], J Hernández[4], A Pérez-Torres[5], H Ramírez-Mendoza[1]

[1]*Departamento de Microbiología e Inmunología, FMVZ, UNAM.* [2]*Centro Nacional de Investigación Disciplinaria en Microbiología Animal, INIFAP.*[3]*Centro de Investigación Biomédica de Oriente, IMSS.* [4]*Centro de Investigación en Alimentación y Desarrollo, A.C.* [5]*Facultad de Medicina, UNAM. México.* betosram@yahoo.es; expide@yahoo.com

Introduction

The *Porcine rubulavirus* (PorPV) is an enveloped, single-stranded, negative-sense RNA virus that belongs to the family *Paramyxoviridae*, and causes blue-eye disease (BED) in pigs (1). PorPV infection remains endemic in the central and western-central regions of Mexico and has only been diagnosed in that country (1, 2). BED is considered one of the four most important viral diseases affecting the Mexican swine industry and it causes important economic losses (1). The viral distribution is influenced by the expression of specific receptors (NeuAcα-2,3Gal) that confer cell and tissue tropism (3). An earlier study has shown that these receptors are abundant in the respiratory tract of pigs (4). The respiratory tract organs have been identified as a site for the abundant replication of PorPV (5, 6). All of these observations were conducted in piglets (3–17 days old). However, there are no studies in which this condition has been described in naturally or experimentally infected growing pigs. The aim of this study was to analyze the pathogenicity and distribution of PorPV in the respiratory tract of experimentally infected pigs.

Materials and Methods

Nine 6-week-old pigs were infected with PorPV and examined clinically. Blood, nasal swab, and tissue samples were collected on different days post-infection (7, 14 and 28DPI). Real-time RT-PCR quantification (qRT-PCR) for N gene of PorPV was performed following a previously described procedure (7).The humoral immune responses and histopathological changes were evaluated. An analysis of variance using the Tukey correction/adjustment procedure was used for mean comparisons of different parameters (rectal temperature and antibody titer) between the days analyzed.

Results

The infected pigs exhibited an increase in the respiratory clinical signs and rectal temperature (Fig 1). In addition, the excretion of PorPV was extended to 23 DPI in the nasal fluid. The distribution of PorPV in the respiratory tract tissues was extended until the end of the experiment; soft palate tonsil and lymph nodes exhibited high viral loads (Table 1). The major microscopic lesions observed in the lungs corresponded to interstitial pneumonia and hyperplasia of the associated lymphoid tissue.

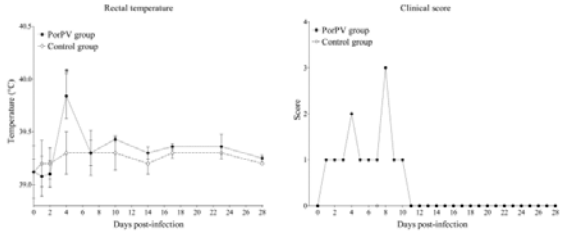

Figure 1. Mean of the rectal temperatures (±SD) and clinical score after infection of 6-week-old growing pigs with PorPV (●) and Control group pigs (○). * $P < 0.05$ between the groups at 4 DPI in the rectal temperature measure.

Table 1. Viral load RNA of PorPV BALT and RT of experimental PorPV-infected growing pigs.

Sample	Number of positive and viral load by qRT-PCR		
	7 DPI	14 DPI	28 DPI
BALT			
Tonsil	3/3 (6.56±0.61)	3/3 (5.89±0.94)	3/3 (6.13±0.02)
ML	3/3 (6.73±0.13)	3/3 (5.58±0.42)	3/3 (6.29±1.21)
TBL	3/3 (6.18±0.28)	3/3 (5.59±0.38)	3/3 (4.92±0.18)
RT			
NM	2/3 (4.70±1.44)	2/3 (4.68±1.62)	2/3 (2.87±0.81)
Trachea	3/3 (4.17±0.30)	2/3 (5.53±0.96)	2/3 (2.65±0.36)
BT	3/3 (4.17±0.63)	2/3 (2.91±0.74)	2/3 (3.56±1.65)
Lung	2/3 (4.66±0.22)	2/3 (3.98±0.64)	2/3 (3.67±1.73)

Conclusions and Discussion

In conclusion, we confirm the clinical disease, seroconversion, excretion, and distribution of PorPV in the respiratory tract of growing pigs. The observations included an increase in the clinical signs, temperature alterations, and microscopic lesions in the PorPV-infected group. The presence of infections in growing pigs is a factor that significantly reduces productive efficiency. The knowledge of the pathogenesis of PorPV may serve to establish programs of prevention, control, and eradication of BED in Mexico.

Acknowledgments

The present study was funded by PAPIIT-IN IN208814-3 and CONACYT AC-90024.

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Serological survey of three emerging swine diseases in volunteer veterinarians in Mexico

JF Rivera-Benitez[1], A de la Peña-Moctezuma[2], H Castillo-Juárez[3], H Ramírez-Mendoza[1]
[1]*Departamento de Microbiología e Inmunología, FMVZ, UNAM. Mexico.* [2]*Centro de Enseñanza, Investigación y Extensión en Producción Animal en Altiplano, FMVZ, UNAM. Mexico.*[3] *Departamento de Producción Agrícola y Animal, UAM-Xochimilco, Mexico,* betosram@comunidad.unam.mx

Introduction

Diseases with zoonotic potential are of paramount importance for personnel in contact with various animal species (5, 6). The main zoonoses affecting personnel involved in swine production are, for bacterial diseases, leptospirosis, brucellosis, streptococcosis, salmonellosis, erysipelas, pasteurellosis, anthrax, campylobacteriosis, and clostridiosis (1; 7); for viral disease, swine influenza (H1N1, H1N2, and H3N2), viral encephalomyocarditis (1; 2; 3), infection by virus from the Paramyxoviridae family (Nipah virus, NiV; and Menangle virus, MenPV) (8), and hepatitis E (4).

Materials and Methods

We conducted an immunological assay of blood samples taken from 85 swine-specialist veterinarians attending the Congress of the Mexican Association of Swine Specialist Veterinarians in Mexico in 2011. Serum samples were assayed for *Porcine rubulavirus* (PorPV), *Encephalomyocarditis virus* (EMCV), and *Leptospira* spp. antibodies.

Results

Using a hemagglutination inhibition test, we registered 2.3% and 27% seropositivity for PorPV and EMCV, respectively. Using viral neutralization tests, we registered 5.8% and 47% seropositivity for PorPV and EMCV, respectively. For *Leptospira* spp., we registered a seropositivity of 38.8%. The variables (sex, age, years of exposure, number of visited farms, biosecurity level, and region) showed no significant effect ($P > 0.05$) on the seropositivity for EMCV, PorPV, and *Leptospira* spp. except for number of visited farms on HI seropositivity for EMCV ($P < 0.05$; odds ratio: 1.38).

Conclusions and Discussion

The emergence of these diseases may be due to processes such as globalization (international commerce, mobilization), expansion of agricultural and farming regions, deforestation, climatic change, and the close proximity of wild and domestic animals, which results in a higher probability of interspecies transmission. The presence of new zoonoses has been associated with changes in the ecology of wild animal populations. In the particular case of zoonotic outbreaks of the HeV, NiV, and MenPV, it has been observed that the introduction of susceptible domestic animals (horses and pigs) enables the adaptation of these viruses from bat populations (Australian flying foxes). In these outbreaks, in Australia (HeV and MenPV in 1994 and 1997, respectively) and Malaysia and Singapore (NiV in 1998), domestic animals served as intermediate hosts for the transmission to humans. In the present study, we detected antibodies against PorPV in 2 (by HI) and 5 (by VN) veterinarians. Infection by PorPV is endemic in the central and west-central regions in which these veterinarians work. The data obtained provide information on the epidemiology of emerging diseases with zoonotic potential in occupational risk groups.

Acknowledgments

The present study was funded by PAPIIT-IN IN208814-3 and CONACYT AC-90024.

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Antigenic variants of the PorPV in sera of field swine and their seroprevalence

AC Escobar-López[1], JF Rivera-Benitez[1], H Castillo-Juarez[2], H Ramirez-Mendoza[1], ME Trujillo-Ortega[3], JI Sanchez-Betancourt[3].
[1]*Departamento de Microbiología e Inmunología, FMVZ, UNAM. México* [2]*Departamento de Producción Agrícola y Animal, UAM-Xochimilco, Mexico.* [3] *Departamento de Medicina y Zootecnia de Cerdos, FMVZ, UNAM.México,*
betosram@comunidad.unam.mx

Introduction

Blue-eye disease (BED) is a viral disease that was first detected in La Piedad in the state of Michoacan, Mexico, in 1980. BED was initially characterized as a neurological and respiratory syndrome in suckling pigs that is accompanied by corneal opacity in 1–10% of cases. Abortions and mummified foetuses have also been found in some pregnant sows, and infertility has been observed in boars (2). *Porcine rubulavirus* is divided into three subgroups based on the sequencing of the HN gene. Group 1 comprises LPMV and PAC-4/1993; group 2 comprises PAC-2/1990, PAC-3/1992, and CI-IV; and group 3 comprises PAC-6/2001, PAC-7/2002, PAC-8/2002 and PAC-9/2003.The groups are classified in this manner because of alterations in the amino acid sequence of the HN-encoding gene, which confers virulence and is associated with clinical signs of neurological involvement in adult animals and in commercial fattening lines (1). The objective of this study was to identify the antigenic variants of the *Porcine rubulavirus* and their seroprevalence in the sera of field swine from different states in Mexico**.**

Materials and Methods

We sampled sera from 1013 non-vaccinated swine from four states in Mexico, Guanajuato, Jalisco, Michoacan and the Estado de Mexico, to analyse anti-*Porcine rubulavirus* antibody titres against three different *Porcine rubulavirus* isolates (PAC-4/1993, PAC-6/2001, and PAC-9/2003) using a hemagglutination inhibition assay.

Results

The results revealed that there were antigenic differences among the isolates assessed. In particular, the estimated correlation between the PAC-4/1993 and PAC-6/2001 (0.50) isolates and between the PAC-4/1993 and PAC-9/2003 isolates (0.56) displayed a moderate positive correlation. In contrast, there was a strong positive correlation between the PAC-6/2001 and PAC-9/2003 isolates (0.73) (Table 1). We also found that in the state of Guanajuato, PAC-4/1993 was the isolate that was most frequently identified; in Jalisco, the isolate was PAC-6/2001; and in Michoacan, the isolate was PAC-9/2003. By contrast, in the Estado de Mexico, all three isolates appeared to circulate with a low seroprevalence. In general, the analysed sera from the four states displayed a *Porcine rubulavirus* serological prevalence ranging from 9% to 23.7%.

Table 1. Sera samples positive for two or three different viral isolates

Site	Positive PAC-6/PAC-4 (%)	Positive PAC-9/PAC-4 (%)	Positive PAC-9/PAC-6 (%)	Positive PAC-4/PAC-6/PAC-9/total (%)
Edo. Mex.	6/23 (26.1)	6/23 (26.1)	3/19 (15.8)	5/62 (8.1)
Guanajuato	7/55 (12.7)	3/55 (5.5)	0/21 (0)	12/91 (13.1)
Jalisco	8/53 (15.1)	0/53 (0)	31/117 (26.5)	38/248 (15.3)
Michoacan	1/32 (3.1)	7/32 (21.8)	13/32 (40.6)	13/112 (11.6)

Conclusions and Discussion

These data indicate that there is not complete antibody cross-antigenicity among the three isolates, and the antigenic variations in the antibody response found in this study implies that the use of a monovalent vaccine would not generate complete protection against the different antigenic subtypes.

Acknowledgments

The present study was funded by PAPIIT-IN IN208814-3 and CONACYT AC-90024.

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Evaluation of relationship between viral loads, viral shedding and productivity measure in PCV2 subclinical infected farm

K Akashi[1], T Matsuda[2]
[1] *Cattle and Swine business Unit, Intervet K.K., Tokyo, Japan,* kyoko.akashi@merck.com, [2]*Farm Consulting Division, Global Pig Farms, Inc., Gunma, Japan,*

Introduction

Porcine Circovirus type2 (PCV2) infection caused a serious problem in the swine industry between 2006 and 2008 in Japan. After commercial PCV2 vaccines became available in 2008, most farms that used PCV2 vaccines showed immediate improvements in mortality and growth rate[1,2]. While high level of PCV2 infection induces severe clinical signs of Post-weaning Multisystemic Wasting Syndrome (PMWS) or Porcine Dermatitis and Nephropathy Syndrome (PDNS), lower level of PCV2 infection results in subclinical disease and economic losses[3]. Also, PCV2 viremia has a negative impact on swine production by reducing average daily gain. However, even it is obvious that the pig infected with PCV2 shed virus in their feces, it is not clear if the fecal virus shedding is correlated to productivity. The objective of the present study was to investigate a relationship between viremia, fecal virus shedding and productivity parameters.

Materials and Methods

The trial was conducted in a Japanese pig farm free of PRRS and Aujezsky's Disease. Data was collected from the litters of 220 sows subjected to 2 different vaccination programs. The initial program was, sow vaccination with Vaccine A, 28 days before farrowing, and piglet vaccination at 21 days post-partum (data set A). Thereafter sows did not receive any more vaccinations and their following litters' were vaccinated with Porcilis PCV at 21 days post-partum (data-set B). The data assessed was; viremia, fecal viral shedding, average daily gain (ADG), feed conversion rate (FCR), post-weaning mortality and Average Days to slaughter. Fecal and blood samples were collected from 5 different pigs at each 30, 60, 90, 120, 150 and 180 days of age. The sera were tested by ELISA and quantitative PCR (qPCR), and the feces were tested by qPCR. The limit of determination of serum PCV2 qPCR was 1.2×10^3 and fecal PCV2 qPCR was 1.2×10^3.

Results

The ADG and FCR were improved by 21g and by 0.03 kg:kg. The average days to slaughter was shortened by 4 days after switching to vaccine B (Table 1). The viruses were detected from the serum and fecal samples between 90 to 180 days of age before changing the vaccine while virus was isolated only at 180 days of age after using Porcilis PCV (Figure 1).

Table 1. Comparison of productivity parameters

	A	B
Number of Animals (heads)	2,428	2,839
ADG (g/day)	734	755
FCR (kg:kg)	2.93	2.90
Average days to slaughter (days)	173	168
Mortality rate (%)	2.7	2.8

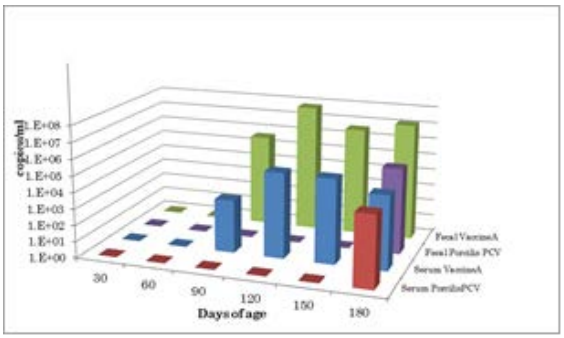

Figure 1. Result of serum and fecal PCV2 qPCR

Conclusions and Discussion

In this study, the higher PCV viremia and fecal shedding with Vaccine A coincided with lower ADG, higher FCR and longer average days to slaughter. These results support the importance of viremia and fecal shedding and the difference between PCV vaccines with respect to impact on viremia and production parameters. As feed cost is 63.5% of the cost to raise a pig according to Japanese ministry of Fishery and Forestly report in 2011, it is critical to improve ADG and FCR.

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Diagnostic evaluation of herds for respiratory diseases revealed high levels of circovirus

H Bak[1], PH Rathkjen[1], R Nielsen[1], M Andreasen[1]
[1]*Boehringer Ingelheim Vetmedica, Copenhagen, Denmark,* hanne.bak@boehringer-ingelheim.com

Introduction

In commercial pig herds, respiratory diseases have a negative effect on growth and feed conversion. Often, the diagnostic focus is on traditional respiratory pathogens such as *Mycoplasma hyopneumoniae*, PRRS and swine influenza. The present study reports findings of Circovirus in herds that were evaluated for respiratory diseases.

Materials and Methods

Practitioners provided contact data for finishing herds wanting an evaluation of coughing among finishers. In each herd, a coughing index (CI) was determined as described by (2), and samples of oral fluid (OF) were collected in the section, where coughing was peaking. On the same day, OF was collected from a section with pigs 2 weeks younger and 2 weeks older (pool of OF from 4 pens/section). OF was examined by PCR for Circovirus (BioScreen GmbH, Hannover, Germany). Coughing was classified as clinically relevant, when max. CI was 2.5 or more, and the level of copies of Circovirus virus in OF was categorised as low (<5 log) or moderate-high (≥ 5 log). The level of Circovirus virus was compared to CI and vaccination against PCV2 with Fishers Exact test, p≤0.05 was considered significant.

Results

In total, 37 herds were included. A high CI was found in 20 herds and a low CI in 17 herds (table 1). Among herds with high CI, 60% had a moderate-high level of Circovirus in OF, and among herds with low CI, 59% had a moderate-high level of Circovirus (p=0.7923). The distribution of CI in herds with moderate-high level of Circovirus in OF compared to herds with a low level is shown in figure 1. Herds with a high CI had a slightly higher vaccination rate against Circovirus than herds with a low CI (35% versus 18%), but the difference was not significant (p=0.4162). Hence, in the present study, CI did not show correlation to Circovirus in OF nor to vaccination against Circovirus.

Table 1. Vaccination status and level of Circovirus in saliva in 37 finishing herds grouped by coughing index[a]

Coughing	No. herds	Circovirus > 5 log [b]	% Circovirus vaccinated
High CI	20	60%	35%
Low CI	17	59%	18%

[a]: Coughing index (CI) as described by (2), with a high CI defined as CI ≥ 2.5.
[b]:The level of PCR copies of Circovirus considered clinically relevant (Bioscreen Lab., Hannover, Germany)

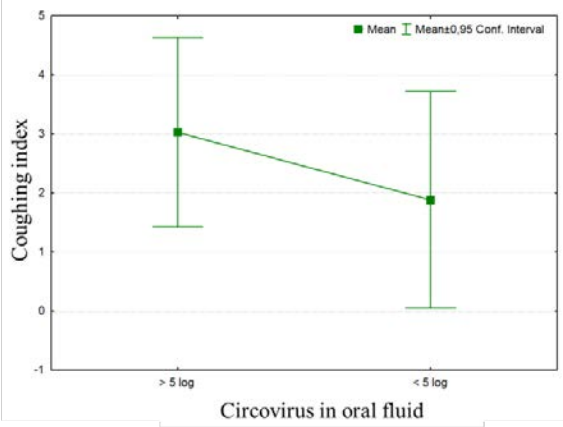

Figure 1. Coughing compared to level of Circovirus in OF Circovirus ≥log 5: Moderate-high; <5 log: Low.

There was, however, a significant difference between vaccinated and non-vaccinated herds regarding Circovirus in OF (p=0.0008). A moderate-high level of Circovirus (5.32 log) was found in only one vaccinated herd, whereas 79% of the non-vaccinated herds had moderate-high levels of Circovirus in OF.

Conclusions and Discussion

A moderate level of Circovirus in OF was not connected to clinical symptoms such as coughing. However, Circovirus has been shown to have a negative effect on productivity in non-vaccinated herds, when the number of copies pr. ml exceeds 5 log units (1).
This critical level of Circovirus was found in 79% of the non-vaccinated herds in this study, and these herds would therefore, despite the lack of reported clinical symptoms, benefit from vaccination of pigs against Circovirus.

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Comparing placenta and presuckle piglet PCV2 status between two breeding sites

T Fangman[1], D Baumert[2], J Rustvold[1]
[1]*Boehringer Ingelheim Vetmedica, Inc, St. Joseph, MO,* [2]Cargill Pork, Marshall, MO,
thomas.fangman@boehringer-ingelheim.com

Introduction

The study objectives were to determine if the placenta PCV2 status could be compared across farms to determine PCV2 status in pre-suckling pigs.

Materials and Methods

Site A (avg. parity 2.8) and B (avg. parity (2.3) are 1,600 and 5,000 head commercial farrow-to-wean herds, respectively, where PCVAD had been diagnosed in weaned pigs from Site A but not Site B. Assorted-parity litters were identified at Site A (n=21) and Site B (n=26). Farrowings were attended and eight pre-suckling pigs per litter were sampled. Piglet handling procedures included: new born piglets were towel-dried, tails were docked using side-cutters and tail-stump blood samples were collected using a flocked swab in Amies' solution. The expelled placental material was collected a minimum of four hours after expulsion at Site A and immediately after expulsion at Site B. Three placental umbilical cords from each placenta were milked out into a single blood tube to create a single sample per placenta. Swiffer® Sweeper Dry Cloths were used to collect environmental samples from the farrowing crate. Blood was collected from the 47 sows and tested on PCV2 PCR.

Results

PCV2 diagnostic results for Site A and Bs' pigs, placentas, dams and farrowing crate environments are shown in Table 1.

Table 1. PCV2 diagnostic results

Sample	PCV2 Test	Site A	Site B
Placental umb. cord serum	PCR	17/21 81.0%	3/26 11.5%
Presuckle piglet serum	PCR	54/168 32.1%	5/246 2.0%
Environmental cloth	PCR	20/20† 100%	10/24† 41.7%
Sow serum	PCR	5/21 23.8%	1/26 3.8%
Litters with ≥ 1 positive pigs	PCR	16/21 76.2%	3/26 11.5%

† 20 of 21 and 24 of 26 crates sampled due to on-site supply avail.

Table 2. Comparing PCV2 PCR results on pig and placenta level per dam

		Litter status*	
		-	+
Dam's placenta	**-**	**24**	**3**
	+	**4**	**16**

*positive litter contained at least one positive pig

Conclusions and Discussion

Differences in PCV2 status of litters, placenta, sows and farrowing crate environment exist between breed-to-wean sites. Placenta and piglet percent positives closely match one another in both herds. This supports the potential use of the placenta as an indicator of the PCV2 status. It is noted that a consistent protocol for placental sampling should be developed as environmental contamination is a possibility. Sow serum PCR was not as sensitive as placenta or piglet testing in determining PCV2 status. PCV2 sow herd testing, utilizing the placenta, may be useful when evaluating interventions that may impact PCV2 stability status. These studies are currently being conducted.

Control of PCV2 subclinical infection in a high sanitary status pig herd: Comparison of two commercial vaccines in a massive field trial

M Atlagich[1], JE Calvo[1], D Guzmán[1], C Briceño[1], F De Grau[2], R Jolie[2]
[1]*MSD, Salud Animal*, [2] *Merck, Animal Health*, rika.jolie@merck.com

Introduction

PCV2 is nowadays regarded as one of the most important pathogens for domestic swine worldwide, causing significant economic losses to the pig industry. Clinical symptoms attributable to PCV2 have been summarized as PCV disease (PCVD) (1). Thus, PCV2 has been associated to play a role in reproductive disorders, the so-called porcine respiratory disease complex (PRDC), enteritis, porcine dermatitis and nephropathy syndrome (PDNS) and proliferativeand necrotizing pneumonia (PNP) (2). Even without overt clinical signs, different field evidences indicate that PCV2 vaccination is able to improve productive parameters (average daily gain (ADG), percentage of runts, body condition and carcass weight) in PCV2 subclinical infection scenarios (3). The success of the commercial vaccines controlling the PCVD is unquestionable. However, their impact on subclinical infection scenarios is still a matter of discussion. Therefore, the objective of the present study was to determine the efficacy of two commercial vaccines to control PCV2 subclinical infection under production conditions.

Materials and Methods

A total of 69,810 pigs were studied, making this trial the biggest up to date according to our knowledge. The pigs originated from 4 sow farms from the same company. The genetics, feeding and handling were the same, certified ISO 9000 and 14000. The pigs were weaned at 21 days of age and raised in wean to finish barns with capacity for 1700 pigs per barn, were separated by sex and origin and maintained with automatic feeding and ventilation, until 180 days of age. Two groups were formed side by side according to the production flow as is shown in Table 1.

Table 1. Experimental groups.

Experimental Group	Circumvent® PCV	Vaccine B
Barns (N°)	20	20
Wean Pigs(N°)	34,989	34,821
Wean Weight	6.15 kg	6.20kg
Wean Age (days)	20.81[a]	21.00 [b]*

([a,b]) Superscripts indicate statistically significant differences * ($p \leq 0.05$)

The compared vaccines were Circumvent® PCV, 2 ml at 3 and 6 weeks of age and Vaccine B, 1 ml at 3 weeks. PCV2 vaccine is the only vaccine in fattening pigs. In addition, 35 pigs from each group were ear tagged and bled at 5, 8, 11, 16, 20 and 25 weeks of age. The samples were processed for detection of PCV2 via qPCR at MSD Service Lab. Boxmeer, NL and assayed for PCV2 antibody using a commercial ELISA (BioChek (UK). The ELISA sample-to-positive (S/P) ratio was used to calculate antibody titers according to the manufacturer instructions. The results were analyzed by t-test analysis.

Results

Table 2. Production data

Experimental Group	Circumvent® PCV	Vaccine B
Slaughter Age (days)	181.7	181.7
Body Weight	129.43 kg	129.20 kg
ADG	0.766g	0.765g
Mortality	5.06%	5.24%
Feed Consumption	314.02 Kg	323.8 kg
Feed Conversion	2.553[a]	2.64[b**]

**($p \leq 0.10$)

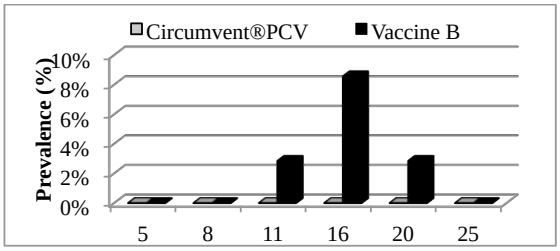

Figure 1. Prevalence of viremia

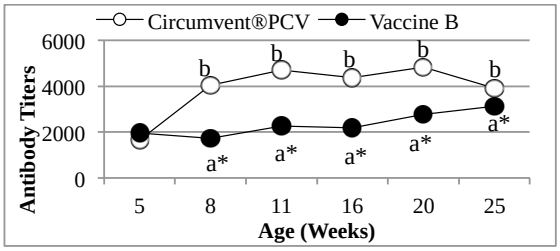

Figure 2. PCV2 antibody titers

Conclusions and Discussion

Circumvent®PCV resulted in a significant better feed conversion than Vaccine B in a high health status farm, which resulted in more than 3.5USD saved per pig.

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Effects of a PCV2 vaccine on detection of ELISA and IFA from porcine serum

S Sub Lee, M-J Kang, M Park, J-H Park, S Dae Jung, C Hee Kweon, W Hur
Daesung microbiological Labs. CO., LTD, 103 Deogyeong-dearo, Uiwang-si, Gyeoggi-do, Korea sslee@dsmbio.com

Introduction

Porcine circovirus type 2 (PCV2) is the causative agent of Postweaning multisystemic wasting syndrome (PMWS), a multifactorial disease of nursery and fattening pigs that causes considerable economic losses to the swine industry worldwide (1, 2, 3).
Immunofluorescent assay (IFA) is the most widely used diagnostic methods for detecting PCV infection. However, this method is labor-intensive and time consuming, and carry the risk of virus contamination. These techniques require experienced technicians who can judge the staining reactions accurately. In contrast, enzyme linked immunosorbent assay (ELISA) can decrease the potential bias that may occur with IFA and is amenable to automation, so it is suitable for large-scale diagnostics.
The objective of pigs sera from each of three vaccinated was measured PCV2 antibody by IFA and ELISA.

Materials and Methods

Serum samples were collected from two wean-to-finish swine farms.
Serum samples were tested using a PCV2 ELSIA kit (Bionote, Hwaseong-si, Ref. of Korea). And postitive-PCV2 samples were carried out IFA diagnostic kit (Daesung microbiological Labs, Uiwang-si, Ref. of Korea).
The PK-15 cell which infected PCV2 were cultured in 96 well plate then fixed with acetone for using IFA test. To measure antibody titer was used Anti-PCV2 MAb (JBT®)
We analyzed the antibody titer and compared to the antibody including three kinds of vaccine efficiency on one dose /1month in piglet groups.

Results

Sera of vaccine pigs made certain positive rate 100% by ELISA (Table 1). But each serum showed variety antibody titer value by IFA.
As showed in Figure 1, mean of vaccine PCV2 antibody titer by IFA are shown. The line A was higher antibody titer and increased constantly. Line A and B is similar to escalating since 8 week of age. Also, line C and D (control) appeared similarly.

Conclusions and Discussion

Porcine circovirus type 2 (PCV2) is recently considered one of the most important viral pathogens in the world (1, 2, 3).
In conclusion, we recommend that sera of vaccined pigs were carried out ELISA and after IFA for PCV2 diagnosis method.

Table 1. The results of PCV2 antibodies titer by IFA

2nd does Pigs groups	ELISA	IFA (log2)
1	+	>11
2	+	6
3	+	11
4	+	9
5	+	10
6	+	7
7	+	8
8	+	9
9	+	>11
10	+	7
11	+	>11
12	+	8
13	+	7
14	+	9
15	+	11
16	+	8
17	+	6
18	+	8

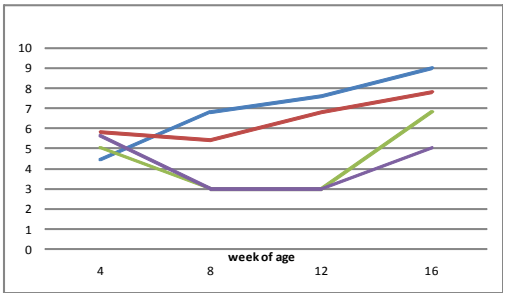

Figure 1. Antibody titer of three each vaccine by IFA
[1]Blue: A, red: B, green: C, purple: saline (control)

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Occurrence of PRRSV and PCV2 infections in wild boars in Poland

K Stępniewska, K Kus, K Szymanek, K Podgórska, A Szczotka-Bochniarz, Z Pejsak
National Veterinary Research Institute, Swine Diseases Department, 57 Partyzantow Str., 24-100 Pulawy, Poland,
kp@piwet.pulawy.pl

Introduction

Wild boars can be a vector of pathogens important for domestic swine such as porcine respiratory and reproductive syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2), which are responsible for economic losses to the swine production (1, 2).
The objective of the present study was to determine the presence of genetic material and specific antibodies against PRRSV and PCV2 in tissue and serum samples collected from wild boars hunted in Poland.

Materials and Methods

Lung, liver, spleen and serum samples from 140 wild boars were collected in 5 hunting regions in 4 provinces in Poland during 2012-2013.
Sera samples were tested with ELISA for the presence of antibodies specific to PRRSV (IDEXX) and PCV2 (IgM/IgG ELISA Ingenasa).
Samples of lungs and spleen from wild boars hunted in the PRRSV seropositive region were also submitted to real-time RT-PCR analysis (Tetracore).
A lung, liver and spleen samples collected from PCV2 seropositive wild boars were tested with real-time PCR for detection of PCV2 DNA (Opriessnig et al. 2003).

Results

Serological and molecular results are shown in Table 1.
Antibodies specific to PRRSV were detected in 3 wild boars from Łódzkie province. However, no genetic material of PRRSV was detected in tissue samples from wild boars from the same hunting region.
Antibodies specific to PCV2 were detected in 48 wild boars. In tissue samples of 45 seropositive animals DNA of PCV2 was detected (43 samples of spleen, 39 of lung and 37 of liver). PCV2 infection status of tested wild boars is shown in Table 2.

Conclusions and Discussion

Our studies show that the PRRSV seroprevalence in Polish wild boars is very low (2,14%). Similar results were obtained by Fabisiak et al. (2), who reported 1 positive result out of 142 analyzed samples.
In opposite to the results of PRRSV ELISA, our study showed that the PCV2 seroprevalence is noticeable. 34,28% of animals were seropositive to PCV2. Higher percentage (47,9%) was shown in the earlier study by Fabisiak et al. (2). In tissues of 93,75% seropositive wild boars DNA of PCV2 was detected.
37 out of 40 samples classified as ”late infection” based on ELISA results were still positive in PCR. Those results may indicate the presence of chronic PCV2 infections in wild boars. No evidence of co-infections with PRRSV and PCV2 was found, which may indicate that the epidemiology of PCV2 in wild boars differs from the one in domestic pigs.
Obtained results suggest that wild boars do not play a major role as a reservoir of PRRSV infections. On the other hand, infections with PCV2 are relatively widespread in wild boars population in Poland.

Table 1. Results of ELISA and (RT-)PCR

Province	Number of wild boars	PRRS		PCV2	
		ELISA	PCR	ELISA	PCR
Pomorskie	62	0	nt*	21	21
Warmińsko-Mazurskie	27	0	nt	7	5
Łódzkie	24	3	0	13	13
Lubelskie	27	0	nt	7	6
Total	140	3	nt	48	45

*not tested

Table 2. PCV2 infection status of Polish wild boars

Province	ELISA positive/ Total	ELISA			PCR
		Active infection	Early active infection	Late infection	
Pomorskie	21/62	2	3	16	21
Warmińsko-Mazurskie	7/27	0	0	7	5
Łódzkie	13/24	0	1	12	13
Lubelskie	7/27	2	0	5	6
Total	48/140	4	4	40	45

Acknowledgements

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Influence of "stimulation" and effect of rope material on detection of total isotype-specific antibodies in porcine oral fluid

I Decorte [a], N De Regge [a], AB Cay [a]
[a] *Operational Direction Viral Diseases, Enzootic and (re)emerging diseases, CODA-CERVA, Ukkel, Belgium,*
inge.decorte@coda-cerva.be

Introduction

Oral fluid collected by means of ropes has the potential to replace serum for monitoring and surveillance of important swine pathogens. Until now, the most commonly used method to collect oral fluid is by hanging a cotton rope in a pen. However, concerns about the influence of rope material on subsequent immunological assays have been raised.
In this study, we evaluated the influence of "stimulated vs unstimulated" oral fluid and the effect of four different rope materials for the subsequent detection of total antibodies of different isotypes in porcine oral fluid collected from PRRSV-vaccinated pigs.

Materials and Methods

Four 8-week-old PRRSV-naïve piglets were housed individually at air-filtered level-2 biosecurity facilities. Three pigs were vaccinated intramuscularly with 2 mL vaccine/pig (Porcilis PRRS, Intervet) at 9 and 11 weeks of age. One pig was not vaccinated and served as a control animal.
"Stimulated" oral fluids (oral fluids collected by masticatory or gustatory stimulation such as chewing (1)) were collected 1 day before the first vaccination and 14 days after each vaccination with four different rope materials according to procedures described by Prickett et al. (2). Animals were presented with the cotton ropes first, followed by hemp, polyamide and polyester. "Unstimulated" oral fluid (oral fluid collected without exogenous gustatory, masticatory, or mechanical stimulation (1)) was collected at the same time points by means of suction with a small suction catheter.
Total amounts of IgA, IgG and IgM antibodies present in oral fluid samples were measured using a commercial direct sandwich ELISA (IgA, IgG and IgM (pig) – ELISA, Celltrend GmbH).

Results

The ELISA results showed that IgA is the predominant antibody isotype in unstimulated oral fluids ($p < 0.1$), as well as in saliva samples collected using synthetic ropes ($p < 0.1$) (Fig 1). Interestingly, there were no significant differences between the IgA, IgG and IgM concentrations in samples collected using natural fibred ropes. When comparing the different rope materials, the results suggest that use of natural fibred ropes yield higher amounts of IgG than synthetic ropes, while synthetic ropes yield higher amounts of IgA than natural fibred ropes. However, these differences were not statistically significant. No significant differences were observed in the total IgM concentrations recovered from different rope types. No significant differences were found in the IgA / IgG / IgM concentrations from samples collected by means of stimulation compared to unstimulated oral fluids.

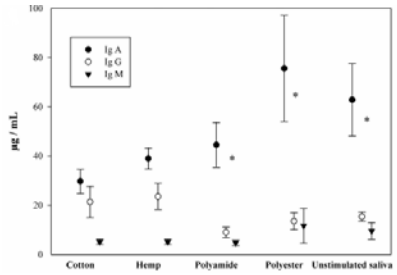

Figure 1. Total IgA, IgG and IgM concentrations in porcine oral fluid samples as determined via ELISA.

Conclusions and Discussion

We determined the total amounts of IgA, IgG and IgM antibodies present in stimulated and unstimulated porcine oral fluid samples. As expected, IgA was the dominant immunoglobulin fraction found in unstimulated oral fluids. Intriguingly, there were no significant differences in the IgA/IgG/IgM concentrations detected in unstimulated oral fluids and oral fluid samples collected using different rope types, indicating that no significant exclusion/retention of antibodies occurs in the rope materials. Despite the absence of significant differences – likely resulting from the high variability among pigs –, the results suggest that natural fibred ropes yield higher amounts of IgG, whereas synthetic fibred ropes are more suitable for IgA collection.

Acknowledgments

This study was funded by the Federal Public Service of Health, Food Chain Safety and Environment 35), Belgium.

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Dynamics of respiratory pathogens in two farms positive to the PRRS through serological profiles

MA Barrera[1], GC Mercado[2],SJ García[2], AVM Carrera[2], PJ Pradal-Roa[2], BJI Sánchez[2]
[1]*Comisión México-Estados Unidos para la prevención de la Fiebre Aftosa y otras enfermedades exóticas de los animales,* [2] *Departamento de Medicina y Zootecnia de Cerdos, F.M.V.Z.–U.N.A.M.*
mcmg_1965@yahoo.com.mx

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most significant viral diseases of pigs, due to the involvement of reproductive, productive parameters and economic losses (1). PRRSV causes a predisposition to infection by different viruses and bacteria (2). The synergy between viruses and bacteria is frequently observed in the field at various stages of swine production, having high impact on the clinical signs and/or mortality, especially in susceptible animals (3). The objective of this study was to evaluate the possible association of the PRRSV with swine influenza (VIP) viruses subtypes H1N1 and H3N2, as well as with *Mycoplasma hyopneumoniae* (MH) and *Actinobacillus pleuropneumoniae* (APP), as well as the prevalence in two farms in the State of Morelos (Mexico), with a history of PRRS, following a model of serological profiles.

Materials and Methods

Two sampling periods with interval of 15 days were carried out. Blood samples of 119 pigs from the production line (3 to 18 weeks of age) and 120 sows of the breeding stock were obtained. Antibodies were identified against the infectious agents listed above. Specific antibodies against PRRSV, MH and APP were detected by commercial ELISA tests. Antibodies against the swine influenza virus (IP) were detected through the hemagglutination inhibition test. The prevalence of pathogens and the association between PRRSV and the other agents was assessed.

Results

Tables 1 and 2 show the percentages of seropositivity obtained by farm and from sampling to the different agents. In farm 1, the prevalence of MH increased, while the prevalence of PRRSV, APP, IP-H1N1 and IP-H3N2 decreased, between sampling periods 1 and 2. Between samplings 1 and 2 in farm 2, the prevalence of PRRSV increased, that of IP-H1N1 remained the same and MH, APP and IP-H3N2 decreased.

Table 1. Percentage of seropositivity for each infectious agent between samplings on farm 1

	PPRS	APP	Mh	H1N1	H3N2
Sampling 1	76.7	33.3	16.7	100	26.7
Sampling 2	56.5	10.3	24.1	93.1	6.9

Table 2. Percentage of seropositivity for each infectious agent between samplings on farm 2

	PPRS	APP	Mh	H1N1	H3N2
Sampling 1	73.3	30	13.3	100	50
Sampling 2	83.3	16.7	3.3	100	40

Conclusions and Discussion

The results are consistent with previous studies that show that infection with PRRSV makes pigs more susceptible to secondary bacterial and viral diseases and has an additive or synergistic effect with some other bacteria or virus to create a more serious disease than any single agent (4). The primary infection of PRRSV may predispose to secondary infections by *Mycoplasma hyopneumoniae*, swine influenza H3N2 or by *Actinobacillus pleuropneumoniae*. Primary infections caused by APP or MH, can predispose to the presentation of other secondary bacterial or viral infections, however, they do not predispose the presentation of PRRS. Serological monitoring will allow us to accurately determine the stages of susceptibility of animals and thus implement appropriate measures for the control of infectious diseases, as well as to assess their results and correct errors, until the establishment of high health and productive herds.

Acknowledgments

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The role of vaccine to control PRDC in a large U.S. single site farrow-finish farm – A case study

T Gillespie[1], M Inskeep[1],
[1]*Rensselaer Swine Services, Rensselaer, IN,*
tom.gillespie@rssvet.com

Introduction

Respiratory co-infections described as Porcine Respiratory Disease Complex (PRDC) are common in modern swine production systems even though technological developments and scientific advancements, as well as, adapting new management technologies to successfully eradicate and control major pathogens. However, even with these improvements, swine producers and veterinarians are continuously being challenged by disease and poor performance. The production and economic impact of Influenza type A virus (IAV), PRRSV and *M. hyopneumoniae*(M hyo) has been shown to have detrimental impact that is greater when these pathogens are combined in production systems causing PRDC.[1] This paper is a case study report of a large farm with a history of endemic PRDC. The intervention program utilizes vaccine to provide population based immunity for control of disease to mitigate the biologic and economic impact of PRDC.

Materials and Methods

The farm is a 2500 head farrow to finish sow farm utilizing continuous flow pig movement in nursery, finishing and wean to market facilities. The farm has a diagnostic and clinical history of endemic IAV, M hyo and PRRSV. In addition, a history of multiple introductions of Type-1 and Type-2 heterologous PRRSV is playing a role in the severity of the PRDC.

Intervention strategies: To provide population based immunity and stabilize the breeding herd to the endemic pathogens of PRDC, mass vaccinations for PRRS were administered three times per year using *Ingelvac PRRS® MLV* (Boehringer Ingelheim Vetmedica, St. Joseph, Mo.) and once per year for PRRS, M hyo and PCV2 using *Ingelvac 3FLEX®* (Boehringer Ingelheim Vetmedica, St. Joseph, Mo.). To control PRDC in growing pigs, *Ingelvac 3FLEX®* was administered to piglets 2 ml, I.M. at weaning (21 days) for PRRS, M hyo and PCV2.

Replacement gilts were vaccinated with 2 doses of *Ingelvac 3FLEX®* and commercial IAV vaccine from different companies while in isolation; one dose at arrival and a second dose 4 weeks later. The sow herd was mass vaccinated twice a year with commercial IAV vaccine.

Diagnostic Monitoring: Routine diagnostics were performed in the breeding herd and growing pig phase of production twice a year (June and December) to assess the status of PRDC pathogens; PRRS, SIV, M hyo, PCV2. Additional diagnostic sampling was performed as needed due to clinical expression of disease.

Measurement of Intervention: Group performance close-outs from nursery, finishing and wean to market production groups were recorded and summarized as quarterly mean performance reports for percent mortality, ADG and feed conversion.

Results

Performance parameters are summarized in Table 1. Results of diagnostic monitoring confirmed the introduction of four wild-type 2 and 1 wild-type 1PRRSVes and the endemic circulation of PRDC co-infection pathogens; M hyo, IAV and PCV2.

Table 1. Summary of Growing Pig Performance

Nursery			
Quarter	Mortality	ADG	FC
2nd-2012	2.73%	0.97	1.66
3rd-2012	1.39%	0.94	1.57
4th-2012	1.51%	1.05	1.55
1st-2013	6.21%	0.82	1.72
2nd-2013	6.35%	0.84	1.71
3rd-2013	5.52%	1.21	1.38
4th-2013	2.56%	0.96	1.89
Farm Goals	2.50%	1.00	1.55
Finisher			
Quarter	Mortality	ADG	FC
2nd-2012	1.50%	1.92	2.83
3rd-2012	2.20%	1.84	2.59
4th-2012	1.79%	1.81	2.55
1st-2013	2.87%	1.77	2.96
2nd-2013	2.23%	1.74	2.94
3rd-2013	2.71%	1.58	2.73
4th-2013	3.64%	1.59	3.08
Farm Goals	2.00%	2.00	2.55
W-Market			
Quarter	Mortality	ADG	FC
2nd-2012	3.35%	1.67	2.54
3rd-2012	4.12%	1.51	2.58
4th-2012	4.68%	1.54	2.48
1st-2013	4.24%	1.72	2.53
2nd-2013	8.46%	1.40	2.52
3rd-2013	7.76%	1.45	2.49
4th-2013	6.45%	1.49	2.22
Farm Goals	4.50%	1.70	2.40

Conclusions and Discussion

The interventions of vaccine targeting endemic PRDC pathogens helped to control and mitigate the consequences of these co-infections. The scope of this paper shows only post intervention strategy monitoring of group close-outs, with performance levels achieving near farm goals over an extended time period. The role of vaccine-derived immunity is one of the important components for control of PRDC.

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Effect of a highly concentrated avian immunoglobulins formulation specific against PRRS in reducing piglet mortality in the production line

W González[1], E Lucio[1], P Ávalos[1], J Munguía[1]
[1] *Investigación Aplicada S.A. de C.V., Tehuacán, Puebla, México.*
wgonzalez@grupoidisa.com

Introduction

The economic impact of a PRSS outbreak in Mexico has been estimated between 80 and 120 million dollars a year, while an acute outbreak has an economic impact between 2,500 and 3,500 pesos per sow a year; in persistant infections the impact is between 60 and 150 pesos per pig in acute cases [(1)].

It is very important to have a PRRS control program in place since it has been demonstrated that the virus produces persistant infections in swine which subsequently become the main virus dissemination sources in farms,[(2)] it causes reproductive problems in sows, respiratory signs in piglets and a notorious weight loss in fattening swine. The aim of this study was to evaluate the efficacy of the application of avian immunoglobulins specific against PRRS in decreasing mortality in a farm with an endemic PRRSV.

Materiasl and Methods

The farm subject of this study is in western central Mexico and is located among 22°27' and 21°38' of latitude and 101°53' and 102°52' of longitude. The farm has 1,100 sows in its reproductive herd and a weekly production of 500 piglets. The farm has an endemic infection of PRRS even though several control strategies have been in place. The company has a replacement system that considers the introduction of sows every four months and the breeding stock includes vaccinations with a modified live virus vaccine against PRRS every three months.

In the seventh week of 2013 a program of applying highly concentrated avian immunoglobulins specific against PRRS to piglets began in the first week after weaning, and again at the tenth week in order to coincide with the transportation to the fattening site. Groups 17, 18 and 19, did not receive immunoglobulins. The information was analyzed using descriptive statistics and compared with historical mortalities in the farm.

Results

The results are shown in figures 1 and 2. Groups 1 to 6 correspond to piglets treated conventionally while groups 7 to 16 received two doses of avian immunoglobulins specific against PRRSV. Groups 17, 18 and 19 were not treated.

MORTALY GROUP

	WEANING							FINISH																	TOTAL					
GROUPS	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	WEAN		FINISH		TOTAL	%
GPO 01	2	3	2	5	14	9	5	8	7	5	3	3	1	2	0	1	1	0	0	0	1	0	1	0	40	9%	33	7%	73	16.0
GPO 02	1	2	3	2	4	6	7	1	6	2	0	1	1	4	1	4	5	2	1	2	5	4	10	4	25	6%	53	12%	78	17.6
GPO 03	1	2	2	6	11	14	5	3	9	2	0	1	0	0	0	0	3	0	2	0	3	0	0	2	41	10%	25	6%	66	16.0
GPO 04	0	0	1	21	14	34	11	1	4	2	3	0	1	1	1	0	0	0	0	1	0	1	0	0	81	18%	15	3%	96	21.7
GPO 05	0	1	2	22	23	15	16	9	12	0	0	0	0	0	1	0	1	1	0	4	1	0	2	4	79	20%	35	9%	124	31.8
GPO 06	0	1	4	14	10	15	13	8	3	3	4	4	0	0	0	2	3	0	0	1	0	0	0	0	57	15%	28	8%	90	24.1
GPO 07	1	1	6	9	12	9	3	3	4	2	3	3	2	2	0	4	0	0	1	1	0	0	3	0	41	10%	28	7%	69	16.4
GPO 08	1	2	2	1	10	4	2	3	2	3	4	0	0	0	0	0	1	0	1	0	0	2	1	0	22	5%	17	4%	39	9.2
GPO 09	0	2	1	3	2	1	1	10	11	5	7	1	1	3	1	0	0	0	0	0	2	1	0	2	10	2%	44	10%	54	11.8
GPO 10	1	0	0	2	3	5	7	1	4	13	4	3	2	2	1	0	2	0	0	0	1	0	2	0	18	4%	35	9%	53	13.1
GPO 11	0	2	0	2	4	1	1	0	6	4	3	1	1	1	0	0	1	1	0	0	0	2	0	0	10	2%	20	5%	30	7.0
GPO 12	1	1	0	2	1	1	2	7	7	6	0	1	1	5	4	0	1	1	0	2	2	1	0	0	8	2%	38	8%	46	10.2
GPO 13	1	0	2	4	3	0	6	5	3	0	0	3	3	6	1	1	0	0	1	0	0	0	1	0	16	3%	24	5%	40	8.2
GPO 14	2	1	0	7	1	9	5	2	3	1	3	4	2	2	0	1	1	1	1	0	0	0	0	0	25	5%	21	4%	46	9.7
GPO 15	0	5	2	9	7	8	9	4	1	3	2	2	0	0	1	0	2	2	0	0	0	0	2	1	40	6%	20	3%	60	8.6
GPO 16	2	4	3	0	0	7	6	5	5	5	0	0	0	0	0	3	0	1	1	0	0	0	0	0	22	4%	20	4%	42	8.5
GPO 17	0	9	13	20	8	12	13	8	9	1	2	0	4	0	1	0	2	0	0	0	0	0	0	0	75	16%	27	6%	102	21.9
GPO 18	1	0	3	19	19	30	12	5	3	12	4	3	4	0	0	0	0	0	0	0	0	0	0	0	84	20%	31	7%	115	27.4
GPO 19	9	5	10	15	9	18	10	16	6	9	5	5	4	3	0	0	0	0	0	0	0	0	0	1	76	18%	49	11%	125	28.9

Figure 1. Accumulated mortality

Average mortality percentage of the untreated groups was 22.8% while the treated groups had an average mortality percentage of 10.27%.

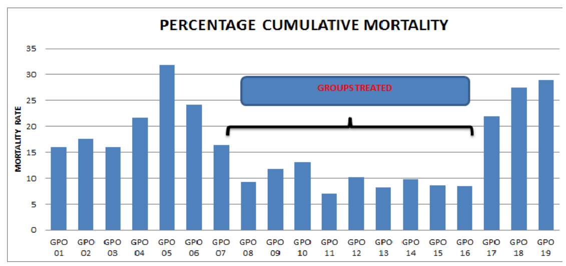

Figure 2. Graphic representation of the mortality per group

Conclusions and Discussion

The results of the use of immunoglobulins show a significant reduction in the mortality percentages, nevertheless there is no previous reference of the use of avian immunoglobulins in piglets, thus the results herein correspond to a new research line in Mexico. As for the treatment of breeding stock sows, other works published in the International Pig Veterinary Society in 2012 described their effect [(3,4,5)].

It can be concluded that establishing programs using avian immunoglobulins specific against PRRS in piglets decreases the mortality percentage and affords the possibility to obtain improvements in other productive parameters.

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One-step multiplex RT-PCR without DNA cross-contamination for differential diagnosis of Swine influenza viruses

H-J Kim[1], Y-K Shin[3], E-M Kim[1], S-H Kim[2], K-K Lee[2], J-Y Song[3], S-Y Kim[4], C-K Park[1]

Department of Infectious diseases, College of Veterinary Medicine, Kyungpook National University, Dae-Ku, Korea[1], Virology Division, Animal and Plant Quarantine Agency, Anyang, Korea[2], Disease Diagnostic Center, Animal and Plant Quarantine Agency, Anyang, Korea[3], RAD Incorporation, Dae-Ku, Korea[4], parkck@knu.ac.kr

Introduction

The pig has been acting as “mixing vessel” of the avian and mammalian influenza viruses as shown in the 2009 pandemic influenza outbreak. Since 2009, the national surveillance program has been promoting in Korean pig population to monitor the swine influenza virus mutation by Animal and Plant Quarantine Agency in Korea. In this program, RT-PCR has been adopted as official diagnostic methods for the detection and differ entiation of swine influenza viruses from nasal swab sam ples collected from pig herds. Currently, a false positive reaction due to DNA carryover contamination was recog nized as a big problem in the diagnostic laboratory.

In order to solve this problem, we would developed a simple one-step multiplex RT-PCR using "UDG system" for the detection and differentiation of swine influenza virus subtypes and 2009 pandemic influenza virus and for the prevention of the false positive reaction by previously amplified DNA products.

Materials and Methods

Swine influenza viruses (Subtype H1N1, H1N2, H3N2 and pandemic 2009 H1N1) were cultured as publ ished by OIE standard procedure. Viral RNA was extra cted by RNA extraction kit (Inclone biotech, Korea). The specific primer sets on swine influenza virus subtypes H1, H3, and pandemic 2009 H1N1 (pH1N1) was designed by DNASTAR® Lasergene (DNASTAR Inc. USA) based on the information of GenBank. One-step multiplex RT-PCR was carried out by mixing purified RNA templates with RT-PCR premix which is included specific primer sets and UDG RT-PCR buffer of commercial kit (RAD, Korea). RT-PCR condition are as follows: reverse-transcription 30 min at 50℃, pre-incubation 15 min at 95℃, 35 cycles of amplification (denaturation 30s at 95℃, annealing 60s at 50℃, extension 90s at 72℃) and final extension 10 min at 72℃. The amplified DNA products have visualized by agarose gel electrophoresis and UV trans-illuminator (Bio-Rad, USA). The sensiti vity and specificity of the test were evaluated on individual and mixed virus RNA samples, and prevention capability of DNA contamination was confirmed by artificially contaminated samples with a previously amplified DNA.

Results

One-step multiplex RT-PCR developed in this study was confirmed to able to detect and differentiate swine influenza virus subtype H1, H3 and pH1N1 on individual or mixed viral samples. The detection limit of the developed RT-PCR was 4 HA unit of each influenza viruses. When the developed RT-PCR with UDG system was applied to artificially contaminated samples with serially diluted previously amplified SIV DNAs, it was proved to prevent a false positive reaction in up to 100pg/ul of DNA contaminated sample (Table 1).

Table 1. One-step multiplex RT-PCR with UDG system on samples contaminated with an amplified DNA of SIV HA gene.

System	Concentration of contaminated DNA (pg/ul)					
	100	10	1	0.1	0.01	0.001
With UDG		-	-	-		-
Without UDG		+	+	+		+

Conclusions and Discussion

In this study, we developed One-step multiplex RT-PCR kit for the simultaneous detection and differentiation of major swine influenza viruses and pH1N1. Compared with the current RT-PCR methods that cannot avoid the carryover DNA contamination, the developed RT-PCR with the UDG system was proven to prevent a false positive reaction by contamination with amplified DNA products.

In conclusion, One-step RT-PCR with UDG system could be applicable to detect and differentiate of swine influenza viruses in clinical laboratories.

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Simple and rapid detection of PCV2 without cross-over contamination by direct PCR amplification of ORF2 gene

E-M Kim[1], S-H Kim[2], K-K Lee[2], S-S Lee[3], H-J Kim[1], M-S Ko[3], S-Y Kim[4], C-K Park[1]

[1] Department of Infectious diseases, a College of Veterinary Medicine, Kyungpook National University, Dae-Ku, Republic of Korea, [2] Virology Division, Animal and Plant Quarantine Agency, Anyang city, Republic of Korea, [3] NANOHELIX Incorporation, Dae-jeon, Republic of Korea, [4] RAD Incorporation, Dae-Ku, Republic of Korea,

graygkdl21@hanmail.net

Introduction

Porcine circovirus diseases (PCVD) are now considered global diseases and the cause of significant economic losses in the swine industry (1).

Laboratory diagnosis of PCV2 is carried out on tissues of infected animals using histopathology associated with the detection of PCV2 DNA by in situ hybridization (ISH) or viral antigens by immunohistochemistry (IHC) or indirect immunofluorescence (IIF). Although these techniques have a good sensitivity and specificity, they must be performed on post-mortem specimens and can be time-consuming. Alternatively, PCR can be used to detect PCV2 DNA in tissue samples and also in a broad range of body fluids such as blood, nasal and semen (6).

The polymerase chain reaction (PCR) assay is widely used as a specific and sensitive diagnostic method for the detection of PCV-2 in field samples (2, 3). However, the PCR amplification that makes so useful also makes it intrinsically susceptible to DNA contamination problems. Especially, contamination by amplification products and primers from previous PCRs is serious (4, 5).

Therefore, the main objective of this study was to develop a direct PCR (dPCR) with UDG for rapid detection of PCV2 and prevention of DNA contamination in entire PCR process.

Materials and Methods

Primer sets that could detect the PCV2 were designed. Nucleotide sequence data for PCV2 strains from GenBank were aligned by using Clone Manager 6 to identify regions that differed between the genotype.

The direct PCR was used as a DNA template that lysates directly extracted from field sample (tissue, blood) without any DNA purification. The conventional PCR was performed with DNA template as genomic viral DNA. Genomic viral DNA from field sample was extracted and purified by using Inclone™ RNA/DNA mini Extraction kit (Inclone biotech, Korea) as described in the manufacturer's manual.

PCR reactions were optimized based on primer concentration selection criteria. The optimized reaction contained 400nM each primer, 12.5µl of Direct PCR kit (Nanohelix, Korea), 2µl of DNA template and was made up to 25µl with sterile water.

Amplification of DNA for PCV2 was achieved by 5 min at 50°C, 5 min at 95°C, 40 repetitive cycles of 20 sec at 94°C, 20 sec at 53°C, and 40 sec at 72°C, and a final extension of 5 min at 72°C. The amplified PCR products were analyzed by 1.5% agarose gel electrophoresis and examined under ultraviolet light.

Results

Detection of PCV2 dPCR method without DNA extraction process was developed in this study. The sensitivity of the dPCR was confirmed that the same level or higher compared to the convention method with DNA extraction process.

DPCR with/without the UDG system were performed on samples containing serially diluted amplified DNA of PCV2 ORF2 gene. The results of dPCR with the UDG system was proven to unaffected by DNA contamination, but the results of dPCR without the UDG system was not (Table 1).

Table 1. Direct PCR with UDG system for prevention of DNA carry-over contamination.

method	concentration of contaminated DNA (copies/µl)							
	10^7	10^6	10^5	10^4	10^3	10^2	10^1	10^0
UDG	-	-	-	-	-	-	-	-
Non-UDG	+	+	+	+	+	+	+	+

Conclusions and Discussion

Detection of PCV2 dPCR with UDG system developed in this study was simple and rapid compared to conventional PCR, because it does not require the DNA extraction process. And it was proven to prevent DNA carry-over contamination that can occur in the PCR process. It is expected that the use of this dPCR method will be very useful and cost-saving for diagnosis of PCV2 in pig disease diagnostic laboratories.

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Performance improvement in SPF animals after PCV2 vaccination in subclinical PCVAD

S Figueras Gourgues[1], V Rodriguez-Vega[1], I Hernandez Caravaca[1]
[1]*Boehringer Ingelheim España S.A., Spain,* sebastian-jose.figueras@boehringer-ingelheim.com

Introduction

The higher the health status of the farm the better the performance parameters will be. The specific pathogen free status (SPF) offers to the farm the opportunity to reach the best performance results.

However, it´s known that PCV2 is a widespread virus which is present in the most of swine populations. Even if the clinical form is not seen it could be there. This subclinical form is able to contribute to a suboptimal performance[1].

In this way, it could be interesting to know if a PCV2 vaccination is able to improve good baseline performances even more, as it has been repeatedly demonstrated[2,3,4].

The objective of this study was to evaluate the profitability of using Ingelvac CircoFLEX® (Boehringer Ingelheim Vetmedica GmbH) in aparently healthy herd conditions. The growth performance and efficiency in subclinically PCV2 infected specific pathogen free pigs (SPF) were compared to those of non-vaccinated pigs.

Materials and Methods

The field observation was carried out in a 750 farrow to finish multiplier sow farm in the north region of Spain weaning at 3 weeks of age. This SPF herd was subclinical diagnosed for PCV2, meaning that it was detected in the laboratory but without clinical symptoms, as well as good production parameters..

Overall 787 pigs were included in the study. Half of the pigs (410) was vaccinated with Ingelvac CircoFLEX® at 9 weeks of age for management suitability. The other half of the pigs remained un-vaccinated.

Results came from fattening data of 400 ear tagged pigs from both treatments, Ingelvac CircoFLEX® (n=173, 136 F1 and GP females and 37 males) and CONTROL (n=227, 194 F1 and GP females and 33 males). Animals were placed in 6 rooms with 12 pens each. Pens 1 to 6 were for vaccinated and pens 7 to 8 for non-vaccinated.

The farm was a genetic nucleus so all the females and few boars were housed in these facilities to be sold to the clients. The other pigs are carried to an external fattening site. The individual recorded variables were weight at start and end of fattening and average daily gain taking into account the date of start and end of fattening. The treatment effect was assessed through analysis of variance (ANOVA) including treatment and sex. The mortality rate was also recorded and assessed by the chi-square test.

Results

Concerning mortality, control treatment had a 2.4% of dead pig during fattening whereas CircoFLEX® group had 1.95%. That means a 19% of mortality rate reduction for the vaccinated group. Average daily gain was improved by 13 grams in the CircoFLEX group as well.

Taking into account 10500 pigs sold per year and the extra payment for these genetic animals, the calculated ROI was 3,95:1 for the CircoFLEX group.

Table 2. Results from fattening in function of the treatment.

	Circoflex	Control	Dif.
N	173	227	54
Weight at start	30.0 ± 0.46	30.7 ± 0.44	-0,7
Weight at end	108.6 ± 1.13	109.0 ± 1.1	-0,4
Days of fattening	181.7 ± 1.13	182.4 ± 1.1	-0,7
Average daily gain (g)	884 ± 8.2	871± 7.8	+13
Mortality%	1,94	2,4	-0,46

Statistical significance could have been shown with a bigger sample size.

Conclusions and Discussion

Under the conditions of this study, CircoFLEX® group improved average daily gain obtaining 2,4 kilos more per pig sold. As well, mortality rate was better in CircoFLEX® group than control.

High baseline performance parameters in a non-vaccinated SPF group were improved in the CircoFLEX® group. In this subclinical PCV2 case, the vaccination increased the profitability of the herd.

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Comparison of real-time reverse transcriptase (RT)-PCR assays for detection of swine HEV in fecal samples reveals an advantage of broadly reactive assays compared to assays targeting specific genotypes

P Gerber[1], T Opriessnig[1,2], C Xiao[1], P Halbur[1], D Cao[3], XJ Meng[3]

[1]*Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA,USA* [2]*The Roslin Institute, University of Edinburgh, Midlothian, UK* [3]*Department of Biomedical Sciences and Pathobiology, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA* Tanja.Opriessnig@roslin.ed.ac.uk

Introduction

Hepatitis E virus (HEV) has been identified in humans and several animal species including domestic pigs (1). There are at least four recognized genotypes capable of infecting humans and genotypes 3 and 4 have been demonstrated to infect pigs (1). Due to its implication in public health and pork safety, several nucleic acid amplification techniques have been developed for HEV RNA detection in various types of samples including sera, feces and environmental samples. The objective of this study was to compare the performance of single-plex real-time RT-PCR assays designed for broadly detection of all recognized mammalian HEV genotypes with duplex real-time RT-PCR assays for detection and differentiation of HEV genotypes 3 and 4 which are found in pigs.

Materials and Methods

Twenty-eight serial fecal samples were collected daily from two pigs experimentally infected with HEV genotype 3 strains from 2 to 14 days post infection. Additionally a total of 186 pig fecal samples were chosen arbitrarily from routine diagnostic cases submitted during May 2013 to the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL). These samples originated on 86 farms located in 12 US states. Viral RNA extraction was carried out on 50 µl of the fecal sample suspensions using a MagMAX 96 Viral Isolation kit (Ambion, Foster City, CA, USA) according to the manufacturer's instructions on an automated extraction platform (KingFisher Flex; Thermo Fisher Scientific). Four different HEV PCR assays were utilized: Two single-plex real-time RT-PCR assays for broad detection of all 4 recognized mammalian HEV genotypes: assays A (2) and B (3), and two duplex real-time RT-PCR assays for detection and differentiation of HEV genotypes 3 and 4: assays C (3) and D (4). Assays B and D utilized the same forward and reverse primers.

Results

In experimental samples, HEV RNA was detected in 96.4% (assay A), 39.2% (assay B), 14.2% (assay C), and 0% (assay D) of the samples. In field samples with unknown HEV exposure, HEV RNA was detected in 67.2% (assay A), 36.4% (assay B), 1.1% (assay C), and 0.5% (assay D) of the samples. Assays showed an overall poor agreement (κ = 0.19 to 0.03) with differences in detection rates between assays ($p < 0.01$). Assays A and B that broadly detect HEV genotypes 1-4 had significantly higher detection rates for HEV RNA than the duplex assays C and D that were both designed to detect and differentiate between HEV genotypes 3 and 4.

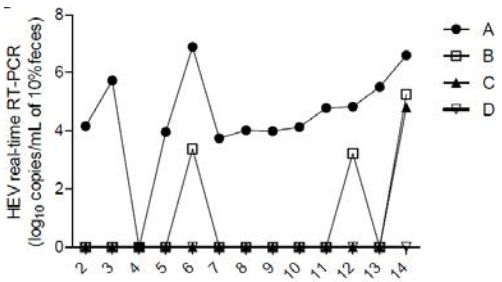

Figure 1. Comparison of the four real-time RT-PCR assays (A, B, C and D) on fecal samples at different days after experimental inoculation of pigs with a swine HEV genotype 3 strain.

Conclusions and Discussion

In this study, two single-plex real-time RT-PCR assays for detection of HEV genotypes 1-4 and two duplex real-time RT-PCR assays for detection and differentiation of HEV genotypes 3 and 4 were evaluated. All assays were compared on the same real-time RT-PCR instrument, on the same day, using the same RT-PCR enzymes, standard curves and nucleic acid extracts. Real-time RT-PCR assays A and B that broadly detect HEV genotypes 1-4 showed better results for RNA detection than the duplex assays C and D that were both designed to detect and differentiate between HEV genotypes 3 and 4. Assay A presented the overall best performance among the tested assays. The results of this study indicate that if reliable detection of HEV in pig samples is desired, broadley reactive PCR assay should be utilized and if desired, positive results can be further follwed up by sequencing to determine the involved genotype.

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Field case report: Efficacy of Ingelvac® PRRS MLV in a Korean swine farm with dual PRRSV infection (NA & EU Isolates)

SW Lee[1], HK Seo[1], CS Shin[1], BJ Cho[1], SY Kang[2]

[1] *Boehringer Ingelheim Vetmedica Korea Ltd,* [2]*Virology Laboratory, College of Science, Chungbuk National University, Korea,* seongwon.lee@boehringer-ingelheim.com

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) causes respiratory disease in nursery and grow-finisher pigs and reproductive failure in sows and boars[1]. Although PRRSV was first isolated in Korea in 1994 and all PRRSV isolates corresponded to the North American genotype (Type II) until 2000, European PRRSV(Type I) has recently emerged in Korea[2]. Since European PRRSV in Korea was isolated in 2000, In this study we evaluate the efficacy of Ingelvac® PRRS MLV in a Korean swine farm infected with PRRS Type I and II (dual infection) .

Materials and Methods

This study was conducted in a commercial 485 sow farrow to finish farm. In 2013, the farm experienced a negative impact in productivity due to infection with EU-type PRRSV. The farm manager decided to execute a control program using Ingelvac® PRRS MLV. In May 2013, the farm implemented a first mass vaccination in the breeding herd followed by a second mass vaccination 4 weeks apart and then every three months (quarterly). Strict needle management as one needle for individual sows was implemented.

Before/After vaccination blood samples were taken for examination by ELISA(IDEXX PRRS 3X) and RT-PCR to determine the variance of exposure level and virus detection. Each 5 blood samples were taken from sows and piglets at the following ages: 3, 30, 50, 70 and 90days of age. At the same time, performance data were analysed before and after vaccination period as well.

Results

Prior to vaccination, PRRSV could be found in sow herd and piglets (Table 1). During this time, there was an early seroconversion in growing pigs between 30~50 days of piglets also, serology profile in breeding herd showed a high variability in SP values, indicating lack of stabilization at the moment.

After breeding herd mass vaccination, the variability of SP values was reduced sugesting a better stabilization process along with RT-PCR negative results in sows, piglets and growing pigs (Table 1, Figure1).

In addition, the farm improved wean to finish mortality from 10% to less than 5% (Figure2) and reduced abortions during the last period of pregnant sows.

Table 1. Results of RT-PCR to detect PRRSV in the serum.

	PRRS ag			
	May 2013		August 2013	September 2013
	Natype	Eutype		
sow	(-)	(+)	(-)	(-)
3day	(-)	(+)	NT	NT
30day	(-)	(-)	(-)	(-)
50day	(+)	(+)	(-)	(-)
70day	(-)	(+)	(-)	(-)
90day	(-)	(-)	NT	NT

(NT; not tested)

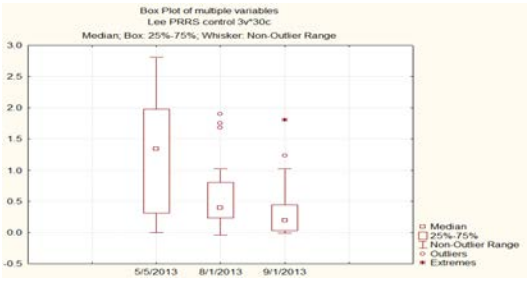

Figure1. Box and whisker plot of PRRSV antibody titer in the 3 different periods of sampling (both sows and piglets)

Figure2. SPC chart of Post-weaning mortality of the herd

Conclusion and discussion

After the breeding herd mass vaccination program was implemented, a clear PRRSV stabilization status was achieved measured by a reduction of variability of SP values from serology profile and no detection of positive results by RT-PCR. A general productivity improvement in reproductive and growing performance was observed confirming Ingelvac® PRRS MLV as a primary tool to control PRRSV even under dual infection scenarios.

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Economic approach of PRRSV stabilization following intradermal mass-vaccination (Porcilis® PRRS) and strict biosecurity measures

V Normand[1], P Berton[1], C Chevance[1], F Bouchet[1], J Metais[1], M Rigaut[2], A Lebret[1]
[1]*Porc.Spective - Groupe vétérinaire Chêne Vert Conseil, Noyal-Pontivy, France*
[2] *MSD Santé Animale, Beaucouzé, France ;*
v.normand@chenevertconseil.com

Introduction

PRRSV stabilization of a herd can be done without depopulation, by combining mass-vaccination of the animals with a MLV (Porcilis® PRRS) and strict biosecurity measures[1]. Aim of this paper is to present an economic approach of a successful stabilization protocol in a herd using intradermal mass-vaccination against PRRSV.

Materials and Methods

The study was done in a 420-production sow, farrow-to-finish farm in Brittany weaning at 21 days, every 2 weeks. In 2010, despite PRRS vaccination in the breeding herd, PRRSV-related clinical signs persisted: cough, poor growth and deteriorated feed conversion.
In January 2011, PRRSV circulation was confirmed in 70 day old growing pigs both by serology and PCR tests (ELISA Herdcheck® PRRS X3, IDEXX and qPCR: Adiavet™ PRRS EU & NA, Adiagene). ORF7 sequencing confirmed that a wild type European strain was circulating (95.1% homology with the Porcilis® PRRS vaccine strain). For those reasons, PRRSV stabilization program was established, following Lebret *et al.* protocol[1].
In May 2011 and 28 days later, two mass-vaccinations of the entire herd (breeding stock and pigs of 21 days and older) were done with Porcilis® PRRS. Thereafter, all breeding animals were vaccinated every 4 months. All mass-vaccinations were done intradermally. Gilts were vaccinated intramuscularly upon arrival and 4 weeks later. For 20 weeks, all piglets were vaccinated intramuscularly at weaning and 4 weeks later. The last batch was vaccinated in November 2011. Biosecurity measures and monitoring of the successful PRRSV stabilization according Batista's protocol[2] have been previously described[3]. This status was confirmed in March 2013[3].
Early 2012, clinical signs sharply reduced. As the objective is to compare economic impact of different technical results, the calculation was done with both fixed price of feed and carcass and the same number of sows (mean observed on this farm during 2010-2011-2012 is proposed).

Results

Economic approach of a PPRSv stabilization program which began in May 2011.

	2010	2011	2012
Number of pig sold/ present sow/year	23.3	26.0	25.5
Live produced weight /present sow /year	2719 kg	2954 kg	2886 kg
Global feed conversion ratio	2.93	2.88	2.74
Extra gain /kg carcass weight (€)	0.156 €	0.154 €	0.143 €
Animal health costs/sow (€) (PRRSV vaccination included)	148 €	188 €	133 €
Number of present sows mean (2010-2011-2012)	452		
Price per kg (carcass weight) mean (2010-2011-2012)	1.30 €		
Price of feed /kg mean (2010-2011-2012)	0.24 €		
Total carcass weight (76,5%/live weight) for 452 present sows (kg)	940 176	1 021 434	997 921
Margin = [Sales] – [(Feed cost + Health cost)] (€)	437 776	477 248	522 063
Increased margin compared with 2010 (€)		39 472 €	84 288 €
Margin/present sow (€)	969 €	1056 €	1155 €
Margin/pig sold (€)	41.57 €	40.61 €	45.29 €

Conclusions and Discussion

Despite high cost of the PRRSV vaccination program (40 €/sow in 2011), return on investment has been observed since the year of PRRS stabilization, thanks to improvement of both herd productivity and feed conversion. In this herd, economic impact of PRRSV circulation was estimated as 185 €/sow or 3.73 € /pig sold (results in 2012 compared with those in 2010), which is consistent with Dykhuis Haden study[4] (5.57 $ US/head placed).

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Effect of oral chitosan on the growth rate of grower pigs

TW Volker[1], G Weber[1]
[1]*Green Bio, Pretoria, South Africa*, info@greenbio.co.za

Introduction

Chitosan is a non-toxic, biocompatible, biodegradable, natural polysaccharide derived from the exoskeletons of crustaceans with the use of alkali sodium hydroxyde.
Chitosan occurs in different molecular weights and each weight range has a different effect on the body. Chitosan acts as a pre-biotic[1] and has also been demonstrated to induce both humoral and cell mediated immune responses[2-4]. We decided to measure the effect of oral administration of chitosan on the growth rate of grower pigs and calculate the cost benefit on a Mycoplasma hyopneumoniae positive swine herd.

Materials and Methods

A farrow to finish unit which was Mycoplasma hyopneumoniae positive was selected for this field trial. Four groups of 10 pigs each, of newly weaned piglets at 21 days of age were randomly selected as follows: C1 boars, T1 boars, C2 gilts and T2 gilts. 1 Liter of a 5% chitosan solution was diluted in 25 Liter water and then dosed with a dosatron at a 1% inclusion in the water line to the treatment groups. (20 ppm Chitosan) The control groups received no medication via the water. Chitosan treatment was continuous for 21 days. All other factors, eg feed, group size, building orientation and management were the same for all groups. The piglets were weighed at the start of the trial, ie 21 days, at 42 days and again at 21 weeks of age. See Table 1. For practical reasons it was not possible to do carcass checks or lung scoring on the test groups, as they were slaughtered at different abattoirs.

Results

Table 1.

AGE	Pig weights kg				$\bar{X}$
	C1M	T1M	C2F	T2F	
21 days	6.4	6.3	5.9	6.1	6.2
42 days	8.7	8.7	8.4	8.3	8.5
147 days	92.5	97.7	94.1	99.8	96.0
Weight gain	86.1	91.4	88.2	93.7	
Difference		5.3		5.5	p<0.01

Table 2.

	Weight advantage	Cost/kg live $	Total gain $
Males	5.3	1.5	7.95
Females	5.5	1.5	8.25
Average	5.4	1.5	8.10

Conclusions and Discussion

The treatment groups had a clear advantage of 5.3 kg and 5.5 kg for the males and females respectively. The difference was significantly different at p<0.01. There was no significant difference between males and females. This equates to an extra income of approximately USD 8.10 per pig, whereas the cost of the Chitosan given for 3 weeks at 1% in the drinking water cost USD 0.15 per pig.

The increased growth can probably be attributed to a combination of the following:
a) Chitosan acting as a prebiotic[1] thereby creating an advantageous environment for an-aerobic bacteria to dominate and improve digestion and absorbtion of nutrients
b) Stimulation of humoral and cell mediated immunity[2-4], thereby enhancing the body's immune reaction towards inherent infections eg Mycoplasma. Improved reaction to the Mycoplasma vaccine, is also a possible strong contribution, whereby Chitosan is acting as an adjuvant.[2]
c) Chitosan activates the dendritic cells i.e. antigen presenting cells, thus the immune response of the host is greatly enhanced.
d) According to K.N. Han et al[3], chitosan improves feed efficiency in young pigs and inhibits the growth of harmful bacteria.
e) The lungs are the first "filter" for antigens as all blood circulates through the lungs before flowing to the rest of the body.
In summary, Chitosan has been shown to have a great cost benefit USD 7.95 per pig, by using it orally in grower pigs as a general pre-biotic[1] and immune enhancer.
Further investigations should include measurement of Feed Intake, Feed conversion ratio's, Lung scores and antibody titre's. The effect of Chitosan on High Health Pigs also needs to be investigated.

Acknowledgments

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Identification of antigenic variation of the PorPV by the hemoagglutination inhibition technique

CV Riaño, NR Carreón, BJI Sánchez
[2]*Departamento de Medicina y Zootecnia de Cerdos, FMVZ, UNAM,* veroni_8705@hotmail.com

Introduction

Blue eye disease of pigs affects all productive stages. This disease has been reported in the central region of Mexico, where it is considered endemic. The aim of this study was show antigenic variability between eight viral isolate of porcine Rubulavirus.

Materiasl and Methods

Isolates were replicated in VERO cells (kidney from African Green monkey) until obtaining an optimal titer of 1:32 to 1:128. Specific sera for each of the viruses were obtained to be used for the hemagglutination inhibition technique aimed at identifying the homologous and heterologous antigen-antibody reaction of each isolate with its respective antiserum. These viral isolates were obtained from the following samples:

Identification	Samples	Region/State	Year
2	Brain of suckling pig	MICH	2008
4	Brain	MICH	2008
5	Semen	unknown	2008
6	Semen	MICH	2008
7	Brain	GTO	2008
11	Brain	MICH	2008
13	Brain	GTO	2008
34	unknown	MEX	2007

Results

Results indicate that there are antigenic variations between virus 34 and viruses 2, 4, 5, 6, 7, 11, and 13. Virus 2 does not present antigenic variation with respect to viruses 4, 5, 6, 7, 11, and 13. Virus 4 does not vary antigenically with respect to viruses 5 and 6. Virus 5 does not present antigenic variation with viruses 6, 7, 11, and 13. Results demonstrate that antigenic relation values of each virus are equal to 1, which indicates the specificity of the antibodies generated by the homologous virus. Titers in heterologous responses, i.e., challenging of one virus with the other antisera, revealed that the reaction differs among all viral isolates, identifying antigenic variation among viral isolates as shown in the following.

Viral isolates are antigenically related when they present a VRA of 0.5 to 1 (Wadey & Faragher, 1981).

VRA	VIRUS							
Anti Sera	**2**	**4**	**5**	**6**	**7**	**11**	**13**	**34**
2	1	1	1.41	2	1	1	1	0.025
4		1	1	1.41	0.70	0.5	0.70	0.70
5			1	1.41	1	1.41	1	0.70
6				1	2	2	1	0.025
7					1	1	0.70	0.70
11						1	0.70	0.70
13							1	0.5
34								1

Discussion

The use of different porcine Rubulavirus isolates in the diagnosis will avoid false negatives, because the antigenic variation demonstrates in this study increases the possibility of finding specific antibodies when using different field isolates in the diagnosis laboratory.

Conclusion

To know the existence of antigenic variation in these isolates that are circulating in the country is important to generate immunogens able to induce an efficient immune protecting response. Evaluation of hyperimmune sera elaborated in a heterologous species to the pig different results were obtained when challenging each of the sera with the different isolates.

Acknowledgments

This work was supported by the Department of Medicine and Zootechnics of Pigs of the UNAM. We thank Laboratorio Lapisa for providing the viral isolates.

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Isospora suis in slaughter house pigs in Mexico

M Trujano[1], Dagnino C[2]
[1]*PHIBRO Animal Health, Querétaro, México,* [2]*Granja Los Olivos, Sonora, México,* margarita.trujano@pahc.com

Introduction

The disease due to *Isospora suis* is common worldwide specially in piglets (1,2,3,4,5). Recent studies in Europe, USA, México and Australia indicates that *Isospora suis* is the most frequent parasite in piglets, the presence of the disease is related to diarrhea (6,1,5).This parasite has a direct life cycle among pigs (there is not an intermediate host) the excreted oocytes by an infested host will depend upon the ambient temperature to maturate before infesting other animals through via oral. The organism colonizes the small intestine then goes through different stages of maturation. The infestation with *Isospora suis* in pigs damages the intestinal epithelium therefore diminishes the nutrients absorption and later diarrhea. There is also growth retardation and low weight gain. Because the mortality rate is low in affected animals, little attention is given to this disease. It has been observed occasionally in growers, fattening and breeding pigs, when they are moved to or inhabit in infected, or continuous flow installations (7,8). The aim of this study is to show the prevalence of this infestation in pigs examined at slaughter houses in Mexico.

Materials and Methods

The work was carried out at slaughter houses in Sonora in 14 farms suspected of Ileitis and diarrhea. 120 Samples of Ileum for histopathology examination were collected and placed in formaldehyde at 10%. 50 samples of ileum were also collected for scrapings of their mucosa epithelium. Histopathology samples were stained with HE and the scrapings were stained with GIEMSA in order to detect *Isospora suis*.

Results

Even though the purpose of examining the animals was to look for any ileitis lesions, the finding of *Isospora suis* in ileums under the microscope was not expected. The samples collected for scrapings corroborate the presence of *Isospora suis*.

Of the 14 farms examined 12 (85 %) showed lesions suggestive of *Isospora suis*. The total number of pigs examined was 630, and 120 (19 %) of them showed *Isospora suis* at different stages of maturation, mainly pair merozoites. The 50 (100 %) scrapping samples stained with GIEMSA were positive to *Isospora suis*; the merozoites stained blue with red nucleus.

Conclusions and Discussion

The studies in this work indicate the presence of *Isospora suis* in adult animals. These results are consistent with those reported by Yaeger et al (2003), they found infestation with Isospora suis in breeding animals. They concluded that the animals of high health status are more vulnerable to parasitic infestations if in the facilities are *Isospora suis* oocysts present. In addition they found that under certain conditions such as high temperatures and farms where the animals have had no contact with these parasites, when these animals were moved to pens with oocysts in the environment they showed diarrhea and sometimes death in adult animals (8).

The conditions of high health status in Sonora as well as the high temperatures represent according to what was described by other authors (8,9) an ideal environment for the presence of *Isospora suis*. These oocysts can be found in floors and facilities.

The use of Salinomycin (30 ppm fed continuously) as a treatment for these infestations was effective to reduce the diarrheas in the farms examined.

When animals in the fattening areas do not respond to antibiotics for gastro-intestinal symptoms *Isospora suis* might be involved

Acknowledgments

Slaughter houses in Sonora, Mexico

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Reproductive disorders related to mycotoxins in swine: A case report

M Trujano
PHIBRO Animal Health, Querétaro, México margarita.trujano@pahc.com

Introduction

The current world pork industry requires greater efficiency in production parameters. Reproductive disorders directly influence the number of piglets produced per sow per year. The risk factors in reproductive problems in pigs are numerous and are correlated with each other. Intoxications with mycotoxins represent a big challenge to the farm practitioner veterinarian. The lesions are usually masked by infectious diseases. This paper describes the first case of abortion related to Ergotoxins in sows in Mexico.

Clinical Case: Two commercial Farms in Mexico with reproductive disorders were studied. Abortions at different stages of gestation were observed. A great variety of clinical signs and symptoms were noticed in sows; abdominal breathing, epiphora, skin erythema, cyanosis and tortuous vessels in the abdominal region, the animals showed low or no feed consumption, some even showed anorexia, and complete feed rejection. Wet gangrene in the abdominal area was observed in some aborted sows. 70% of sows had either dysgalactia or agalactia. In the farrowing areas piglets were weak, small and 50% in each litter showed necrosis of tail. Blood samples were taken and sent for serological analyses; they were negative for PRRS, Parvovirus and Influenza.

Materials and Methods

Mycotoxins Analysis: Determination by enzyme immunoassay (ELISA): Aflatoxin B1, Ochratoxin A, Deoxynivalenol, T-2, Fumonisin B1, Citrinin and Zearalenone in feed samples from Gestation and Farrowing areas.

Qualitative determination of Ergotoxins (1): Amoniacal ether was added to the sample for the extraction of alkaloids, tartaric acid was added to the ammonia extract to form the tartrates, p-dimetil benzaldehide sulphuric was added for the reaction of devan of urk. The formation of a blue-violet complex is considered positive.

Results

Post-mortem findings in fetuses and sows: Petechial, ecchymosis and bleeding were observed in kidney, heart, liver, spleen, skin, lung, subcutaneous tissue and placenta, hemorrhagic ascitis, subcutaneous edema and reddish amniotic fluid in embryos.

Histopathology Findings: The lesions most commonly observed in the organs examined were thrombi in blood vessels and hemorrhages.

Mycotoxins**:** Tables 1 and 2 show the presence of DON Ergotoxin and Zearalenone in the feed samples analyzed.

Table 1. Gestation feed results

Samples	ZEA (ppb)	OA (ppb)	DON (ppb)	T-2 (ppb)	Ergotamine Ergotoxin**
Gestation	**82.0***	**4.8***	565.0*	49.5	**(+)**
Limit rates	**<50**	**<5**	**<300**	**<50**	**Test (-)**

Table 2. Lactation feed results

Samples	ZEA (ppb)	OA (ppb)	DON (ppb)	T-2 (ppb)	Ergotamine Ergotoxin**
Lactation	**95.5***	**5.5***	672.0*	45.0	**(+)**
Limit rates	**<50**	**<5**	**<300**	**<50**	**Test (-)**

Methods ELISA (r-Bopharm) * Above limit rate
** Van Urk positive reaction to p-dimetil aminobenzaldehido sulfuric from the extracts with amoniacal ether

Conclusions and Discussion

One common mycotoxin in our environment but little studied is Ergot (2), there are not reports in Mexico that relate this mycotoxin to Abortions.

In this study the lesions, as well as the analyses of Mycotoxins verified Ergotoxins's presence.

These Mycotoxins are powerful initiators of the contraction of the smooth muscle present in uterus and in the muscular layer in arteries (3,4). They simulate the action of the dopamine in the nervous central system; inhibit the liberation of prolactin, disables the lacteal secretion. The abortions can be explained as a result of the vasoconstriction in the arteries which produces ischemia, lack of irrigation and nutrients to the embryos or fetuses. Ergot related lesions impact the most since they culminated in abortions.

The feed was removed and the animals were fed a different kind of feed, yeast cellular wall was added.

Mycotoxins should be considered when no response to any kind of treatment is observed in reproductive problems in pigs.

Acknowledgments

Alejandro Caro, Jalisco, Mexico. LFA, Mexico

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Respiratory problems related to *Aacaris summ* in pigs. A case report.

M Trujano[1], J López[2]
[1]*Phibro Animal Health, Queretaro, Mexico,* [2]*DESPPO, Jalisco, Mexico* margarita.trujano@pahc.com

Introduction

In general thanks to the health status in many farms around the world, the prevalence of different species of parasites is very low. However, *Ascaris suum* remains in almost any farm, it is considered among the most common parasite in the United States of America (1,2), and in Western Europe (3). *Ascaris suum* affects mainly pigs between 2 and 5 months of age (1) it causes economic losses in the period of fattening and seizures in slaughter houses (4). The adult ascaris compete for food with the infested animals and can dramatically reduce the feed conversion. In a study carried out in the USA states they concluded that infestations with Ascaris produced in the 1994, an estimated $174 million in economic losses.

This work describes a case of respiratory problems associated with *Ascaris suum* in Mexico.

Materials and Methods

Commercial Farm (Sites 2 and 3) located in the state of Jalisco with a total of 2,700 animals was studied. The fattening area showed severe mortality (10 %) in 23 weeks old animals. They signs, we were told, were related to respiratory problems, i.e.: more than 40 coughs per minute, abdominal respiration and 20 sneezes per minute. The animals were treated with antibiotics, mainly enrofloxacin and florfenicol, without good results. Sudden death was commonly observed in animals in good conditions.

Results

Post-mortem examination. The dead animals were examined; Table 1 shows the quantity of parasites found in different areas and organs.

Table 1. Average number of parasites found in the animals examined.

ORGAN	FINDINGS
Cavities	> 20 Parasites, even in mouth
Larynx, pharynx	> 20 parasites
Lungs	> 10 Parasites
Esophagus	> 6 parasites
Gall bladder	> 2 parasites
Stomach	> 5 parasites
Intestines	>10 Parasites, also one in common biliary duct

In the post-mortem examination of dead animals, large quantities of parasites were observed in different organs, mainly in the larynx and trachea, which caused suffocation and therefore sudden death in animals.

Conclusions and Discussion

The Ascaris infestation was so sudden and severe in this farm that there was no other type of signs in addition to severe coughing and lack of breathing. The animals were in good condition; therefore, there was no suspicion of possible parasitic infestation. These findings differ from what has been observed by other authors (5) who reported that the clinical manifestations in massive infections by *Ascaris suum*, cause irritation, diarrhea and weight loss.

The sudden death in animals observed in the present work coincides with what was reported in a work done in the USA, where it was found that serious infections caused by this parasite could cause death (1,5) When performing movements of animals with a high health status to old installations with holes in the floor or bad disinfection, it is possible to suspect of infestations. It is recommended to examine, when possible the dead animals post-mortem to corroborate the lesions with the signs observed and reach a definitive diagnosis. The use of Oxibendasole 15mg per Kg during 10 days helped to solve this problem.

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Jalisco, Mexico swine producers

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Influence of doxycycline on the postvaccinal immune response in pigs

M Pomorska-Mól, K Kwit, E Czyżewska, A Dors, Z Pejsak
Department of Swine Diseases, National Veterinary Research Institute, Pulawy, Poland
mpomorska@piwet.pulawy.pl

Introduction

Doxycycline (Doxy) belongs to the tetracycline antibiotic family (1). Earlier studies have shown that some antibiotics, including doxy may negatively influence the immune response (2, 3), but to date there are limited information how antibiotics affect the effector arms of adaptive immunity after its administration in therapeutic doses. This study was undertaken to examine the influence of doxy given in therapeutic doses on the postvaccinal immune response in pigs vaccinated during antibiotic therapy.

Materials and Methods

Fifty pigs were divided randomly into three groups: control not vaccinated (C, n=10), control vaccinated (CV, n=20), and experimental (DOXY, n=20). Feed and water were offered *ad libitum*.

The commercial product containing doxy was used (Soludox 50%, Eurovet Animal Health BV). For vaccination of pigs attenuated gE- deleted vaccine against pseudorabies (PR) and inactivated vaccine against swine influenza (SI) were used (Akipor 6.3, Gripovac, Merial, France).

From day -1 to day 5 pigs from DOXY group received doxy orally with drinking water, at the recommended dose 12.5 mg doxy hyclate/kg b.w./day. Antibiotic was given for 7 following days. Pigs from DOXY and CV groups were vaccinated at 8 and 10 weeks of age (0 and 14 day of study). Pigs from C group were not vaccinated and did not receive doxy.

Blood samples for evaluation of T-cell antigen-specific response (lymphocyte proliferation assay (LPA), in vitro IL-4 and IFN-γ secretion) were taken on days: -1, 0, 2, 5, 8, 14, 16, 28, 42. Serum samples, for evaluation the development and persistence of SIV and PRV-specific antibodies (HerdChek*Anti-PRVgB or HerdChek*Anti-PRVgp1, IDEXX Laboratories, USA; hemaglutinin inhibition assay) were taken 1, 2, 3, 4, 6, 8, 10 and 12 weeks after first vaccination.

Results

None of the non-vaccinated pigs had antibodies against PRV and H1N1, H1N2 and H3N2 influenza viruses during study. No significant differences were found between ELISA S/N ratio and HI titre in pigs from CV group and in pigs vaccinated during antibiotic therapy. Eleven days after first vaccination, in 1 pig from CV group the PRV-specific proliferation was observed. Two days after the second dose of PRV vaccine, 40% of animals from CV group developed a PRV-specific proliferation, while in DOXY group only 20% of pigs responded. 2 and 4 weeks after booster dose, all pigs from vaccinated groups responded specifically in LPA, however the mean SI in DOXY group was significantly lower compared to CV group ($p<0.05$) (Figure 1).The mean as well as individual SI value in pigs from all groups did not reached value pointing to SIV-specific stimulation (1.89).

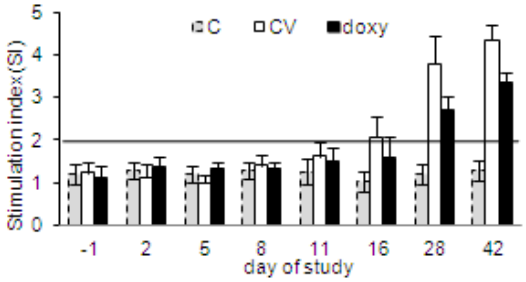

Figure 1. The mean (±SD) SI after vaccination against PR. *The line at 1.93 SI indicates border-line between nonspecific and antigen-specific stimulation.*

In group C there was no significant increase of INF-γ concentration in culture supernatant after PRV stimulation. In contrast, PRV restimulation of PBMC from vaccinated animals resulted in the release of high amounts of INF-γ. The highest concentrations of INF-γ in culture supernatants were observed in vaccinated pigs that not received doxy. *In vitro* H1N1 and H3N2 stimulation of PBMC did not induce detectable production of INF-γ in pigs from all groups. The IL-4 was below limit of detection (<15.6 pg/ml).

Conclusions and Discussion

The results reviled the negative impact of doxy on the T-cell postvaccinal response after vaccination with attenuated vaccine against PR. Simultaneously, no significant influence on the humoral immunity against PRV and SIV was observed. Although the exact mechanism of T-cell response suppression remains to be elucidated, the present observations should prompt further studies on the practical significance of such phenomena in terms of clinical implications.

Acknowledgments

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Monitoring PRRSV sero-conversion by using oral fluid sample

A Martos-Raich[1], M Badosa-Brossa[1], E Coma-Oliva[1], J Serra-Martínez[2], X Barrera-Toro[3], L Planasdemunt-Regàs[3], L Porquet-Garanto[1], X Rebordosa-Trigueros[1]

HIPRA 17170 Amer, Girona, Spain[1]; Biofar Laboratoris, S.L 08261 Cardona, Barcelona, Spain[2]; AVP Planasdemunt i Associats 17400 Breda, Girona, Spain[3], alba.martos@hipra.com

Introduction

Serology is one of the most important methods for PRRS diagnosis. Since now, the only way to do it is bleeding some animals at different age. However, pig oral fluid (OF) is a suitable sample for monitoring both viremia (PCR) and sero-conversion (ELISA) after PRRSV infection. In this study a new methodology to adapt the existing CIVTEST® SUIS PRRS E/S kit (HIPRA) to be used with OF matrix instead of serum was evaluated under field conditions. The ultimate goal of this study was to follow the PRRSV post-infection sero-conversion by analyzing Oral Fluid (OF) sample at different times post-challenge by ELISA and PCR.

Materials and methods

The study started with 30 groups of 9 gilts from PRRSV-free farms (as confirmed by PCR and ELISA). After week 2 (wk2) 10 groups were selected until the end of the study. Each group was infected in isolation at wk0 using a field strain of PRRSV Type 1. The study lasted for 8 weeks with weekly sampling of OF. The presence of antibodies to PRRSV was evaluated by using the CIVTEST® SUIS PRRS E/S (HIPRA) and different conjugates (IgG, IgA or IgA+IgG). OF samples were also analyzed by qPCR. Individual serum samples of all animals were also analyzed at wk3 and wk7.

Results

The use of a combined conjugated anti-(IgG + IgA) gave the greatest precocity in detecting post-infection seroconversion. Using this conjugate 100% of the OF-samples analyzed at wk1 were positive. This value remained positive until the end of the study. The use of anti-IgG conjugate also gave good sensitivity. So, 80% of the initial 30 samples gave positive at wk2 (50% for the groups selected until the end of the study). At wk3 100% of the samples were positive using the anti-IgG conjugate, remaining also all positives at wk8. In contrast, the use of the anti-IgA conjugate affected the specificity of the test, since positive OF results appeared at wk0. The conjugate used in this study was a monoclonal anti-IgG, while as anti-IgA was used a polyclonal sera. It is possible that the use of a higher quality reagent could improve the specificity results. Both responses, the (IgG + IgA) and IgG alone, presented the same kinetic with two peaks at wk3 and wk7. This pattern was clearer in the IgG response than in the (IgG + IgA). The results indicate that OF sample is a good alternative to the classical serology even in terms of sensitivity.

Conclusions and discussion

CIVTEST® SUIS PRRS E/S presented good diagnostic performance with OF. The results indicate that ELISA is showing adequate sensitivity even as an alternative to PCR. The kind of conjugate used in the test has a certain effect on the earliness of detection of sero-conversion and its specificity.

Acknowledgments

Biofar Laboratoris, AVP Planasdemunt i Associats

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Intestinal gene expression involved in innate and acquired immune responses of pigs is affected by *Salmonella* infection and diets supplemented with gut health-enhancing feed additives

M Lessard[1, 2], N Bergeron[2], K Deschêne[1], JJ Matte[1], F Guay[3], N Bissonnette[1], G Talbot[1], J Gong[4], Q Wang[4], S Quessy[2], A Letellier[2]

[1]*Dairy and Swine R & D Centre, Agriculture and Agri-Food Canada (AAFC), Sherbrooke, QC Canada;*
[2] *Swine and Poultry Infectious Disease Research Centre, Université de Montréal, St-Hyacinthe, QC, Canada;*
[3]*Department of Animal Sciences, Université Laval, Québec, Canada;* [4]*Guelph Food Research Centre, AAFC, Guelph, ON, Canada,* martin.lessard@agr.gc.ca

Introduction

As a tool to reduce the use of antibiotics as growth promoter and to minimize incidences of enteric infections caused by different pathogens such *E. coli* and *Salmonella* Typhimurium (1), a cocktail of micronutrients and feed additives (CFA) was designed to modulate the development of systemic and mucosal immune system, barrier function of intestinal wall and bacterial populations in the gut. Its efficacy was assessed in weaning piglets infected with *Salmonella*.

Materiasl and Methods

Composition of CFA added to diet: Cranberry extract rich in polyphenol, encapsulated calvacrol, an essential oil, *Pediococcus acidilactici* MA18/5M and yeast-derived products of mannans and glycans were included. Diets supplemented with CFA were also enriched with selenium yeast and vitamins (A, D and B complex).

Treatments and measurements: At weaning, 32 litters of 12 piglets each were allocated to four dietary treatments: control diet (CTRL), CTRL diet supplemented either with chlortetracycline (ATB), CFA or CFA and bovine colostrum (COL) in replacement of spray-dry animal plasma. After 28 days of feeding, 32 pigs per group were orally inoculated with *S.* Typhimurium DT104. Half of them were euthanized 3 days post-infection (dpi) and the other half, 7 dpi. Ileal mucosa samples were taken for RNA extraction. Gene expression of cytokines, enzymes and other molecules involved in intestinal defenses was determined by real-time PCR using *HPRT* and *PPIA* as housekeeping genes. Statistical analyses were performed using the MIXED procedure followed by contrasts to evaluate time and diet effects (SAS Institute Inc., Cary, NC, USA).

Results

The expression of most genes showed a peaked response at 3 dpi (Table 1). Only *IL-1β* gene expression was reduced 3 dpi. At 7 dpi, expression levels of *TNFα* and *IFN-γ* were still comparable to 3 dpi level while all others were reduced compared to 3 dpi but were still higher than on day 0 for *IL-8*, *COX2* and *GPX2* ($P < 0.05$).

Dietary treatments only influenced expressions of *βDef-2* and *GPX2* ($P < 0.02$). Results indicated that *βDef-2* expression was higher at 3 dpi in CTRL and CFA groups than in ATB ($P = 0.01$ and 0.06, respectively). On days 0 and 7 dpi, there was no difference among treatments. *GPX2* gene was also more expressed at 3 dpi in pigs fed CFA diet compared to pigs fed ATB or COL ($P = 0.005$ and 0.05, respectively).

Table 1. Intestinal gene expression of immune factors involved in innate and adaptive immunity after *Salmonella* challenge

	RELATIVE EXPRESSION[1]			*P* value	
GENE	Day 0	3 dpi	7dpi	0 vs 3	3 vs 7
IL-1β	0.444	0.216	0.447	0.004	<0.001
IL-6	0.773	0.941	0.825	0.001	0.02
TNFα	0.798	0.911	0.839	0.04	0.35
IL-8	0.265	0.594	0.419	<0.001	0.005
MCP-1	0.448	0.648	0.470	<0.001	<0.001
IFN-γ	0.162	0.589	0.522	<0.001	0.55
COX2	0.284	0.484	0.360	<0.001	0.009
GPX2	0.422	0.849	0.579	<0.001	<0.001
βDef-2	0.477	0.690	0.453	<0.001	<0.001

[1]The Ct of genes of interest were normalised to Ct values of housekeeping genes and the relative mRNA expression of each gene were calculated as $2^{-\Delta\Delta Ct}$.

Conclusions and Discussion

Our results showed that *Salmonella* infection induced proinflammatory cytokine signalling in the ileum, as reported previously (2, 3) and other immune factors, such as *COX-2*, *GPX2* and *βDef-2*. Dietary supplementation with feed additives and bovine colostrum as alternative to antibiotics influenced the expression of some genes after *Salmonell*a infection compared to pigs fed CTRL or ATB diets.

Acknowledgments

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Flexible adjuvants for combined live Aujesky's disease and inactivated swine influenza vaccines

J Ben Arous[1], A Shevtsov[2], S Remyga[2], O Goryushev[2], S Deville[1], L Dupuis[1]
[1] *SEPPIC, 22 Terrasse Bellini, Paris La Défense, 92806 Puteaux Cedex, France,* [2] *FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"), Yur'evets, 600901 Vladimir, Russia,* juliette.benarous@airliquide.com

Introduction

Live attenuated and inactivated antigens are both widely used for pig vaccination. In the field, animals receive multiple vaccines against diverse diseases in a short time, and there is a strong demand for compatible and mixable vaccine formulations comprising diverse types of antigens. However, as inactivated vaccines usually contain vaccine adjuvants, whereas live vaccines are usually not adjuvanted and not compatible with adjuvants, these types of vaccines are not mixable. Diverse adjuvants technologies are used for swine inactivated vaccines, such as oil emulsion adjuvants, aluminium hydroxide and polymeric adjuvants.

We have shown previously that Montanide™ adjuvants based either on polymer technology or oil in water emulsions were compatible with a PRRS live vaccine, and enhanced the protection to challenge of vaccinated animals. These adjuvants also allowed the reduction of the antigenic load in a live PRRS vaccine (1).

Here we show that these Montanide™ adjuvants can be used to formulate highly efficient inactivated swine influenza vaccines, and are also compatible with an Aujesky's disease live vaccine. They can therefore allow the formulation of efficient combined inactivated/live vaccines for swine against swine influenza and Aujesky's disease.

Materials and Methods

Inactivated vaccines against Swine influenza virus (SIV) were formulated with either the polymer adjuvant Montanide™ Gel 01 (Gel), an oil in water emulsion adjuvant Montanide™ ISA 15A VG (ISA 15A) or without adjuvant (Table 1). All vaccines contained the same antigenic dose. A non vaccinated group was included as a negative control.

Table 1. Vaccination groups

Groups	Adjuvant	% of use
GEL	Montanide™ Gel 01	10
ISA 15A	Montanide™ ISA 15A VG	15
ANTIGEN	No adjuvant	/
CONTROL	Not vaccinated	/

At day 0 and day 21, 10 seronegative pigs (15 kg) were vaccinated in each group intramuscularly in the neck simultaneously with 2ml of the corresponding inactivated SIV vaccine and 2ml of Aujesky's disease attenuated live vaccine.

Safety properties of the vaccines (systemic and local reactions) were assessed at vaccination, during the trial and at slaughter. Efficacy was followed both by serological analysis and application of a challenge procedure. Antigen specific ELISA analyses against Aujesky's virus and SIV were performed at day 0 (before vaccination), day 21 (before revaccination), day 42 (before virulent challenge) and day 56 (at slaughter).

At day 42, 5 animals in each group were infected with Aujesky's virus, and 5 other animals were infected with SIV. Clinical signs and hyperthermia after challenge were measured for 14 days, and nasal viral loads were scored. Bacterial over-infections of the lungs were scored at slaughter at day 56.

Results

All vaccines tested were safe. Antibody titers against swine influenza virus were significantly superior for Gel or ISA 15A adjuvanted formulations compared to the non adjuvanted vaccine. Immune response after vaccination with adjuvanted vaccines reached positive protective threshold at 21 days after only one injection, whereas in the non adjuvanted group, protective antibody level was not reached before the infective challenge.

Protection against SIV challenge was also improved, as hyperthermia after infection, lung lesions at slaughter and nasal viral shedding were reduced compared both to non vaccinated and non adjuvanted groups.

Moreover, both adjuvants were compatible with Aujesky's disease live vaccine. Protection conferred by the attenuated Aujesky's disease part of the vaccine was not reduced by the presence of either adjuvant. Moreover, viral shedding after Aujesky's challenge was reduced in the Gel group.

Conclusions and Discussion

These results show that relevant aqueous adjuvants such as Montanide™ Gel 01 or Montanide™ ISA 15A VG are compatible with both inactivated and attenuated viral vaccines for swine. These adjuvants are highly effective in improving SIV vaccine protective efficacy and do not impair the efficacy of co-administered live vaccine.

Therefore, such adjuvants can allow the formulation of multivalent combined inactivated/live vaccines. Such combined vaccines could lead to the reduction of the number of injections given to pigs in the field.

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The experience of using diferent vaccines against PRRS in a 800-sow farm in South China

Dedicated in the memory of Yang Xianjin

B Fang [1], Z Ao [2], L Zhu[1], T Tan [1] G Chen[1]

[1]*Boehringer Ingelheim Int'l Trading (Shanghai) Co. Ltd., Beijing100004, China*

[2]*Shantou Dongjiang Animal Husbandry Co., Ltd. China* tao.tan@boehringer-ingelheim.com

Introduction

Highly pathogenic porcine reproductive and respiratory syndrome (HP-PRRS) is one of the important swine diseases for Chinese pig farming industry, causing high loss for both sows and growing pigs [1]. PRRS vaccines have been considered the most useful tool to control the fatal disease but not all PRRS vaccines in China are efficacious. This study focuses in the result of using several kinds of PRRS vaccines in an 800-sow farm in south China from 2008 to 2013.

Materials and Methods

The study was conducted in an 800-sow farm in south China with a continuous flow system. The farm applied different vaccines in breeding herd and piglets during the period from 2007 and 2013. A Time series plot was used to monitoring mortality in suckling pigs, nursery and fattening pigs. Also a SPC-Individual Chart was analyize with Born to Market mortality. no other significant changes occurred in the farm in regards health programs implementation (vaccine or medication) during the period monitored. PRRS vaccines used in this farm was listed belowAug. 2007-Aug. 2008: Inactivated vaccine (strain CH-1R), produced by a local company, ;

Sep.2008-Jul.2010: Live vaccine (VR2332), Boehringer Ingelheim,

Aug.2010- Sep.2011:Live attenuated vaccine (JXA1), produced by a local company,

Oct.2011-Dec.2012; Live attenuated vaccine (VR2332), produced by Boehringer Ingelheim,

Jan.2013-May.2013; Live attenuated vaccine (TJM-f92), produced by a local company.

The mortality rates in lactation, nursery and fattening period were recorded in a quarterly basis

Results

In August 2008, highly pathogenic PRRS affected the herd in this farm. The abortion and mortality of sows were around 30%, 10% respectively while the mortality in suckling, nursery and fattening pigs were about 20%, 30% and 10%, respectively. The production got back to levels before the outbreak (baseline) after vaccination with Ingelvac® PRRS MLV. When the farm changed VR2332 to other PRRS vaccines, the mortality in suckling, nursery and fattening were increased sharply According to the mortality monitoring and vaccines logged at each period, Ingelvac® PRRS MLV showed better efficacy performanced than the other vaccines implemented even when it is compared with vaccine HP PRRS (TJM-f92) based vaccine.

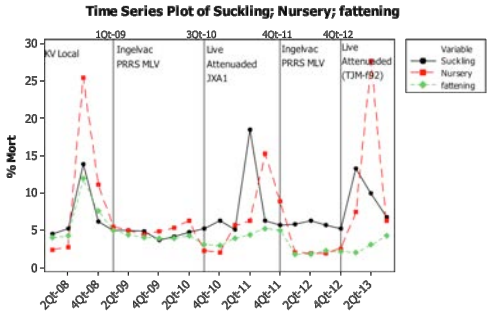

Figure 1. Mortality in suckling, nursery and fattening pigs from 2008 to 2013

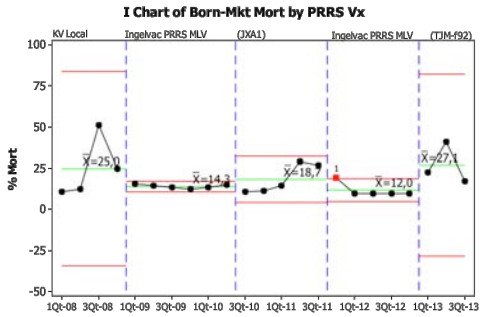

Figure 2. SPC chart of born to market mortality

Discussion

This study mainly focused on the mortality of pigs in this farm after using different PRRS vaccines from 2008 to 2013. The monitoring results showed poor efficacy performance using CH-1R, JXA1 and TJMF92 based vaccines compared to VR2332 based vaccine,. This also marks the importance ofconsistency in PRRS vaccines related to have impact inefficacy expressed by mortality reduction. Also, this re-confirms the relevance of implementing a good measurement process during implementation of control strategies.

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Safety and efficacy of an intramuscular vaccination against *M. hyopneumoniae* using needle-free injection devices

D Mouzin[1], S Wu[1], J Escala[2], G Labarque[3]
[1]*Elanco,Greenfield, Indiana, United States,* [2]*Elanco, Basingstoke, United Kingdom,* [3]*Elanco, Antwerp, Belgium,* mouzin_douglas_e@elanco.com

Introduction

The injection of veterinary medicinal products in pigs using a needle may cause safety issues for pigs (abscesses, iatrogenic transmission of pathogens), swine holders and their staff (needle-stick injuries), and consumers (residual needle fragments in carcasses) (4). Several studies have shown that the efficacy of either vaccines (5,6), antibiotics (3,7), or iron (1,2) following an intramuscular administration using a needle-free injection device was at least equivalent or even superior than the one following an intramuscular administration of the same veterinary medicinal products using a needle. The aim of the present vaccination-challenge study was to assess the safety and efficacy of an intramuscular vaccination with Stellamune® One at 21 days of age using two needle-free injection devices.

Materials and Methods

A total of 108 *M.hyo*-seronegative pigs were randomly divided into 6 groups and vaccinated at 21 days of age following the experimental design as shown in Table 1. Rectal temperatures and injection sites were measured for 5 days post-vaccination (DPV). At 14 DPV, 2 randomly selected pigs from each group were euthanized for histopathological examinations of injection sites. The remaining pigs underwent an intratracheal challenge with a lung homogenate of a virulent *M.hyo* strain at both 14 and 15 DPV (35 & 36 days of age). Another 8 pigs were not challenged and served as negative controls. Pigs were necropsied at 28 post-challenge (DPC) and the extent of their lung lesions was determined, using the scoring method described in the *European Pharmacopoeia* monograph 2448. Broncho-alveolar lavage fluids (BALF) were collected to determine *M.hyo* quantities by polymerase chain reaction (PCR). Arcsine square root-transformed data of the lung lesion scores and *M.hyo* quantities in BALF were analyzed by a mixed linear model (SAS version 9.3, SAS Institute, Cary, NC, USA) that included the fixed effect of treatment and the random effect of room.

Results

None of the pigs displayed general and local reactions following the vaccinations and the histopathology of the injection sites at 14 DPV did not reveal pathological changes. The non-challenged control pigs did not display lung lesions and no *M.hyo* was detected in their BALF. Both lung lesion scores and *M.hyo* quantities in BALF were significantly ($P<0.05$) lower in vaccinated pigs when compared to control pigs within device. Lung lesion scores of vaccinated pigs were reduced 81%, 68%, and 85% when compared to those of control pigs, when using a traditional needle, the AcuShot™ Needle-Free Injector and the Pulse® 250, respectively. Similarly, *M.hyo* quantities in BALF of vaccinated pigs were reduced 77%, 61%, and 63% when compared to those of control pigs.

Table 1. Lung lesion scores and *M.hyo* quantities in BALF at 28 days post-challenge.

Group	No. of pigs	Vaccination		Lung lesion score	*M.hyo* genomic copies/ml BALF
		Vaccine	Device		
A	12	Saline	Needle	14.43^{A}	95540^{A}
B	24	Stellamune®	Needle	2.76^{B}	22268^{B}
C	12	Saline	AcuShot™	14.22^{A}	75750^{A}
D	24	Stellamune®	AcuShot™	4.59^{B}	29697^{B}
E	12	Saline	Pulse® 250	19.07^{A}	80967^{A}
F	24	Stellamune®	Pulse® 250	2.85^{B}	29786^{B}

^{A,B}: Superscripts indicate statistically significant differences between groups within device ($P<0.05$).

Conclusions and Discussion

The results of this study indicate that the intramuscular administration of Stellamune® One using both a conventional needle and needle-free injection devices is safe and efficacious, as demonstrated by the absence of general and local reactions following vaccination and significantly lower lung lesions and *M.hyo* quantities in BALF in vaccinated than in control pigs upon challenge.

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Comparison of the field efficacy of two commercial APP vaccines in a large pig farm with acute outbreaks of pleuropneumonia

V Goman[1], M Orlov[1], S Kukushkin[2], N Nikulin[2], S Chernyshov[2], I Samsonov[2]
[1]*Doronichi farm, Kirov region, Russia,* [2]*Department of Animal Health, Boehringer Ingelheim LLC, Moscow, Russia,*
sergey.kukushkin@boehringer-ingelheim.com

Introduction

Actinobacillus pleuropneumoniae (App) is the etiologic agent of pleuropneumonia in pigs (1). The goal of this study was to compare the field efficacy of two commercial APP vaccines in a large pig farm with acute outbreaks of pleuropneumonia.

Materials and Methods

The study was conducted in a large multisite farrow-to-finish farm (6,000 sows) with circulation of virulent APP (serotype 2) and acute outbreaks of pleuropneumonia in the fattening site. All pigs in the farm included this trial were vaccinated against PCV2 with CircoFLEX® (Boehringer Ingelheim) at 21 days old and APP at 35 and 55 days old as routine. Traditionally pigs demonstrated typical clinical and postmortem lesions of APP at 100-110 days old. According to a serological study (IDEXX APX IV ELISA test kit) after 120 days old pigs became positive to toxin APX IV of APP. Before this trial all pigs vaccinated against APP with Coglapix vaccine (CEVA Sante Animal) for the last two years. The trial included 5398 pigs which were vaccinated with Ingelvac APPX® (Boehringer Ingelheim) and other 5430 pigs vaccinated with Coglapix as control. Main characteristics of APP vaccines to present in table 1.

Table 1. Main characteristics of APP vaccines in trial

Vaccine	Dose	Composition
Coglapix	2 ml	5 strains of APP (data about the serotypes not available) and toxoids APX I, II, III
Ingelvac APPX	2 ml	serotypes 1, 2, 3, 4, 5, 7 of APP and toxoids APX I, II, III

Control groups were kept under same management conditions as treated pigs, in different rooms on the same buildings and site (side-by-side). The Chi-square test was applied to analyse the results. Lung lesions score were estimated in slaughterhouse according to Christensen G. et al. (1999).

Results

The trial results show in table 2 and figure.

Conclusions and Discussion

This results demonstrate good efficacy of Ingelvac APPX® vaccine which provided more market pigs than routine vaccine used before. Pigs from treated groups demonstrated less affected lungs than in Coglapix groups.

Table 2. Main results for nursery and finishing sites

Parameters	Coglapix	APPX	X^2
Nursery (28-80 d.)			
Number of pigs, n	5430	5398	
Died, %	1.27%	0.76%	7.04*
Cull, %	1.79%	1.48%	1.57
Total losses, %	3.06%	2.24%	6.98*
Finishing (81-180 d.)			
Number of pigs, n	5069	5101	
Died, %	3.10%	2.57%	2.59
Cull, %	2.56%	1.98%	3.92*
Total losses, %	5.66%	4.55%	6.51*
Slaughter weight, kg	104.2±0.8	104.6±1.3	
Slaughter age, days	178.0±3.0	179.3±4.0	
Total mortality nursery-finishing, %	4.16%	3.19%	7.28*
Total losses nursery-finishing, %	8.38%	6.54%	13.27*
Average % of affected surface out of all lungs	6.43	1.08	
Average % of affected surface out of lungs with active pneumonia	9.26	5.57	
Lungs with pleurisy, %	78.6%	62.5%	5.27*

* The results are significantly different.

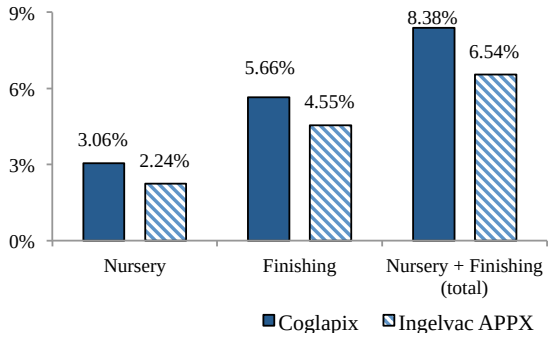

Figure 1. Total losses of pigs in nursery and finishing sites in treated and control groups.

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Effect of sample investigation of economic losses of FMD vaccination-related abnormal meats at the injection site collection material on detection of PRRSV antibody in oral fluid

EY Ko[1], HK Jeong[1], DK-Lee[1], JH-Han[2]

[1]*Dodram Pig Farmer's Cooperative, Icheon city, Gyeonggi-do, Republic of Korea*

[2]*College of Veterinary Medicine, Kangwon National University, Chuncheon, Gangwon-Do 200-701, Republic of Korea,* misty_olive@naver.com

Introduction

Foot-and-mouth disease (FMD) caused by FMD-type O virus was occurred in South Korea from Nov. 2010. Early on The Ministry of Food, Agriculture, Forestry and Fisheries implemented slaughter policy, but it failed. The government determined to use of nationwide vaccination from Dec. 2010 until now. However, vaccination-related side effects, abnormal meats such as intramuscular abscess and granuloma at the injection site produced critical problems. Degraded pork quality due to vaccination-related side effects led to huge economic losses in Korean pork industry .

This survey investigated economic losses due to abnormal meat from 2010 before vaccination to 2012 after FMD vaccination in South Korea and experimental relationship between needle type (needle and needle-free) and injection site (brachiocephalic and gluteal m.)

Materials and Methods

The incidence of abnormal meats was investigated 243,234, 177,783 and 283,383 pigs from 2010 to 2012, respectively. The pigs were vaccinated with 2 ml at 8 weeks old by brachiocephalic muscular injection. And the cost of economic losses due to abnormal meats was calculated. It is calculated by multiplying the weekly sale price by weight of abnormal meat from the injection site. To prevent further economical losses, following study was conducted. Total 8 weeks old 276 pigs were classified into 3 different groups; A) brachiocephalic region with needle, B) gluteal region with needle, and C) brachiocephalic region with needle-free, and each group received single dose of FMD vaccination at 8 weeks old. The examination on the injection sites was performed at 25 weeks old to confirm the occurrence of abnormal meat associated with economic loss.

Results

Table 1. Economic losses due to abnormal meats by FMD vaccination from 2010 to 2012 (Unit: million won)

M / Yr	1	2	3	4	5	6	7	8	9	10	11	12	Total
10	20	28	44	36	28	18	24	16	18	26	22	26	306
11	18	16	221	261	212	148	191	158	168	153	175	285	2006
12	284	264	200	216	256	212	248	260	240	212	241	212	2845

Table 2. Total economic losses by FMD vaccination with injection site and needle type

Group	Total No. tested	Abnormal meats (kg)	Abnormal meats/pig (kg)	Economic losses (won)	Economic losses/pig (won)
A	71	10.9	0.15	41,418	**583**
B	70	3.3	0.05	12,458	**178**
C	135	91.0	0.67	343,525	**2,545**

Conclusions and Discussion

In the investigation of abnormal meats by FMD vaccination from 2010 to 2012, The cost of economic losses was 306 million won in 2010 before FMD vaccination but increased to 2,006 million won in 2011 and 2,845 million won in 2012 after FMD vaccination. The resultings were marked increase of economic losses (about 6.5 fold). In the experimental study, Group B was the lowest economic losses per pig among experimental groups. Group C was higher than those of other groups and economic losses were higher about 14 times than group B. According to the results, gluteal muscular injection with needle is recommended.

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Early vaccination with Stellamune® once enhances finishing herd performance compared to a two-dose *M. hyopneumoniae* vaccination

A Hidalgo[1], A Cox[2], G Labarque[3]
[1]*Elanco AH, UK,* [2]*Adrian Cox Consultancy, UK,* [3]*Elanco AH, Belgium,* hidalgo_alvaro@elanco.com

Introduction

Mycoplasma hyopneumoniae (*M.hyo*) is the primary pathogen of enzootic pneumonia (EP), a chronic respiratory disease in pigs causing major economic losses to the pig industry worldwide (1). Infection by *M.hyo* may occur before weaning (2). Early vaccination with Stellamune® Once at 7 days of age has been shown to provide a protective immunity starting from 21 days of age following a single 2-ml administration (3). This study aims to compare the efficacy of a single-dose vaccination with Stellamune® Once at 7 days of age with a two-dose *M.hyo* vaccination regime at 7 and 23 days of age.

Materials and Methods

This study was conducted in a *M.hyo*-positive 400-sow farrow-to-finish herd between November 2012 and June 2013. Piglets from 8 consecutive batches of production were randomly allocated into two treatment groups. Stellamune group (n=667 pigs) was vaccinated with a single dose of Stellamune® Once (Elanco AH) at 7 days of age. Group B (n=664 pigs) was vaccinated with M+PAC® (MSD AH) at 7 and 23 days of age, being the routine vaccination regime in farm. Ten blood samples were collected per treatment group at 4, 13 and 20 weeks of age and tested using DAKO® Mhyo ELISA (Oxoid Limited, Hampshire, UK); moreover samples from 20 week old pigs were tested for antibodies against swine influenza (SI) virus using HAI test. The efficacy of both *M.hyo* vaccines was evaluated using performance parameters [average daily weight gain (ADWG); days to slaughter; number of light pigs, defined as pigs not reaching slaughter weight within the first 5 weeks of the selling process; mortality] and lung lesion examinations at slaughter as previously described (4).

Results

A summary of ADWG by experimental group is presented in Table 1. Overall, pigs vaccinated with Stellamune® Once performed better, gaining 13.2 g/d more from weaning to slaughter than pigs in group B. Statistically significant differences in ADWG could not be confirmed due to the limited number of batches analyzed. However, during the finishing period, the pigs in the Stellamune group gained 33 g/d more. Pigs vaccinated with Stellamune® Once reached slaughter weight 4.3 days earlier than pigs in group B, at 155.3 and 159.6 days respectively (p=0.08). In addition, vaccination with Stellamune® Once reduced the number of light pigs compared to vaccination with the two-dose *M.hyo* vaccine, from 42% to 34% (p<0.05). Blood tests confirmed a serological response to *M.hyo* exposure during the finishing stage and excluded SI. A total of 591 pigs were investigated at slaughter. There was no statistical difference between the average EP-like lung lesion scores in the experimental groups (p=0.236; 3.28 Stellamune group and 2.87 group B). 1.33% of the lungs in group B was attached to the carcass and could not be assessed. The involvement of *M.hyo* in the EP-like lesions was confirmed by histopathology. Mortality in the Stellamune group was 1.5% during the study, increasing up to 2.56% in group B (p=0.179).

Table 1. ADWG by vaccination group.

	ADWG (g/d)		
	Stellamune	Group B	difference
Wean-slaughter	574.7	561.5	13.2
Finishing period	713.4	680.4	33.0

Conclusions and Discussion

Under the conditions of this study, vaccination with Stellamune® Once at 7 days of age was effective in controlling lung lesions due to *M.hyo*. In addition, when performance parameters were examined, early vaccination with Stellamune® Once produced more heavy pigs and saved an average of 4.3 days of finisher accommodation costs when compared to a two-dose *M.hyo* vaccination. Similarly, an increase in carcass weight has been described before when Stellamune® Once was compared to another vaccination regime (5). It is noteworthy that during this study, the levels of pleurisy detected at slaughter dropped markedly when compared to historical data and then peaked again after the study, when historical routine vaccination continued. Together with improved performance, vaccination with Stellamune® Once had the benefit of reducing labor, being more convenient and being easier to implement in routine farm practices than a two-dose vaccination regime

In conclusion, early intervention with Stellamune® Once enhanced performance of the finishing herd while effectively controlled lung lesions due to *M. hyopneumoniae*.

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Comparative effects of vaccination against PCV2 and PRRSV in a PCV2-PRRSV challenge model

C Chae[1], C Park[1], HW Seo[1], DD Tien[1]
[1]*Department of Veterinary Pathology, College of Veterinary Medicine, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-742, Republic of Korea,* swine@snu.ac.kr

Introduction

PRRSV increases the PCV2 DNA load in the sera and tissues of co-infected pigs (1,2). Based on these results, one possible way to minimize the effects of the PRRSV-associated enhancement of the replication of PCV2 and the induction of PMWS may be the use of a PRRSV-based vaccination in preweaned pigs. However, there are no reports in the literature describing the effects of PCV2 and PRRSV challenges on pigs that have been immunized with either the PCV2 or PRRSV vaccines. Therefore, the objective of the present study was to determine the effects of PCV2 and PRRSV vaccinations in an experimental PCV2-PRRSV challenge model.

Materials and Methods

A total of 72 pigs were randomly divided into 9 groups (8 pigs per group). At 21 days of age, pigs in groups 1 and 2 were immunized with the PCV2 vaccine(Fostera PCV™ vaccine), pigs in groups 3 and 4 were immunized with the PRRSV vaccine(Ingelvac® PRRS MLV), pigs in group 5 were immunized with both the PCV2 and PRRSV vaccine. At 49 days of age (0 dpc), every pigs excepting group 9 were intratracheally administered an 3 ml dose of PCV2b (strain SNUVR000463) containing 1.2 x 10^5 $TCID_{50}$/ml and/or 3 ml of PRRSV (strain SNUVR090851; North American genotype) containing doses of 1 x 10^5 $TCID_{50}$/ml. Pigs in groups 1 and 6 were challenged with PCV2. Pigs in groups 3 and 7 were challenged with PRRSV. Pigs in groups (2, 4, 5, and 8) were challenged with both PCV2 and PRRSV. Blood samples from each pig were collected by jugular venipuncture at -28, 0, 10, and 21 dpc for detecting viremia. All pig was necropsied at 21 dpc. And immunohistochemistry and histopathological analysis were conformed. The continuous data for PCV2 DNA and PRRSV cDNA quantifications were analyzed using an ANOVA for each time point. Discrete data were analyzed by the Chi-square and Fisher's exact tests. A value of $P < 0.05$ was considered to be significant.

Results

Vaccination against PCV2 significantly reduced PCV2 viremia, PCV2-induced lesions, and PCV2-antigens in the dual infected pigs. However, vaccination against PCV2 did not affect PRRSV viremia, PRRSV-induced lesions, and PRRSV antigens in the dual infected pigs. But vaccination against PRRSV did not reduce PRRSV viremia, PRRSV-induced lesions, and PRRSV antigen in the dual infected pigs. In addition, vaccination against PRRSV increased PCV2 viremia, PCV2-induced lesions, and PCV2-antigens in the dual infected pigs.

Conclusions and Discussion

The impairment of the protective cell-mediated immunity against PRRSV by PCV2 in the PRRSV vaccine-PCV2-PRRSV model may allow for the increase in PRRSV viremia, PRRSV-associated lesions, and PRRSV antigens rather than their reduction following the PRRSV vaccine. Because these 3 parameters were significantly reduced in pigs which received PRRSV vaccine and followed by PRRSV challenge only compared with pigs which challenged with PRRSV only, our results ruled out its possible limited efficacy against a heterologous virus.

These results suggest that the reduction of PRRSV viremia by the PRRSV vaccine is affected by the pig's PCV2 infection status. Our results are clinically meaningful, indicating that swine practitioners should check PCV2 infection statuses in pigs before PRRSV vaccines are used to control PRRSV infection in swine herds.

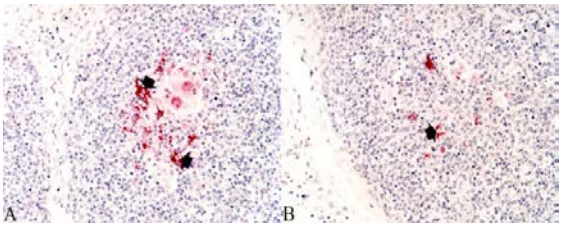

Figure 1. Immunohistochemistry for the detection of PCV2 antigen in lymph node. **A:**PCV2 antigen from pigs which received PRRSV vaccine followed by dual challenge (group 4) at 21 dpc. **B:**PCV2 antigen from pigs which were challenged with PCV2 (group 6) at 21 dpc.

Acknowledgments

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Efficacy and non toxicity of a bivalent acellular vaccine formulation of proteoliposome against *Leptospira spp* serovars of epidemic interest in swine populations

DF Arencibia Arrebola[1], LA Rosario Fernández[2], N Batista Santiesteban[1], JF Infante Bourzac[1], Y Valdés Abreú[1], B Tamargo Santos[2], VG Sierra Gonzalez[1]

[1]*Finlay Institute. 198 street and 27 avenue. Number 19805. La Lisa. Havana. Cuba.* [2]*Institute of Pharmacy and Food Science (IFAL, U.H). 222 street between 25 and 27 avenue. La Lisa. Havana. Cuba,* darrebola@finlay.edu.cu

Introduction

Leptospirosis constitutes one of the zoonosis with more impact in the veterinary and human health. The available leptospiral vaccines are fundamentally of inactivated whole cells and most for veterinary use. Although these formulations are effective they have multiple limitations, among those that it not stands out the strait margin of crossed protection against serovars included in the vaccine, low immunologic memory, absence of cellular immune response and reactogenicity. It is that the serovars of more incidences in pig populations in the word are Canicola, Ballum, Arborea, Mozdoc y Cophenageni. The aim of this work was evaluated the effectiveness and toxicity of a new bivalent acellular vaccine formulation (*Leptospira spp* Canicola and *Leptospira spp* Mozdok).

Materials and Methods

In the candidate's vacunal evaluation it was used pigs of Yorkshire breed for the study of immunologic characterization and Syria hamsters for the challenge and toxicity studies. During the study it was evaluated the antibodies response and generated cytokines as well as the protection for *in vivo* lethal challenge and the toxicity to single, repeated dose and local tolerance.

Results and Discussion

The immunization (schedule of two doses with an interval of three weeks) in Yorkshire pigs generated a potent response of IgM after the first dose which it is maintained for 8 weeks after the second dose, the IgG response it increases significantly regarding the values of the first dose and it is maintained for 16 weeks. The cellular response was potent when being increased the cytokines expression as IFN γ and IL1 from the first dose. The determination of CD45+ as indicator by immunologic memory it showed that this memory was present until 16 months (last determination). The *in vivo* evaluation against lethal challenge with 100 000 LD_{50} of the serovares Canicola, Ballum, Mozdok and Cophenageni in Syrian hamsters it evidenced a protection of 100% in the immunized animals and challenged. In all cases the elimination of carrier state was also verified in kidneys, liver and lungs. The evaluation in toxicity studies reveals the absence of damage in the main immunologic organs, while the evaluation of the damage in the immunization area through histopathological studies only evidences damage associated to the use of the needles**.**

Conclusions

The obtained results endorse the effectiveness and non toxicity of the new vacunal candidate evaluated that it includes: immunogenic, generator of immunologic memory, crossed protection, non systemic and local toxicity, with regard to the traditional vaccines; it is recommended the continue studies of effectiveness against other serovars not included in this study and the beginning of the clinical trials in pig populations affected with Leptospirosis.

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Comparison of Porcilis® *M. hyopneumoniae* once administered at three weeks of age with a classical intramuscular vaccination in a field study

F Voisin[1], A Trotel[1], E Pagot[1], D Roudaut[2], L Volant[2], M Rigaut[2]
[1]*CTPA Department, ZOOPOLE développement, Ploufragan, France* [2]*MSD, Beaucouzé, France,*
florian.voisin@zoopole.asso.fr

Introduction

Different vaccination protocols are used worldwide against *M. hyopneumoniae* (M.h) caused diseases in pigs. Intramuscular vaccination is the most common route.

The objective of this study was to compare an intramuscular (Suvaxyn® M.Hyo mono), and intradermal vaccination (Porcilis® M Hyo ID once) applied with the IDAL® intradermal injector under field conditions.

Materials and Methods

For this multi-centric, contemporary, controlled, randomized and blinded trial, three farrow-to-finish farms were selected in France (Brittany). Per farm, 2 to 3 batches were included during winter 2012-13 (7 batches in total). The 2,348 included piglets were individually identified, weighed and randomized at 21 days of age (Table 1). A negative control group, injected intramuscularly with Diluvac®, was also included.

Table 1. Treatment groups

	N
Porcilis® ID once (Porc)	941
Suvaxyn® M.Hyo mono (Suv)	937
Diluvac® (Cont)	470

The pigs were weighed individually before the first departure to the slaughterhouse of their batch. Lung lesions at the slaughterhouse were scored according to Madec[1] (scale of 28). Individual treatments and mortalities were recorded.

Adjusted analysis of variance and Pearson Chi-square and a Kruskall-Wallis non-parametric test were used to test respectively the average daily gain (ADG), the pneumonia, treatment and mortality rates and the pneumonia scores.

Results

In total 2,348 pigs were included, and 2,212 weighed at the first departure to the slaughterhouse. The growth results were not different between groups (average ADG=691.8 g/d, p = 0.975).

The percentage seroconverted control animals was 31.7% as a whole, with 0 and 14% in two batches and 33 to 60% for the 5 remaining batches.

A total of 1,570 lungs were observed at the slaughterhouse, corresponding to 50 to 80% of animals in each batch. The number of lungs with 7 lobes was 1,265, while 305 lungs were missing one or more of the lobes due to removal at the slaughter line. Lung lesions, mortalities and individual treatments are presented in Table 2.

Table 2. Lung lesions, individual respiratory treatments and mortalities

Group	Porcilis	Suvaxyn	Control	p
% Pneumonia free lungs	49.1	47.4	46.1	0.716
Pneumonia score of all lungs*	1.8[a]	2.1[ab]	2.7[b]	0.144
Pneumonia score of affected lungs only	3.6[a]	3.9[a]	4.9[b]	0.006
% of lungs with lesion score >5	8.9[a]	11.6[ab]	16.7[b]	0.007
% Treatment	4.3[a]	6.1[ab]	7.0[b]	0.083
% Mortality	5.0	4.2	6.4	0.192

*average based on all scored lungs, including those where 7 lobes were not present [a,b] different superscript within a row is significantly different.

Conclusions and Discussion

Although the study was designed to be able to detect a 15 g/d difference in ADG between vaccinated groups, such difference was not detected. The observed ADG is in the Breton average[2].

The seroconversion rate in the control group was extremely variable, supporting a batch effect of M.h circulation. Pneumonia rate is also lower than in other studies[3,4], confirming the low M.h. pressure in the included batches. Despite this, both vaccines significantly reduced pneumonia score and Porcilis also significantly reduced rates of severely affected lungs and treatment. In addition, Porcilis Mhyo ID Once has several benefits over intramuscular vaccination, including no needle breakage or carcass damage, lower vaccine volume and operator/animal friendly.

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Effect of vaccination with FLEXcombo® on productivity comparing with conventional vaccination program in a south China farm

Dedicated to the Memory of Xianjin Yang

S Sun[1], L Chen[2], G Chen[1], T Tan[1]
[1]*Boehringer Ingelheim Int'l Trading (Shanghai) Co. Ltd, Beijing100004, China*
[2]*Henan Agricultural University, Zhengzhou450008, China*
tao.tan@boehringer-ingelheim.com

Introduction

The benefits of combined vaccination against separate vaccination have been demonstrated previously[1, 2]. The objective of this study was to compare the efficacy of of FLEXcombo (mixing of Ingelvac CircoFLEX® and Ingelvac® MycoFLEX) to the separated vaccination by measuring fattening performance on a farm in the south of China.

Materials and methods

The study was performed in a 1200-sow commercial farm located in the south of China with a continuous flow. The breeding herd was vaccinated against CSF, PR, FMD, AR, JE and PPV while the piglets were vaccinated against CSF, PR, FMD and HP. Although there had not been obvious clinical signs of PCV2 and M. hyopneumoniae, PCR test showed these two pathogens were circulating in this farm. The farm decided to implement PCV2 and M. hyopneumoniae vaccines and a field trial was conducted in April 2012 to evaluate the efficacy of FLEXcombo in comparison to the separated vaccination with CircoFLEX and MycoFLEX as well as a non-vaccinated group.

A total of 312 piglets from 30 sows were divided into 3 groups. In group 1, 106 piglets were vaccinated with FLEXcombo at 14 days of age, 2ml per pig; in group 2, 108 piglets were vaccinated with CircoFLEX (1ml per pig) at 2 weeks of age and MycoFLEX (1ml per pig) at 3 weeks of age. 112 piglets in the control group were treated with physiological saline at 2 and 3 weeks of age, 1ml per pig each time. The bodyweight in group1, 2 and control group at 2 weeks of age were 5.10 kg, 5.03 kg and 5.12 kg, respectively.

Pigs in these three groups were raised in different barns on the same site under the same management and housing conditions like ventilation and temperature. Bodyweight was recorded at 160 days of age while mortality and average daily gain (ADG) were also documented.

Results

Mortality in group 1 and 2 were 2.83% and 2.77%, respectively, which was lower than 3.57% in the control group. Average daily gain (ADG) in group 1 and 2 were 693g and 686g, respectively, which were higher than 658g in the control group (Table1). The mortality and ADG in both group 1 and 2 were not significantly different.

Table 1. Relevant parameters in the control and the vaccinated groups.

	Group 1 FLEXcombo	Group 2 Separate Circo+Myco	control
Number of pigs	106	108	112
Survival	103	105	108
Mortality	2.83%	2.77%	3.57%
Average body weight at day 14 (KG)	5.10	5.03	5.12
Average body weight at day 160 (KG)	106.25	105.19	101.19
Weight gain(KG)	101.15	100.16	96.07
ADG (g)	693	686	658

Conclusions and Discussion

In this study, vaccination of CircoFLEX and MycoFLEX improved the productivity of pigs comparing with the control group. There was a slight difference (+0.07 g/day) between group 1 (FLEXcombo) and group 2 (separate CircoFLEX and MycoFLEX vaccination) which may be due to a reduced stress in vaccination and handling, which is very important for Chinese pig industry where pigs have been vaccinated with many kinds of vaccines, The cost of labor savings should be evaluated in future studies.

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Field evaluation on the effect of antibiotics and *Mycoplasma* vaccination for the control of respiratory diseases in a thai PRRSV positive herd

P Sitthicharoenchai[1], S Jittimanee[1], Y Woonwong[1], K Poonsuk[1], J Arunorat[1], U Kanyook[1], K Kaewkawin[1], M Watcharathai[1], W Buthasane[1], M Lumyai[3], M Makhanon[2], R Thanawongnuwech[1]

[1]*Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand,* [2]*Novartis Animal Health (Thailand) Co., Ltd., Bangkok, Thailand,* [3]*Thai-Denmark Swine Breeder PCL, Bangkok, Thailand,* roongroje.t@chula.ac.th

Introduction

Respiratory disease is a major problem in the swine industry. The common respiratory disease pathogens present in swine farms include PRRSV and *M. hyopneumoniae*(M hyo). When occurring simultaneously or in combination, it results in severe clinical respiratory manifestation in pigs known as porcine respiratory disease complex (PRDC). To prevent and control M hyo in pig farms, good management practice and housing conditions, antibiotics and vaccines are routinely used in most commercial swine farms (1). The study objective was to determine the effect of tiamulin hydrogen fumarate (THF) in combination with inactivated M hyo vaccination and to obtain a suitable protocol for antibiotic and vaccine use in controlling PRRSV-positive swine herds.

Materials and Methods

To evaluate the efficacy of the combination of antibiotic and vaccine usage, clinical signs, the production indices, serological analysis, bacteriological analysis and lung lesions (2) were evaluated. A total of 150 pigs in a PRRSV positive commercial swine farm were divided into 3 groups (A, B, C) and housed in separate pens in the same building. All animals were given 200 ppm of tiamulin hydrogen fumarate (Denagard®, Novartis Animal Health, Switzerland) coated premix added to pelleted feed for 4 weeks at 3 to 7 weeks of age. M hyo bacterin (MycoShield™, Novartis Animal Health, USA) were administered 1 shot (1ml) in group B at 3 week-old (0 day post vaccination, dpv) and 2 shots in group C at 3 (0 dpv) and 5 (14 dpv) week-old pigs. The animals in group A received no vaccination.

Results

The average daily gain and feed conversion ratio of pigs in group A, B and C during nursery period (3-10 week old) were 282.72, 330.49, 365.92 g/d and 1.65, 1.43, 1.42, respectively. The average lung lesion scores are displayed (Table 1). The serological analysis of M hyo is shown in Figure 1. Bacterial culture and PCR results verified the high prevalence of *M. hyorhinis* (data not shown).

Table 1. Average lung scores (ALS)

Group	ALS[1] (56 dpv)	ALS[1] (Abattoir)
A (THF)	1.33	1.88
B (THF + 1 shot)	2.33	2.29
C (THF + 2 shot)	0.83	1.86

[1]Score range from 0 to 5: 0 = no remarkable lesion, 1 = less than 5%, 2 = 5-25%, 3 = 25-50%, 4 = 50-75%, and 5 = greater than 75% lung damage.

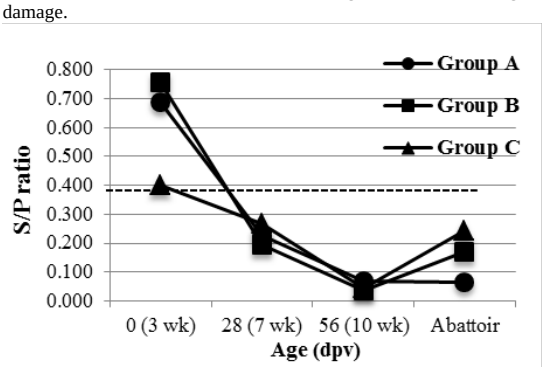

Figure 1. M hyo ELISA (IDEXX) (Positive S/P ratio ≥ 0.4)

Conclusions and Discussion

Obtained results indicate a better productive performance (ADG, FCR & mortality rate) in pigs with M hyo vaccine administration, particularly when receiving the 2-shot vaccination. The average lung lesion scores were not significantly different among the groups. The serological analysis showed no M hyo antibody response after the first and second shot of vaccination. This may be due to high maternal derived antibodies and the high prevalence of PRRSV in the herd. High prevalence of *M. hyorhinis* was also found based on PCR and bacterial culture, which might contribute to the high mortality rate of pigs in group A and group B (data not shown). In conclusion, this study proves that the combined use of vaccination and medication assist in the prevention and control of M hyo in a farm with high prevalence of bacteria and PRRSV particularly when using the 2-shot vaccination. Usage of this program will improve the productive performance of infected herds. In addition, the impact of PRRSV in nursery period may have continuous effect through slaughter. Delaying PRRSV infection will benefit the growth performance.

Acknowledgments

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Livability improvement by vaccination with Ingelvac® PRRS MLV and Ingelvac® CircoFLEX

Dedicated to the memory of Xianjin Yang

H Qiu[1], J Wu[2], A Wang[3], L Zhu[3], T Tao[3], G Chen[3]
[1]*Shandong branch of ZHENGBANG GROUP co., ltd, Shandong, 251101, China*
[2]*Shandong Academy of agricultural Sciences, Shandong, 250100, China*
[3]*Boehringer Ingelheim Int'l Trading (Shanghai) Co. Ltd., Beijing100004, China*
tao.tan@boehringer-ingelheim.com

Introduction

Porcine Circovirus type 2 (PCV2) and Porcine Reproductive and Respiratory Syndrome virus (PRRSV) are two of the major pathogens in Chinese swine industry. PCV2 is associated with systemic wasting, dermatitis and enteritis [1], while PRRSV can cause reproductive failure in pregnant sows and respiratory problems in growing pigs [2]. The co-infection of PRRSV and PCV2 is a common observation in Chinese farms, which leads to huge economic losses. The objective of this study was to evaluate the productivity improvement after vaccination with Ingelvac® PRRS MLV and the added value of including Ingelvac® CircoFLEX as part of vaccination scheme.

Materials and Methods

This study was conducted in a farrow-finish farm in Shandong province (Northern China) with 1200 sows. The farm was built in 2002 with a strict all-in-all-out system. The Breeding herd was routinely vaccinated against Classical Swine Fever virus (CSFv) Foot and Mouth Disease virus (FMDv) and Pseudorabies virus (PRv), 3 times a year; The vaccination program for piglets was the following: CSF, at 25 and 55 days of age; PRv, nasal vaccination at 3 days of age and a second dose intramuscular at 60 days of age; FMD, 70 and 90 days of age. The productivity was not good enough from the farm owner's point of view therefore a vaccination program against PRRS and PCV2 was implemented: December 2010, piglets were vaccinated with Ingelvac® PRRS MLV at 14 days of age while sows were vaccinated four times a year. In September 2011, the farms decided to add Ingelvac® CircoFLEX in pigs at 14 days of age and in breeding herd 5 weeks pre-farrow.
Farrow-finish Livability in pigs from January 2011 to December 2012 was recorded to evaluate the efficacy of Ingelvac® PRRS MLV and the additional potential benefit of adding Ingelvac® CircoFLEX in the vaccination program.

Results

Farrow-finish livability in the period with PRRS MLV and PCV2 vaccination was significantly better than the period from PRRS MLV alone and was statistically significant in the growing period only. This improvement was expected due to the PCVAD clinical presentation in pigs before the implementation of PCV2 vaccine.

Table 1. Summary of livability at different stages

Livability	January to August 2011	Sept 2011 to 2012	Diff	P Value
Suckling	95.3375	96.14375	0.80625	0.458413
Nursery	96.0375	97.06875	1.03125	0.099676
Grow-Finish	84.975	91.31875	6.34375	0.039423
Farrow to Finish	76.35	84.53125	8.18125	0.024463

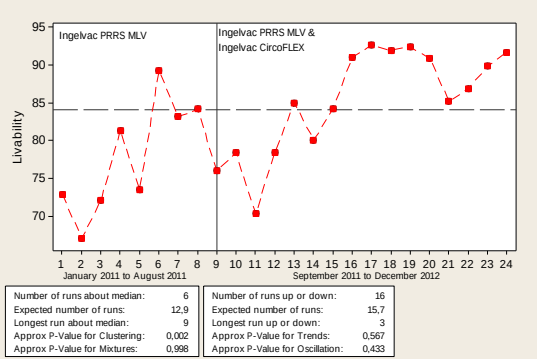

Figure 1. Run chart of farrow to finish livability showing in the 2 periods.

Conclusions and Discussion

This study demonstrates the added value of Ingelvac® CircoFLEX in finishing pig performance resulting in a significant positive impact in overall livability. Also, the study stresses the importance of a correct strategic vaccination program, focusing on current disease dynamics, monitoring and evaluation.

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Comparison of field efficacy of three commercial single dose PCV2 vaccines for pigs

V Poydenko[1], S Kukushkin[2], V Gaponenko[1], A Korolkov[1], T Bondarenko[2]
[1]*Kubanskiy bacon farm, Krasnodar region, Russia,* [2]*Department of Animal Health, Boehringer Ingelheim LLC, Moscow, Russia,* sergey.kukushkin@boehringer-ingelheim.com

Introduction

In recent years vaccination against PCV2 became a routine measure worldwide (1). Vaccination schemes of different producers originally had significant differences, including vaccination only reproductive herd or a single or double vaccination of piglets. Currently, there is a trend to a single vaccination of pigs against PCV2 at weaning time. The main aim of this study was a comparison of field efficacy of three PCV2 vaccines for pigs after single dose.

Materials and Methods

The study was conducted in a two site farrow-to-finish farm (3,618 sows). One site included farrowing and nursery pigs and at 77 days old piglets were transferred to the finishing site. Routinely all piglets were vaccinated against PCV2 with Ingelvac CircoFLEX (Boehringer Ingelheim) at 21 days old and gilts and sows at 2-3 weeks before insemination with the same vaccine. This trial included three PCV2 vaccines used according to manufacturer's recommendation: 1) Ingelvac CircoFLEX (Boehringer Ingelheim) at 14 or 21 days old, dose 1 ml; 2) Porcilis PCV (MSD) at 21 days old, dose 2 ml; 3) Circovac (Merial) at 21 days, dose 0.5 ml. All PCV2 vaccinated groups were kept under the same management conditions in different rooms on the same buildings and site (side-by-side). The Chi-square test was applied to analyse the results.

Results

The trial results show in tables 1-3.

Table 1. Main results for Ingelvac CircoFLEX

Parameters	CircoFLEX, 14 days	CircoFLEX, 21 days
Nursery (24-77 d.)		
Number of pigs, n	1084	2972
Av. start weight, kg	6.9	7.5
Died, %	2.31%	1.55%
Transferred to sanitary pens, %	0%	0.37%
Finishing (78-165 d.)		
Number of pigs, n	1059	2915
Died, %	2.74%	1.92%
Cull, %	1.89%	2.37%
Total losses, %	4.63%	4.29%
Av. slaughter weight, kg	118	118
Total mortality nursery-finishing, %	4.98%	3.43%
Total losses nursery-finishing, %	6.83%	6.12%

Table 2. Main results for Porcilis PCV and Circovac

Parameters	Porcilis PCV, 21 days	Circovac, 21 days
Nursery (24-77 d.)		
Number of pigs, n	3162	1136
Av. start weight, kg	7.0	7.1
Died, %	2.25%	4.49%
Transferred to sanitary pens, %	0.73%	1.67%
Finishing (78-165 d.)		
Number of pigs, n	3094	1080
Died, %	3.78%	4.17%
Cull, %	4.82%	2.78%
Total losses, %	8.60%	6.94%
Av. slaughter weight, kg	114	114
Total mortality nursery-finishing, %	5.95%	8.45%
Total losses nursery-finishing, %	11.39%	12.76%

Table 3. Statistical analysis of trial results (X^2)

Compared groups	Total mortality nursery-finishing, X^2	Total losses nursery-finishing, X^2
CF, 14d. vs. Porcilis	1.48	18.48*
CF, 14d. vs. Circovac	10.61*	22.01*
CF, 14 d. vs. CF, 21d.	4.99*	0.62
CF, 21d. vs. Porcilis	21.51*	52.68*
CF, 21d. vs. Circovac	44.69*	49.13*
Porcilis vs. Circovac	8.34*	1.49

* The results between groups are significantly different.

Conclusions and Discussion

These results demonstrate good efficacy of PCV2 vaccination as a control measure of disease. In comparison, during 5 weeks before start PCV2 vaccination by Ingelvac CircoFLEX total losses for nursery and fattening were 15.87% and 6.31%, respectively. Ingelvac CircoFLEX demonstrated the best efficacy among tested vaccines after a single dose and provided more market pigs with higher final weight than the other products. It was equally effective when administered at 2 or 3 weeks of age (there were no significant differences in the total losses for nursery and finishing).

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Intramuscular vaccination against *M. hyopneumoniae* of swine using a live attenuated vaccine

Q Xiong, Y Wei, Y Gan, L Hua, Z Feng, M Liu, F Bai, G Shao

Institute of Veterinary Medicine, Jiangsu Academy of Agricultural Sciences. Key Laboratory of Veterinary Biological Engineering and Technology, Ministry of Agriculture. National Centre for Engineering Research of Veterinary Bio-products, Nanjing 210014, China, gqshaojaas@gmail.com

Introduction

Attenuated *Mycoplasma hyopneumoniae* live vaccine strain 168 is a commercial available vaccine against mycoplasmal pneumonia of swine in China (1). To change the present intrapulmonical inoculation route, the aim of this study is to evaluate the intramuscular immunization effect of this live vaccine.

Materials and Methods

A mixed adjuvant consisting of astragalus polysaccharide and carbomer was prepared, and subsequently tested for its toxicity for the live vaccine. After that, the live vaccine containing adjuvant was used to inoculate pigs intramuscularly. Immune response was evaluated by detecting lymphocyte proliferation, serum IgG antibody, SIgA antibody in nasal and saliva swabs. Challenge was performed 8 weeks after the first immunization to evaluate the protective efficacy of the vaccine.

Results

Intramuscular inoculation with the adjuvanted live vaccine induced obvious specific lymphocyte proliferative response. The serum IgG antibody increased after two times immunization, and more distinctly after the challenge. However, no mucosal SIgA was detected in both nasal and saliva swab samples during the whole experiment. After challenge, animals from the live vaccine inoculated group exhibited less severe pulmonary lesions (median score 5.98) than unvaccinated pigs (median score 12.12) ($P < 0.05$). A much slighter degree of cilia lost was observed in the pigs treated with live vaccine compared with the unvaccinated pigs.

Conclusions and Discussion

The live vaccine containing a mixture adjuvant of astragalus polysaccharide and carbomer could provide good protection against mycoplasmal pneumonia of swine after intramuscular injection. It indicates that the current intrapulmonically inoculated live vaccine has the potential to be developed into a commercial intramuscularly inoculated vaccine.

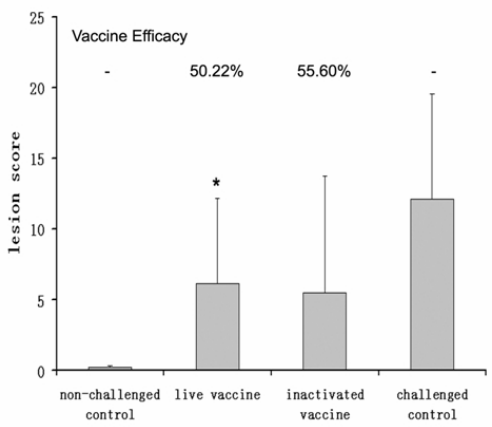

Figure 1. Lung lesion scores after challenge. *The lung lesion scores were determined using the method described by Madec et al. (2), with the score potentially ranging from 0 to 28. The efficacy of the vaccine was calculated as follows: vaccine efficacy = [(challenged control group median score - vaccinated group median score) / (challenged control group median score – non-challenged control group median score)] × 100%.*

Acknowledgments

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Field efficacy of Vaxsafe MHP, a live thermosensitive attenuated vaccine against *M. hyopneumoniae*

PJH Lara[1], G de AR Echeveste[1], MF Quezada[1], FR Cortes[1], AB Reyes[1], LE Adame[1], CR Rodríguez[2], DB Lozano[1], MD Sarfati[1], PE Soto[1], AJM Wence[1]

[1]*Laboratorio Avi-Mex, S.A. de C.V., México D.F.* [2]*Granja La Laguna, Jalisco, México,* horacio.lara@avimex.com.mx

Introduction

Enzootic pneumonia (EP) also called Mycoplasmal pneumonia, is globally recognized as one of the most economically significant swine respiratory diseases (1). The etiological agent of this disease is *Mycoplasma. hyopneumoniae* (MH) which attaches to the ciliated epithelial lining of the swine respiratory tract, resulting in ciliary destruction and impairment of the mucociliar defense mechanisms, predisposing the lungs to secondary bacterial infections (2). The presence of MH in animals suffering of any respiratory viral disease usually magnifies clinical signs, lesions and decrease productivity. A novel type of live vaccines (thermosensitive attenuated vaccines) for Mycoplasmas has been developed based on a chemical mutagenic change. These vaccines were developed for the prevention and control of specific Mycoplasma in other animal species, and have been used in the field for more than fifteen years with great success. One single administration in non-infected animals is enough to colonized the epithelial cells of the upper respiratory tract and provide specific protection against clinical signs, lesions and the effects produced by the challenge with a wild Mycoplasma.

Materials and Methods

A single group of 553 commercial pigs - 7 days old, from a full cycle farm (650 sows) negative to PRRS (serology, PCR), SIV positive (IH) and also positive to MH (bacteriology, ELISA) were used in this field trial. A group of 378 piglets were vaccinated (V) with Vaxsafe® MHP (1.0 mL, IN, at 7 days of age). 175 pigs were left un-vaccinated (NV) as a control group. Both groups were subjected to the farm´s management usual practices, but never received an anti-mycoplasmal antibiotic therapy. When piglets were 5 weeks old, the farm suffered a severe outbreak of SIV (H3N2), affecting both groups in a similar way, but again, no antibiotics were use. At the end of the productive cycle, animals were sacrificed at the abattoir. Macroscopic lesions in lungs (pneumonic areas) were determinate by planimetry (3, 4). The T test (two tails) was used for the statistical analysis.

Results

Lung lesions in all animals from both groups were collected (Table 1). Both groups showed pulmonary lesions, varying in incidence and severity degree.

Pulmonary lesions were lower in pigs from the vaccinated group, with a 14% less in the incidence of pneumonia and a 40.9% less in the severity of lesions compared with the un-vaccinated control group.

Table 1. Summary of lung lesions collected at slaughter time

Group	Incidence of pulmonary lesions	Severity of pulmonary lesion
NV	87%	11%
V	73%	6.5%

p = 0.000034 (T test performed on raw data)

Conclusions and Discussion

One single application of this novel live attenuated thermosensitive vaccine in non-infected commercial fattening pigs at 7 days of age produced a protective lung response against *Mycoplasma hyopneumoniae* lesions by a competitive colonization of the respiratory epithelia with a good immune protection. In this case, animals in both groups were naturally infected in the farm with a SIV, but pulmonary lesions were statistically reduced in animals vaccinated against MH.

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Field evaluation of the productive performance of pigs immunized with Vaxsafe® MHP a live thermosensitive attenuated vaccine

PJH Lara[1], G de AR Echeveste[1], MF Quezada[1], AB Reyes[1], LE Adame[1], CR Rodríguez[2], DB Lozano[1], Sarfati[1], PE Soto[1], AJM Wence[1]

[1]Laboratorio Avi-Mex, S. A. de C. V., México D. F., [2]Granja La Laguna, Jalisco, México, horacio.lara@avimex.com.mx

Introduction

Enzootic pneumonia (EP) also called Mycoplasmal pneumonia of swine (MPS), is globally recognized as one of the most economically significant swine respiratory diseases (1) due to the reduction in the rate gain and feed efficiency as well as for medication costs. The presence of *M. hyopneumoniae*(MH) in animals suffering of any respiratory viral disease usually magnifies clinical signs, lesions and decrease productivity.

A novel type of live vaccines (thermosensitive attenuated vaccines) for Mycoplasmas has been developed based on a chemical mutagenic change. These vaccines were developed for the prevention and control of specific Mycoplasma in other animal species, and have been used in the field for more than fifteen years with great success. One single administration in non-infected animals is enough to colonized the epithelial cells of the upper respiratory tract and provide specific protection against clinical signs, lesions and the effects produced by the challenge with a wild *Mycoplasma hyopneumoniae*.

Materials and Methods

A single group of 553 commercial pigs - 7 days old, from a full cycle farm (650 sows) negative to PRRS (serology, PCR), SIV positive (IH) and also positive to MH (bacteriology, ELISA) were used in this field trial.

A group of 378 piglets were vaccinated (V) with Vaxsafe® MHP (1.0 mL, IN, at 7 days of age). 175 pigs were left un-vaccinated (NV) as a control group. Both groups were subjected to the farm´s management usual practices, but never received an anti-mycoplasmal antibiotic therapy. When piglets were 5 weeks old, the farm suffered a severe outbreak of SIV (H3N2), affecting both groups in a similar way, but again, no antibiotics against MH were use. At the end of the productive cycle (155 days), animals were sacrificed at the slaughterhouse.

Productive performance for Market Weight (MW) in Kg and Daily Gained Weight (DGW) in grams was evaluated based on farm data. The T test (two tails) was used for the statistical analysis. Also, a Rate of Return (RoR) was estimated using the farm data and the probable cost of the vaccine.

Results

At slaughter time, the productive performance was evaluated (Table 1). Vaccinated pigs resulted with an improvement of 1.86% in MW, as the average in body weight was 2.0 kg heavier in vaccinated pigs. The DGW was improved in vaccinated pigs by a 2.6% as they finished with an average of 708g compared with the control group with 690g. Also, with the available, data the RoR was estimated to be 1:4.46.

Table 1. Productive performance evaluation.

Indicator	NV	V	Difference	Statistical Difference (T test)
MW	107 Kg	109 Kg	2.0 Kg	0.004408
DGW	690g	708g	18g	0.002497

MW = Market Weight ; DGW = Daily Gain Weight

Conclusions and Discussion

Immunization of commercial piglets with one single dose of the live thermosensitive attenuated vaccine Vaxsafe® MHP at 7 days of age and raised in a contaminated farm with pathogenic *M. hyopneumoniae* is clearly justified by the productive performance as vaccinated pigs showed an improvement of 2.0 extra Kg at slaughter time, by an extra 2.6% in DGW and by an estimated RoR of 1:4.46. In this case, animals in both groups were naturally infected in the farm with a SIV, but the performance parameters were statistically better in animals vaccinated against MH.

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Study on the impact of v accination with AR and PCV2 combined vaccine (AR-X®) to breeding herds on two large farms in Korea

JS Yeo[1], KJ Kim[2], JE Ryu[2], KW Lee[1], HW Choi[1], HK Won[1], IJ Yoon[1]
[1]*Choong Ang Vaccine Laboratories Co., Ltd. (CAVAC), Daejeon, Republic of Korea*
[2]*Pig & Health Vet Group, Chungcheongnam-do, Republic of Korea,* jsr35@cavac.co.kr

Introduction

Atrophic rhinitis (AR) is a typical respiratory disease affecting the feed efficiency and weight gain rate in pigs. On the other hand, porcine circovirus type 2 (PCV2), an important causative pathogen of PCVAD, causes systemic infection and digestive, respiratory, and reproductive problems. Both of these diseases cause significant economical loss to the pig industry[1,2]. In this study, we attempted to verify the prevention of AR as well as reproductive problems such as mummification and stillbirth caused by PCV2 by using a combined vaccine (AR-X®).

Materials and Methods

This study was carried out in 2 pig farms (Farm M, 1,030 sows F-W and Farm L, 1,150 sows F-F) from December in 2012 to December in 2013. Both farms were using AR vaccine but not PCV2 vaccine to breeding herds.

During one year, AR-X® was administered once before farrowing and twice for gilts before mating in Farm M. On the other hand, the vaccine was administered by mass vaccination in Farm L, and then 3 weeks after the mass vaccination, it was administered once before farrowing and twice for gilts before mating. Comparison of the reproductive performance before (2012) and after (2013) vaccination was carried out in both farms. In addition, side effects such as anorexia, fever, abortion, purulence, and occurrence of atrophic rhinitis were observed.

Results

Vaccination in the two farms with AR-X® showed apparently healthy sows after vaccination as confirmed by the absence of stress-related clinical signs such as anorexia, fever, abortion and purulence. Also atrophic rhinitis did not occur from any animals after the vaccination.

In Farm M, an increase of 0.8-total born per litter, 0.5-born alive per litter, 0.7 pig per sow per year (PSY), and a decrease of 3.4 non-productive days was shown. In case of Farm L, an increase of 0.9-total born per litter, 0.5-born alive per litter, 2.0 PSY, and a decrease of 6.4 non-productive days was shown (Table 1).

Consequentially, AR-X® brought great result through the simultaneous administration of AR and PCV2 vaccines, and the excellent convenience of use such as reduction in the number of vaccinations, labor, and time.

Table 1. Reproductive data in 2 farms

	Farm M			Farm L		
	Before AR-X® (2012)	After AR-X® (2013)	Differ-ence	Before AR-X® (2012)	After AR-X® (2013)	Differ-ence
No. of sows	1,031	1,020	-11	1,148	1,153	5
Average parity no.	4.3	3.1	-0.2	2.29	3.2	0.91
Farrowing rate	83	84.2	1.2	84.7	88	3.3
TBL	11.4	12.2	0.8	10.4	11.3	0.9
BAL	10.5	11	0.5	9.7	10.2	0.5
Stillborn (%)	5.7	6.9	1.2	5.9	7.8	1.9
Mummified fetus (%)	2.6	2.5	-0.1	0.9	1.3	0.4
No. of weaned pigs	9.7	9.9	0.2	9.1	9.7	0.6
Non-productive days	39.9	36.5	-3.4	37.4	31	-6.4
LSY	2.31	2.33	0.02	2.33	2.38	0.05
PSY	22.4	23.1	0.7	21.06	23.07	2.01

* TBL- total born per litter, BAL- born alive per litter

Conclusions and Discussion

This study demonstrated that AR-X® can improve the reproduction performance in swine farms. Both farms showed increased total born per litter, born alive per litter, and PSY. Furthermore, with the assumption of 95% growing rate, an increase of 0.67 market pig per sow per year (MSY) in the Farm M and 0.91 MSY in Farm L was shown. The number of increased MSY eventually would be 690 heads per year in Farm M and 2,196 heads per year in Farm L.

Through the result of our study, the necessity of regular PCV2 vaccination on breeding herds in order to increase the farm performance and production of healthy piglets is verified. The AR-X® used in the study is not only effective in preventing AR and PCV2 infection, but also has the advantage of decreasing the number of inoculation, labor, and stress following vaccination. Therefore, the vaccine is expected to be effective in protecting breeding herds from the aforementioned diseases.

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Economic benefits of Ingelvac Circoflex® comparing to Korean local PCV2 vaccine

HJ Chae[1], CB Han[2], YH Lee[1], JR Lee[1], BJ Cho[1], YS Oh[1]
[1]*Boehringer Ingelheim Vetmedica Korea Ltd., Seoul, South Korea*, [2]*Sol animal health trading, Hwa-Sung, South Korea*
Heejin.chae@boehringer-ingelheim.com

Introduction

Porcine Circovirus (PCV2) causes various diseases like PDNS and PMWS, known as PCV2 associated diseases (PCVAD). In Korea, PCV2 was isolated for the first time in 1998, and the first PCV2 vaccine was introduced in 2008. Seventy three percent of Korean swine farms vaccinated against PCV2 in 2009. In Korea, there are many local PCV2 vaccines as well as global vaccines like in other countries. And currently several local PCV2 vaccines have changed from 2 shots to 1 shot to reduce stress of pigs and working hours by vaccination. The benefits of PCV2 vaccination are already well known in Korea, especially the reduction of mortality and improvement in production performance. However, differences between global and local PCV2 vaccines are not known in relation to mortality, production performance and economic benefit. The objective of this study was to compare local and global PCV2 vaccines in both production performance, including mortality, as well as economic aspects.

Materials and Methods

The field observation was conducted on a 2site production farm with 800 sows. Pigs were weaned at 28 days of age, and transferred to the nursery house. Around 70 days of age, pigs were transferred to the grower/finisher house. In this trial, we used Ingelvac CircoFLEX® as a global PCV2 vaccine in 'A' group and one of the local PCV2 vaccines in 'B' group. In the 'A' group, we vaccinated 1126 pigs from 3 batches at 25 days of age. In the 'B' group, we vaccinated 1053 pigs from 3 batches at the same age as 'A'. Mycoplasma vaccine (Ingelvac MycoFLEX®) was administered at 25 days of age in 'A' group (as FLEXcombo) and 18 days of age in 'B' group. Both the local PCV2 vaccine and Ingelvac CircoFLEX® were injected one time in each batch. Prior to this trial, the farm had successfully utilized Ingelvac CircoFLEX® since 1998, reducing high mortality prior to PCV2 vaccination; the main clinical signs were emaciation and growth retardation in the nursery house and respiratory symptoms in the grower/finisher house. In this trial, we also evaluated economic benefits based on BECAL (Boehringer Ingelheim Economic CALculator) (1).

Results

By comparing Ingelvac CircoFLEX® as'A' group and local PCV2 vaccine as 'B' group, we detected a difference in mortality. In 'A' group, average mortality of 3 batches is 9.07%. And mortality of 'B' group is 15.23%. So mortality of 'A' group is 6.16% lower than 'B' group.(Table.1). Other indicators of production, such as ADWG (average daily weight gain), FCR (feed conversion rate) and age of slaughter, were also different in favor of Ingelvac CircoFLEX (Table.2). FCR and age of slaughter of 'A' group are 0.077 and 8.87 lower than 'B' group, respectively. Also, ADWG of 'A' group is 36g higher than 'B' group.

Table 1. Number of dead pigs per production phase and wean-to-finish mortality in 'A' and 'B' group.

		Ingelvac CircoFLEX® (group 'A')	local PCV2 vaccine (group 'B')
Number of pigs		1126	1053
Number of dead pigs	nursery	53	90
	grower-finish	107	173
Mortality (%)		9.07%	15.23%

Table 2. FCR, ADG (average daily gain), age of slaughter.

	Ingelvac CircoFLEX® (group 'A')	local PCV2 vaccine (group 'B')	Diff.
FCR	2.87	2.94	-0.07
ADG	797g	761g	+36g
Age of slaughter	186.07	194.93	-8.86

The ROI (return on investment) of Ingelvac CircoFLEX® compared with local PCV2 vaccine was calculated by using BECAL. The result of ROI is 9.55: 1in case of carcass price 4,000 KRW, feed price 550 KRW, and difference of vaccine price per pig 1,200 KRW.

Conclusions and Discussion

As many other cases demonstrated before in Korea (2, 3), PCV2 vaccine can reduce mortality effectively (4). Especially in this comparative trial, the Ingelvac CircoFLEX® group demonstrated higher performance than the local PCV2 vaccine group. Even though the price of local PCV2 vaccine is much cheaper, the Ingelvac CircoFLEX®-vaccinated group provided better value because of improved mortality, FCR, ADG and age at slaughter. When selecting a PCV2 vaccine, the most important point is not the price of vaccine but its effectiveness.

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High risk of discontinuing PCV2 vaccination in the male line on a Korean GP farm

YS Oh[1], SH Lee[2]
[1]*Boehringer Ingelheim Vetmedica Korea Ltd., Seoul, South Korea,* [2]*Woorison Agricultural Co. Chun-An, South Korea,*
yusik.oh@boehringer-ingelheim.com

Introduction

PCV2 vaccination in Korea has started from end of 2008. From that time, vaccination rate against PCV2 increased rapidly and up to 90% of Koran farms are vaccinating based on the report of government's tender support. As a result of high rate of PCV2 vaccination, the productivity has improved before vaccination (1).
In early 2013, the price of pork plunged 30% less than last year so farmers have tried to reduce production cost. With high and stable performance and newly established farm, farmers might try stopping vaccination against PCV2. This case report describes the high risk of stopping vaccination against PCV2 in males to reduce production cost in Korean GP farm.

Materials and Methods

The farm is a farrow to finish farm with 850 sows in the central part of Korea. It was restarted on 2012 and produced piglets from Oct. 2012. The farm is PRRS, APP and M.hyo negative but has subclinical PCVAD.
In the farrowing unit, piglets were vaccinated against PCV2 at 3 weeks of age with Ingelvac CircoFLEX® (Boehringer Ingelheim). PCV2 vaccination was applied for 3 months from restarting and decided to stop vaccination from Feb. 2013 in the male line. Vaccination for the female line continued. The field observation was done only up to the end of the nursery period due to batch mixing in the grow-finish. To evaluate the risk of stopping vaccination against PCV2, culled pigs, dead pigs were recorded in each batches and genders. Mortality data of each gender was compared in order to calculate the benefit-to-cost ratio (BCR) of the vaccinated group.

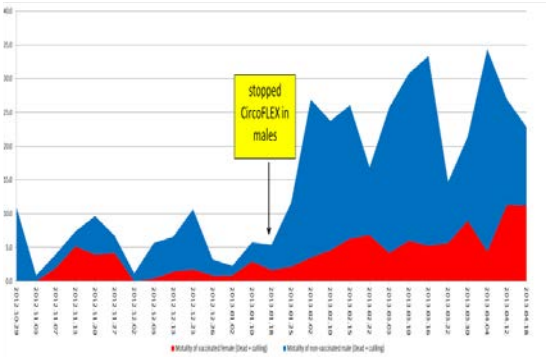

Figure 1. Comparison of mortalities of males (blue shade) and females (red shade) shades in 2 periods

Results

Figure 1 shows the comparison of mortalities between males and females in the period when Ingelvac CircoFLEX was being used in the entire herd and when vaccination was stopped in the males. After stopping PCV2 vaccination in males from 25th of Jan, the maximum mortality of male increase up to 30%. The clinical signs, and diagnostic results in the male mortalities were consistent with PCVAD
Figure 2 shows the combined impact of male and female mortality where there is a dramatic increase in average combined mortality when males were not vaccinated.

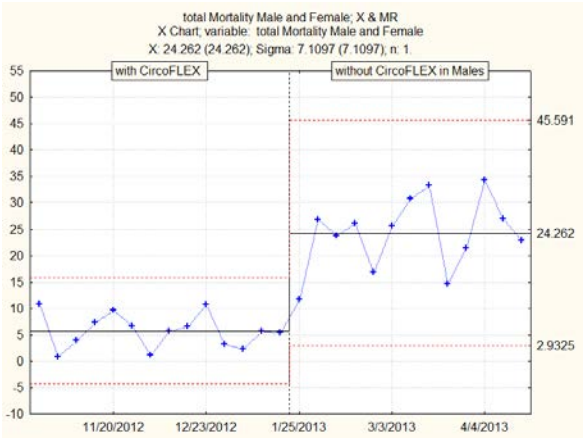

Figure 2. SPC Chart of combined mortality rate (%) of males and females comparing periods with and without Ingelvac CircoFLEX® vaccine in males.

Conclusions and Discussion

Based on the mortality change, stopping PCV2 vaccination resulted in severe loss on Korean GP farm. The benefit-to-cost ratio between vaccinated female and non-vaccinated male is 3.7:1, based on mortality alone. The mortality of vaccinated female is also increased than whole vaccinated period. It can be elucidated that PCV2 infection pressure was higher by shedding from infected male.

Acknowledgments

The author thanks Dr. SH Lee Woorison Agricultural for the collaboration.

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Comparative study of efficacy PCV2 vaccines commercially available in Russia

S Raev[1], K Alexeev[1], B Orlyankin[1], A Zaberezhny[2,3], T Aliper[1]

[1]Independent Non-Profit Organization "Diagnostic and Prevention Research Institute for Human and Animal Diseases", Moscow, Russia, [2]The D.I. Ivanovsky Institute of Virology of Ministry of the Ministry of Health and Social Development of the Russian Federation, Moscow, Russia, [3]Y.R.Kovalenko All-Russian Research Institute of Experimental Veterinary Medicine (VIEV), raevsergey@mail.ru

Introduction

PCV-2 is considered to be one of the most economically important viral pathogens essentially in all major swine producing countries. First PCV-2 vaccine was made in France in 2004. Subunit vaccine «VERRES-CIRCO» was developed in 2009 in Russia. As another commercial subunit vaccines against PCV2 «VERRES-CIRCO» based on the capsid protein (open reading frame 2 (ORF-2) of PCV-2a was isolated from pigs with PMWS) expressed in the baculovirus expression system with some particular qualities in materials and methods used (insect cell line, antigen preparations, and so on). The primary goal of the present study was to compare laboratory parameters (amount of antigen, antibody response in lab animals) and production parameters of «VERRES-CIRCO» with another vaccine from an international manufacturer.

Materials and Methods

The amount of antigen was detected in both vaccines by SERELISA PCV2 Ag Capture ELISA. To detect anti-Cap PCV2 antibody responses in guinea pig serum samples was performed a commercial ELISA «CIRCO-serotest» following manufactures' instructions. To detect PCV2 genome in pig sera was performed a commercial PCR test-kit.

The study was carried out on a large Russian pig herd. The main clinical signs in this farm were growth retardation and high mortality rate associated with PCV-2 infection which was confirmed by ELISA and PCR. Piglets in the 1st group were vaccinated with «VERRES-CIRCO» (Vaccine A), 1 ml per pig; piglets in the 2nd group were vaccinated with vaccine-analog (Vaccine B), 1 ml per pig; piglets in the 3rd group remained unvaccinated.

The animals were observed for local and systemic effects after vaccination.

Results

Comparison of antigen amount, mean ELISA antibody titers are shown in Table 1.

All pigs were healthy before and after vaccination. No local reactions were observed. The mortality rate and ADWG (average daily weight gain) comparison of the control group and the PCV2 vaccinated groups are shown in Table 2. The pigs vaccinated with Vaccine A had a higher ADWG (+9 and +27 g/d for post weaning period; +8 and + 33 g/d for fattening period) than in Vaccine B and control groups respectively; and lower mortality rate (-1,8 and -2,5 % for post weaning period; -1,2 and -3,5 % for fattening period) than in Vaccine B and control groups respectively.

Table 1. Amount of capsid protein and mean group anti-PCV2-IgG antibody response

Parameter/sample	Vaccine A	Vaccine B	Wild-type baculovirus
Antigen titer in ELISA	1:6400	1:6400	1:10
Antibody titer in guinea pigs	1:12300	1:11800	1:20

Table 2. Production parameters

Parameter	Vaccine A	Vaccine B	Control
Nursery			
Pigs, n	7927	10718	15690
Mortality rate (%)	9,2	11,0	11,7
ADWG (g/d)	406	397	379
Fattening			
Pigs, n	3748	5760	11408
Mortality rate (%)	4,9	6,1	8,4
ADWG (g/d)	660	652	627

Conclusions and Discussion

PCV2 piglet vaccination has become global routine use. The data of this field observation indicates that the choice of vaccine might significantly influence production performance. «VERRES-CIRCO» vaccinated pigs performed better than pigs vaccinated with other PCV2 vaccine. The economic value of the improvements exceeded the vaccine cost, with a positive return of investment (ROI) of 1:5,25.

Acknowledgments

To, E. Shemelkov, M. Musienko and A. Mishin for technical assistance.

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PCV2 vaccine protocols reduce PCV2 viremia in low PCV2 challenges

K Bretey, A Sponheim, K Saddoris-Clemons, B Payne
Boehringer Ingelheim Vetmedica, Inc, St. Joseph, MO, keith.bretey@boehringer-ingelheim.com

Introduction

Previous studies indicate that PCV2 viremia levels do not influence ADG in PCV2 vaccinated pigs; however, veterinarians still inquire about viremia.[1,2,3] An objective of these trials was to compare the average PCV2 log levels in non-vaccinated and vaccinated pigs under field conditions.

Materials and Methods

Two trials were conducted. Both included non-vaccinated control groups and two PCV2 vaccination protocol groups (Table 1). Vaccination occurred per label instructions for all products. All pigs were serially sampled at 6 time points (Table 1). Serum was evaluated using an updated PCV2 qPCR protocol (Health Management Center, Ames, IA). The low end cut-off for percent positives of the updated protocol was 2 logs at 45 cycles. Average log level of viremia was calculated for each group at each time period. Results were analyzed by MANOVA repeated measures. Preset contrast were utilized to compare differences between vaccinated and non-vaccinated pigs and to compare vaccinated groups in trial A and Tukey's HSD was used to discern differences in trial B.

Table 1. Trial group description

Trial	Group	N serially bled	Weeks of age sampled
A	NVC†	60	3, 10, 13, 16, 19, 22
	FLEX‡	60	
	PCVM^	60	
B	NVC†	10	3, 7, 10, 14, 19, 23
	CF*	30	
	PCV#	30	

†Sterile saline; ‡Ingelvac CircoFLEX-MycoFLEX® (Boehringer-Ingelheim, St. Joseph, MO); ^Circumvent® PCVM (Merck Animal Health, Summit, NJ); *Ingelvac CircoFLEX® (Boehringer-Ingelheim Vetmedica, In., St. Joseph, MO); # Fostera® PCV (Zoetis,Florham Park,NJ)

Results

Vaccination statistically decreased viremia compared to non-vaccinates in trial A on weeks 10, 13, 19, and 22 (P=0.01, P=0.02, P<0.0001, P<0.0001, respectively) and in trial B, week 23 (P<0.0001). In trial A, percent positive samples peaked at 19 weeks and was 75% (NVC), 41% (FLEX) and 39% (PCVM). Peak percent positive in trial B occurred at 23 weeks and was 71% (NVC), 19% (CF) and 20% (PCV). Viremia between the vaccinated groups was not different (P>0.10) at any time points measured in either trial (Tab. 2 and Fig. 1).

Table 2. Trial A average serum quantitative PCV2 PCR by group at specified weeks of age

Age, wks	Treatment			P-value	
	NVC	FLEX	PCVM	Vx vs. NVx	FLEX vs. PCVM
3	0.00	0.00	0.00	-	-
10	0.68	0.07	0.36	0.01	0.19
13	1.27	0.75	0.62	0.02	0.68
16	1.74	1.26	1.42	0.19	0.64
19	2.86	1.41	1.26	<.0001	0.63
22	2.60	1.03	0.99	<.0001	0.90

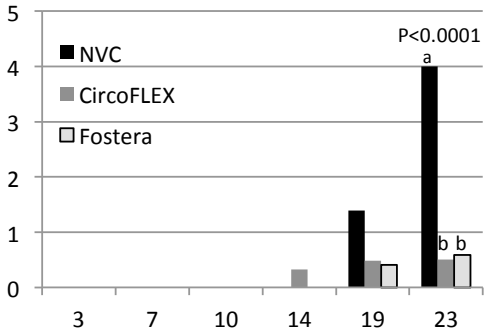

Figure 1. Trial B average serum quantitative PCV2 PCR, log level, by group at specified weeks of age.

Conclusions and Discussion

Both studies represent low PCV2 field challenges (<4 logs average). However, commercial PCV2 vaccines continue to significantly reduce viremia compared to non-vaccinate controls and reduce viremia by the same magnitude. These viremia studies also indicate that vaccines do not completely eliminate viremia. More sensitive quantitative PCV2 tests (< 4 logs detection limit) should be considered in field trials to more accurately assess PCV2 status of pigs. This study continues to add to the body of evidence the industry requests on PCV2 viremia.

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Relative reduction in PCV2 viremia in viremic replacement females, comparing two vaccine protocols. A pilot study

K Bretey[1], J Hocker[2], B Payne[1]
[1]*Boehringer Ingelheim Vetmedica, Inc, St. Joseph, MO,* [2]*AMVC Management Services, Audubon, IA,*
keith.bretey@boehringer-ingelheim.com

Introduction

The objective of this study was to observe the potential efficacy of PCV2 vaccines in reducing or eliminating PCV2 viremia in replacement gilts when vaccinated at weaning and boostered at a typical selection age and weight of 22 weeks of age and 200-275 pounds.

Materials and Methods

Pigs were weaned from a commercial sow farm and randomly allocated to one of three treatment groups at weaning. Viremic animals (n=81) weighing between 200-270 lbs (22 weeks of age) were selected from the commercial finishing facility and administered the appropriate booster vaccination (Table 1). All pigs were commingled and serially bled at 22, 27, 28, 29, 30 and 31 weeks of age. PCV2 qPCR viremia levels were analyzed. The positive/negative cut-off was 2 logs, 100 viral equivalents (HMC, Ames, IA). Lung and lymphoid tissue were collected at marketing for PCV2 tissue burden assessment (ISU VDL, Ames, IA).

Table 1. Trial group description

Group	N	Treatment	Timing (Wk of age)	Dose
A	27	Non-Vac Controls[a]	3, 6, 22	3 x2 ml
B	27	FLEX[b]	3	1 x 2ml
		CF[c]	22	1x 1 ml
C	27	PCVM[d]	3, 6	2x 2 ml
		PCV[e]	22	1 x 2 ml

[a]Sterile Saline, [b]Ingelvac® CircoFLEX-MycoFLEX®, [c]Ingelvac CircoFLEX® (Boehringer Ingelheim Vetmedica, Inc., St Joseph, MO); [d]Circumvent® PCVM, [e]Circumvent® PCV (Merck Animal Health, Summit, NJ)

Results

Total and percent of positive pigs are displayed in Table 2. Figure 1 shows the average of log level for positive pigs. Histopathology and IHC results were negative on all tissues from all animals.

Table 2. Total and percent positive PCV2 qPCR

	Treatment			Contrast P-value	
Age wks	**A**	**B**	**C**	**A vs. B/C**	**B vs. C**
22	27/27 (100%)	27/27 (100%)	27/27 (100%)	-	-
27	14/27 (51.9%)	11/27 (40.7%)	6/27 (22.2%)	0.09	0.24
28	17/27 (63.0%)	8/27 (29.6%)	4/27 (14.8%)	**0.0005**	0.33
29	16/27 (59.3%)	9/27 (33.3%)	6/27 (22.2%)	**0.008**	0.54
30	22/27 (81.5%)	9/27 (33.3%)	13/27 (48.2%)	**0.008**	0.41
31	13/27 (48.2%)	6/27 (22.2%)	3/27 (11.1%)	**0.004**	0.47

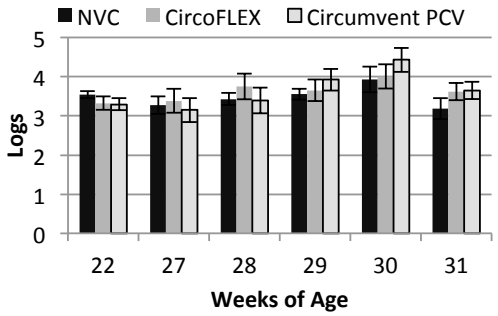

Figure 1. Average log qPCR by weeks of age (only positive animals included)

Conclusions and Discussion

Even with lower PCV2 field challenge (<4.5 logs maximum average in any one treatment group) commercial PCV2 vaccines significantly reduced the percent of PCV2 qPCR positives compared to non-vaccinated controls. While both vaccines reduced viremia by the same magnitude, neither vaccine eliminated viremia. In this study, viremia was not a predictor of tissue PCV2 burden. Further field trials are underway to understand PCV2 vaccine use in replacement animals and the role of PCV2 in the breeding herd.

Reduction of PHE case after implementation of ileitis vaccination in a multiplier breeding farm

YD Yoon[1], HK Seo[2]
PigCare Animal clinic, Boehringer-Ingelheim Vetmedica Korea[2]
pic268@hanmail.net, hkseo@seo.boehringer-ingelheim.com

Introduction

PHE is an acute clinical form of proliferative enteritis that is often associated with young adult pigs 4 to 12 months of age and is commonly found when replacement gilts form a high health site are introduced into a new farm site. Especially, gilt suppliers are very concerned about PHE because many complaints after delivery of gilts are related to PHE outbreaks. The present paper describes the incidence of PHE cases after placement in a commercial herd..

Materials and Methods

This field case was recorded in a farrow to finish multiplication herd with 500 sows. The herd is PRRS negative and PCV2 and Mycoplasma hyopneumoniae vaccination is done routinely in piglets. Most of the replacement gilts are delivered to other breeding farms at 95 kg of body weight and 150 days of age. Since 2004 the multiplier farm recorded the number of PHE cases in gilts they delivered to other breeding farms. Additionally for some of the animals that broke with PHE, the time after introduction into the new herd was recorded. Ileitis vaccination was implemented at the beginning of 2010. Since then pigs are vaccinated at 4 weeks of age via drench. The number of PHE case was recorded per year prior and after vaccination the implementation of ileitis vaccination..

Results

Most of the animal where the time after introduction was recorded broke with PHE between day 31 and 70 after introduction (62,1 %). Only 22 % broke with in 30 days after introduction and 16 % of the animals showed clinical signs of PHE more then 71 days after introduction..

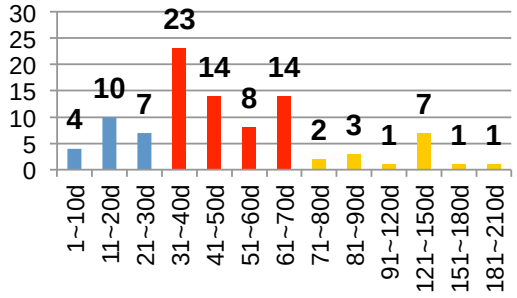

Figure 1. PHE cases according to the days after introduction

The number of PHE cases was 15 in 2004, 80 in 2005, 45 in 2006, 18 in 2007, 24 in 2008, 12 in 2009, 4 in 2010, 1 in 2011 and 3 in 2013 respectively(Fig 2)

Before ileitis vaccination was implemented the mean annual number of the PHE case was 32.3 compared to 2.7 in vaccinated animals which means a 94% reduction after vaccination.

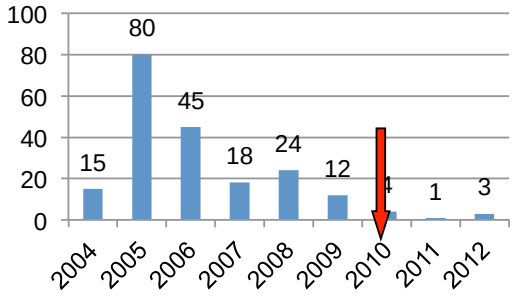

Figure 2. PHE case incidence changes by years

Conclusions and Discussion

Most PHE cases in gilts occurred within 70 days after introduction. It is common practice in Korea that during the first 30 days in the commercial sow herd gilts are kept in groups pen with other (cull) sows for the acclimatization and adaptation to the new environment. In this period antibiotics are used frequently to cover different pathogens. After then, they are moved into individual stalls for breeding. This procedure might be the reason why most PHE cases are occurring during this period.

Ileitis vaccination was effective in reducing the number of PHE cases in replacement gilts. However there were still very few cases of PHE in vaccinated animals. This might be due to the fact that these animals did not react to vaccination (non-respondests) or that they were missed duringe the vaccination procedure. Therefore the general recommedation for ileitis control in gilts is to vaccinate them once around weaning or during nursery and for a second time 3 weeks before shipment to the commercial herd or at least at placement in the commercial farm.

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Optimization of immune strategy for a new live vaccine candidate against porcine pleuropneumonia in a murine model

J Hur, JY Moon, JH Lee
College of Veterinary Medicine and Bio-Safety Research Institute, Chonbuk National University, Jeonju 561-756, South Korea, hurjin@jbnu.ac.kr

Introduction

Actinobacillus pleuropneumoniae (*A. pleuropneumoniae*) is the causative agent of porcine pleuropneumonia. Porcine pleuropneumonia is a highly contagious endemic disease, which causes significant losses in large pig farms worldwide (2,3). There are a few key virulence factors of *A. pleuropneumoniae*, such as outer membrane proteins (OMP), repetitive glycine-rich sequences in repeats-in-toxins (RTX toxins) and fimbriae (3). Among these, Apx toxins (RTX toxins) are substantially involved in pathogenesis (5).

The importance of Apx toxins as vaccine candidates has been reported, in which antibodies neutralizing Apx toxins can protect macrophages or neutrophils from necrosis. In addition, animals vaccinated with Apx toxins were protected against bacterial infection (1). The highly conserved protein OmpA is an integral component of the outer membranes of gram-negative bacteria (4).

Materials and Methods

The *Actinobacillus pleuropneumoniae* antigens ApxIA, ApxIIA, ApxIIIA and OmpA were expressed in an attenuated strain of *Salmonella* (Δ*lon*Δ*cpxR*Δ*asd*) to construct a novel vaccine candidate against porcine pleuropneumonia. In order to evaluate the immunization strategy of the vaccine candidate, a total sixty BALB/c mice were equally divided into four groups (n = 15). Group A mice were intranasally immunized only at 6-weeks-of-age, while group B mice were intransally primed and boosted at 6- and 9-weeks-of-age, respectively, and group C mice were intransally primed at 6-weeks-of-age and subsequently boosted twice at 9- and 12-weeks-of-age. Group D mice were used as a control, which were inoculated with sterile PBS.

The individual antigen-specific IgG and IgA titers were determined in serum and fecal samples, respectively, by enzyme-linked immunosorbent assay (ELISA).

For challenge experiment, the challenge strain was prepared and intranasally inoculated at 10 WPPI. All challenged mice were monitored daily for mortality and abnormal behavior till day 14 after the challenge.

Results

Groups A, B, and C showed significantly higher serum IgG and fecal IgA immune responses than those of the control group. After virulent challenge with a wild type A. pleuropneumoniae, the immunized groups A, B and C showed 33.3%, 13.3% and 26.7% mortality as the control group showed 60% mortality.

Conclusion and Discussion

In this study, a new live attenuated *Salmonella* expressing ApxIA, ApxIIA, ApxIIIA and OmpA antigens was constructed as a vaccine candidate for the prevention of porcine pleuropneumonia. In the murine model, the fecal IgA and serum IgG titers in the immunized groups A (single administration), B (double administration) and C (triple administration) were significantly increased compared to those in the control group D. The fecal IgA and serum IgG titers of the groups B and C were especially elevated. These results indicated that the double and triple immunized group mice induced higher protective IgG and IgA. In addition, the double immunized group mice defended most optimally against the challenge. Thus, these results showed that the double intranasal immunization with the vaccine candidate can optimally induce systemic and mucosal immunity, which can effectively protect against porcine pleuropneumonia.

Acknowledgments

This study was supported by Technology Development Program for Agriculture and Forestry, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.

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Protection against neonatal piglet enterotoxigenic *E. coli* (ETEC) diarrhea by vaccination of pregnant sows with a *Salmonella* ghost expressing ETEC fimbrial antigens

J Hur, Y J Kwon, J H Lee
College of Veterinary Medicine and Bio-Safety Research Institute,
Chonbuk National University, Jeonju 561-756, South Korea, hurjin@jbnu.ac.kr

Introduction

Enterotoxigenic *Escherichia coli* (ETEC) causes diarrheal disease, which is the most common enteric colibacillosis encountered in neonatal piglets (Harmsen et al., 2005; Vu-Khac et al., 2007). Many ETEC strains produce one or more adhesins, which promote bacterial colonization of the small bowel, allowing expression of the toxins such as heat-labile enterotoxin and/or heat-stable enterotoxin in close proximity to the intestinal epithelium (Nagy and Fekete, 1999; Vu-Khac et al., 2007). K88 (F4), K99 (F5), F6 (Fas) and F41 are mainly important adhesins of neonatal porcine ETEC. In the present study, a *Salmonella* ghost was used to express ETEC fimbrial adhesins such as K88ab, K88ac, K99, F6 and F41. The systemic and colostral immune responses in the pregnant sows and their piglets were evaluated. In addition, the protection efficacy was also evaluated against an experimental *E. coli* colibacillosis in piglets.

Materials and Methods

To construct the ghost, the genes encoding ETEC fimbrial antigens were cloned into a ghost cassette plasmid, which was subsequently electroporated into a *Salmonella* Typhimurium strain. In order to investigate the efficacy of the ghost as a vaccine candidate for protection against neonatal piglet ETEC diarrhea, pregnant sows were primed and boosted with the candidate.

To determine the fimbria-specific IgG and IgA concentrations in serum and colostrum, a standard ELISA was performed using the pig IgG or IgA ELISA Quantitation kit according to the manufacturer's instructions. Serum samples from both pregnant sows and piglets were diluted to 1:200 for IgG and 1:8 for IgA titers. Subsequently, the colostrum samples were diluted to 1:320 and 1:16 for measurement of mucosal IgG and sIgA titers, respectively. Enzymatic reactions were developed with o-phenylenediamine and measured with an automated ELISA spectrophotometer at 492 nm. A standard curve representative of the relationship between the concentration of standards and their absorbance values was generated. The antibody concentration for each sample was measured in nanograms per milliliter (ng/ml) or microgram per milliliter (μg/ml).

JOL489, JOL564 and JOL599 were grown in LB broth overnight at 37 °C, diluted 1:20 in fresh LB broth, and grown at 37°C to an OD600 of 0.8. Bacteria were harvested by centrifugation at 3,400 × g for 20 min. Each challenge strain was resuspended to approximately 3×10^9 CFU in 1 ml of sterile PBS-sucrose after washing twice with sterile PBS. All five-day-old piglets were orally challenged with a total of 1 ml mixture containing 1×10^9 CFU of each challenge strain on the day of preparation. All piglets were monitored daily for diarrhea and mortality until day 14 after challenge. The rectal swabs from all sows were collected weekly from 10 weeks of pregnancy to 4 weeks post-farrowing, and examined to determine whether wild type ETEC strains were shed in the feces. The rectal swabs were transported to the laboratory on ice and processed on the same day. Isolation of the vaccine candidates was performed according to the previously described method (Hur and Lee, 2010). The attempts to isolate challenge strains from rectal swabs of the diarrheic piglets were made by previously described methods (Hur and Lee, 2012). The swabs were inoculated directly onto Eosin Methylene Blue. Approximately 30 of the typical E. coli colonies (large, blue-black and green metallic sheen isolates) per piglet were selected. The isolates were cultured on blood agar containing 5% sheep blood for 18 h at 37°C, and the presence of hemolysis was determined visually. Hemolytic colonies were confirmed by PCR using the K88, K99, F6 and F41 primer sets, as previously described (Kim et al., 2010) and identified using the API 20E system. The challenge strain induced diarrhea was confirmed by isolating the challenge strain from the rectal swab.

Results

Serum IgG and colostral IgA titers against all the individual adhesin antigens were significantly increased in groups C and D sows compared to those in group A at week 6 PPI ($p \leq 0.05$). Serum IgG and colostral IgA titers in group B sows were slightly higher than those in group A. In addition, colostral IgG titers in all immunized group sows were significantly increased compared to those of group A sows on the day of farrowing ($p \leq 0.05$). Serum IgG and IgA titers of all suckling piglets from groups C and D sows were significantly higher than those of control piglets on day 4 after birth ($p < 0.01$). Serum IgG and IgA titers in group B piglets were slightly higher than those in group A. Five days aged suckling piglets were orally challenged with approximately 3×10^9 CFU of ETEC wild type strains (Table 1). Groups C and D piglets did not exhibit clinical signs such as diarrhea up to day 14 after challenge. In contrast, diarrhea was observed in 16 of 18 group A piglets beginning on day 3 after challenge, and 3 group A piglets died due to severe diarrhea. Among 17 group B piglets, diarrhea was observed in 10 piglets.

Conclusions and Discussion

Table 1. Clinical signs in suckling piglets post challenge with virulent strains.

Sow group[a]	No. of Piglet	Diarrhea (%)	Death (%)
A	18	16	3
B	17	10	0
C	20	0	0
D	23	0	0

[a] The sows of group A were administered with PBS as a control, Group B were immunized with 2 x 10^9 cells of the ghost vaccine candidate , Group C were immunized with 2 x 10^{10} cells of the ghost vaccine candidate, and Group D were immunized with 2 x 10^{11} cells of the ghost vaccine candidate.

In this study, systemic and colostral immune responses were markedly induced by oral immunization with 2 x 10^{10} or 2 x 10^{11} cells of the ghost vaccine candidate. Furthermore, clinical signs of suckling piglets from sows immunized with 2 x 10^{10} or 2 x 10^{11} cells were rarely observed after challenge with the virulent E. coli strains, while diarrhea and mortality were observed in approximately 90% and 20% of control group piglets, respectively. Therefore, these findings indicated that the oral immunization of pregnant sows with either 2 x 10^{10} or 2 x 10^{11} cells of the candidate may effectively protect neonatal piglets from diarrhea caused by ETEC.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MISP) (No. 2013R1A4A1069486).

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Comparison of two PCV2 piglet vaccination programmes on performance in Spain

R Bernal
Bigvete, S.L. Spain, ruben@bigvete.es

Introduction

It has been repeatedly demonstrated that piglet vaccination against PCV2 is the best tool to decrease the economic losses caused by this disease[1,2,3] . The objectives of this evaluation were to compare the growth performance and efficiency in commercial pigs using Ingelvac CircoFLEX® (Boehringer Ingelheim Vetmedica GmbH) compared to another PCV2 vaccine.

Materials and Methods

The field observation was carried out in a 2.350 sow farm in the central region of Spain weaning at 3 weeks of age. The herd was positive for PCV2, Mycoplasma hyopneumoniae, APP and SIV and negative for PRRS. The total number of piglets included in this study was 59.494. In the first period, between July 2011 and October 2011, an amount of 18.390 piglets were vaccinated with 1ml of Ingelvac CircoFLEX® and moved to 7 independent fattening units (group A1). Afterwards, the vaccination program was changed and between November 2011 and April 2012 an amount of 29.390 piglets were vaccinated with 0,5ml of an inactivated PCV2 vaccine and moved to 10 independient fattening units (group B). Then between May 2012 and July 2012 an amount of 11.714 piglets were vaccinated again with 1 ml of CircoFLEX® and moved to 6 independent fattening units (Group A2). Serum samples were taken in March 2012 from ten pigs each at 3, 5, 7, 9, 12, 15, 18, 21 and 24 weeks of age. Infection was confirmed in group B by clinical signs, ELISA (IgG, IgM), histopathology and in situ hibridization. Average daily gain (ADG, g/d), feed conversion rate corrected from 18 to 100 kg ($FCR_{18\text{-}100}$), mortality (%) medication costs, culls (%) and weight gain (kg) were analyzed by statistical process control (SPC; R sofware, qcc package) and analysis of variance (ANOVA; SAC, cary, NC).The fattening unit was considered as the statistical unit. The economic difference between the two vaccination programmes was calculated using the BECAL calculator provided by Boehringer Ingelheim, taking into account a feed price of 336€/ton, and meat price of 1.35 €/kg

Results

Figure 1 shows the SPC chart of the mortality rate and FCR in the different batches. Average mortality was 48% less in the addiction of groups
A1+A2 (3.38%) vs. group B (6.52%) and the difference of FCR (18-100) was 148 grams between groups A1+A2 and group B. Performance results of the different parameters are summarized in table 1. Animals in the addiction of groups A1 + A2 grew 67 grams more per day.

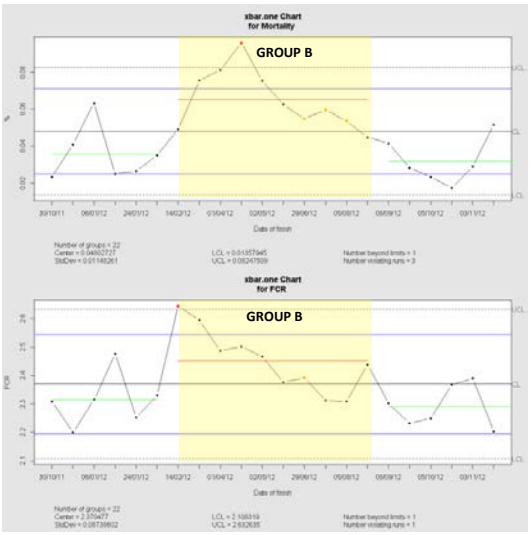

Figure 1. SPC (Statistical Process Control) graph showing the impact of the two different vaccinations on mortality and FCR *The medication costs were 50% lower in groups A (1+2) (0.87€/pig) vs. group B (1.73€/pig) and the average percentage of culls was 28.6% lower in groups A(1+2) too.*

Table 1: Performance parameters.

	Group A1+A2 (CircoFLEX®)	Group B	P-value
ADG (g/d)	717.0 ± 10.1	649.8 ± 11.5	0.0002 (**)
FCR	2.29 ± 0.027	2.45 ± 0.028	0.0016 (**)
Mortality (%)	3.38± 0.40	6.52 ± 0.46	0.0001 (***)
Medication cost (€)	0.87 ± 0.19	1.72 ± 0.22	0.007 (**)
% Tail enders	1.00 ± 0.25	1.4 ± 0.29	0.3205 (NS)
Fattening days	122.6 ± 1.78	124.4 ± 2.03	0.5173(NS)

NS: non significant; * p<0.05; ** p<0.01; *** p<0.001

Conclusions and Discussion

Under the conditions of this study, the Ingelvac CircoFLEX® vaccinated group had a lower mortality, higher weight gain, better FCR and less medication costs compared to the group B. Taking into account the production costs, the use of Ingelvac CircoFLEX® showed a return of investment of 9,32€ compared to the other PCV2 piglet vaccination program.

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Expression of a truncated E2 protein of classical swine fever virus in *E. coli*

C-Y Fang[1], J-F Lai[1], H-Z Zeng[1], W-Z Huang[1], H-J Lin[1], Z-W Chen[1], J-P Wang[1], J-H Lin[1,2]

[1]*Division of Animal Medicine, Animal Technology Laboratories, Agricultural Technology Research Institute, Taiwan, ROC,* [2]*School of Veterinary Medicine, National Taiwan University,* duncanfang@gmail.com

Introduction

Classical swine fever (CSF) is an infectious and highly contagious viral disease in the swine industry, which may cause severe economic losses in endemic countries. The disease can produce clinical signs and lesions ranging from acute to a subacute, chronic, and/or inapparent. Previous studies showed that the E2 outer envelope protein of CSF virus (CSFV) can elicit a protective immunity in pigs against CSFV. The objective of this study was to express recombinant E2 protein in *Escherichia coli* in a soluble form and evaluate the protective efficacy of recombinant E2 in pigs.

Materials and Methods

The gene encoding E2 protein which truncated in its signal peptide and C-terminal transmembrane domain was optimized based on the codon bias of *Escherichia coli* and synthesized by overlap extension polymerase chain reaction (OEPCR). The synthetic gene was inserted into pET-F which contained a fusion partner gene. The resulting plasmid was named pE2H and transformed into *E. coli* BL21 (DE3). *E. coli* transformants were incubated at 37°C with shaking to OD_{600} = 0.6 and then induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). The cell pellet of *E. coli* was collected and analyzed by SDS-PAGE. Fractionation of soluble and insoluble proteins was performed by using Easy-Lyse Bacterial Protein Extraction Protocol (Epicentre, CA, USA). Recombinant E2 protein was purified from soluble fraction of *E. coli* by immobilized metal-ion affinity chromatography (IMAC).

Results

The gene encoding truncated E2 protein was synthesized by OEPCR and used to construction of E2 expression vector pE2H (Figure 1). The expression vector was further transformed into *E. coli* host and the expression of recombinant E2 in *E. coli* was analyzed. Our results showed that utilization of the fusion protein strategy lead to high level soluble expression of truncated E2 in *E. coli* (Figure 2) and the recombinant E2 protein could be recognized by the swine anti-CSFV hyperimmune serum (Figure 3). The soluble recombinant E2 could be purified from *E. coli* by IMAC. The protective efficacy of purified recombinant E2 protein will be evaluated in the near future.

Conclusions and Discussion

In this study, we reported the soluble expression of E2 in *E. coli*. The purified recombinant E2 protein will be used to develop subunit vaccine or to develop enzyme-linked immunosorbent assay (ELISA) for the detection of E2-specific antibody in pig sera.

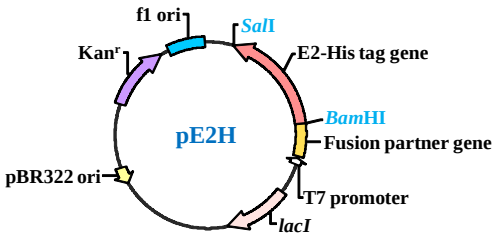

Figure 1. Schematic representation of pE2H.

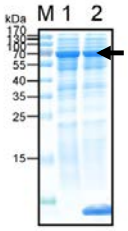

Figure 2. Fractionation of recombinant E2 expressed in *E. coli* (pE2H). 1: total cell lysate of *E. coli* (pE2H); 2: soluble faction of *E. coli* (pE2H).

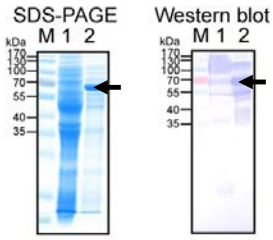

Figure 3. Western blot analysis of recombinant E2 expressed in *E. coli*. 1: total cell lysate of *E. coli*; 2: total cell lysate of *E. coli* (pE2H).

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Prokaryotic expression and vaccine efficacy of PCV2 ORF2 protein

T-T Peng[1], C-Y Fang[1], Z-W Chen[1], J-P Wang[1], M-W Hsieh[1], C-Y Yang[1], C-M Chen[1], S-R Wang[1], J-H Lin[1,2]
[1]*Division of Animal Medicine, Agricultural Technology Research Institute, Taiwan, ROC,*
[2]*School of Veterinary Medicine, National Taiwan University,* ptttammi@gmail.com

Introduction

Porcine circovirus type 2 (PCV2) is the etiologic agent of the porcine circovirus-associated disease (PCVAD) that causes great economical loss in swine industry (1). A plethora of studies have showed that vaccination is an effective way to control the disease (2). The efficacy of PCV2 vaccine is based on serological conversion against PCV2 and to reduce viral replication *in vivo* (3). Commercial vaccines can reduce mortality of pigs and increase average daily weight gain (ADWG) rather than preventing PCV2 infection and transmission (4, 5). ORF2 protein (the capsid protein) of PCV2 is demonstrated to elicit protective efficacy in pigs. However, the ORF2 antigen is produced by an eukaryotic expression system. This study demonstrated a low cost *Escherichia coli* expressed ORF2 subunit vaccine that can provide good protective efficacy in pigs.

Materials and Methods

The recombinant ORF2 (rORF2) protein was expressed in *E. coli* and was purified, emulsified with an oil or aqueous adjuvants (SEPPIC). Four weeks old specific pathogen free (SPF) pigs were randomly assigned into three groups, vaccinated at 4 and 6 weeks of age. Two weeks after secondary immunization, all pigs were challenged with 2 x 10^5 $TCID_{50}$/mL PCV2 virus. All pigs were sacrificed at 12 weeks old. Anti-PCV2 IgG antibody was measured by a commercial PCV2 ORF2 ELISA kit and a quantitative real-time PCR was used to determine the PCV2 virus loads in plasma samples.

Results

All pigs showed healthy and no adverse effect to the PCV2 subunit vaccine during the experiment period. Significant seroconversion was observed after secondary immunization in compared to control group (Figure 1). The average PCV2 ELISA S/P ratio was no significant differences between vaccinated groups, while the control group remained serologically negative until three weeks after challenge. After challenge, vaccinated groups had slightly increased level of viremia but significantly lower than control group and there was no significant differences between vaccinated groups (Figure 2).

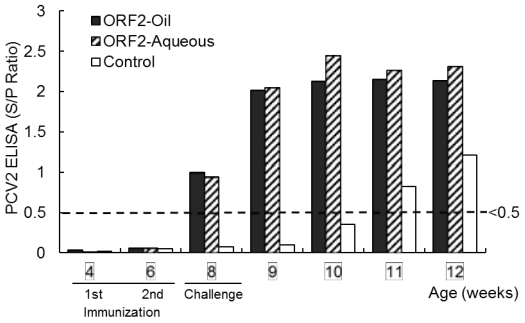

Figure 1. Average anti-PCV2 IgG antibody response. Pigs were vaccinated with rORF2 emulsified with oil adjuvant (n=2; filled bar), aqueous adjuvant (n=2; slashed bar) or non-vaccinated control group (n=3; un-filled bar).

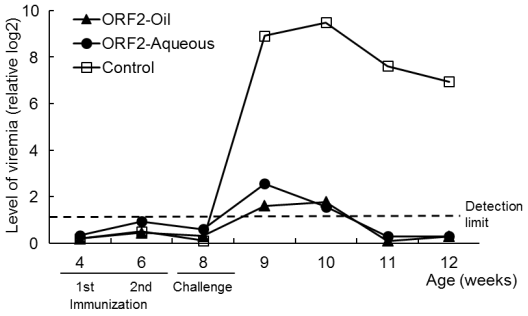

Figure 2. Level of virus load of plasma sample. Pigs were vaccinated with rORF2 emulsified with oil adjuvant (n=2; filled triangle), aqueous adjuvant (n=2; filled circle) or non-vaccinated control group (n=3; un-filled square).

Conclusions and Discussion

The study demonstrated that two dose immunization schedule for *E.coli* expressed PCV2 ORF2 protein is efficacious for protecting and controlling PCV2 infections in SPF pigs. The low cost and easy production of ORF2 is competitive to generate a new PCV2 vaccine.

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P.160

Efficacy of the single dose (One-shot) *M. hyopneumoniae* inactivated bacterin vaccine in a swine farm in Taiwan

C-W Hsu, Z-W Chen, J-P Wang, C-S Chang, Y-C Huang, W-Y Liu, K-M Huang, J-H Lin
Division of Animal Medicine, Agricultural Technology Research Institute, Taiwan, ROC., d3474037@hotmail.com

Introduction

Mycoplasma hyopneumoniae (*M. hyo*) is the causative agent of swine enzootic pneumonia (SEP), one of the most important chronic respiratory diseases that affects swine production worldwide. The pathogen results in significant economic losses through retarded growth, poor food conversion and increased susceptibility of pigs infected with other pathigen. Prevention of SEP may be achieved by improving hygiene and management in pig farms (1,2). In addition, vaccination is the most effective strategy for control and prevention of SEP. Commercial *M. hyo* vaccines were prepared from killed bacteria (bacterins). Single dose vaccine is attractive to users since it can reduce labor work and is beneficial to farm management. This study developed single dose (One-shot) *M. hyo* inactivated bacterin vaccine. The objective of the study was to evaluate the efficacy of the vaccine a commercial farm in Taiwan.

Materials and Methods

In this study, the trial carried out in a farrow-to-finish commercial farm in Taiwan. Total 210 Pigs in immune group (n=180) were immunized intramuscularly once with 2 ml ATRI One-shot *M. hyo* inactivated vaccine at 3 weeks old and the control group (n=30) were not immunized. Serum samples were collected at 3, 7, 11, 15, 19, 23 and 27 weeks old. Serum antibodies were tested by a commercial *M. hyo.* antibody ELISA test (IDEXX Laboratories, Inc., Westbrook, ME, USA). The result is interpreted as sample-to-positive (S/P) ratio using the standard protocol described in the kit. Error bars are standard error of mean (SEM). Also, body weights were recorded in this trial to calculate the daily body weight of pigs.

Results

During the immunization period, all pigs appeared healthy and showed no adverse effect to the prototype vaccine including abscess or necrosis response at the injection sites. The data showed that immune group elicited significant seroconversion at 4 weeks after immunization, The immunity persisted for at leat 4 months (Figure 1). The Average daily weight gain (Figure 2B) between immunized and control group were observed that immunized with One-shot *M. hyo* vaccine had better performance.

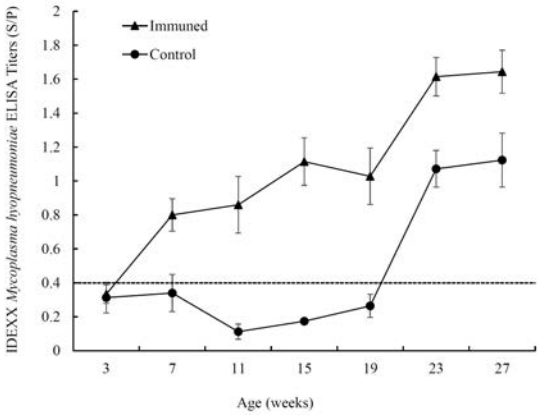

Figure 1. Mean *M. hyo.* ELISA S/P result on serums collected from in a commercial pig farm. (Cutoff S/P ratio ≥0.4)

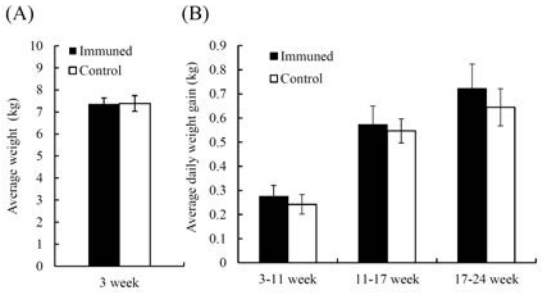

Figure 2. (A) Average weight and (B)Average daily weight gain from in a commercial pig farm.

Conclusions and Discussion

These results showed that the vaccines used in this study were safe to pigs. The One-shot *M. hyo* vaccine should preferably before 7 weeks of age and showed seroconversion after vaccination. Duration of the immunity lasted until the finishing phase.

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Safety and efficacy of an experimental live vectorized H1N1 Swine Influenza vaccine

PJH Lara[1], G de AR Echeveste[1], MF Quezada[1], FR Cortes[1], PF Castro, DB Lozano[1], MD Sarfati[1], PE Soto[1].
[1]*Laboratorio Avi-Mex, S.A. de C.V., México, D. F.*, horacio.lara@avimex.com.mx

Introduction

Swine Influenza Virus (SIV) causes great losses, either by the disease *per se*, by secondary infections or as a possible zoonosis.

Due to its high mutagenic capacity, it becomes difficult to produce updated killed vaccines produced with whole virus and OIE´s normative ban the production of live SIV vaccines. Employing Genetic Engineering techniques, a SIV recombinant vaccine was constructed using a live viral vector (2, 3, 4, 5, 6, 7, 8). This report presents the results of the safety and efficacy tests of an experimental live vectorized vaccine (rPMV-SIV/HA/H1/Avimex) produced with a SIV subtype H1N1 isolated in Mexico in 2002.

Materials and Methods

Three weeks-old SPF piglets (Avifarma, S. A. de C.V.) were housed in positive pressure isolation units.

Table 1. Group Distribution

Group	Objective	Challenge	piglets
1	Negative Control	No	10
2	Positive Control	Yes	10
3	Vaccinated	Yes	10

Piglets in group 1 remained un-vaccinated and un-challenged as a negative control group. Piglets in group 2 remained un-vaccinated but were challenged as a positive control group. Piglets in group 3 were vaccinated at day 4 (0 DPV) by the IM route with 2.0 mL of the experimental live vectorized vaccine. They were check daily for adverse reactions due to the vaccine or vaccination procedures (safety test). After two weeks (14 DPV) piglets of groups 2 and 3 were challenged with a known SIV pathogenic strain subtype H1N1 isolated in Mexico in 2002, in a specially designed nebulization chamber using $10^{6.0}$ $TCID_{50\%}$/mL/piglet (efficacy test). All animals were checked daily for clinical signs. Animals in all the groups were humanely euthanized 14 days PC and an evaluation of pulmonary lesions was performed on each animal (9).

Results

No adverse reactions were detected in vaccinated animals during the observation period (14DPV). No clinical signs were observed in animals in group 3 (vaccinated and challenged) or in group 1 (un-vaccinated and un-challenged). Clinical signs induced by the SIV challenge were detected in group 2 (positive control, un-vaccinated but challenged) and were observed as ear erythema, ocular secretion, ocular edema, conjunctivitis, cough and sneezing, fever was also detected during 10 days post PC. The pulmonary lesion percentage found at the post mortem exam is shown in table 2.

Table 2. Pulmonary lesion evaluation

Group	Percentage
Negative Control (group 1)	0.1 a
Positive Control (group 2)	10.8 b
Vaccinated (group 3)	5.7 c

Different literals indicate significative statistical differences ($p=<0.05$). Turkey's test.

Conclusions and Discussion

The vectorized live vaccine didn't cause any sign or lesion in vaccinated pigs, meaning that the vaccine is safety.

The efficiency of the live vectorized vaccine against the challenge with SIV subtype H1N1 was demonstrated by the evaluation of the pulmonary lesions as previously demonstrated (10). Vaccinated challenged piglets (group 3) were protected against clinical signs and the pulmonary lesions were notably reduced as compared to the positive control group.

The safety and efficacy tests of the experimental live vectorized vaccine against SIV subtype H1N1 were satisfactory in SPF piglets.

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Live virus determination of PRRSV vaccines on primary porcine alveolar macrophages

DS Pearce[1], JG Calvert[1], RG Ankenbauer[1], JRD Allison[2]
[1]*Zoetis, Kalamazoo, Michigan, USA,* [2]*Zoetis, Florham Park, NJ, USA,* jim.allison@zoetis.com

Introduction

In the pig, the porcine alveolar macrophage (PAM) is the primary target for replication of the Porcine Respiratory and Reproductive Syndrome virus (PRRSV). In the development of a modified-live PRRSV vaccine the strain selected is attenuated by progressive passage on a non-PAM cell line. During the process of attenuation the virus loses pathogenicity as it accumulates mutations that favor its replication in a different host cell. The majority of live PRRSV vaccines have been produced using cells of simian origin (e.g. MA-104 and derivatives such as MARC-145). Fostera™ PRRS, however, was attenuated using a unique line of baby hamster kidney cells engineered to express the CD163 PRRSV receptor. Use of a different cell is likely to lead to different selection pressures and may result in different characteristics in the attenuated virus. This study compared the ability of the Fostera PRRSV to grow on primary PAM cells with that of two other commercial vaccine strains.

Materials and Methods

Three modified-live virus vaccines were tested: Fostera PRRS (Zoetis), Ingelvac® PRRS MLV and Ingelvac PRRS ATP (Boehringer Ingelheim). Serial 10-fold dilutions, prepared using a standard diluent, were added to 96-well microtiter plates previously seeded with PAM cells (Rural Technologies Inc). Plates were incubated at 37°C under 5% CO_2 for 1-2 days, then centrifuged. The supernatants were discarded and cells were fixed with ice-cold 80% acetone for 15 minutes. The fixative was discarded and the plates air-dried for 15 minutes. Monoclonal antibody MAb SDOW17-F (Rural Technologies Inc.) was diluted 1:100 in PBS and 0.1 mL added to each well. The plates were examined using a fluorescent microscope and the viral titers calculated using the Spearmen-Karber method (1,2,3)

Results

The live virus titers for the 3 vaccines are shown in Figure 1. Fostera PRRSV had the highest titer, followed by Ingelvac PRRSV ATP. Ingelvac PRRSV MLV did not have a detectable live virus titer on PAMs (≤1.5 $\log_{10}$ $TCID_{50}$/mL). To verify the presence of live virus in Ingelvac PRRSV MLV, the vaccine was titrated on the MARC-145 monkey kidney cell line. This showed a titer of 4.33 $\log_{10}$ $TCID_{50}$/mL, demonstrating that live virus was present. Figure 2. shows the detection of PRRSV nucleocapsid using fluorescent antibody. Fluorescence was detected with Fostera PRRS at a dilution of 1:20 through to 1:20,000. It was not detected in any dilution of the Ingelvac PRRS MLV vaccine, while fluorescence was detected with Ingelvac PRRS ATP only at dilutions up to 1:200.

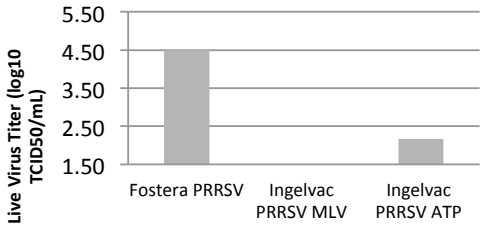

Figure 1. Live virus titer comparison for the 3 vaccines tested using PAMs as cell substrate

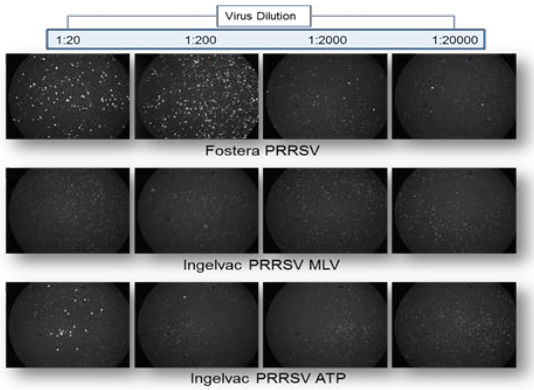

Figure 2. Detection of PRRSV nucleocapsid using fluorescent antibody.

Conclusions and Discussion

The results confirm that the Fostera PRRSV differs from the other vaccine strains in that it can replicate efficiently in the target host cell without any re-adaptation, a property that may potentially influence the speed and nature of the immune response. Modified-live vaccines, particularly those produced using different methods of attenuation, may have fundamentally different characteristics.

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Efficacy and non toxicity of a bivalent acellular vaccine formulation of proteoliposome type against *Leptospira spp* serovars of epidemic interest in pig populations

DF Arencibia [1]*, LA Rosario[2], N Batista[1], JF Infante[1], Y Valdés[1], B Tamargo[2], VG Sierra[1]

[1]Finlay Institute. 198 street and 27 avenue. Number 19805. La Lisa. Havana. Cuba. [2]Institute of Pharmacy and Food Science (IFAL, U.H). 222 street between 25 and 27 avenue. La Lisa. Havana. Cuba, darrebola@finlay.edu.cu

Introduction

Leptospirosis constitutes one of the zoonosis with more impact in the veterinary and human health. The available leptospiral vaccines are fundamentally of inactivated whole cells and most for veterinary use. Although these formulations are effective they have multiple limitations, among those that it not stands out the strait margin of crossed protection against serovars included in the vaccine, low immunologic memory, absence of cellular immune response and reactogenicity. It is that the serovars of more incidences in pig populations in the word are Canicola, Ballum, Arborea, Mozdoc y Cophenageni. The aim of this work was evaluated the effectiveness and toxicity of a new bivalent acellular vaccine formulation (*Leptospira spp* Canicola and *Leptospira spp* Mozdok).

Materials and Methods

In the candidate's vacunal evaluation it was used pigs of Yorkshire breed for the study of immunologic characterization and Syria hamsters for the challenge and toxicity studies. During the study it was evaluated the antibodies response and generated cytokines as well as the protection for *in vivo* lethal challenge and the toxicity to single, repeated dose and local tolerance.

Results and Discussion

The immunization (schedule of two doses with an interval of three weeks) in Yorkshire pigs generated a potent response of IgM after the first dose which it is maintained for 8 weeks after the second dose, the IgG response it increases significantly regarding the values of the first dose and it is maintained for 16 weeks. The cellular response was potent when being increased the cytokines expression as IFN γ and IL1 from the first dose. The determination of CD45+ as indicator by immunologic memory it showed that this memory was present until 16 months (last determination). The *in vivo* evaluation against lethal challenge with 100 000 LD_{50} of the serovares Canicola, Ballum, Mozdok and Cophenageni in Syrian hamsters it evidenced a protection of 100% in the immunized animals and challenged. In all cases the elimination of carrier state was also verified in kidneys, liver and lungs. The evaluation in toxicity studies reveals the absence of damage in the main immunologic organs, while the evaluation of the damage in the immunization area through histopathological studies only evidences damage associated to the use of the needles**.**

Conclusions

The obtained results endorse the effectiveness and non toxicity of the new vacunal candidate evaluated that it includes: immunogenic, generator of immunologic memory, crossed protection, non systemic and local toxicity, with regard to the traditional vaccines; it is recommended the continue studies of effectiveness against other serovars not included in this study and the beginning of the clinical trials in pig populations affected with Leptospirosis.

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Cost of pneumonia due to chronic pleurisy and increased use of antibiotic after different vaccination strategies against *M. hyopneumoniae*

ED Sorensen[1], P Astrup[2], J Haugegaard[2]
[1]*OE-VET, Swine advisory service, Naestved, Denmark;*
[2]*MSD Animal Health, Copenhagen, Denmark,* Peter.astrup@merck.com

Introduction

Pneumonia in growing pigs is often caused by *Mycoplasma hyopneumoniae* (Mhyo) followed by secondary infection with bacteria like *Actinobacillus pleuropneumonia* serotype 6 (AP6). If Mhyo is not controlled, an increased frequency and severity of pneumonia and chronic pleurisy at slaughter may occur. Both frequency and severity of pneumonia and chronic pleurisy is related to a reduced ADG and a subsequent increase in FCR. On top of the loss from ADG and FCR, impact during growth is direct loss due to quality impairment of the carcass. The value loss for a carcass with chronic pleurisy is 63 € cent (Danish Crown Oct. 2013). In addition, clinical pneumonia also results in increased antibiotic use. The aim of this case description is to use automatically generated data like slaughterhouse reports and antibiotic use data to calculate the cost that results from pneumonia on top of loss from ADG and FCR impact.

Materials and Methods

A Danish finisher farm vaccinated pigs shortly after entering the weaning facility with M+Pac and Porcilis PCV, to prevent disease caused by Mhyo and PCV2 virus. The pigs were transferred from the sow farm in an AI/AU system in batches of 390 every 4th week, reared from 7-30 kg and moved into a continuous flow. The farm was infected with PCV2, Mhyo and AP6. The vaccination program was changed in the beginning of Dec2012 to a 2 ml combination of PCV2 and Mhyo vaccine. During early Spring, coughing frequency increased, resulting in a severe pneumonia problem due to a growing endemic AP6 infection. The number of daily doses of penicillin (Ethacilin inj. recorded in vetstat) to treat pneumonia increased from 0,9 to 3,5 per pig. In the beginning of July, a ResPig investigation was initiated. Serology results showed Mhyo seroconversion, PCV2 in moderate numbers and ApX-II toxin in high levels in older pigs. PRRS Elisa was negative. An extended slaughterhouse investigation (ESI) of 32 lungs demonstrated a high frequency and a high average distribution of catarrhal pneumonia (Table 1). SIV was not suspected in the farm. In week 18, the farm returned to using M+pac. Later, the PCV2 vaccine was again shifted to Porcilis PCV. In week 43 and week 48, two new extended slaugterhouse examinations of a total of 53 lungs were done (Table 1).
Weekly slaughterhouse reports of pigs with pleurisy supported development of poor lung health, as the frequency of pleurisy increased after switching to the non-MSD Mhyo vaccine. Pleurisy dropped significantly after returning back to M+pac (Figure 1).

Results

Table 1. Results from extended slaughterhouse examination.

Lesion	32 lungs Vac 1	53 lungs Vac 2
Catarrhal pneumonia - % lungs affected	72%	47%
Catarrhal pneumonia in affected lungs– average % of lung with lesion	16%	8%
Catarrhal pneumonia in all lungs– average % of all lungs	11%	3,6%
% of lungs with >15 % of lung affected	47%	6%
% of lungs with pleurisy	75%	13%

Vac 1: non-MSD vaccine; Vac 2: M+pac

The weekly pleurisy recordings at the slaughterhouse are summarized in Figure 1.

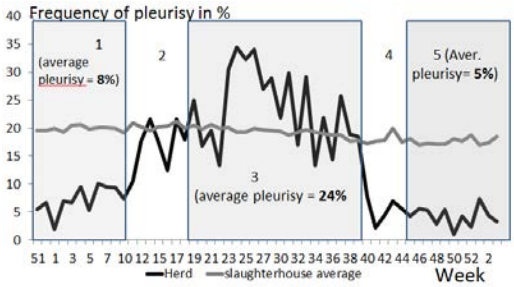

Figure 1. Numbers 1-5 reflect different periods: 1) pigs vaccinated with M+pac, 2) Transition, 3) Non-MSD Mhyo vaccine, 4) transition, 5) M+pac.

Conclusions and Discussion

In this case study, pleurisy severity and pneumonia antibiotic treatment increased after switching the vaccination program from M+pac and Porcilis PCV to a combination vaccine and dropped after returning to the original vaccination program. Lungs were severely affected by catarrhal pneumonia during non-MSD vaccine use and both frequency and severity dropped after returning to M+Pac. With the limitations of this being a case study, it can be concluded, that the increased frequency of 16%-points in chronic pleurisy had a cost of 10 € cent per pig. Furthermore, the cost of antibiotic treatment increased from 15€ cent to 58€ cent. On average, the direct cost and loss was 53€ cent higher per pig in the period of vaccinating with the non-MSD vaccine compared to M+pac. In Return Of Investment (ROI) calculations, the cost of antibiotic use and loss from impaired slaughter quality is often overseen due to solely focusing on production parameters like ADG and FCR.

Development of a *C. perfringens* type A/C toxoid vaccine for sows to protect piglets against the Necrotic Enteritis and negative effects of an infection with *C. perfringens* type A

S Springer, J Finzel, G Hagemann, V Florian, N Hitzel, HJ Selbitz
IDT Biologika GmbH, Animal Health, R & D Department, Dessau-Rosslau, sven.springer@idt-biologika.de

Introduction

Clostridium perfringens Type C (CpC) causes necrotising enteritis (NE) in suckling piglets which can result in a high mortality (1). However, *C. perfringens* Type A (CpA) belongs to the normal intestinal microbiota of piglets within the first days of life (2). Strains producing high quantities of toxins (α- und β2-toxin) though are able to cause diarrhea in suckling piglets under unfavourable conditions (3). In order to control the disease a CpA-toxoid-vaccine (Enteroporc A) was developed, a CpA-CpC-toxoid-vaccine (Enteroporc AC) is currently under development. Aim of the studies was to examine the efficacy of the vaccines by using intoxication models under laboratory conditions.

Materials and Methods

Sows were vaccinated with the respective vaccine batches (Enteroporc A, Enteroporc AC) 5 and 2 weeks prior to farrowing. The corresponding control groups received physiologic saline solution at the same times. Prior to the 1st and 2nd vaccination blood samples and at the time of farrowing colostrum samples were taken from each sow. Sera and colostrum samples were analysed for antibodies (ab) against α, β1 and β2 toxins by ELISA technique. For the evaluation of the efficacy 2 piglets of each litter were challenged with a sterile supernatant of CpA resp. CpC i.p. on day 1 after farrowing. After the CpA challenge the clinical symptoms were evaluated by a score and the mortality was determined. After the CpC challenge the mortality and the number of runts were determined.

Results

All sows reacted with a significant rise in ab (Mann Whitney U test, one tailed, $p<0.05$) according to the vaccination against α- and β2-toxin (Enteroporc A) resp. α-, β1- and β2-toxin (Enteroporc AC) in the sera (at the time of farrowing) and in the colostrum. After challenge with the CpA supernatant animals of the unvaccinated group partially became severely ill. Vaccinated animals (Enteroporc A) did not become ill (see Table 1). The differences (score) between both groups were significant (Mann Whitney U test, one tailed, $p < 0.05$). After i.p. challenge with the CpC supernatant 78.6 % of the animals of the control group died, 7.1 % showed a reduced development (runts). 14.3 % of the animals of the vaccinated group died, runts did not occur (see Table 2). The differences in mortality were significant (Fisher exact test, one tailed, $p< 0.05$).

Table 1. Results of the clinical score (mean and SD) and the mortality after vaccination with Enteroporc A and i.p. challenge of the piglets with the CpA supernatant

Group	N (sows)	N (piglets)	Mean Score± SD	Dead piglets
Vacc.	8	16	0[a]	0
Placebo	8	16	2.81 ± 2.01	1 (6.25%)

Vacc. = Vaccinated, [a] $p < 0.001$ (Mann- Whitney U test, one tailed)

Table 2: Results of the mortality and number of runts after vaccination with Enteroporc AC and i.p. challenge of the piglets with the CpC supernatant

Group	N (sows)	N (piglets)	Dead piglets	Runts
Vacc.	7	14	2 (14.3%)[a]	0
Placebo	7	14	11 (78.6%)	1 (7.1 %)

Vacc. = Vaccinated, [a] $p < 0.011$ (Fischer's exact test, one tailed)

Conclusions and Discussion

The vaccine Enteroporc A resulted in the development of ab against α- und β2-toxin in the serum and colostrum and reduced significantly the morbidity after i.p. challenge with a CpA supernatant. Enteroporc AC led to the development of ab against α-, β1- und β2-toxin and significantly reduced mortality. For the future trials are planned to prove the efficacy under field conditions.

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Modified-live PRRSV vaccine at weaning reduces shedding of wild-type virus in aerosol of growing pigs

S Dee[1], J Nerem[1], T Wetzell[2], JP Cano[2], J Rustvold[2]
Pipestone Applied Research, Pipestone,MN[1], Boehringer Ingelheim Vetmedica Inc, St. Joseph, MO[2], sdee@pipevet.com

Introduction

The risk of area-spread of porcine reproductive and respiratory syndrome virus (PRRSV) continues to be high in swine-dense regions[1] potentially because of PRRSV shedding from large populations of growing pigs. The therapeutic use of modified-live virus (MLV) vaccine in infected pigs has been shown to reduce the duration of wild-type virus (WTV) shedding to sentinels and in aerosol.[2,3] The objective of this study was to quantify the effect of MLV vaccine on performance and measure WTV shedding in pigs vaccinated at weaning and challenged 4 weeks later.

Materials and Methods

A total of 2100 PRRS-negative weaned pigs were randomly allocated to either a non-vaccinated control (NVC) or to a MLV vaccinated group, each housed in separated rooms. Biosecurity protocols were implemented to avoid PRRSV transmission between rooms. Pigs in the MLV group were IM vaccinated with Ingelvac PRRS® MLV (Boehringer Ingelheim Vetmedica, Inc, St Joseph, MO) at 4 weeks of age. Four weeks post-vaccination 10% of the pigs in each group were IM inoculated with 1 mL of PRRS WTV RFLP pattern 1-18-2 at a concentration of $4.2x10^7$ RNAc/mL. Infection dynamics was monitored by PCR and ELISA tests on serum and oral fluid (OF) samples collected at 3, 7, 14, 23, 29, 37,64, 93 and 118 days post-vaccination (DPV). Daily air samples were collected from each group at 8 AM using Liquid Cyclonic Collectors (Midwest MicroTek, Brookings, SD) placed in front of exhaust fans for 30 minutes and tested by PCR. Mortality, cull rate, average daily gain (ADG) and feed conversion (FC) were recorded to compare wean to finish performance between groups.

Results

Pigs in the MLV group were PRRSV PCR and ELISA negative before vaccination as the pigs in the NVC group were negative before inoculation. Mild clinical signs developed in both groups following the inoculation with WTV. There was no significant difference between groups in the duration and magnitude of viremia and seroconversion detected in serum or OF samples. MLV vaccine was not detected in the NVC group. The frequency of detection of PRRSV RNA in air samples was significantly higher (P<0.0001) in the NVC than in the MLV group (Table 1). The duration of detection of PRRSV RNA in air samples was shorter in the MLV group than in the NVC group (Table 1). Performance parameters per group are summarized in Table 2.

Table 1. Detection of PRRSV in air samples by PCR

Parameter (days)	NVC	MLV
Frequency post-vaccination	0/28[a]	5/28[b]
Duration post-vaccination	0	6
Frequency post-inoculation	21/118[b]	4/118[a]
Duration post-inoculation	36	6

Different superscripts indicate significant differences (p<0.0001) with McNemar's test

Table 2. Vaccination to market performance

Parameter	NVC	MLV
Mortality %	4.8	5.1
Cull rate %	5.9	2.8
ADG (lbs)	1.57	1.63
FC (lbs)	2.38	2.40

Conclusions and Discussion

PRRS MLV vaccination at weaning reduced the frequency and duration of WTV shedding in aerosol. If the room conditions did not affect performance, MLV vaccine reduced the percentage of culled pigs by 3.1 percentage units and improved ADG by 0.06 lbs/d. The observed performance benefits as well as shedding reduction in MLV vaccinated pigs challenged with WTV support the recommendation of MLV vaccination of growing pigs at risk of infection. The prophylactic use of PRRS MLV vaccine in growing pigs at risk of infection represents a valuable tool to reduce the risk of transmission between herds in swine-dense-areas.

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Piglet vaccination against circovirus reduces antibiotic use in weaners

H Bak[1]

[1]*Boehringer Ingelheim Vetmedica, Copenhagen, Denmark,* hanne.bak@boehringer-ingelheim.com

Introduction

In several European countries, pig producers are under severe political pressure to reduce the consumption of antibiotics. In earlier studies, vaccination of piglets against Circovirus has shown to reduce the use of antibiotics in pig herds (1, 2, 3). The present study was carried out in a large sample of pig herds and compared consumption of antibiotics in weaners that had been vaccinated against Circovirus to the consumption in non-vaccinated weaners.

Materials and Methods

From the Danish National database, a random sample of sow herds with piglet production was drawn. The sow herds were sorted according to their number in the central husbandry register (CHR), and the prescriptions for each sow herd were looked up in the Vetstat Database. Based on the prescription of vaccines against Circovirus from May 1 2012 until April 30 2013, the sow herds were allocated to one of two groups:

Group 1: Piglet vaccination against Circovirus.

Group 2: No piglet vaccination against Circovirus.

A total of 50 herds in each group were included.

Data for antibiotic use was extracted from the Vetstat database from June 1 2012 until May 31 2013 for piglet producing herds receiving piglets from the 100 sow herds. For each piglet producing herd, the number of prescribed doses of antbiotics (ADD, Animal daily doses) per 100 pigs per day was recorded on a monthly basis throughout the year, and a yearly average for each herd was calculated. These yearly averages were then used to calculate a yearly average for each sow herd, weighted according to the number of weaners in each piglet producing herd receiving piglets from the sow herd.

Statistical analysis was carried out using the weighted averages for each of the 50 sow herds in each group. Mann-Witneys U-test was used to compare the mean number of prescribed doses in the two groups, with the level of significance set at p=0.05.

Results

In the herds with vaccination of piglets against Circovirus, the mean consumption of antibiotics for weaners was 10.6 ADD/100 pigs/day., This represents a reduction of 10% or 1.18 ADD/100 pigs/day compared to the consumption in herds with no piglet vaccination against Circovirus. Figure 1 shows the distribution of the antibiotic consumption in herds with and without piglet vaccination. Some non-vaccinated herds had an extremely low consumption. Figure 2 shows the mean and standard deviation of the antibiotic consumption in vaccinated compared to non-vaccinated weaners. The difference between vaccinated and non-vaccinated weaners was not statistically significant.

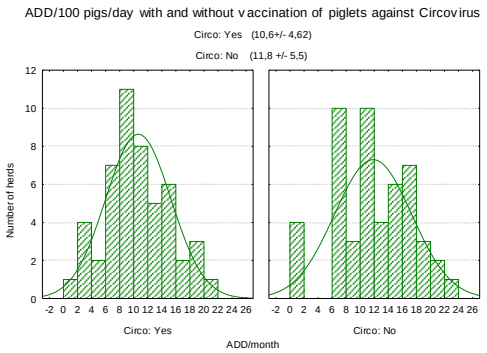

Figure 1. Distribution of sow herds according to mean consumption of antibiotics; Circovaccinated piglets compared to non-vaccinated, 50 sow herds per group.

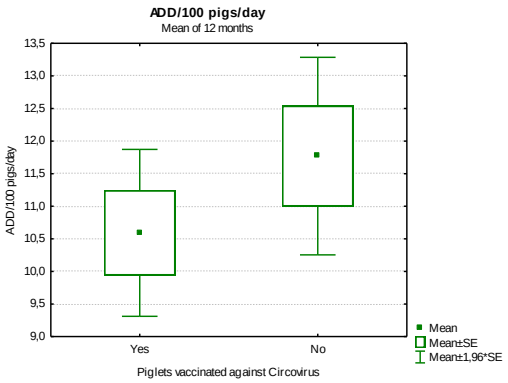

Figure 2. Mean consumption of antibiotics with and without vaccination of piglets against Circovirus.

Conclusions and Discussion

In this study, the consumption of antibiotics was reduced 10% in weaners vaccinated against Circovirus compared to non-vaccinated weaners. The difference was not statistically significant but still, it can be regarded as valid, considering the fact that the sample was drawn without any knowledge of diagnosis in the herds. Some herds appear to be skeewing the data in the non vaccinated group. A number of the non-vaccinated herds might have a very low prevalence of Circovirus in weaners or freedom from other diseases, possibly shown by the extremely low consumption of antibiotics.

Hence, the present study confirms that vaccination of piglets against Circovirus can be considered as a tool to reduce consumption of antibiotics in weaners.

Acknowledgements

LandIT is thanked for the initial sample of sow herds.

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Effects of routine administration of Meloxicam (METACAM®) in sows after farrowing on piglets performance

S Andreoni[1], O Vischi[1], F Persico[2]
[1]*Boehringer Ingelheim Italia spa Divisione Vetmedica,* [2]*DVM Vet practictioner,*
simone.andreoni@boehringer-ingelheim.com

Introduction

Swine is the specie that suffers from the highest lactation losses, from 12% to 25% (1).
PPDS (post partum dysgalactia syndrome) in sows is associated with economic losses in piglets with decreased growth and increased mortality; the use of NSAIDS as an adjunct therapy in PPDS is described to alleviate effects of inflammation and endotoxiemia in sows and to increase piglet survival (2).
The use of NSAIDs during lactation is limited to clinical PPDS in sows while is not common to treat both clinical as well as subclincal PPDS.
The aim of the study is to investigate the effects of a systematic administration of Metacam® (Boehringer Ingelheim Vetmedica GmbH) in sows under Italian swine production management conditions.

Materials and Methods

The field trial was conducted in two farms with different PPDS incidence (Farm A: 700 sows farrow-to-wean, Farm B: 250 sows farrow-to-wean) located in the center and north of Italy respectively. Overall, 203 sows were randomly allocated on the day of farrowing, to group "treated" (93 sows: 42 Farm A, 51 Farm B) or group "control" (110 sows: 49 Farm A, 61 Farm B). All sows were uniquely identified by numbered ear tag and their litters by farrowing pen number. No cross-fostering was allowed between the two groups, while it was possible within the same group.
In the control group only clinical cases of PPDS were injected with Metacam® while all sows in the treated group received a Metacam® injection IM at 0,4 mg/kg b.w. (2 ml/100 kg b.w.).
Referred to sanitary prevention procedure active in the farms, all the sows received a parenteral antibiotic treatment at farrow.
The efficacy of the treatment was evaluated by comparing mortality rate and body weight at weaning date (21d Farm A, 25d Farm B). Body weight data were analyzed by Student`s test. Mortality rate was analyzed using the Chi-Square-test. Significance level was $p<0.05$.

Results

Overall, 2.743 born alive piglets were included in the trial (1.241 treated vs 1.502 control). Total piglet mortality rate up to weaning was significantly lower in the Metacam® group compared to control group (7.57% vs. 12,12%; p=0.0001) (figure 1). This resulted in higher survival rate of treated group (92,43% vs 87,88%)
In the control group 10/49 sows of farm A and 1/61 sow of farm B were treated for clinical PPDS.

Regarding litter performances, piglets from treated sows showed higher weight at weaning than the control group (Farm A 6.06 vs 5.67 Kg p<0,05; Farm B 6,86 vs 6,59 Kg p>0,05) (Figure 2).

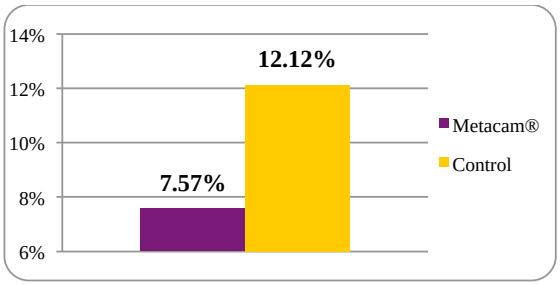

Figure 1. Mortality rate % (p=0,0001)

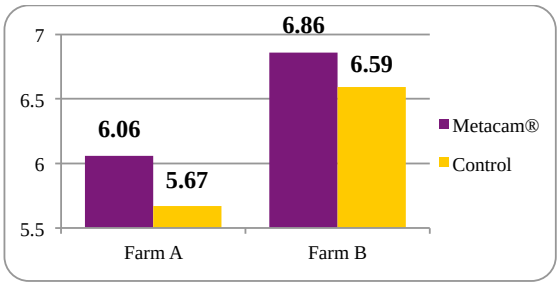

Figure 2. Body weight at weaning (Kg) (A p<0,05; B p>0,05)

Conclusions and Discussion

Not all sows exhibit the same range of intensity of PPDS clinical signs and number of affected sows may vary (2).
Is reported that with a 1% improvement in piglet mortality assumed from the treatment, the benefits outweigh the treatment cost (3).
In this trial, in presence of different PPDS incidence, routine administration of Metacam® in sows at farrowing, significantly decreased piglet mortality rate and increased piglets weight at weaning .

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Demographics and spatial trends of PRRS in swine herds from two regions of Ontario, Canada

AG Arruda[1], Z Poljak[1], R Friendship[1], J Carpenter[2], K Hand[3]
[1]*Department of Population Medicine, University of Guelph, Guelph, ON,* [2]*Ontario Swine Health Advisory Board, Stratford, ON,* [3]*Strategic Solutions Group, Puslinch, ON,* arrudaa@uoguelph.ca

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is the most costly and complex disease affecting swine herds in North America (1). In eastern and central Canada, it has been estimated that between 40 to 80% of the swine herds are seropositive (2). PRRS Area Regional Control and Elimination (ARC&E) projects began in Ontario in 2010 with the aim of monitoring disease trends and decreasing the pressure of infection in specific areas. Currently, more than 20% of Ontario swine herds are participating in ARC&E projects (3). Two of those projects include the areas of Niagara and Watford. The objectives of the present study were to describe demographics and biosecurity practices of swine herds participating in control programs and investigate the presence of spatial clustering and clusters of positive herds.

Materials and Methods

Swine herd demographics and biosecurity information was collected using a standard questionnaire administered by project personnel. Sampling was conducted by veterinarians and included blood and/or oral fluids (n= 9 to 11 samples per herd). All samples were processed in the Animal Health Laboratory at the University of Guelph and included ELISA, PCR and sequencing of the ORF5 gene. A herd was considered positive if at least one of the samples was positive. Descriptive analysis was conducted using SAS version 9.3 and ArcGIS 10.1, clustering analysis was conducted using the D-function on R version 2.15.0 and cluster analysis was conducted using R version 2.15.0 and SaTScan version 9.1.1.

Results

A total of 73 and 77 sites were enrolled from the regions of Niagara and Watford, respectively. The two regions had approximately 1,700 and 510km^2 and low and mild density of pigs, respectively. The distribution of production types by region is shown in Table 1. For the Watford region, 58.8% of all herds reported using an all-in all-out pig flow, 50% reported having a shower-in facility in place and from the herds that did not have it, approximately 70% reported having at least *Danish entry* in place. The mean prevalence of PRRS in swine herds at the end of 2013 was 16.7% and 48.2% for the regions of Niagara and Watford, respectively. Spatial analysis showed no evidence of spatial clustering of PRRS-positive herds for either of the regions in this time period. Risk maps showed that herds located at the eastern region of Niagara and south western region of Watford are at a higher risk to be positive for PRRS compared to the other areas. Clusters of positive herds were found in both regions using the scan statistic and spatial relative risk methods.

Conclusions and Discussion

Preliminary data analysis using two regions in Ontario that are characterized by a relatively moderate pig density does not support location as a significant risk factor for the presence of PRRSV infection. However, clusters of positive herds could be detected for both regions. Regions where the risk is increased should be targeted for surveillance and disease control, but further elucidation, including ones based on phylogenetic and social network analysis, are needed on what are the main contributors to this finding.

Table 1. Distribution (%) of production types for herds participating in the PRRS ARC&E programs for two regions of Ontario

Production type	Niagara[1]	Watford[1]
Farrow-wean	5.5 (5)	8.0 (6)
Farrow-finish	11.0 (8)	11.7 (9)
Farrow-feeder	9.6 (7)	6.5 (5)
Nursery	8,2 (6)	5.2 (4)
Wean-Finish	6.8 (5)	10.4 (8)
Finish	57.5 (42)	55.8 (43)
Isolation/ Acclimatization	0 (0)	2.6 (2)
Missing information	1.4 (1)	0 (0)
Total	**100 (73)**	**100 (77)**

[1]The number correspond to the percentage of total herds, and in brackets the actual number of herds.

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Ontario Ministry of Agriculture and Food and Ontario Ministry of Rural Affairs, ON, N1G 4Y2
Ontario Swine Health Board Advisory, ON, N5A 6S8
Canadian Swine Health Board, ON, K1P 5Z9
Ontario Pork, ON, N1G 5G6
Natural Sciences and Engineering Research Council, ON, K1A 1H5
Agricultural Adaptation Council, ON, N1G 5L3

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Effect of Aivlosin®/Chlortet® pulse program on the frequency of respiratory illness and growth performance in pigs of a commercial farm

A Ruiz[1], V Medina[1], J Uriarte[2], R Cerdá[2]
[1]*Departamento de Patología y Med. Preventiva, Facultad de Cs. Veterinarias, Universidad de Concepcion, Chile,* [2]*Eco Animal Health, UK,* aruiz@udec.cl

Introduction

Despite all the changes in production systems and deployment of high health status, respiratory problems continue to have great importance in the swine industry today (1). There are many microorganisms that can affect the respiratory tract of pigs, being currently accepted that respiratory disease is the result of a complex interaction that may involve a large number of agents. Respiratory disease with multiple etiologies, in which *Mycoplasma hyopneumoniae* plays an important role is usually known as Porcine Respiratory Disease Complex or PRDC (2). The objective of the present study was to evaluate the effect of Aivlosin®, a macrolide antibiotic, and Chlortet®, a premix chlortetracycline calcium complex, on productive performance and the appearance of respiratory symptoms in pigs reared on a commercial farm.

Materials and Methods

A commercial farm of approximately 5000 sows, with segregated weaning, three site production system, all-in all-out management and automatic feed system was used. From this farm, 2500 piglets of two consecutive weeks of production, housed in the same type of building and with the same management, were followed from weaning age to slaughter, 20 to 167 days respectively. Animals from one production week were randomly assigned to the control group, whose pigs received a normal antimicrobial product used regularly for respiratory problems in the industry (Group A), while pigs the other production week were treated with the Aivlosin®/Chlortet® mix (Group B), as summarized: Group A received pulses of Chlortetracycline between 21 to 30, 31 to 70, 71 to 85 and 86 to 108 days of life at 500, 400, 500 and 100ppm respectively; Tylosin between 71 to 85 and 86 to 108 days of life at 110ppm and Lincomicin 4,4% between 109 to 130 days of life at 100ppm. Group B received pulses of Aivlosin® between 21 to 40, 70 to 77 and 98 to 104 days of life at 20, 50 and 50ppm respectively; and Chlortet® between 70 to 77 and 98 to 104 days of life at 400ppm.

Both groups were fed *at libitum* with the different diet that are normally used in the swine industry. The animal's feed consumption and weight were recorded throughout the study and feed conversion efficiency (FCE) and average daily gain (ADG) were calculated. Any treatment and clinical signs were recorded. Necropsy was carried out on animals that died or had to be euthanized for welfare reasons. The cost and profit margins were calculated on the basis of the variables described. Approximately two hundred lungs per group were randomly selected at the slaughterhouse, and the location and size of lesions were recorded.

Results

At the weaning age, no statistical differences were observed between groups, therefore they were comparable. By the end of the growing period, day 73 approximately, pigs from both groups had similar production parameters, which did not show statistically significant differences in average weight, ADG and FCE. However, by the end of the fattening stage, the animals of group B reached an average weight of 109.2 kg 2.1 days earlier than the pigs in group A (166.7 days v/s 168.8 days respectively), they were 1.5% more efficient, with 1.3% more pigs classify as a premium, better ADG in 0.019 Kg, 3 cents less feed cost per kg of pork produced and better FCE (2.79 and 2.70 for Groups A and B respectively), differences that are profitable.

The causes of death of the animals from both groups during the study were not unusual for a commercial farm. However, at the fattening period, 25 pigs fewer died before reaching slaughter weight in the Aivlosin®/Chlortet® treated group, a 2% decrease in mortality for group B.

Inspection at the slaughterhouse showed 30.8% of lungs with lesions compatibles with M. hyopneumoniae infection in the animals of group A, while only 16.5% of the lungs of pigs in group B were affected.

Conclusions and Discussion

The Aivlosin®/Chlortet® pulse programme did not show a better performance in the pigs during the growing period compared with the other antibiotics routinely used on the studied farm. However, it showed a significant improvement in efficacy in the fattening phase (lower mortality and lower feed consumption and greater ADG, resulting in better FCE) which resulted in a greater amount of kilos sold at a lower cost. Along with this, Aivlosin®/Chlortet® had a significant effect on reducing the number of animals with lung lesions and the extent of these injuries.

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P.171

Veterinary safety issues in Georgia

K Nnadiradze, N Phirosmanashvili
Association for Farmers Rights Defense, AFRD; foodsafetyge@gmail.com

It is common knowledge that climate change particularly affects developing countries like Georgia, but its effects on health, nature and environment are still very hard to predict. In a joint effort to bridge this gap, we worked on research project to assist risk management and health decision –makers in allocating resources and implementing preventative measures ahead of disease epidemics.

Whilst climate-change predictions depends on many variables, making forecasting a real conundrum for scientists across the world, the impact of a changing weather on human health is even more uncertain, Its is now largely accepted that global warming increases the concentrations of air and water pollutants, and affects the seasonality of certain epidemic diseases in Georgia, but main question is how can such changes be predicted, especially in Georgia, where local knowledge is hardly used in forecasting methods. Our research project brought together researchers from different Institutes and Universities in Georgia to increase data from climate-change –modeling and disease –forecasting systems. The Projects was focused on risk management and disease control in Animals, aiming giving to decision-makers the necessary recommendations to deploy intervention methods and help prevent large-scale spread of zoonotic diseases and different pollutants affecting food and feed for human ad animals. The overall objective of our research project was to combine climate models, weather-dependent infection-control data for key diseases, and local knowledge of Farmers about population behavior, disease control and transmission patterns. We learned by these researches that there was a clear lack of use of climate-model data sets for impact studies, assessments and evaluations.

We investigate the conditions of the animals diseases like: brucellosis, bovine tuberculosis, echinococcosis, leishmaniosis, listerias and zoonotic trypanosomes. These diseases pose a direct risk to human health's in Georgia, and can also have a serious impact on livestock productivity – and hence the livelihoods of Farmers and their families. By helping farmers implement effective zoonosis controls and for increasing awareness of the problems they cause, we had success. Our team now is developing new practical, cost-effective and sustainable strategies for keeping the diseases in check. We built a unique database to provide detailed information on location, scope and type of research currently being carried our on these zoonoses. AFRD has identified an overwhelming need for capacity building in Country side of Georgia, where most farmers are focused more on veterinary medicine, than human health.

Risk of pig farm manure management on Mexican disease crackdowns

MA García[1], R Olea[1]
[1]Departamento de Medicina y Zootecnia de Cerdos, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de Mexico, miguel_gag@hotmail.com

Introduction

During the last 40 years, pig manure management and disposal regulation have been evolved in Mexico. Since the beginning, these regulations have been focus on microbial content of the sludge discarded of pig farms (1, 2). Main microbial quality control is linked to human health risk, but poor attention has been on farm manure management and disposal (MM&D) linked to intra or inter-farm pig disease spreading risk. Probably the success of Mexican classical swine fever eradication crackdown has contributed to minimise the importance of microbial survival in organic matter during MM&D.
The main objective of this paper is to emphasise the risk that MM&D have on the success of actual and future Mexican campaigns on disease control and eradication.

Materiasl and Methods

A literature review was developed to characterise virus inactivation time of Aujeszky Disease Virus (ADV) and Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) during the in and out of farm MM&D. Collection, storage and disposal characteristics were modelled according to MM&D practice in main pig farms in Mexico (3). Specific fermentation processes were considered to model MM&D characteristics (4) including changes in pH, temperature and middle time storage of organic matter. Then reported surviving conditions for ADV and PRRSV were contrasted with MM&D conditions.

Results

The main results of summarised findings are shown in Table 1.

Table 1. Influence of Manure Management and Disposal (MM&D) on time (days) of viruses inactivation

System	Colection	Storage		Disposal
Temp, °C	5 to 25	5 to 25	30 to 50	5 to 25
ADV	9 ± 6	13 ± 0.7	3 ± 3	9 ± 6
PRRSV	7 ± 6	NR	1 ± 1	7 ± 6

Conclusions and Discussion

In the literature review of available papers was included viruses inactivation time in laboratory and field conditions. Even fermentation organic matter into every process is expected to reduce viruses amount, it is unknown the minimum infected dose in manure (5), thus in this study was considered maximum time of active virus recover. Another concern is that field conditions farm´s MM&D is to variable then more of literature data are not applicable to predict viruses spreading with epizootic propose. Then in this study virus recovery data were contrasted for expected manure conditions on Mexican farms. Table 1 show that in more of MM&D process is possible to find active viruses and these could be spread into or out of farm facilities. Even this study is only a model, this highlight the risk of minimise internal movement of photogenes for routine activities and could be one of the possible sources of outbreaks into or between farms (6, 7).
Modelling MM&D conditions for different environments under Mexican swine regions variations aimed to predict the risk of internal and external practice on MM&D. Therefore, MM&D practice should be consider as important risk point in the successful of National Mexican disease crackdowns.

Acknowledgment

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Evaluation of the pig health in Northern Ireland during the last ten years

J Borobia, J Cottney
MOSSVET, 34 Seagoe Industrial Estate, Portadown, Craigavon, Co. Armagh, Northern Ireland, BT63 5QD,
borobia@hotmail.com

Introduction

Recording lesions in slaughtered pigs has been carried out in many pig producing countries in order to provide information to the farming industry. The aim of the study was to assess the evolution of pig health in Northern Ireland over 10 consecutive years by evaluating lesions of the skin, thoracic and abdominal cavities and its correlations.

Materials and Methods

A total of 252,017 pigs from an average of 142 producers were examined twice a year from 2004 to 2011 and three times a year from 2012 to 2013. Data was not collected in 2007 by lack of funding. This sampling represented 95% of the pig producers in Northern Ireland (1). Lungs were scored for pneumonia consolidation using the Muirhead 55 point scoring system (2). Lesions of pleurisy, pericarditis, necrotising pneumonia, lung abscesses, liver spot (ascariasis), papular dermatitis (mange) and tail biting were also recorded. Data was analysed using JMP® version 9.0.3 (SAS Institute Inc., Cary, NC, USA) at a significant level of 0.05.

Results

A summary of lesions by period and season is shown in Tables 1 & 2. A weak positive correlation between pleurisy and pericarditis ($r=0.17$, $p<0.001$); pleurisy score >10% and necrotising pneumonia ($r=0.66$, $p<0.001$) were moderately correlated.

Table 1. Lesions by period.

	2004-2006	2008-2010	2011-2013
EP%	24.7^{A}	15.4^{B}	8.7^{C}
PL%	8.8^{B}	10.9^{A}	10.2^{A}
NP%	0.7^{B}	4.1^{A}	0.4^{B}
Per.%	2.4^{B}	2.1^{B}	3.5^{A}
Abs.%	0.5^{A}	0.4^{AB}	0.2^{B}
LS%	16.8^{A}	16.1^{A}	15.4^{A}
PD%	3.6^{A}	3.1^{A}	0.8^{B}
TB%	0.3^{A}	0.3^{A}	0.8^{B}

EP= Enzootic Pneumonia *Pl.*= Pleurisy *NP*=Necrotising Pneumonia *Per.*=Pericarditis *Abs.*=Abscess *LS*=Liver Spot *PD*=Papular Dermatitis *TB*=Tail Biting
Levels not connected by same letter are significantly different.

Conclusions and Discussion

The results of the present study show for the first time the trend of the pig health in Northern Ireland during the last 10 years. There was a significant reduction of enzootic pneumonia lesions over the years without a clear seasonal effect when individual years were considered (data not shown). On the other hand, pleurisy lesions have increased since 2008. A marked increase in lung lesions (EP and NP) was identified in the colder seasons of 2008 - 2010. Pandemic (H1N1) 2009 Influenza A arrived to Northern Ireland in September 2009 (3). This viral infection could have contributed to the increase of pleurisy since (4).

Table 2. Lesions by period and season: warmer months (Q2-Q3)/colder months (Q4-Q1).

	2004- 2006		2008-2010		2011-2013	
	Q2-Q3	Q4-Q1	Q2-Q3	Q4-Q1	Q2-Q3	Q4-Q1
EP%	25.2	24.32	14.4^{A}	16.1^{B}	9.4	8.3
PL%	7.9^{A}	9.5^{B}	10.6	11.2	10.8	9.8
NP%	1.1	0.5	1.58^{A}	5.8^{B}	0.3	0.4
Per.%	2.3^{A}	2.6^{B}	2.1	2.1	3.8	3.5
Abs.%	0.2^{A}	0.8^{B}	0.5	0.4	0.3	0.3
LS%	16.2	17.3	15.1^{A}	16.8^{B}	14.8	15.9
PD%	2.9^{A}	4.3^{B}	3.3	2.9	0.7	0.9
TB%	0.2^{A}	0.5^{B}	0.3	0.3	0.8	0.8

Multiple interactions between other lesions and pleurisy were confirmed in this study. Interestingly, in cases of severe pleurisy (>10%), NP seemed to play a major role. These associations points towards the multiple aetiology of pleurisy lesions and the need of tailored control programs in farm. Liver spots did not experience a significant change in trend over the last 10 years, being more prevalent during the colder season (data not shown), contrary to (5). Papular dermatitis lesions were more common during the colder months, as seen in the Netherlands (6) and Australia (7). The reduction of skin lesions during the last 3 years could be linked to mange control programmes. The increase of tail biting during the last 3 years could be related to an increase of productivity of the sow herd leading to overcrowding and resulting in increased stress for the animals (8)

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The influence of probiotic folder additive on the morpho-functional state of pig duodenum

H Jang[1], KH Heo[1], HG Jung[1], GI Kotsjumbas[2], VM Lemishevskyj[2]
[1]*WOOGENEBNG,* [2]*Lviv National University of Veterinary Medicine and Biotechnologies*
hjang@woogenebng.com

Introduction

The normal function of the gastrointestinal tract of animals, despite the continuous intake of pathogenic bacteria can be maintained only if the natural balance of gastrointestinal micro flora. Influence of some external and internal factors (poor quality food, non- sanitary standards, desultory and inappropriate use of antibiotics), leads to disruption of the dynamic equilibrium of microbial associations in a healthy organism.

Probiotic drugs can optimize the environmental conditions in the intestine for the development of their own micro flora of the host, and the number of which is growing in Ukrain. However, there is still a need to clarify the effectiveness of probiotics in pigs breeding and basic mechanisms of their action.

Materials and Methods

The experiment was carried out in educational and research and production center " Komarnivskyj" Gorodok district, Lviv region, Ukraine on piglets of "Large White" (Velyka Bila), at the age of 28 days. According to the principle of analogues two search groups of piglets were formed per 30 heads in each. Piglets of the first group were fed with mixed fodder according to the rules recommended for "Large White" (Velyka Bila) breed taking into account age categories. On 42nd day of the experiment per 5 heads from each group were withdrawn from the experiment, pathological and anatomical cutting was conducted with the selection of material. Histological, immunohistological and mucosa ultrasuructure analysis were conducted by conventional methods.

Results

At light-optical study of histological preparation of duodenum of pigs it was indicated that in the control group of animals mucosal villi tight were placed tightly, not high, had a fingerlike shape A moderate infiltration of the mucosa of lamina propria of lymphocytes was noted. In the first group of piglets, which were fed probiotic "Probion-forte" together the villi in the form of leaves, were well structured and slightly higher relatively to the control group of piglets. Prismatic epithelial cells with marked acidophilus border on the apical surface.

By the morph metrical research, villus height of duodenum mucosa of pigs of the control group was 315.13 µm, and in pigs of the first group villus height was significantly increased and reached 374.64 µm, which is more than 59.51 µm in piglets of the control group (Tab. 1). Duodenal villus width of the control group of piglets was 164.96 µm, and in piglets of the first group - 166.32 µm. By increasing the height and width of the villi of the mucosa, are area was increased of contact with the chimes, which contributed to increased activity of digestion and absorption in the intestines of piglets, which were fed probiotic feed additives with the fodder.

The depth of the crypts of the mucous membrane of the duodenum of piglets from the controls group of oval shape and their depth was 122.71 µm, and in piglets of the first group crypt depth was significantly increased to 18.26 µm and was 140.97 µm. However, it was noted a modest increase of bowled exocrinocytes in crypts compared with the control group of animals. Width of crypts significantly increased in the mucosa of the duodenum of piglets of the first group and was 39.87 µm, relatively similar parameter of the control group 39,87 µm.

Conclusions and Discussion

The aim of the work was to study morphometric parameters, ultrastructure and content of nucleic acids in the wall of the duodenum of pigs by forage feeding with the addition of probiotic fodder additive "Probion-forte" in dose of I g/kg of fodder. The hright of villus of "Probion-forte" feding group is increase, crypt depth and a number of plasma cells in the lamina propria of mucosa of the duodenum, which helps the digestive process and increase the area of nutrient absorption in the intestines. The number of plasma cells are increased in the lamina propria of mucosa and testify immunomodulatory effect of fodder additives. Ultra structural alteration of microvilli and changes in the nuclei of duodenal enterocytes of piglets of the first group indicates a more pronounced acfunctional activity of enterocytes and thereby increases the activity of parietal digestion in the intestine.

Table 1. Morphometric indices of piglets duodenum on the 42nd day of the experiment. (M±m, n=5)

Tissue	The control of group pigs	Probion-forte 1g/kg
Villus height, µm	315,13±1,00	374,64±2,23***
Width villi, µm	164,96±1,31	166,32±1,08
Crypt depth, µm	122,71±1,93	140,97±2,50**
Width crypt, µm	39,87±0,50	46,34±0,53***
Index of villi, con.un.	2,56	2,65***

*Note: * - p<0,05; ** - p<0,01; *** - p<0,001.*

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The prevalence of lung lesions in pigs at slaughter in Ireland

A Hidalgo[1], A Cox[1], P Kirwan[2]
[1]*Elanco Animal Health, UK & Ireland,* [2]*Pat Kirwan & Associates, Dublin, Ireland,* hidalgo_alvaro@elanco.com

Introduction

Abattoir inspections are a useful tool for monitoring pig health and a source of data for epidemiological studies (1). In a recent report, pneumonia was identified as the most common cause of death in pigs submitted for laboratorial diagnosis in Ireland (2). As a result, detailed information on lung lesions at slaughter is desirable to monitor associated diseases. This study aims to investigate the prevalence of lung lesions in pigs at slaughter in Ireland.

Materials and Methods

A total of 12,597 finishing pigs sent to slaughter to a specialised pig abattoir in Ireland during the last weeks of March 2012 and 2013 were included in this study. A detailed description is presented in Table 1.

Table 1. Description of pigs and batches studied by year.

	2012	2013	Total
Pigs assessed	7,043	5,554	12,597
Batches	63	52	115
Pigs/batch	111.8	106.8	109.5
Herds	45	40	85

Enzootic-pneumonia (EP)-like lesions (Scale from 0 to 55), viral-like pneumonia (presence or absence), pleuropneumonic (PP) lesions (presence or absence), lung abscesses (presence or absence), pyaemic lung lesions (presence or absence) and pleurisy (mild: adhesions between lung lobes only; severe: adhesions involving the visceral pleura and the parietal pleura; 0 for absence) were assessed individually in the processing line. The scoring system used for these pathological lesions has been described before (1).

Results

A summary of lung scores results by year is presented in Table 2 and 3.

Table 2. Mean value of pathological lung lesions.

	Year		p-value
	2012	2013	
EP-like	1.090	1.367	0.289
Viral pneumonia (%)	2.592	0.530	<0.001
PP lesions (%)	1.265	0.603	0.052
Abscesses (%)	2.492	2.175	0.577
Pyaemia (%)	0.034	0.198	0.356
Pleurisy mild (%)	12.54	14.55	0.288
Pleurisy severe (%)	8.074	4.371	<0.001

Table 3. Prevalence of positive batches by lesion.

	Prevalence (%)		
	2012	2013	Total
EP-like	85.7	78.8	82.6
Viral pneumonia	65.0	23.0	46.0
PP lesions	52.3	30.7	42.6
Abscess	74.6	63.4	69.5
Pyaemia	4.7	3.8	4.3
Pleurisy mild	90.4	92.3	91.3
Pleurisy severe	85.7	82.6	84.3

Conclusions and Discussion

Respiratory disease in finishing pigs is prevalent among Irish pig farms, as showed by prevalence of lung lesions at slaughter. In this study, more than 80% of the batches analyzed were positive for EP-like lesions and more than 90% presented pleurisy lesions. Moreover, the number of herds investigated per year in this study was considered to be representative of commercial Irish pig farms, representing approximately 15% of them (3).

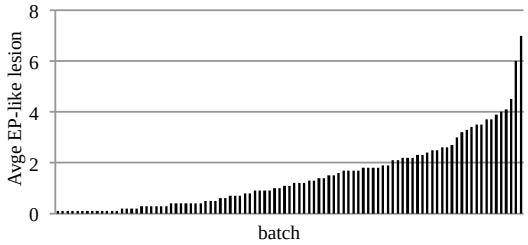

Figure 1. Distribution of average EP-like lesion score for EP-like positive batches.

While the mean value for EP-like lesions indicated a stable situation in 2012-2013, it is noteworthy that 28.4% of affected batches presented an average EP-like lesion score >2 (Fig. 1). Conversely, the high level of pleurisy detected in this survey indicates, overall, the need for better control measures in farm.

This abattoir survey identified a high prevalence of respiratory disease among commercial Irish pig farms. EP-like lesions and pleurisy were the most prevalent lung lesions. Overall, these findings suggest the need for improved control measures on farm and for continued monitoring programmes.

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Ileitis clinical form is age related; results of *Lawsonia* serological profiling compared to age and clinical signs

M Steenaert
Boehringer Ingelheim, Alkmaar, The Netherlands, martijn.steenaert@boehringer-ingelheim.com

Introduction

Ileitis caused by *Lawsonia intracellularis* (Li) has several clinical forms, from subclinical disease to the chronic from (known as PIA) to acute signs (known as PHE) (1, 4). In a herd clinical signs may differ in time and in subpopulations, but on a farm often only one or two clinical forms are recognized over a period of time. In epidemiological studies is shown that Li spreads slowly in a herd (6) and is mainly a consequence of animal replacement and movement of contaminated feces. Suggestions are made that the main clinical form is age dependent (3). If so, one might expect a different Li antibody serological profile at different ages compared to the predominant clinical presentation on a farm. The aim of this study was to compare farm serological profiles to the observed clinical signs.

Materials and Methods

In 2011-2013 serological samples were collected by practitioners/ local veterinarians at different farms in the Netherlands. On these farms ileitis was suspected. In the Netherlands fattening pigs in general arrive at a fattening farm at 9-10 weeks of age. Pigs are usually slaughtered at the age of 26-28 weeks. The standard sampling scheme used was taking blood at the age of about 10 weeks (beginning of the fattening period, shortly after arrival), at about 16 weeks of age and at about 22 weeks of age. Mean sample size per batch at 10 weeks of age was 7.9, at 16 weeks of age was 6.4, at 22 weeks of age was 5.4. Sample size varies at different ages assuming the prevalence of positive samples in a population increases with age. Test results were compared to age and clinical sign. The serological tests were performed at different laboratories, using either the bioScreen Ileitis Antibody ELISA or the Indirect Fluorescent Antibody Test (2). Test results of both testing methods were regarded as comparable, although the ELISA test is considered to be more sensitive (5). The main clinical signs as registered in this paper are based upon: the experienced view of the farmer and/ or the diagnosis made by the veterinarian and/ or by the author at the farm visit. We defined PHE as a sudden onset of bloody to black diarrhea, paleness with low morbidity and high mortality. We defined PIA as growth retardation, lack of uniformity and/ or too little feed intake.
Overall comparisons of the median proportions of infected animals at each time point were performed using Kruskal-Wallis tests. Post-hoc comparisons between groups were performed by pairwise Wilcoxon tests.

Results

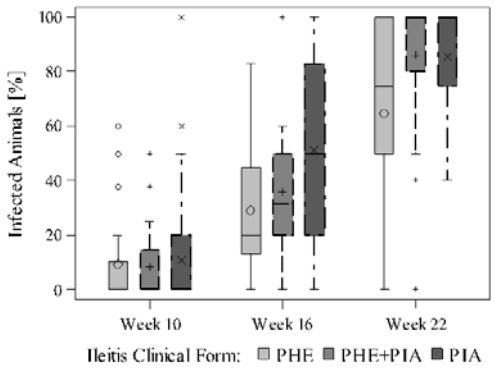

Figure 1. Distribution of the proportion of infected animals per batch by age (week) and clinical form of ileitis.

Significant overall differences in the median proportion of infected animals exist at age 16 weeks (p=0.041) and 20 weeks (p=0.023).
At the age of 16 weeks the median proportion of infected animals is significantly higher in the PIA group compared to the PHE group (P=0.021).
At the age of 22 weeks the median proportion of infected animals is significantly higher in the group PHE+PIA (p=0.018) and the group PIA (P=0.019) compared to the PHE group.

Conclusions and Discussion

The data presented in this paper show that the predominant clinical form of ileitis is correlated to the age of the animals when the batch is infected: PIA is mainly correlated with Li infection before 16 weeks of age, PHE is mainly correlated with Li infection from about 16 weeks of age.

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P.178

Characterization of *Leptospira spp* isolated of clinical swine cases from Nicaragua Republic for the development of future antileptospirosic vaccines

DF Arencibia Arrebola[1], LA Rosario Fernández[2], JF Infante Bourzac[1], Y Valdés Abreú[1], N Batista Santiesteban[1]
[1]*Finlay Institute. 198 street and 27 avenue. Number 19805. La Lisa. Havana. Cuba.*
[2]*Institute of Pharmacy and Food Science (IFAL, U.H). 222 street between 25 and 27 avenue. La Lisa. Havana. Cuba,*
darrebola@finlay.edu.cu

Introduction

Leptospirosis constitutes a current problem of animal health. Although, it exist available vaccines in the market, they have as main disadvantage the low cross-protection serogroup/serovar specific. Due to this situation it is important to have virulent and characterized strains of the serogroup or serovar of epidemic interest. In the 2007 year they were isolated by specialists of Finlay Institute 8 strains of leptospira pig clinical cases in Nicaragua republic with evidence and symptoms characteristic of leptospirosis.

Materials and Methods

With the aim of characterizing new isolated of *Leptospira spp* for the development of future antileptospirosic preparations, we were studied 8 clinical isolated obtained of clinical pig isolated in 2011 year.

Results and Discussion

The 8 isolated were classified as pathogens by means of 3 phenotypic assays and the amplification by PCR of virulence genes (*ompL1* and *lipL32*). Was determined by means of microscopic microagglutination (MAT) the serogrupos of these strains: 3507 (Icteroahemorrhagiae), 6307(Pomona), 8807 (Ballum), 4207 and 7407 (Sejroe) and 8707, 3907 and 5007 (Pyrogenes). When determining the virulence qualitatively in Yorkshire pigs as biomodel it was evidenced that the 3507, 6307, 8807, 4207 and 7407 strains were highly virulent. On the other hand the 8707, 3907 and 5007 strains were classified as non-virulent. With the LD_{50} determination it was demonstrated that the 7407, 6307 and 8807 strains were less virulent than the 4207 and 3507 strains. The 8807 and 6307 strains also produced haemorrhages focuses in kidneys and lungs; the renal haemorrhages were more frequent in the 6307 strain. Finally the analysis of whole cells extract it allowed to identify the antigenic bands expression of common for the selected strains with molecular weight between 11 and 94 kDa. These antigenic bands were equally identified in the 3 autochthonous strains of our country.

Conclusions

The characterization of this isolated it will allow the use of these strains as future vaccine candidates in pig epidemic buds of Leptospirosis.

P.179

Prevalence of antibodies to selected viral and bacterial pathogens in domestic swine and the feral swine in Mexico

NR Carreón[1], RCM Pérez[2]; BI Sánchez[1], AJF Morales[3], LM San Vicente [4]
[1]*Departamento de Medicina y Zootecnia de Cerdos. Facultad de Medicina Veterinaria y Zootecnia. Av. Universidad 3000 Col. UNAM Ciudad Universitaria Del. Coyoacán, México, D.F. C.P. 04510.* [2]*CIBNOR, La Paz, Baja California Sur, México.* [3]*CENID-Microbiología INIFAP* [4]*ECOSUR, México.* rcarreonn@prodigy.net.mx

Introduction

"One world one health" is a worldwide movement that includes several organizations working in global health. The birth of this movement was due to many controlled or extinct diseases that have been reappearing with catastrophic effects for human beings, live stock, wild life, and domestic animals. Several of these diseases are the result of the interaction between animal and human pathogens, but wild life animals are important in the spread and reservoir of these diseases. Nowadays, we know that 60% of disease outbreaks were caused by zoonotic agents, and 72% were triggered by wild life populations. Pigs play an important role in spreading pathogens; they are usually reservoirs of many diseases. The aim of this work was to determine the prevalence of viral and bacterial diseases in domestic swine and the feral swine inhabiting Sierra la Laguna in Baja California Sur, México.

Materials and Methods

The diagnosis was performed with serological tests by antibody detection for swine flu (Sf), salmonellosis (Sl), brucellosis (Br), leptospirosis (Lp) and tuberculosis (Tb).

Results

The average prevalence was 37.14% to Sf, 25.7% to Lp, 28.7% to Sl, and 14.2% to Br. These results suggest a constant pathogen flow in this population representing a very important risk factor for the spread and transmission of these diseases to other wild life animals and human beings living in Sierra la Laguna.

Conclusions and Discussion

The presence of these diseases in domestic and feral swine populations can affect their population dynamics. Moreover, the flow of relevant diseases between the feral swine populations of Sierra la Laguna might also be occurring in other animal populations putting their health in risk. Therefore, it a priority to develop management plans and epidemiologic surveillance of these diseases in the animals of Sierra la Laguna.

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Use of endemic corridors for animal health assessment and epidemiologic surveillance in swine diseases

L Galindo[1], J Sanchez[1]
[1]Department of Swine Medicine, FMVZ , UNAM . lumariong@gmail.com

Introduction

The endemic corridor was created by Selwyn Collins in 1932 for the surveillance of the influenza epidemic. It is a tool to understand the behavior and evaluate the nature of endemic or epidemic disease. It consists of a graphic representation of the actual incidence over the historical incidence of approximately 5 to 7 years, and it allows the detection of high numbers of cases of the disease under study. For its elaboration, is essential to use accurate and quality information. Currently in Mexico, Aujeszky's disease is the only disease of pigs which is under national regulation for its eradication, because of this the detection of an increase in cases or outbreaks are significant to its elimination, making it essential the use of epidemiological tools such as the endemic corridor.

Materials and Methods

For the elaboration of the endemic corridor we used the weekly database reports of the "Epidemiological Surveillance System" for the years 2007 to 2012, and the census of the "Agricultural Information Service"
The endemic corridor calculation was performed following seven steps:
1. Database annually per week.
2. Calculating rates (using geometric mean rates for = 0, these are joined 1)
3. Logarithm of rates.
4. Get mean, standard deviation and confidence intervals at 95 % confidence based on a logarithmic
5. Return to original unit data and calculating the number of cases.
6. Calculate the differences between the mean and the confidence intervals.
7. Create an area chart.

Results

In the corridor we can see how the endemic index of the week 19 of 2012 trespass the alert zone trough the epidemic zone (see figure 1).
According to the endemic corridor of cumulative incidence, the area of success at week 52 is between 0 and 139 cases, the security zone between 140 and 316 cases and alert zone between 317 and 735 cases, more than 736 cases can be considered in epidemic area for week 52. By 2012 the number of cases at week 52 was 59 cases of AD (see figure 2).

Conclusions and Discussion

The epidemiological surveillance needs appropriate tools for de detection of a high incidence of cases and the broadcast alert. This is possible using time series made with good quality information. The correct processing and interpretation of the endemic corridors at a farm, region or country favors the process of monitoring at all levels and the establishment of animal health status.
As seen in the example, the actions taken to control and eradication of AD in Mexico have been effective, and to test its effectiveness we have endemic corridors that demonstrate the actual status compared with the historical. Also these can be made at the farm level and be useful for the control of various diseases in pigs and other species.

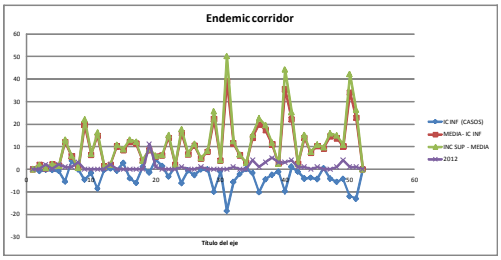

Figure 1. Endemic corridor

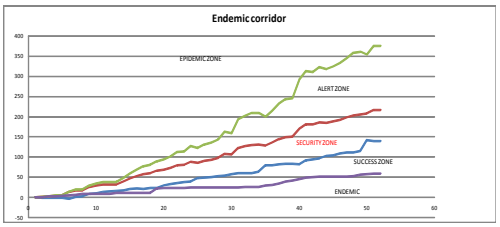

Figure2.Endemic corridor of cumulative incidence

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Percentage of sample positivity to PRRSV in Mexico

A Sotomayor[1], LM Galindo[1], J Amador[1], ME Trujillo[1], JI Sánchez [1]
[1] *Depto. de Medicina y Zootecnia de Cerdos, FMVZ, UNAM;* mvzaliciasotomayor@gmail.com

Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) is considered one of the most important diseases affecting swine industry. (1, 2,) It is characterized by reproductive failure in the last third of gestation and respiratory disease in pigs of all ages. (3, 4) Due to this nonspecific presentation of the disease, it is necessary to strengthen the diagnosis to enhance control. The objective of this study was to evaluate the ability of detecting the presence of PRRSV in different types of samples with the RT-PCR test.

Materials and Methods

The samples used in this study came from slaughterhouses and farms of some states of the Mexico. Sampling was conducted on animals with respiratory nasal mucus and visible lung lesions. The samples used were: serum , lung, lymph node , trachea, nasal turbinate , nasal swab and semen. The RNA extraction process was performed by the method of phenol (Gibco Life Technologies , 1996) . The RT-PCR was performed using the RT-PCR kit One Step Invitrogen ® using the following conditions : a cycle of 50 ° C for 30 minutes , a cycle of 95 ° for 15 minutes , 40 cycles : 94 ° C for 30 seconds , 54 ° C (ORF 5) or 58 ° C (ORF 7) for 60 seconds and 72 ° C for 60 seconds , with a final cycle of 72 ° C for 10 minutes and kept at 4 ° C until used. The product run in a 2% agarose electrophoresis gel running horizontally and the amplicons visualize.

A total of 204 samples from the states of Hidalgo, Guanajuato, Puebla , Veracruz , Morelos, Jalisco, Michoacán, State of Mexico, Oaxaca , Sonora, Yucatan, Mexico City, Guerrero, Tlaxcala and Coahuila were processed . In some cases RNA pools including up to 5 samples were formed.

Results

From the 155 processed samples, 90 were positive, which indicates a frequency of positivity of 58% of the disease (Figure 1).

Figure 2 shows the type of samples that were processed and the frequency of positive and negative samples.

Conclusion and Discussion

The data obtained in this study suggests that 58% of clinical cases observed were due to PRRS disease, being present at least one positive case in all the sampled states and with every type of sample. With this, we can conclude that the PRRSV is one of the most frequent health problems in some swine farms in the country, causing economic losses. It also represents a problem of significant health because of their wide distribution and their occasional failure diagnosis, for this reason we consider the implementation of important regional control measures to reduce the spread of the disease and its economic impact on the Mexican swine production.

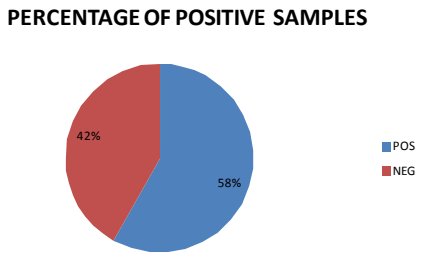

Figure 1. Percentage of positive samples

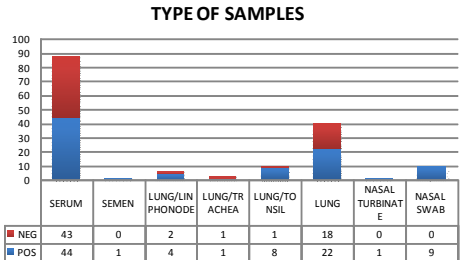

Figure2. Type of samples processed.

Acknowledgements

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Epidemiological study for determinate CSFV circulation as a tool for the vaccination suspecting in the North Coast and Central zone of Colombia

ME Peña[1], MA Rincon[1], CP Calderon[1], A Castillo[1]
[1]*Instituto Colombiano Agropecuario – ICA, Bogotá.* mario.pena@ica.gov.co, marioe.pena@gmail.com

Introduction

In accordance with the OIE procedure for official recognition of disease status for free regions, is necessary to suspend the vaccination against the virus, at least one year before (1).

This study was made in four weeks, in order to obtain samples that mean a major representation of the population and the samples collected were tonsils, because the zone was vaccinated, and they were processed by RT-PCR (2).

The main purpose of this study was to determinate the classical swine fever virus circulation in the North Coast and Central zone of Colombia as tool for the vaccination suspecting.

Materials and Methods

The present study was made in the North Coast and Central zone of Colombia, a geographic zone that includes 19 departments, 1.464.680 pigs and 35.969 producers, of which 28.597 are backyards producers.

The study considered two stratums, backyards producers and intensive producers and the sampling size was calculating under the Cannon Formula (2001) and use the following parameters: expected prevalence 0,3%, level of reliability 95% and sensibility 99%. With these parameters the sampling size was 1.113 tonsils. In order to distribute them in the two defined stratums, it was considered to give the 80% of the sample to backyards producers and 20% to intensive producers (Figure 1 and 2).

The tonsils were recollected in 223 not official slaughter sites, like road restaurant and premises for stratum 1 (backyards producers) and in 13 official slaughter houses for stratum 2 (intensive producers). For the proportional distribution of the sample it was considered the number of pigs slaughtered in a month in each official and not official slaughter sites and the number of sites per department.

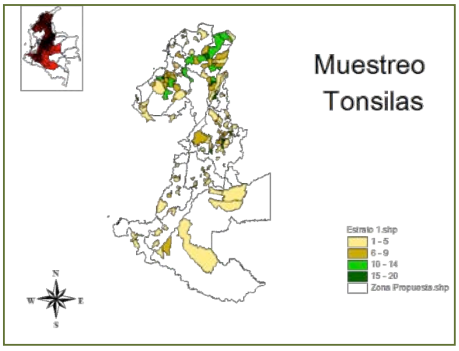

Figure 1. Stratum 1 distribution

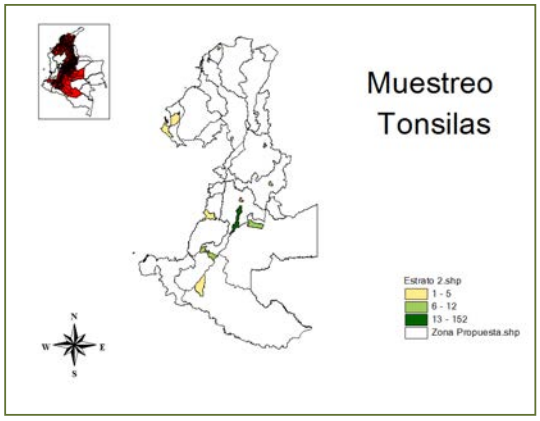

Figure 2. Stratum 2 distribution

Results

The results of this study yielded 1.113 tonsils with negative results under the RT-PCR test.

Below is a table in which the results of the sampling are summarized.

DEPARTMENT	ESTRATUM 1			ESTRATUM 2			TOTAL
	Samples	Positives	Negatives	Samples	Positives	Negatives	SAMPLES
ANTIOQUIA	20	0	20	4	0	4	24
ATLANTICO	20	0	20	12	0	12	32
BOLIVAR	22	0	22	0	0	0	22
BOYACA	15	0	15	4	0	4	19
CALDAS	4	0	4	10	0	10	14
CAQUETA	14	0	14	5	0	5	19
CASANARE	4	0	4	4	0	4	8
CAUCA	7	0	7	0	0	0	7
CESAR	162	0	162	0	0	0	162
CORDOBA	124	0	124	0	0	0	124
CUNDINAMARCA	68	0	68	156	0	156	224
HUILA	18	0	18	7	0	7	25
LA GUAJIRA	18	0	18	0	0	0	18
MAGDALENA	52	0	52	0	0	0	52
META	7	0	7	8	0	8	15
NORTE DE SANTANDER	6	0	6	0	0	0	6
SANTANDER	252	0	252	9	0	9	261
SUCRE	62	0	62	0	0	0	62
TOLIMA	15	0	15	4	0	4	19
TOTAL	890	0	890	223	0	223	1113

Conclusions and Discussion

The results from the study on the viral circulation for Classical Swine Fever conducted within the North Coast and Central Zone of Colombia are satisfactory and allow assuring that within these swine populations there is no viral circulation. The inclusion of the not offical slaugther sites in the sampling, ensure the search of the virus in the higher risk population.

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Adoption of sustainable assessment strategies to reduce public health risk in suburban pig family farms

R Olea-Pérez[1], M Pérez-Cardenas[1], E Celaya-Mendoza[2]
[1]*Departamento de Medicina y Zootecnia de Cerdos, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de Mexico,* [2]*Comité para el Fomento y Protección Pecuaria del Distrito Federal, Sociedad Civil,*
perol@unam.mx

Introduction

Urban expansion areas have been an increasing phenomenon in the last half century for main big cities of Mexico. Urbanisation created a suburban family farms (SFF), where agricultural land areas were transformed on mixed animal and/or crop family productions into small and irregular fractioned housing developments(1). This SFF try to maintain traditional rural customs, such as growing animal and planting vegetable. However, rural-urban balance has moved on the urban side and many SFF, which grow up pigs face great pressure on management and disposal of pig manure. Animal Protection and Promotion Committee in Mexico City (CFCC-DF, Spanish acronym) as Civic Society among other activities customise animal disease prevention and control national campaigns and adopt several initiatives to reduce public health risk in Mexico City suburban areas.

Veterinary Faculty and CFCC-DF implementing an initiative to encourage pig-SFF owners to adopt sustainable development strategies, taking advantage of the main interest of producers: The manure management and disposal alternatives for Mexico City pig-SFF.

Materials and Methods

In the three main pig-SFF areas of Mexico City were formed local groups of SFF owners interested in alternative technologies to change management and disposal of pig manure. Three actions were carried out in the initiative: Surveying owners, assessment of nutrient stream case studies (2) and knowledge transfer sessions. The survey was carried out in the three groups of SFF owners, the survey included facilities, productive, feeding and manure management data accounting for March 2012 to February 2013 period. Four farms were used to assess the greenhouse gases emission for the nutrients stream through animal production and manure management according to Tier 2 of IPCC guidelines, (2). Training strategies included group sessions, demonstration modules and knowledge spreading activities (3).

Results

The mainly pig herd size of SFF was 4 sows and they litters, with two annual fatting cycles. All farmers use partially bakery or corn-mills feedstuffs as feeding complements. There were solid separation on 85% of SFF and potential production of 51.54m^3 of methane gas. Methane gas production was the equivalent of potential reduction of greenhouse gas emissions for manure management. Main group meetings were focus on public health risk of actual manure management and adoption of alternative technologies. Pros and cons of anaerobic reactors (Biodigestor), compost and vermicompost were the main discussed technologies. Even biodigestor looked attractive for methane use there were more disadvantage for adoption in the SFF. As result of these meetings anaerobic reactors were discarded and 80% of producer preferred compost and vermicompost procedures. Two farmers implemented composting modules for training proposes. One compost manual with one thousand printing for SFF owners was the final product.

Conclusions and Discussion

Assessment of animal environmental impact on controversial places, such as animal manure management and disposal in suburban areas is a difficult task, mainly because animal producers feel attacked by urban expansion. Therefore, an initiative based on sustainable assessment strategies to undertake actions for public health risk reduction showed good result. Using knowledge transfer as corner stone allowed more active participation of main pig-SFF stakeholders on technological adoption of manure management with less environmental and health risk.

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Arsenic, cadmium and lead residues in kidneys of Venezuelan slaughter pigs

J Guevara[1], J Riera[1,2]
[1]Universidad Central de Venezuela. Facultad de Ciencias Veterinarias. Maracay, Edo. Aragua. Venezuela.
[2]Laboratorio Sedicomvet. Maracay, Edo. Aragua. Venezuela., jennerguevara@yahoo.com

Introduction

Pollution of the environment with heavy metals is a serious problem, which is recognized in most countries of the world. Content of metals in foods of plant origin as animal depends on many factors, among which must be emphasized, environmental conditions, production methods and the composition of the soil (2). Metals accumulate in the liver, and particularly in the kidneys (1). The aim of this study was to determine the presence of arsenic, cadmium and lead in Venezuelan slaughter pigs.

Materials and Methods

A total of 600 kidneys of slaughtered pigs from 20 farms of the central region of Venezuela (Aragua State and Carabobo State) were analyzed for arsenic, cadmium and lead during October 2010-March 2011. Atomic absorption spectrometry was used to estimate and evaluate the levels.

Results

The results are shown in tables 1 and 2:

Table 1. Prevalence of heavy metals according provenance.

	Arsenic	Cadmium	Lead
Aragua Farms	11/11	11/11	11/11
Carabobo Farms	3/9	9/9	5/9

Table 2. Average levels detected residues of arsenic, cadmium and lead in samples of kidney of pigs according to the region of provenance.

	Average **mg/L**		
	Arsenic	**Cadmium**	**Lead**
Aragua a	0,7826	0,0139	0,0893
Carabobo a	1,041	0,0308	0,3283

a P>0,05

Conclusions and Discussion

The results show that cadmium was heavy metal more prevalent (100%), followed by the lead (80%) and arsenic (70%).

Mean levels of arsenic and lead were below official tolerance of 2 mg/L for arsenic and 0,5 mg/L for lead, however, cadmium levels showed values higher than the allowed limit of 0.001 mg/L.

The results suggest that exposure of animals to dietary or environmental arsenic and lead is not significant. In contrast, the average concentrations detected for cadmium can have a potential public health impact.

On the other hand, according to the region of provenance (Aragua and Carabobo), is observed that there were no significant statistical difference between them. This is possibly because to Aragua and Carabobo States are States bordering with a similar climate and who share the same sources of water and food for their pigs.

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Some risk factors associated with the occurrence of the second litter syndrome in sows

R Santos-Ricalde[1], A Alzina-Lopez[1], J Segura-Correa[1]
Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán, km 15.5 Carretera Mérida-Xmatkuil, Apdo. Postal 4-116, Mérida, Yucatán, México, rsantos@uady.mx

Introduction

Litter size commonly increases with the parity number in sows. However, in some sows the number of piglets in the second litter is lower or similar to that of the first litter, phenomenon known as second-litter syndrome (3). The second-litter syndrome negatively affects the pregnancy rate of second parity sows and sows productive lifetime in the farm, since reproductive failure is one of the main reasons for culling young sows. Factors such as litter size at first parity, herd size, season of farrowing, and weaning to service interval have been reported as risk factors for the second litter syndrome (1). Therefore, the objectives of this study were to estimate the incidence of sows with the occurrence of the second-litter syndrome and to determine the effect of some factors, in three farms in the south-eastern of Mexico.

Materials and Methods

Data from Four commercial farms of the state of Yucatan, Mexico, were used. Farms 1, 2 and 3 were full cycle farms with 3000, 1200 and 500 sows, respectively. The three farms produced their own replacements and practiced the quarantine of the gilts. Sows were fed commercial feed according to the productive stages. In all farms, breeding was carried out mainly by artificial insemination. Data from 2003 to 2011 recorded in the PIGCHAMP program were used. Sows were categorized into two groups: 1 if sows had similar or lower number of pigs at the second parity than that at the first parity and 0 for sows that increased litter size at the second parity. The data from 8592 farrowing records for 4296 sows were analysed using binary logistic regression procedures. The risk factors evaluated were season of farrowing (Dry, rainy and windy), number of pigs born alive (≤ 8, 9-10, 11-12 and ≥ 13 piglets) and weaning to conception intervals (≤ 3, 4-11 and ≥ 12 days). All statistical analyses were carried out with the SPSS program.

Results

The overall frequency of sows with the second litter syndrome was 55.8%. The odds of the second litter syndrome were 1.56 and 2.01 times higher for the sows farrowing during the dry and rainy season versus those farrowing in the windy season. Sows with large litters (> 12 pigs) had higher odds (33.2) showing the second litter syndrome than sows with small litters (< 9 pigs). Sows with shorter weaning to conception intervals (<4 and 4-11 days) had higher odds showing a decrease in litter size at the second parity in comparison with sows with longer weaning to conception intervals.

Table 1. Factors associated with the second litter syndrome.

Factor	Estimate	SE	Odds ratio	95% Confidence limits
Season				
Dry	0.180	0.087	1.20	1.01, 1.42
Rainy	0.212	0.090	1.24	1.04, 1.47
Windy	0		1	
Number of pigs born alive				
<9	0		1	
9-10	1.29	0.096	3.63	3.01, 4.38
11-12	2.25	0.105	9.51	7.75, 11.7
>12	3.50	0.134	33.24	25.6, 43.2
Weaning to conception interval (days)				
<4	0.577	0.091	1.78	1.49, 2.13
4-11	1.01	0.092	2.74	2.29, 3.28
>11	0		1	

Conclusions and Discussion

The higher odds of the second litter syndrome for sows farrowing in the dry and rainy season may be attributed to the high temperature and/or high humidity in those seasons as compared with windy season. The higher probability of a reduction in the size of the second litter, as the size of the first litter increases could be associated to an excessive weight loss during first lactation (4). The results obtained in this study showed an increased of litter size as weaning to conception intervals increased. Similar results have been reported before (2). However that delaying of the breeding of the first parity sows after weaning will increase non-productive days of the sows.
From the results of this study, it can be concluded that a high proportion of sows showed second litter syndrome (55.8%). Sows with large litters at the first parity, those farrowing in the dry and rainy seasons and those with shorter weaning to conception intervals, had higher odds showing the second litter syndrome.

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Genetic characterization and pylogenetic analysis of *S. hyicus* field strains isolated from sows in Korea

JY Jung[1], BE Park[1], JH Jo[1], SM Kim[1], EY Ko[2], HK Jeong[2], DK Lee[2], JH Han[1]
[1]*College of Veterinary Medicine and Institution of Veterinary Science, Kangwon National University, Chuncheon, Gangwon, Republic of Korea,* [2]*Dodram Pig Farmer's Cooperative, Icheon, Gyeonggi-Do, Republic of Korea,*
neves7@naver.com

Introduction

Staphylococcus(*S.*) *hyicus* is known to cause exudative epidermitis, which is generalized infection through the skin of sucklings and weaned pigs. Mortality and morbidity in infective pigs can reach 90%. Furthermore, abortion with endometritis and polyarthritis can be caused. High *S. hyicus* colonization rate can be founded in vagino-uterine samples of healthy sows and in skin samples of their piglets. *S. hyicus* isolates from the skin of piglets were of the same type as their sows, indicating that vertical transmission could have taken place. This study was conducted genetic analysis of *S. hyicus* based on the nuc gene to evaluate genetic characterization of Korean isolates from the sow's reproductive system.

Materials and Methods

A total number of 186 estrous sows from parity ≥2 were used for detecting of *S. hyicus* by swab. Samples were collected from cervico-uterus when artificial insemination was performed. Six *S. hyicus* isolates were detected from samples by PCR. A full-length nuc gene of *S. hyicus* was amplified by PCR with forward primer, hy-F1(5'-CAT TAT ATG ATT TGA ACG TG-3') and reverse primer, hy-R1(5'-GAA TCA ATA TCG TAA AGT TGC-3'). The PCR was performed in 20㎕ premix mixtures(2㎕ of DNA extracted, 1㎕ of each primer, 16 ㎕ of free water). The amplification reaction was performed with an initial step at 95℃ for 2min, followed by 30 cycles of denaturation at 95℃ for 30sec, annealing at 52℃ for 30sec and extension step at 72℃ for 30sec, and final extension step at 72℃ for 2min. Six *S. hyicus* isolates of 793bp were used for DNA sequencing. DNA sequencing was carried out by Macrogen (Korea) with primers used in the previous PCR. The 6 *S. hyicus* sequences were analyzed together with 7 representative nuc gene sequences reported in GenBank including the strain of the former Japan isolate(AB465332) in 2010 and France isolate(L23973) in 1994 from piglet's skin. A figure showing alignment of amino acid and a phylogenetic tree was constructed by MEGA 5.2 software.

Results

The results of nucleotide sequences and phylogenetic tree of Korean S. hyicus isolates were as follows (Figure 1 and 2).

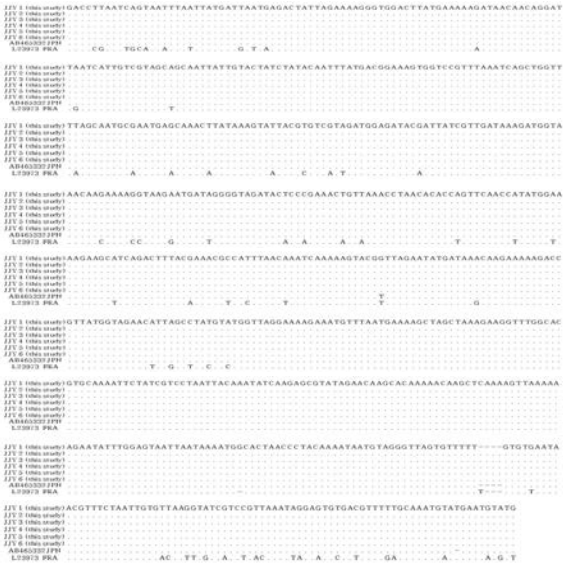

Figure 1. The nucleotide sequences of Korea isolates(JJY1-6) and some published *S. hyicus* strains.

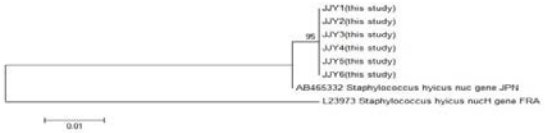

Figure 2. The phylogenetic tree of Korea isolateds(JJY1-6) was produced by MEGA5.2 software based on the nucleotide sequences, presenting the relationship of some published S. hyicus strains from Japan and France.

Conclusions

All 6 nuc genes of *S. hyicus* Korean isolates were completely identical, and revealed nucleotide identities ranged between 99~100% compared with the strain isolated in Japan, and 93-94% compared with the strain isolated in France.

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Reproductive performance by cervical and post-cervical artificial insemination in sows

JY Jung[1], BE Park[1], JH Jo[1], SM Kim[1], EY Ko[2], HK Jeong[2], DK Lee[2], JH Han[1]
[1]*College of Veterinary Medicine and Institution of Veterinary Science, Kangwon National University, Chuncheon, Gangwon, Republic of Korea,* [2]*Dodram Pig Farmer's Cooperative, Icheon, Gyeonggi-Do, Republic of Korea*
neves7@naver.com

Introduction

Artificial insemination(AI) methods include depositing the semen within the cervix or uterine body. The former is cervical AI (CAI) and the latter is post-cervical AI(Post-CAI); depositing the semen closer to the site of fertilization. The difference between both methods is the region where the semen is deposited. Both methods are known to useful tool to increase breeding grade and decrease disease transmission. However, in spite of several studies, there are few reports to compare reproductive efficiency between CAI and Post-CAI. This study was to evaluate multiparous sow's reproductive performance and compare in reproductive performance such as conception(%), return-to-estrous(%), abortion(%), culling(%), death(%), farrowing(%), total born litter size(n), live born litter size(n) and fecundity index(n) followed by CAI and post-CAI.

Materials and Methods

A total number of 186 estrous sows from parity ≥2 were used for comparison of reproductive performance. 72 sows performed CAI and 114 sows performed Post-CAI, respectively. Conception was detected by ultrasound (Songgang, Korea), and the return-to-estrous was evaluated using sexual stimulation about 18-24 days after AI. Abortion was detected by direct visualization from 40 to 60 days after AI. Culling was performed when continuous infertily was observed. During the farrowing period, total born litter size and live born litter size were checked.

Results

The results of reproductive performance in sows performed CAI and post-CAI were table 1. Conception rates were 84.72±0.36% and 90.35±0.30%, return-to-estrous rates were 15.28±0.36% and 9.65±0.30%, abortion rates were 3.28±0.18% and 0%, culling rates was 4.17±0.20% and 0.88±0.09%, death rates were 0% and 2.63±0.16%, and farrowing rates were 76.39±0.42% and 87.72±0.33%, respectively. Total born litter sizes of sows which CAI and Post-CAI were performed were 12.42±0.57 and 11.70±0.42, live born litter sizes were 11.55±0.52 and 10.75±0.38, and fecundity index were 882.30 and 942.99, respectively.

Table 1. Reproductive performance in sows by CAI and Post-CAI

Contents	CAI	Post-CAI
Conception (%)	61/72[a] (84.72±0.36)*	103/114[a] (90.35±0.30)
Return-to-estrous (%)	11/72[a] (15.28±0.36)	11/114[a] (9.65±0.30)
Abortion (%)	2/61[a] (3.28±0.18)	0/103[a] (0)
Culling (%)	3/72[a] (4.17±0.20)	1/114[a] (0.88±0.09)
Death (%)	0/72[a] (0)	3/114[a] (2.63±0.16)
Farrowing (%)	55/72[a] (76.39±0.42)	100/114[b] (87.72±0.33)
Total born litter size (n)	12.42±0.57[a]	11.70±0.42[a]
Live born litter size (n)	11.55±0.52[a]	10.75±0.38[a]
Fecundity index (n)**	882.30	942.99

* Data are expressed as the mean ± SEM
** Fecundity index(not included in statistical analysis): farrowing rate multiplied by average number of live piglets born per litter (total number of live piglets born per 100 inseminations).
a, b Different superscripts in the same row indicate significantly different values ($p < 0.05$).

Conclusions

Post-CAI reproductive parameters such as conception rate, return-to-estrous rate, abortion rate, culling rate, death rate, total born litter size, and live born litter size were similar to those of CAI, but farrowing rate and fecundity index were greater in Post-CAI.

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Survey on bacterial isolation of reproductive system after cervical or post-cervical artificial insemination in sows

JY Jung[1], BE Park[1], JH Jo[1], SM Kim[1], EY Ko[2], HK Jeong[2], DK Lee[2], JH Han[1]

[1]*College of Veterinary Medicine and Institution of Veterinary Science, Kangwon National University, Chuncheon, Gangwon, Republic of Korea,* [2]*Dodram Pig Farmer's Cooperative, Icheon, Gyeonggi-Do, Republic of Korea*

neves7@naver.com

Introduction

Artificial insemination(AI) is very useful tool to introduce superior genes into sow herds, with minimal risks for disease transmission when compared with natural mating. Recently, new strategy which is to deposit the semen within the uterine body of sows, post-cervical artificial insemination(Post-CAI), receives attention, which takes only a few seconds and uses a lower number of spermatozoa per dose compared with cervical artificial insemination(CAI). However, it has not been reported that isolation of bacteria such as *Staphylococcus*(*S.*) *hyicus*, *Escherichia*(*E.*) *coli* and *Achromobacter*(*A.*) *xylosoxidans* that can cause endometritis are different depending on AI methods in sow. The objective of our study was to compare isolation rate of endometritis-inducing bacteria by swab from cervico-uterine region performed Post-CAI and CAI. And to investigate the resistance on antibiotics, antibiotic susceptibility tests were carried out.

Materials and Methods

A total number of 186 estrous sows from parity ≥2 were used for isolation of bacteria by swab. Seventy two sows performed CAI and 114 sows performed Post-CAI with first sampling, respectively. Second sampling for isolation of bacteria was carried out when sow showed return-to-estrous within 18 to 24 days after AI . Samples were enriched in nutrient broth (Difco) at 37°C for 24hr, and streaked on the selective agars such as *S. hyicus* with mannitol salt agar(MSA), *E. coli* with eosin methylene blue agar (EMBA) and *A. xylosoxidans* with Burkholderia cepacia selective agar (BCSA). Isolated bacteria were confirmed by PCR. *S. hyicus* was amplified by PCR with forward primer, 5'-CAT TAT ATG ATT TGA ACG TG-3' and reverse primer, 5'-GAA TCA ATA TCG TAA AGT TGC-3'. *E. coli* K88 was amplified with forward primer, 5'-TGA ATG ACC TGA CCA ATG GTG GAA CC-3 and reverse primer, 5'-GCG TTT ACT CTT TGA ATC TGT CCG AG-3'. *A. xylosoxidans* was amplified with forward primer, 5'-CGC ATC CTG TTC CAG CA-3' and reverse primer, 5'-GTG CCG GTC TTG CCA TAC-3'. And antibiotic susceptibility tests were performed followed by Bauer-Kirby method.

Results

The results of isolated bacteria and antibiotic susceptibility were as follows (Table 1 and 2).

Table 1. Isolation of *S. hyicus*, *E. coli* K88 *and A. xylosoxidans* by PCR in sows at estrous and return-to-estrous performed CAI and Post-CAI.

Bacteria	AI methods	Contamination (%)	
		Estrous	Return-to-estrous
S. Hyicus	CAI	0/72 (0)[a*]	3/11 (27.3)[b]
	Post-CAI	1/114 (0.9)[a]	2/11 (18.2)[b]
E. Coli K88	CAI	4/72 (5.6)[a]	3/11 (27.3)[b]
	Post-CAI	3/114 (2.6)[a]	3/11 (27.3)[b]
A. xylosoxidans	CAI	0/72 (0)	0/11 (0)
	Post-CAI	0/114 (0)	0/11 (0)

a, b Different superscripts in the same row indicate significantly different value ($p < 0.05$).
*No. of the sows detecting bacteria by PCR/No. of sows tested

Table 2. Antibiotic susceptibility of S. hyicus and E. coli K88 at estrous and return-to-estrous from the sows performed CAI and post-CAI

Sample No.	Bacteria	Phase	AI methods	ENO	D	GM	COL	AM	AmC	E	AK	SXT	NOR	G	TET
1	*S. hyicus*	Estrous	Post-CAI	S	S	S	S	S	S	I	S	S	S	R	S
2		Return-to-estrous	CAI	S	S	S	I	S	S	S	S	S	S	I	S
3				S	S	S	I	I	S	S	S	S	S	S	S
4				I	S	R	R	S	S	I	I	S	S	I	S
5			Post-CAI	S	S	S	I	I	S	S	S	S	S	I	S
6				S	S	S	S	S	S	R	S	S	S	R	S
1	*E. coli*	Estrous	CAI	S	R	S	S	R	S	R	S	S	S	R	R
2				S	I	S	S	R	S	R	S	S	S	R	R
3				I	I	I	R	R	S	R	I	S	S	R	R
4			Post-CAI	S	I	S	R	R	S	R	S	S	S	R	R
5				S	R	R	R	R	S	R	S	S	S	R	R
6		Return-to-estrous	CAI	S	I	S	S	R	S	R	S	S	S	R	R
7				S	I	I	I	I	S	R	I	S	S	R	R
8				S	R	R	R	I	S	R	S	S	S	R	R
9			Post-CAI	I	I	I	R	R	S	R	I	S	S	R	R
10				I	R	S	R	R	S	R	S	S	S	R	R

ENO, enrofloxacin; D, doxycycline; GM, gentamicin; COL, colistin; AM, ampicillin; AmC, Amoxicillin; E, erythromycin; AK, amikacin; SXT, trimethoprim/sulfamethoxazole; NOR, norfloxacin; G, sulfisoxazole; TET, tetracycline; CIP, ciprofloxacin
S, susceptible; I, intermediate; R, resistant

Conclusions

The isolation rate of endometiritis-inducing bacteria such as *S. hyicus*, *E. coli* K88 and *A. xylosoxidans* and antibiotic resistance of *S. hyicus* and *E. coli* K88 showed no significant difference between sows performed CAI and Post-CAI.

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Effect of Altrenogest treatment on the homogeneity of follicular development in sows

S Kitkha[1,2], A Boonsoongnern[3], N Ratanavanichrojn[3], P Jirawattanapong[3], A Pinyopummin[3]

[1] Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand. [2] Center of Excellence on Agricultural Biotechnology: (AG-BIO/PERDO-CHE), Bangkok, Thailand. [3] Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Sean Campus, Nakhon Pathom, Thailan. fvetacp@ku.ac.th

Introduction

The variation of follicular development and ovulation time were related to the diversity of embryo during pre-implantation stage. The later-ovulating follicles resulted in less-developed embryos which were eliminated due to the limitation of uterine space (1). Synthetic progestin (altrenogest) feeding during lactation and post weaning in multiparous sows enhanced reproductive performance in subsequent litter. Many studies revealed a higher ovulation rates and embryo survival (2). These advantages might be related to an improvement of follicular growth/homogeneity. Therefore, the objective of this study was to evaluate the effect of altrenogest on the homogeneity of follicular development.

Materials and Methods

Fifty-nine lactating sows, parity 6 – 8, were divided into 4 groups (T1 – T4). T1 was a control group. The sows were not received altrenogest (AG) (Altresyn®, Ceva Animal Health). Other groups were administered orally with AG in different doses and duration (T2: 20 mg/d, 5 days; D-4 – D0; T3: 20 mg twice a day (40 mg/d), 7 days; D-4 – D2; T4: 20 mg/d, 7 days; D-4 – D2) (D-4: 4 days before D0; D0: weaning day; D2: 2 days post D0). After AG, the sows in each group were separated into 2 subgroups (A and B). The sows in subgroup A were slaughtered on D1 post weaning (T1) or D1 post AG (T2 – T4) whereas the sows in subgroup B were slaughtered on D4 post weaning (T1) or D4 post AG (T2 – T4). Ovaries were obtained after slaughter. All follicles were counted and measured for their size (diameter).

Number of follicles (NF), follicular size (FS) and coefficient of variance (%CV) of FS among 4 groups and 2 subgroups were compared by two-way ANOVA and followed by Bonferroni and simple effect when it had interaction.

Results

NF decreased significantly from D1 to D4 post AG in T3 and T4 (Table 1). On D4 post AG, T4 had less NF than T2. FS increased from D1 to D4 post AG. On D1 post AG, FS of T2 – T4 was smaller than T1 (control). However, the follicles in T3 and T4 grew rapidly to a larger diameter than T1 on D4 post AG. Moreover, %CV of FS in T4 had the least value on D4 (p<0.05).

Table 1. Number of follicles (NF), follicular size (FS) and coefficient of variance of follicular size (%CV) among 4 groups and 2 subgroups (Mean ± S.E.M.).

			NF		FS (mm)		%CV	
	N		Mean		Mean		Mean	
Gr	Subgr A	Subgr B	Subgr A	Subgr B	Subgr A	Subgr B	Subgr A	Subgr B
T1	9	5	78.78 ± 7.97	60.60 ± 10.69^{12}	4.09 ± 0.09^{1a}	4.61 ± 0.13^{3b}	39.17 ± 3.66	42.01 ± 5.24^{1}
T2	6	7	97.50 ± 9.76	77.86 ± 9.03^{1}	3.39 ± 0.05^{23a}	4.44 ± 0.08^{3b}	36.91 ± 2.45	38.39 ± 3.24^{12}
T3	8	8	84.63 ± 8.45^{a}	58.50 ± 8.45^{12b}	3.10 ± 0.06^{3a}	5.20 ± 0.11^{2b}	43.07 ± 3.79	40.55 ± 5.27^{1}
T4	7	8	80.57 ± 9.03^{a}	41.88 ± 8.45^{2b}	3.41 ± 0.08^{2a}	6.48 ± 0.14^{1b}	45.01 ± 4.39^{a}	25.32 ± 3.46^{2b}

(1, 2, 3) values were differ significantly among groups (p<0.05).

(a, b) values were differ significantly between subgroups (p<0.05).

Conclusions and Discussion

During the progress of follicular phase, some small and medium follicles were atresia. Dose and duration of AG treatment influenced follicular growth rate which reflected the number of escaped follicles from atresia. AG prevent the growth of medium to large follicles, so only the small follicles could reach medium size. Because of a larger proportion of medium follicles, the average follicle size in treatment group (T2 – T4) on D1 (subgroup A) was less than control (T1). Subsequently, pool of medium size follicles developed to be the large size follicles on D4 (subgroup B). Incomplete suppresion of LH release in T4 might allow follicular growth from small to medium size during altrenogest treatment, therefore most of follicles were large follicles at D4 with more homogeneous sizes (the least %CV). In contrast, twice a day of altrenogest treatment (T3) might inhibit LH completely which resulted in a slower growth rate of follicles in T3 than T4. This study demonstrated that altrenogest treatment protocol affected the number of follicles, follicular size and homogeneity of follicles.

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VEGF-receptor system immunoreaction in subepithelial endometrium area in healthy and arresting Iberian pig attachment sites

A Palomo, MA Sanchez, RA Garcia-Fernandez, B Sanchez, P Garcia-Palencia, C Naranjo, JM Flores
Dpt Animal Medicine and Surgery. Faculty Veterinary Sciences. Complutense University Madrid (Spain),
jflores@ucm.es

Introduction

The Iberian pig is an autochthonous Mediterranean breed characterized by a lower prolificacy. Recently, it has been reported that 23 % of the conceptuses die during the first 30 days of gestation (1).VEGF (Vascular Endothelial Growth Factor)-receptor system seems to play an important role in the vascular events during the peri-implantation period in pigs (2,3). The objective of this study was to analyze the expression of VEGF-receptor system in the vessels of subepithelial area of endometrium of Iberian pigs at healthy and arresting embryo attachment sites at gestation day (gd)22 and gd32.

Materials and Methods

The reproductive tracts of Iberian sows from Guijuelo (Salamanca, Spain) at gd22 (n=20) and gd32 (n=20) were collected after slaughtering. Embryos were grouped as healthy (H) or arresting (A) based on standard criteria (4,5). Endometrial samples were obtained from H and A attachment sites. Expression of VEGF, VEGFR1 and VEGFR2 were analyzed by immunohistochemistry using anti-VEGF antibody (Santa Cruz Biotechnology), anti-VEGFR1 antibody (Abcam®) and anti-VEGF R2 antibody (Bioss Inc.,). Quantification was performed in 10 fields, counted at 400X magnification. Results obtained were analyzed using SPSS Statistics 19 Software for Windows (SPSS-Ibérica Inc., Spain).

Results

Results regarding VEGF-receptor system vascular density are compiled in Figure 1. The density of VEGF positive capillary vessels in the subepithelial area of endometrium increased in healthy conceptus-attachment sites and decreased in arresting fetal sites as pregnancy advanced. VEGFR1 expression was higher in the subepithelial capillary endometrium from healthy sites and increased from gd22 to gd32. The density of VEGFR2-positive capillary vessels decreased slightly in healthy attachment sites from gd22 to gd32, whereas in arresting sites an obvious decrease in VEFGR2 immunostaining was observed on gd32 as compared with gd22.

Conclusions and Discussion

VEGF, VEGFR1 and VEGFR2 were detected in endometrium samples at gd22 and gd32 in lean breeds (3,4,6). Our results confirm that VEGF system is expressed in a similar way in Iberian pigs. Immnunoreaction from VEGF and VEGFR1 in the subepithelial capillary vascular network increased in healthy conceptus-attachment sites as pregnancy advanced. This may show a significant role for VEGF system in embryo viability.

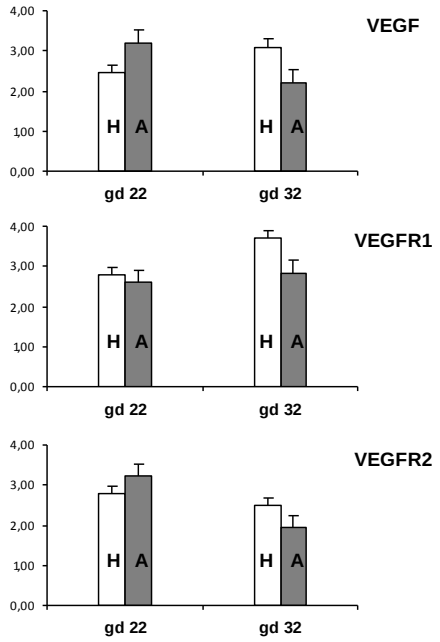

Figure 1. VEGF-receptor system positive vessels/mm^2 in subepithelial capillary area of endometrium associated with healthy (H) and arresting (A) conceptuses at gd22 and gd32 (mean ± s.e.m).

Acknowledgments

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Use of synthetic seminal plasma, Predil® MR-A®, by two-phase insemination technique in Iberian sows

RT Pallás[1], G Fernández[1], R Hernández-Gil[1], S Martín[1], FJ Rodríguez[2], P Martínez[1], JA García Ruvalcaba[1]
[1]*KUBUS, S.A., Madrid, Spain,* [2]*Solano Veterinaria y Nutrición, S.L., Mérida, Spain,* rtpallas@gmail.com

Introduction

Due to its composition, the seminal plasma (SP), provides protection to the spermatozoa against inflammatory reaction once they come in contact with the uterus (immunological reaction), thus assuring sufficient spermatozoa population arrives at the fertilization site (5). The biochemical composition of SP includes organic and inorganic compounds that are important not only to maintain the spermatozoa viability but also to fertilize the oocyte (4). The most important effects of SP are, due to its influence in the fertilization process, improving sperm transport by stimulating uterine contractions, isthmus relaxation and promoting uterus immune modulation. Therefore, using the two-phase insemination technique applying synthetic seminal plasma before insemination (3), the SP exerts its effect on the spermatozoa, stimulates uterine contractions and relaxes the oviduct isthmus. Moreover, most importantly, the synthetic seminal plasma, Predil® MR-A®, increases the viability and motility of the spermatozoa, enhancing their fertilization capacity (1, 2, 3). Until now, it has been demonstrated that the use of Predil® MR-A®, produces an improvement in both, reproductive and productive parameters, in white sows in intensive production (improvements both in farrowing rate and prolificacy) (1, 2, 3). The aim of this study is to evaluate whether the positive effects of synthetic seminal plasma in white sows can be transferred to Iberian sows.

Materials and Methods

The study was carried out between August and November 2012 in a 750 Iberian sow farm (intensive production) in the province of Badajoz (Spain). 317 weaned sows cycling spontaneously within the first week post-weaning were randomly allocated into two groups: the control group with 188 sows artificially inseminated in the traditional way, and the Predil group with 129 sows inseminated using the two-phase insemination technique by administration of 35 cc de Predil® previously to the application of the semen dose. For both groups, all semen doses, 85 cc, were bought to Tecnogenext Boar Stud in the same province of Badajoz. All sows were inseminated using a conventional spiral catheter.

Fertility rate, number of piglets born alive, dead and total born were registered from both of these groups.

Litter size data were analyzed by GLM procedure of SAS. Fertility data were analyzed as a binary variable using the GLIMMIX procedure of SAS.

Results

Mean results found for each group are shown in Table 1.

Table 1. Mean results using Predil® MR-A® in Iberian sows.

	Number of sows	Born Dead	Born Alive	Total Born	Farrowing Rate (%)
Predil	129	0.15	7.76	7.91	91.47 [a]
Control	188	0.11	7.53	7.64	84.57 [b]
SEM		0.057	0.265	0.25	0.045
Difference	-59	0.04	0.23	0.27	6.90

(a, b) Superscripts indicate statistically significant differences within main effect (p = 0.015).

Given the low number of piglets farrowed by the Iberian sows naturally, although the best results were always found in the Predil group, the differences found were not statistically significant. However, the difference found in farrowing rate was statistically significant (p=0.015).

Conclusions and Discussion

These results show the effectiveness of using synthetic seminal plasma in weaned Iberian sows inseminated using the two-phase insemination method. The Iberian sow, like many other European rustic sows have very limited reproductive capacity, particularly in relation to prolificacy, so the use of Predil® MR-A® is perfectly suitable. Although the differences in litter size were not statistically significant, always the Predil group had better results, which gives us an idea about the positive effect of Predil®. Its use has greater benefit during summer time where the effect of high temperatures affects both the sow and the boar by decreasing the semen quality.

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An evaluation of testes size as a method of predicting future semen production in boars

MG Marchesi[1], F Cesarini[1]
[1]Gruppo Martini, Longiano, Italy, m.marchesi@martinigruppo.com

Introduction

Recent new technologies including post cervical insemination and synchronised fixed time single insemination will mean that in the future there will be a need for fewer boars of an even higher genetic merit. Inevitably these boars will have an even higher cost. The introduction of boars with low production capability must be avoided and this study investigated the relationship between the size of testes and future production capability.

Materials and Methods

On arrival in quarantine, an assessment of the size of testes was carried out on 75 young boars of the same breed. A scale of 1 to 5 was used, with score 1 being small and score 5 very large. The subsequent productive career of the boars were monitored in terms of volume per ejaculate, concentration, total sperm numbers and forward motility over a period of 18 months. The semen was analysed with a CASA system (SpermVision®) and the average data of Volume (mV, ml), Concentration, total spermatozoa (mspz, bil) and progressive motility (mMotp, %) were related to the size of testicles. A total of 3328 ejaculates (from 16 to 68/boar) were assessed (table 1).

Table 1. Distribution of data

Score	N° boars	N° ejac. avg
1	6	18
2	25	40
3	24	35
4	10	50
5	10	46

Results

There is no correlation between progressive motility and size of testes, neither with concentration. Boars with score 5 on average produced nearly twice as many total spermatozoa than those with score 1 (table 2)

Table 2. Semen quality of score 1 and score 5

score=1	Mean	SD	Min	Max
mV	95	44	26	160
mspz	52,3	24,6	16,1	98,3
mMotp	88,1	4,5	69,1	92,3
score=5	Mean	SD	Min	Max
mV	287	57	134	405
mspz	96,5	29,3	46,2	152,6
mMotp	84,8	7,9	69,9	93,0

Conclusions and Discussion

Genetic suppliers have payed little attention to this important aspect of conformation.

The effect of the litter size in which a boar is raised and consequential weaning weight also plays an important role in the future reproductive career. Boars raised in smaller litters (1) were shown to have larger testes than those reared in larger litters, probably due to the extra nutrition received in the active period of Sertoli cell mitosis. There is a high correlation (r=0,79) between weaning weight and adult sperm production. The same study also highlighted that spring born boars have larger testes than autumn born boars. Long term selection for lean growth rate over the years has had a negative effect on testicular growth rate and increased age at puberty (2). The heritability of selection of testes size shown to be 0,5 (3). A heritability of 0,35 with an increase in testes weight of 18,7 g /generation was shown in study of selection over nine generations for increased testes weight (4). Novel methods of influencing the size of the mature testes have been studied by reducing the estrogen synthesis in developing boars (5). The use of an aromatase enzyme inhibitor, Letrozole , given orally from 1 week of age generated larger testes and more Sertoli cells by seven months of age. Reducing the endogenous estrogen delays the puberty and allows a longer window for the proliferation of Sertoli cells.

This present study showed a significant difference (P<0,001) in the volume of semen produced between boars with testes score of 1 and 5, with a r value of 0,85. Similarly total spermatozoa numbers showed a correlation of r = 0,82.

Pressure must be borne on genetic suppliers to apply selection pressure on testes dimension and boar studs must clearly assess new arrivals in the quarantine.

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Post mortem anatomic study of boar testicles from Spanish AI centers

JL Úbeda[1], R Ausejo[1], Y Dahmani[1], MV Falceto[2],B Moreno[2], JYeregui[1]
1 Magapor SL. Parque Científico Tecnológico Agroalimentario Valdeferrín-Aula Dei, Calle 5. 50600 Ejea de los Caballeros (Zaragoza) Spain. sveterinarios@magapor.com
2 Departamento de patología animal, Facultad de Veterinaria Zaragoza. Miguel Servet, 177. Zaragoza Spain.

Introduction

Recently, an increase of slaughtered young boars because of their low semen quality has been observed. This fact diminishes animal amortization and decreases productivity of boar studs. Environmental factors like temperature, conditioned air, management, nutrition, staff, water, diseases, etc., may affect sperm quality. The main objective of this work was to elucidate anatomic and histological indicators related to bad semen quality, and to setup early diagnosis for treatment using *in vivo* biopsy, allowing longer maintenance of boars in AI centers.

Materiasl and Methods

Testicles from 23 young Pietrain boars (<2 years) derived from 4 different boar studs were picked from slaughter house. In total, 67% of the 23 slaughtered animals exhibited more than 30% of abnormal sperm, 5% had lameness, 10% were sent to the slaughter house because of their decreased genetic value, 13% due to lack of libido and 5% were slaughtered because of low semen concentration or volume. Boars jumped once per week and semen quality was assessed in all of them during a month. Motility (subjective), abnormal forms and acrosomes (eosin-nigrosin staining), as well as volume and concentration (Bürker chamber) were performed at the boar studs. Low semen quality was reported when an ejaculate had less than 70% spermatozoa motility, more than 30 % total abnormal forms, less than 80% of normal acrosomes, and/or less than 50 ml of volume. All these boars studied had their last 5 collections as "low". Samples from the slaughter house and biopsy samples were analyzed in the department of Pathology and Anatomy, Veterinary Faculty of Zaragoza University. Macroscopic studies included visual examination, transversal and longitudinal cuts of testicle and epididymis (head, body and tail), as well as palpation results and pictures of interesting details. Histological studies with Hematoxilin-Eosin staining were done to the testicle and to the head, body and tail of the epididymis.

Results

In 90% of cases microscopic or macroscopic injuries were identified. The most frequent injuries at the testicle were related to abnormalities by palpation (9%), edema (13%), inflammation (13%), and fibrosis (35%). Moreover, epididymis and pampiniform plexus suffered from edema (13%) and varicocele (22%), respectively.

Conclusions

The results confirm that boars were properly slaughtered, and that most of lesions were chronic and diffuse. However, due to the impossibility to undertake epididymal biopsies, microscopic diagnoses have to be mainly focused on testicles, losing diagnostic sensitivity.

Semen quality in boar studs in Central Western Colombia

G Gómez[1], H Mesa[1], J Sánchez-Osorio Moreno[2], F Henao Uribe[1]
[1]*Departmento Producción Agropecuaria- Universidad de Caldas, Manizales, Colombia.* [2]*Departamento de Medicina y Cirugía Animal, Universidad de Murcia, Murcia, España,* fhenao@ucaldas.edu.co

Introduction

Ignorance of the dynamics of semen quality hinders the development of controlled reproduction programs to improve fertility and prolificacy. Therefore, objective assessment of semen quality and male reproductive behavior are necessary for boar studs to make rational and efficient use of boars (1). Cytoplasmic droplets (CD) are the most common morphological alteration in males destined to controlled reproduction processes (2).

Materials and Methods

For nine months, with intervals of 45 days, semen quality of 48 males in six farms was evaluated. All farms were located in the western-central region of Colombia, between 920 and 1844 MASL. The tests performed were: concentration (CON) and progressive (PM) and total motility (TM) assessed using a Sperm Class Analyzer ® (SCA®) equipment (3); morphology (4); acrosome integrity (ACRI) (5); membrane structural integrity (MSI) through propidium iodide fluorescence (6); membrane functional integrity (MFI) using hyposmotic shock test (7); and DNA fragmentation (IDF) using Halotech dna ® kit (8). The effects of age group and racial group on the variables of semen quality were evaluated by means of a log-linear model, consisting of a *Poisson* regression with a link logarithmic function using the PROC GENMOD-SAS (SAS Inst. Cary, NC). The statistical model included the random effect of individual for repeated measures in the same boar. The Spearman correlation coefficients between seminal variables were calculated.

Results

Descriptive statistics of the variables analyzed are shown in Table 1. Morphological variables other than CD had prevalence lower than 1%. Boars more than 37 months old had higher MFI than those 19 to 36 months old and those younger than 18 months (67 ± 3% vs. 59 ± 3% and 62 ± 3%, respectively; P<0,05). The correlation of ACRI with PCD and TCD was -0.13 and -0.16, respectively (P<0.05).

Table 1. Descriptive statistics of seminal variables

Variables	Mean	Standard Error
PCD, %	7.1	0.6
DCD, %	8.3	0.6
TDC, %	15.4	0.9
MSI, %	85.7	0.6
MFI, %	62.5	0.9
ACRI, %	88.9	0.4
PM, %	65.7	0.8
TM, %	80.3	0.7

Conclusions and Discussion

The variables PCD, DCD and TDC were similar to other reports (10, 11). The correlation of ACRI with PCD and TCD was similar to other reports (2, 6, 8). Likewise, the normal sperm correlation with PCD and TCD was in agreement with (9, 10). Morphological variables other than CD had prevalence lower than 1%, similar to other studies (9, 11). The values found for MSI, MFR, and ACRI agree with those reported previously (12, 13).
Cytoplasmic droplets are the most common morphological alteration in semen samples from farms in the western-central region of Colombia; so far, the causes and effects of these on the fertilizing capacity of sperm in Colombia are not clearly known.

Acknowledgments

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Effect of seminal plasma on pig semen freezability

C Niño[1], J Valencia[1], H Mesa[1], G Gómez[1], F Henao[1]
[1]*Departamento Producción Agropecuaria-Universidad de Caldas, Manizales, Colombia,* fhenao@ucaldas.edu.co

Introduction

Intrauterine deep insemination [1] and FlatPack™ packages [2] have allowed the use of frozen-thawed pig semen with acceptable results but little consistent due to the high variability of freezing ability among males [3, 4]. This variability has been related to genetic factors [4] not yet associated to the composition of seminal plasma (**SP**) or sperm cell (**SC**) functionality [3]. This work was oriented to discriminate the effects of the origin of SP and Es on freezability of pig semen.

Materials and Methods

Semen from eight males from two farms was evaluated fresh and thawed to identify one male per farm with extreme values of freezability: high freezability (**HF**) and low freezability (**LF**) according to the percentage of functionally competent sperm cells (**FCS**) after thawing. The FCS population is defined as those normal for: membrane structural integrity (**MSI**), membrane functional integrity (**MFI**), acrosome resistance (**AR**), and sperm morphology (**SM**), after correcting for pre-freezing agglutination and tail round abnormalities (folded and coiled). Semen was frozen in 0.5 mL straws using Androhep Plus® y Androstar CryoPlus®. The proportion of FCS was evaluated using a combined test with interference contrast and fluorescence at 1000X [5].
In a second moment, to assess the effect of SP and SC origin on freezability, all four possible combinations of those were frozen after being separated through centrifugation at 800 G for 20 minutes immediately after collection. Variables evaluated were: MSI, MFI, SM, FCS, acrosome integrity (**ACRI**), total motility (**TM**) and progressive motility (**PM**).
The effects of SP and SC origin, and their lineal interaction, on the variables of semen quality were evaluated by means of a log-linear model, consisting of a Poisson regression with a link logarithmic function using the PROC GENMOD-SAS (SAS Inst. Cary, NC). Results are presented as least-squares means ± standard error.

Results

Centrifuged semen showed lower values than non-centrifuged for: MFI (53.6 ± 0.03 vs. 55.0 ± 0.03, respectively; P<0.01), ACRI (13.1 ± 0,07 vs. 20.5 ± 0.06, respectively; P<0.01), PM (13.0 ± 0.08 vs. 20.5 ± 0.06 respectively; P<0.01), TM (27.1 ± 0.05 vs. 36.3 ± 0.04, respectively; P<0.01), and FCS (8.8 ± 0.09 vs. 13.0 ± 0.08, respectively; P<0.01).
Samples with SP from HF males showed higher MSI than that from LF males (53.5 ± 0.03 vs. 8.8 ± 0.09 respectively; P<0.01). None of the other variables measured showed a significant effect for SP. The SC from HF males showed higher values than from LF males for: MFI (62.3 ± 0.04 vs. 47.9 ± 0.04, respectively; P<0.01), SM (89.5 ± 0.03 vs. 72.0 ± 0.03 respectively; P<0.01) and FCS (13.8 ± 0.08 vs. 5.4 ± 0.12, respectively; P<0.01).
There was a significant SP×SC origin interaction for ACRI and PM (P<0.01; Table 1).

Table 1. SP×SC origin interaction for seminal variables.

	SC-HF SP-HF	SC-HF SP-LF	SC-LF SP-HF	SC-LF SP-LF
IAC	24.3±0.08[a]	19.2±0.09[b]	10.5±0.12[c]	6.1±0.16[d]
MP	13.8±0.1[a]	13.5±0.10[b]	7.1±0.15[c]	12.0±0.10[d]

a, b, c y d indicate significant differences (P≤0.01)

Conclusions and Discussion

As shown in other studies [6], variability among males and a possitive effect of SP from HF males was detected when added at thawing. Other researchers [7] found differences in plasmatic proteins on males of differing freezability, a possible cause for the differences in MFI, SM, and FCS detected in the present study. A positive effect of SP from HF males was detected on SC from LF males.

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Evaluation of the supplement VIUSID vet powdered of pigs in fatten in breeding systems of low inputs

JC Rodrígez-Fernández[1], V Mendez-García[1], I Calero-Herrera[1], L Suarez[1], K Peña[1], J Gómez[2]
[1]*Departamento de Medicina Veterinaria y Zootecnia, Universidad de Sancti Spíritus, Cuba,* [2]*Instituto Provincial de Medicina Veterinaria, Sancti Spíritus, Cuba,* jcarlos@uniss.edu.cu

Introduction

Although the swinish production has been intensified more and more, in many regions pigs are raised in conditions of intermediate or low specialization, for that reason new products and/or technologies should be evaluated in these ambient. The objective of this work was to evaluate the effect of the supplement VIUSID vet Powder, on the productive performance of pigs in fatten, in systems of breeding of low inputs.

Materials and Methods

For this work a total of 20 pigs in fatten were used. Ten of them received daily 2 g of VIUSI vet powdered per kg of food, the rest animals were used as control. The experiment lasted 50 days.

The supplement evaluated is developed by Catalysis, S.L and it contains: extract of the root of the plant Glycyrrhiza glabra, Malic acid, Glycyrrhicine acid, Ascorbic acid, Folic acid, Cyanocobalamin, Pyredoxine, Arginine, Wisteria, Pantothenate of calcium and Zinc. The product undergo a biocatalytic process of molecular activation, which improves its biological activity and the biochemical reactivity of all antioxidant molecules.

Statistical analysis: for data with homogeneous variance the T-test was used, with previous confirmation of the normal distribution (test of Kolmogorov-Smirnov) and homogeneity of variance (test of Levene).

Results

It was not found any difference in the variables initial weight (table 1), what demonstrated the homogeneity among the groups. The supplement VIUSID vet Powder, in dose of 2 g/kg of food, influenced positively ($p < 0,05$) on the variables increase of weight and on nutritive efficiency

Conclusions and Discussion

In Mexico was reported that the supplement with VIUSID of pigs in fatten, in dose of 2 kg/ton of food, improved the productive indicators, the daily gain in 89 grams and the nutritive efficiency in 90 grams (1).

Neither deaths nor diarrheas were found in both groups.

Table 1. Effect of the treatments on the variables studied (mean value).

Variables	Viusid	Control	p =
n initial	10	10	
n final	10	10	
Initial weight, (kg).	46,30	41,42	0,646
Increase of weight, (kg).	20,16	17,33	0,000
Alimentary efficiency.	6,06	7,08	0,001
Improve of increase of weight, (%)		16.33	
Improve of the nutritive efficiency (%)		14,41	

The increment of weight of the animals was due to the best efficiency in the conversion of the food for the pigs treated with VIUSID vet Powdered. It was observed an improvement of 16 and 14% or higher in the variables that differed statistically. The absolute values reached in the conversion were not good for the potential of the animals, because of the given food was below the requirements in energy for this category of pigs (2,66 Mcal/kg.), although it had a high contribution of gross protein.

It was proven that, under conditions of breeding of low inputs, the supplement VIUSID vet Powdered reduced significantly the deaths, and preserved the count of leukocytes and lymphocites at the normal levels in the treated animals but they diminished significantly in those of control, also, it was improved the gain of weight and the nutritive efficiency significantly.

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Effect on the postpartum behavior of the supply of VIUSID vet Powder to gestated sows

JC Rodrígez-Fernández[1], V Mendez-García[1], I Calero-Herrera[1], L Suarez[1], K Peña[1], RJ Marín[2], Y Bernal[1,2], J Gómez[3]
[1]*Departamento de Medicina Veterinaria y Zootecnia, Universidad de Sancti Spíritus, Cuba,* [2]*Centro Genético Porcino "Cabaiguán", Cuba,* [3]*Instituto Provincial de Medicina Veterinaria, Sancti Spíritus, Cuba,* jcarlos@uniss.edu.cu

Introduction

When the pregnant sow doesn't have in good body condition at farrow, its postpartum behavior is unfavorable and its economic efficiency is affected. The dietary supplement can have different characteristic, but all of them have the same end, to improve the productive results. For that reason, this experiment was conducted to evaluate the effect of the nutritional supplement VIUSID vet Powder given to pregnant sows, on its postpartum behavior.

Materials and Methods

For this work were formed three homogeneous groups of pregnant Yorkshire sows. Group I: The control (n 21), Group II: (n 21) this group received one gram of VIUSID vet Powder blended with the food, from 15 days before the farrow and up to 15 days postpartum, and Group III: (n 25), idem to the previous one, but the dose of the supplement was of 2 g/kg of food. The evaluated product is developed by Catalysis, S.L and it contains: extract of the root of the plant *Glycyrrhiza glabra*, Malic acid, Glycyrrhicine acid, Ascorbic acid, Folic acid, Cyanocobalamin, Pyredoxine, Arginine, Wisteria, Pantothenate of calcium and Zinc. For the hematologic study were taken samples of 16 sows for each treatment. For the statistical procedure an multipurpose analysis of the general lineal Pattern was used To the variables that differed statistically among treatments, were applied the multiple range test.

Results

The supplement VIUSID vet Powder, in dose of 2 g/kg of food, influenced positively on the variables, piglet born live (PBL), piglet alive to the 21 days (PA21) and on the weight of the litter to the weaning (WLW) (Table 1). The rest of the variables studied were not affected by the treatments. The group II with one gram of supplement didn't differ from the control group nor from those treated with 2 grams.

Table 1. Effect of the treatments on the variables in study (mean value).

Variables	(I)	1g (II)	2g (III)	p =
PBL	9,33 b	9,95 ab	10,44 a	0,013
PA21	8,47 b	9.14 ab	9,28 a	0,030
WLW (kg.).	77,43 b	81,60 ab	83,56 a	0,036

(a, b) indicate statistically significant differences within main effect ($p < 0,05$).

Conclusions and Discussion

It is well-known that most of the deaths that occur in the first hours of the newly born are due to the mother. The parturition requires of an intense physical activity during a lingering period, which can derive in oxidative stress, for what an option would be the administration of substances with anti-oxidizer properties that can attenuate the damage. The VIUSID vet Powder contains substances like the Malic acid and the Ascorbic acid with anti-oxidizer effects that by a process of molecular activation, may improve considerably its biological activity and the chemical reactivity of all the anti-oxidizer molecules. The hematologic studies didn't show results of interest, nor the variables studied differed statistically among them. It was concluded that the dietary supplement VIUSID vet Powder influence positively on the postpartum behavior of the sows.

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The influences of the supply of VIUSID vet powdered, on the productive performance of the sows for replace

JC Rodrígez-Fernández[1], V Mendez-García[1], I Calero-Herrera[1], L Suarez[1], K Peña[1], RJ Marín[2], Y Bernal[2], J Gómez[3]

[1]*Departamento de Medicina Veterinaria y Zootecnia, Universidad de Sancti Spíritus, Cuba,* [2]*Centro Genético Porcino "Cabaiguan", Cuba,* [3]*Instituto Provincial de Medicina Veterinaria, Sancti Spíritus, Cuba.* jcarlos@uniss.edu.cu

Introduction

The performance of the sows for replace should be taken in consideration; a good preparation of the future breeders is reflected in the cost, since a wrong handling of the same ones contributes in 30% or more than the nonproductive days (1). The objective of this work was to evaluate the influence of the supply of VIUSID vet Powdered, on the productive behavior of sows for replace.

Materials and Methods

For these work 64 Yorkshire sows of 97 days of age were used; 38 of them were treated and 26 were as control. The treatment consisted on giving VIUSID vet Powdered in dose of 2 g/kg of food, during 70 days.

Because of the existence of significant differences ($p < 0.05$) among the initial weight of the two groups, the increment of weight was adjusted according to the effect of the initial weight as covariant. For the hematological study they were taken samples of 26 sows from the beginning to the end of the experiment (12 treated and 14 as control) Statistical analysis.

Variables	Performed test
Initial weigh Increase of weight Daily mean gain. Alimentary efficiency. Final Hematocrit Initial Protein plasmatic Final plasmatic protein Final count of leukocytes. Difer. among proteins.	Test t for equal variances
Initial Hematocrit. Initial count of leukocytes. Difer. among hematocrit. Difer. among leukocytes.	Test t for no equal variances

For the study before and later, the test T for related samples was used. To evaluate the percent of animals with leukocytosis, the differences between proportions was used.

Results

In tables 1 and 2 are shown the results obtained in the productive behavior and in the hematological study, the Viusid influenced significantly ($p < 0,05$) on the increment of weight, the daily mean gain and the alimentary efficiency, from the same manner acted on the hematocrit and the plasmatic protein.

Table 1: Results of the indicators related with the gain in weight of sows for replace, (means).

Variables	Control	Viusid	p =
Initial weigh (kg).	42,11	36,86	0,000 *
Increase of weight (kg)	45,78	48,85	0,008 *
Daily mean gain (kg)	0,654	0,698	
Alimentary efficiency.	3,91	3,66	0,006 *
Improve of increase of weight (%).		6,70	

Table 2: Behavior of the hematological variables, from the beginning to the end of the experiment (values mean).

Variables / Samples		Control 14	Viusid 12	p =
Hematocrit x 10^{-2} l/l	Initial	33,64	30,66	**0,019**
	Final	37,28	36,91	0,681
	Difer.	3,64	6,25	**0.020**
	p =	**0,000**	**0,000**	
Plasmatic protein g/dL	Initial	6,30	5,96	0,163
	Final	6,20	6,20	1,000
	Difer.	-0,09	0,24	0,150
	p =	**0.038**	0,336	
Leukocytes x 10^9 /L	Initial	14,41	18,17	0,111
	Final	16,28	18,22	0,212
	Difer.	1,87	0,04	0,483
	p =	0,189	0,986	

Conclusion and Discussion

With the supply of 2 grams VIUSID for kilogram of food was possible to improve both, the productive performance and the hematologic values of the sows for replace.

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Evaluation of the supplement VIUSID vet to recently weaned pigs in breeding system of low inputs

JC Rodrígez-Fernández[1], V Mendez-García[1], I Calero-Herrera[1], L Suarez[1], K Peña[1].
[1]*Departamento de Medicina Veterinaria y Zootecnia, Universidad de Sancti Spíritus, Cuba,* jcarlos@uniss.edu.cu

Introduction

In the swinish breeding two moments exist in which important changes take place in their feeding, the first one is to the birth and the second is to the weaning, due to the suppression of the milk. The weaning is the more stressed moment for the life of the pig, in which should adapt quickly to the environmental and nutritional changes (1).

In some places the pigs are raised in systems of breeding that don't have a high specialization, for that reason this work had the objective to evaluate the influence of the nutritive supplement VIUSID vet Powdered on the productive performance of recently weaned pigs, in a system of breeding of low inputs.

Materials and Methods

For this work a total of 31 recently weaned pigs were used. Sixteen of them received daily 2 g of VIUSI vet powdered per kg of food, the rest animals were used as control. The experiment lasted 50 days.

The supplement evaluated is developed by Catalysis, S.L and it contains: extract of the root of the plant Glycyrrhiza glabra, Malic acid, Glycyrrhicine acid, Ascorbic acid, Folic acid, Cyanocobalamin, Pyredoxine, Arginine, Wisteria, Pantothenate of calcium and Zinc. The product undergo a biocatalytic process of molecular activation, which improves its biological activity and the biochemical reactivity of all antioxidant molecules.

Statistical analysis: for data with homogeneous variance the T-test was used, with previous confirmation of the normal distribution (test of Kolmogorov-Smirnov) and homogeneity of variance (test of Levene).

The incidence of diarrheas was analyzed by means of the proportion hypothesis test

Results

It was not found any statistical differences between initial weights (table 1), but there were differences among the rest of variables ($p<0,05$).

The diarrheas decreased significantly in the treated animals (Fig. 1).

Conclusions and Discussion

The increment of weight of the animals was higher in those consumed supplement Viscid this effect had influenced by the best nutritive efficiency (21,29%). The incidence of diarrheas was 36,7 % lower in the treated animals with regard to the controls.

It was concluded that the supplement VIUSID vet Powder decreased significantly the incidence of diarrheas in the recently weaned pigs and was better the increase of weight and the alimentary efficiency.

Table 1. Effect of the treatments on the variables studied (mean value).

Variables	Control	Viusid	p =
Initial weight, (kg).	7,98	7,70	0,580
Final weight, (kg).	15,25	18,17	0,031
Increase of weight, (kg).	7,26	10,47	0,009
Alimentary efficiency.	5,87	4,62	0,002
Improves of the increase of weight, (%)		44,21	
Improves of the nutritive efficiency (%)		21,29	

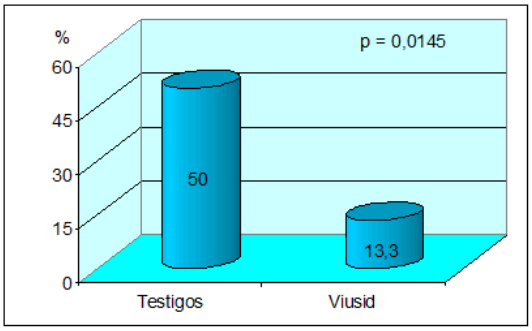

Figure 1. Incidence of diarrheas in the experiment.

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Effect of dietary organic minerals on sow reproductive performance

JK Yeh[1], SH Kim[1], AJ Frio[2], JY Yeh[3]
[1]*Alltech Korea,* [2]*Alltech Asia and Pacific,* [3]*Division of Life Sciences, Incheon National University, Korea*
jyeh@alltech.com

Introduction

There are many forms of trace minerals available for use in swine nutrition. Some studies have shown organic trace minerals can be introduced in livestock diets and be more bio-available than inorganic forms (1,2). Several trace minerals can be produced with peptides, amino acid or carbohydrates. Such mineral products are available for feeding swine. Adding organic trace minerals to the diet may minimize chelation interactions with minerals and other dietary factors (5). The effect of organic trace minerals on sow reproduction has been widely investigated (3,6). The objective of this study was to evaluate the dietary organic minerals on the number of litter size in the high performance sow.

Materials and Methods

Total of 600 sows (Yorkshire x Landrace) were obtained for this trial in one commercial farm located in Chungbuk province, Korea in 2012. Dietary trace minerals (Cu, Fe, Mn, Se, Cr, I and Zn) of organic or inorganic origins were fed to sows divided into 2 groups. The level of Zn was adjusted by Korean feed regulation. Organic trace minerals were metal proteinates, whereas the inorganic minerals were provided in salt form (4). The organic mineral source was Bioplex, Alltech Inc. (Nicholasville, KY, USA). The organic trace minerals were sequestered with enzymatically hydrolyzed soybean protein, whereas organic Se was a yeast protein largely as selenomethionine (Sel-Plex, Alltech Inc.). Most inorganic trace minerals were salts in the sulfate form, except Se, which was as Na selenite and Mn oxide. All sows were housed in individual gestation stalls with concrete slotted floors with each stall containing a concrete feeder and nipple waterer. At 110 days gestation sows were moved to individual farrowing crated and fed their lactation treatment diets. In farrowing, we calculated the live piglet number within one day. After weaning, also checked the number of weaner piglets in the trial 2 groups each.

Results

The total number of live born piglet and weaning piglet are shown in Table 1. Reproductive performance response to dietary trace mineral source demonstrated more number of piglets born and weaning piglets when sows were provided organic minerals rather than the inorganic minerals. The proteinate organic mineral group obtained average 13.1 piglets as live born and average 11.3 piglets as weaning.

Conclusions and Discussion

Sows fed proteinate organic minerals farrowed more total (average 13.1 vs. 12.4) and weaning piglet (average 11.3 vs. 10.7) compared with sows fed inorganic minerals in this trial. Peters and Mahan (2) reported that sows fed organic trace minerals farrowed more total (average 12.2 vs. 11.3) and live pigs (average 11.3 vs. 10.6) compared with sows fed inorganic trace minerals.

Table 1. Feeding effects of proteinate organic minerals and inorganic minerals on the litter size (average) in sow

Item	Proteinate organic minerals	Inorganic minerals
No. of sow	300	300
Total no. of live born piglet/litter	13.1	12.4
No. of neonatal lactating piglet/litter	12.2	11.6
No. of weaning piglet/litter	11.3	10.7

Wedekind et al. mentioned that organic minerals can be more bio-available than inorganic minerals forms (7). The key organic minerals on the reproductive function are Mn, Zn, Se, Cr and I. This data suggested that proteinate organic minerals can increase the total number of live born piglet and number of weaning piglet for reproductive performance in sow.

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The impact of enzyme inoculation on fermentation and aerobic stability of ensiled maize cobs

AT Kanengoni[1#], BD Nkosi [1], RS Thomas[1], SP Ndou[2], M Chimonyo[2], B Ndimba[3], K.Dzama[4]
[1]ARC- Animal Production Institute, Private Bag X2, Irene, 0062, South Africa, [2]Discipline of Animal & Poultry Science, University of KwaZulu-Natal, P. Bag X01, Scottsville, 3209, South Africa, [3]Agricultural Research Council, Proteomics Research and Services Unit, Infruitec. Nietvoorbij Institute, [4]Department of Biotechnology, University of the Western Cape, Private Bag X17, Bellville, Cape Town, 7535, South Africa, d Department of Animal Sciences, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa, Arnoldk@arc.agric.za

Introduction

Maize is a staple crop in Southern Africa and after harvesting; maize cobs become available and are either ploughed back into the field or used as a fuel for fire. Maize cobs are readily available and can be incorporated into pig diets to offset the high feed costs (K), optimize animal welfare, reduce gut diseases and minimize nutrient losses to the environment (B). Although unprocessed maize cobs have been included in pig diets, efficient utilization by growing pigs is not achieved due to high dry matter and lignocellulose content (885 gDM/kg; 930 gNDF /kg DM; 573 gADF/kg DM) (K). Utilisation of the maize cobs by pigs can be improved if the lignocellulosic bonds can be disrupted sufficiently by fermentation and use of exogenous enzymes (Z). The addition of cell wall degrading enzymes to maize forage at ensiling improved the chemical characteristics of the resultant silages and reduced fibre content (C). The aim of this study was to evaluate the impact of ensiling maize cob with exogenous enzymes on silage quality and fibre levels.

Materials and Methods

Maize cobs (920 g/kg DM) were ground to pass through a 5 mm sieve and treated with or without enzyme inoculation, as follows: CON (no additives), ENZ1 (molasses, whey and 0.5 g/kg enzyme) and ENZ2 (molasses, whey and 1 g/kg enzyme). The mixtures (567 ± 2.2 g/kg DM) were ensiled in 1.5 L anaerobic glass jars that were kept at 26 ± 1.5 ^{0}C room temperature for 32 days. Samples were collected on days 0, 1, 4, 15 and 32 for determination of fermentation characteristics and nutritive value of the silage. Samples of day 32 were also subjected to an aerobic stability test, whereby the samples were exposed to air for 5 days and pH together with CO_2 production were measured. Fermentation, aerobic stability and nutrient composition parameters of the silage were analysed in a completely randomized design by ANOVA in SAS (2012).

Results

Control had higher (P<0.001) WSC and NH_3-N but had lower (P<0.001) final pH. Lactic acid production was increased (P<0.001) with ENZ1. There were no differences (P > 0.05) in aerobic stability among the treatments. After 32 days of ensiling, ENZ1 had higher (P<0.001) NDF and ADF concentrations. Although there were no differences (P > 0.05) in DM and GE MJ/kg DM between the treatments, control had higher (P<0.001) CP and EE. Mean DM, NDF and ADF losses are shown in Table 1.

Table 1. Mean DM, NDF and ADF losses in maize cobs ensiled enzymes for 32 days (n=3)

Treatment	$DMl_{os}s$ (%)	NDF_{loss} (%)	ADF_{loss} (%)
CON	49.26[a]	10.97[a]	5.54[a]
ENZ1	49.36[b]	10.85[a]	5.38[a]
ENZ2	49.44[b]	14.48[b]	8.54[b]
Treatment	0.0258	0.0149	0.0025
SEM	0.072	2.07	1.345
R^2	0.99	0.817	0.855
CV	0.146	19.8	24.9

Conclusions and Discussion

Good quality silage is characterized by a concentration of lactic acid (3 to 14%) and butyric acid (<0.1 g) of the total silage acids. All the treatments in this study had a lactic acid concentration in the range 30.9 to 44.7 g/kg DM, butyric acid (<0.1 g) and NH3-N concentrations less than 100g/kg TN at 30 days, it could be interpreted that the fermentation was good (N). In this study, enzyme addition did not significantly reduce pH of the maize cob silage. The low CO_2 production (<1.6 g/kg DM), obtained in this study indicate better aerobic stability of the maize cob silages compared to 4.66 g/kg obtained by (N). It was concluded that addition of enzymes when ensiling maize cobs does not improve the fermentation quality and addition of exogenous enzyme at 1 g/kg reduced the fibre fractions of the silage. Further work is needed to investigate the effect of these findings on animal performance.

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Field evaluation of Calsporin®, a probiotic based on viable spores of *Bacillus subtilis* C-3102: Health and performance of sows and suckling piglets

SK Kritas[1], T Marubashi[4], G Filioussis[1], E Petridou[1], G Christodoulopoulos[2], AR Burriel[2], A Tzivara[2], E McCartney[3]
[1] Veterinary Faculty, University of Thessaloniki, Thessaloniki, and [2] University of Thessaly, Karditsa, Greece, [3] Pen & Tec Consulting SL, Barcelona, Spain [4] Calpis Co Ltd., Tokyo, Japan; skritas@vet.auth.gr

Introduction

Breeding sows are subjected many stressors that negatively affect reproductive performance (1-4,7). Bacillary probiotics, given orally for short or longer time periods, have exhibited variable beneficial effect in sows and their litters (5,6). The aim of this study was to investigate the efficacy of a probiotic based on viable spores of *Bacillus subtilis* C-3102 (Calsporin®, Calpis, Japan) on health and reproductive performance of sows during 2 consecutive reproduction cycles.

Materials and Methods

The study was performed on a commercial 350-sow farrow-to-finish pig farm, practising all in/all-out flow, using artificial insemination and weaning piglets at approximately 28 days of age. Breeding animals were vaccinated against pseudorabies, parvovirus, erysipelas, atrophic rhinitis, enteropathogenic *E. coli*, *Clostridium perfringens* and PRRS (modified live vaccine). Piglets were vaccinated against enzootic pneumonia and porcine circovirus type 2. All adults were treated with ivermectin twice a year.

Litters were weaned and dams moved to the gestation unit in weekly lots. In the gestation unit, sows were housed in individual stalls for approximately 30 days for service and pregnancy testing. Thereafter, lots of 14 pregnant sows were placed in gestation pens with separate feeding-resting cubicles. Each morning, feed dispensers delivered a pre-weighed amount of feed appropriate for each sow. Approximately 7 days prior to farrowing, pregnant sow lots were moved to the farrowing unit and housed individually in farrowing crates within a farrowing pen.

Four sequential lots of sows were studied for a full reproductive cycle (1 pregnancy and 1 lactation period). There were 2 treatments, each employing two lots of sows: Control (T1), fed basal feeds, and a probiotic (T2) treatment, fed basal feeds with added *Bacillus subtilis* C-3102, 3 x 10^5 CFU/g. In addition, T1 and T2 piglets from T1 and T2 sows, respectively, were offered creep feed: T1 (basal Control) or T2 Calsporin® (*Bacillus subtilis* C-3102, 3 x 10^5 CFU/g). Each dam with her litter represented an experimental unit. Sows on trial that were neither culled nor showed signs of infertility remained in the same gestation lot and carried on trial for a 2nd reproduction cycle. Health and zootechnical data were recorded for sows and litters and subjected to Pearson chi-squared test (proportions), and ANOVA (continuous variables).

Results

The effects of Calsporin® on sow and litter performance during two successive reproduction cycles are presented in Table 1. In both reproduction cycles, Calsporin® a) improved sow body condition during pregnancy significantly ($p<0.05$), b) reduced bodyweight loss during lactation significantly ($p<0.05$), and c) reduced weaning-to-oestrus interval significantly ($p<0.05$).

Table 1. Calsporin® efficacy in sows and litters

	1st cycle		2nd cycle	
	T1	T2	T1	T2
SOWS	n=27	n=28	n=21	n=23
Parity	3.3	3.6	4.2	4.2
Lactation (days)	30.1	29.7	26.7	27.1
Sow weight at study start (kg)	239	242	238	237
Backfat loss over lactation (mm)	3.9	3.4	3.8	2.7*
Sow weight loss over lactation (kg)	46.9	35.0*	40.1	25.2*
Sow feed intake during lactation (kg)	219	237*	220	230*
Wean-oestrus (days)	6.5	5.3*	6.8	6.0*
LITTER				
N° piglets born	12.3	12.3	12.2	12.6
Weight of piglets at birth (kg)	1.6	1.5	1.4	1.5*
N° pigs weaned	11.2	11.2	11.1	11.9*
Weight piglets at weaning (kg)	7.5	8.0*	7.7	8.1*
Piglet mortality (%)	8.4	6.3	9.1	5.7
Piglet diarrhoea score	5.2	4.6	6.4	4.2
Mean daily gain (g/piglet/day)	195	217*	236	244

*Figures in same row, within same cycle, differ significantly ($p<0.05$)

The probiotic improved mean piglet weight at weaning in both cycles ($p<0.05$). In the 2nd cycle, the probiotic improved mean weight of piglets born and mean n° piglets weaned. Microbiological examination of faecal samples suggests that the probiotic reduces intestinal *E. coli* and *Clostridium* spp. in piglets, especially after longer-term administration (2nd cycle).

Conclusions and Discussion

A continuously-fed probiotic (*B. subtilis* C-3102) is beneficial in sows and piglets, especially when administered over the longer-term. Benefits noted in these studies included better body condition of sows and piglets, improved sow conception and farrowing rates, and enhanced piglet viability, vigour and growth (1-7).

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Field evaluation of Calsporin®, a probiotic based on viable spores of *Bacillus subtilis* C-3102: Health, performance, and carcass quality of grower-finisher pigs

SK Kritas[1], T Marubashi[5], G Filioussis[1], K Papageorgiou[1], A Govaris[2], AR Burriel[2], D Valoumas[3], E McCartney[4]
[1]*Veterinary Faculty, University of Thessaloniki, Thessaloniki,* [2]*University of Thessaly, Karditsa,* [3]*Creta Farm, Rethymno, Greece;* [4]*Pen & Tec Consulting SL, Barcelona, Spain,* [5]*Calpis Ltd., Tokyo, Japan*; skritas@vet.auth.gr

Introduction

Increased interest in probiotics evolved from problems and concerns relating to antimicrobial resistance. The EU ban on antimicrobial growth promoters also stimulated interest in probiotics as an alternative way to reduce disease associated with enteropathogens, especially around weaning (4). There are numerous publications documenting beneficial effects of probiotics on health, growth and feed efficiency in nursery pigs (3,4). However, few published studies show positive effects of probiotics on zootechnical performance in growing-finishing pigs (1,2). The aim of this study was to assess the efficacy of Calsporin® (Calpis, Japan), a probiotic containing viable spores of *Bacillus subtilis* C-3102, in fattening pigs (88 to 186 days of age). This probiotic strain is approved in the EU and many other geographical regions for use in young pigs and poultry.

Materials and Methods

The study was performed on a commercial 1,800-sow farrow-to-finish pig farm, practising all in-all out flow and artificial insemination. Pigs are routinely vaccinated against enzootic pneumonia, PRRS (modified live vaccine) and porcine circovirus type 2. All fattening units on this farm contain separate rooms, each with 12 pens that house a maximum of 25 pigs/pen. In this study pigs were grouped by size, allocated to mixed-sex pens (male to female ratio 1:2), and thereafter weighed by pen. Two or 3 rooms were filled per week.

The study was run in 2 time blocks. In the first time block, young pigs derived from 4 sequential (weekly) weaning batches were used. Pigs of batches 1 and 3 were housed in one room and fed basal T1 control (untreated group), whereas pigs of batches 2 and 4 were housed in a separate room and fed T2 diets (probiotic group, basal feeds containing *Bacillus subtilis* C-3012 at 1.5 x10^5 CFU/g). This separation prevented cross-contamination from probiotic to control treatments. The same procedure was used for the second time block, but the room allocations were reversed to compensate for any rom effect (cross-over design). In total, 89 T1 and 89 T2 pens (replicates) were used, with 1,923 and 1,869 pigs in T1 and T2 treatments, respectively. At study start, mean pen age, nº pigs/pen and pen bodyweight did not differ significantly between treatments. Health, zootechnical and carcass quality parameters were recorded for the entire experimental period and subjected to Pearson chi-squared test (proportions), and ANOVA (continuous variables).

Results

No significant differences between treatments were observed for bodyweight, feed consumption, feed efficiency, or mortality. However, probiotic supplementation improved growth significantly, by 17 g/pig/day ($p<0.05$).

Microbiological examination of faecal samples indicated that the probiotic treatment tended to reduce *Escherichia coli* and *Clostridium* spp. excretion, at both 50 days on trial, and at study end.

Table 1. Effect of probiotic on fattener performance

Treatment	T1	T2
Nº replicates (pens)	N=89	N=89
Mean age, study start (days)	97.9	99.1
Mean pigs/pen, study start	20.7	21.0
Mean pen weight, study start (kg)	1021	1033
Mean pig weight, study start (kg)	49.0	49.2
Days on trial	88.3	86.5
Mean age, study end (days)	186.3	185.5
Mean pigs/pen, study end	20.3	20.7
Mean pen weight, study end (kg)	2,078	2,127
Mean pig weight, study end (kg)	102.4	103.0
Mean daily gain (kg/pig)	0.608	0.625*
Runts culled/mortality (%)	2.0	1.7
Mean pen feed consumption (kg)	3,231	3,299
Mean pig feed consumption (kg)	159.8	160.8
Mean feed consumption (kg/pig/day)	1.83	1.88
Mean feed:gain	3.02	3.01

* Figures in the same row differ significantly ($p<0.05$)

With regard to carcass quality at slaughter, the probiotic treatment significantly improved backfat thickness ($p<0.05$), and there was a tendency for a better overall meat score ($p=0.058$) in the probiotic compared to the control treatment. In addition, significantly more carcasses from probiotic-treated pigs were classified in the highest E category (63%) in comparison with control pig carcasses (49.5%) ($p=0.007$).

Conclusions and Discussion

The results of this large study indicate that supplementation of *Bacillus subtilis* C-3012 at 1.5 x10^5 CFU/g feed improves growth in fattening pigs under commercial farm conditions and tends to reduce faecal excretion of *Clostridium spp.* and *E. coli*. An extra 17 g/pig/day growth is equivalent to 1.5 kg extra bodyweight per pig over the whole study period. In addition, the probiotic treatment improved carcass quality, to the economic benefit of the farmer. These data further support the view that probiotics can help reduce the use of growth promoting antimicrobials in pigs, thus minimizing public health risks, such as drug residues and antimicrobial resistance.

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Bacterial communitties in feces of hairless Mexican pigs

R Santos-Ricalde[1], L Sarmiento-Franco[1], R Rojas-Herrera[2]
[1]*Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán, km 15.5 Carretera Mérida-Xmatkuil, Apdo. Postal 4-116, Mérida, Yucatán, México,* [2]*Facultad de Ingeniería Química, Universidad Autónoma de Yucatán, Apdo. Postal 1226-A, Mérida, Yucatán, México,* rsantos@uady.mx.

Introduction

There is considerable interest in understanding and influencing the intestinal micriobiota, notably because of the need to replace antibiotics as growth promotes in animal production (2). Manipulation of the nonstarch polysacarides that escape digestion in the small intestine, but undergo bacterial fermentation in the large intestine, and positively affect the intestinal micriobiota has been suggested as a way to change the microbiota and promote intestinal health. The local breed of pigs in Mexico is the hairless Mexican pig (HMP), which is considered an unimproved genotype (3). Bearing in mind that the HMP is a rustic breed genetically operate under a system of grazing low-tech, this breed may be an option aimed to creation of alternatives to the production of high quality products, like organic pig production, where antibiotics can be restricted. This study tested the hypothesis that diets high in forages increase the diversity of the microbial community in the posterior gut of pigs.

Materials and Methods

Fifteen HMP barrows (12.0 ± 0.51 kg) were housed individually in pens. The pigs were randomly allotted to one of three treatment diets, based on sorghum and soybean: A) Control diet; B) diet containing 15% of Ramon leaf meal (RLM) (*Brossimun alicastrum*) and C) 30% of RLM. All diets were formulated to be similar in nutrient content. Food was offered ad libitum and food refusals were weighed daily. Fecal samples from all pigs in the three treatments were collected on day 1, 14, 28, 42, 56 and 70. The fecal samples were snap frozen and stored at -20 °C until DNA insolation. Microbial DNA was extracted from feces, and the 16S rRNA gene was amplified by PCR and analyzed by Denature Gradient Gel Electrophoresis (DGGE) technic (1). Diversity indexes, including evenness (E), Shannon´s index (H´), richness (R), Simpson´s index (S) and Sorenson´s pairwise similarities coefficient (S_D) were calculated.

Results

The marker band obtained from *F. succinogenes* was representative for the Operational Taxonomic Unit (OUT) 32, yielding the same DGGE fingerprint and was only present in faeces from pigs fed 15% RLM diet; this population was apparent at day 4 and 70. The frequency and density of OUT 8, which was obtained at the same position of marker band from *R. flavefaciens*, was higher in faeces from pigs fed 30% RLM diet that in those pigs fed on either the control or 15% RLM diet. Dietary effects on fecal microbial communities were pronounced, with higher R values ($P<0.05$) in fecal bacterial populations from pigs fed control diet than those of pigs fed on either RLM diets. There were higher H´ values ($P<0.05$) in fecal bacterial populations from pigs fed control diet than those of pigs fed on either the 15% or 30% RLM diets in three of the sampling points. Among diets treatments and across sampling days, the greater ($P<0.05$) intragroup S_D value was in fecal bacterial populations from pigs fed 15% RLM diet (93%) at day 42. The S_D values in samples from pigs fed 15% RLM diet were less stable, having three significant ($P<0.05$) different S_D values across sampling days. However, in samplings from pigs fed 30% RLM diet, S_D values were more constant ($P>0.05$). The pairwise comparison control–30% RLM showed greatest percentage of similarity (72.36%) at day 42.

Table 1. Intestinal microbial community diversity indexes from the fecal samples.

Diversity Index	Control	15% RLM	30% RLM
E	0.98±0.006	0.98±0.005	0.99±0.005
H´	3.01±0.283	2.93±0.221	2.90±0.246
R	21.83±5.901	19.93±3.754	19.40±5.422
S	20.54±5.753	18.71±3.752	18.44±5.371

Conclusions and Discussion

Results in this experiment indicate that changes in the fiber composition of the feed affected the composition of the intestinal microbiota; therefore dietary fiber may be practical tool in terms of selective stimulation of particular bacteria in the gut. Further studies are needed to identify organisms whose appearance or loss is associated with diet, wit the goal of defining causal effects as assessment of their activities in order to fully explore the effect of dietary fiber on the intestinal microbiota.

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Effect of antioxidant supplementation to the sows at peripartum on piglets survival at birth

F Barbé[1], A Sacy[1], E Chevaux[1], Y Le Treut[1], B Ramirez[1]
[1]*Lallemand SAS, 19 rue des Briquetiers – BP 59, 31702 Blagnac, France*
fbarbe@lallemand.com

Introduction

Pregnancy and farrowing are periods of oxidative stress, not only for the mother, but also for the newborn (1,2). Intensity of oxidative stress is high at the beginning of gestation, moderate at the moment of histological differentiation of organs and tissues in the fetus, and then increased progressively at the end of gestation. An adequate supplementation of antioxidants is therefore of interest for maximizing reproductive performances for improving vitality, development and growth of newborns (3). A previous study already showed a beneficial effect of distributing antioxidants to the sow during weaning-estrus interval on the percentage of immature piglets per litter and on the within-litter variation of birth weights (4). The purpose of this study was to assess the effect of sow's antioxidant supplementation (SOD, organic selenium) on the piglets survival at birth.

Materials and Methods

The tested antioxidants were organic selenium, acting as a cofactor of glutathione peroxidase (Alkosel; 1 g/sow/day) and superoxide dismutase provided by a melon-freeze dried juice concentrate (Melofeed: 0.3 g/sow/day), given in top-feeding to the sow from 8 days before farrowing until piglets weaning. The field trial was performed on 169 sows: 63 sows were supplemented with Alkosel (A), 54 sows were supplemented with a combination of Alkosel and Melofeed (A+M) and 52 sows were not supplemented (control group: C). The percentage of stillborn piglets at birth and the percentage of sows having 2 or more stillborn piglets were calculated. To take into account the litter size effect, the percentage of stillborn piglets in the trial was also compared to the equation established from several farms: *% of stillborn = 0.87 × litter size – 4.04* (Lallemand, internal data, n = 66 sows). The data were analyzed by a linear mixed model with SPSS Statistics 21, according to the supplemented group (C, A, A+M) and the sow's parity (from 1 to 5, with parity 1 being analyzed separately).

Results

The percentage of stillborn piglets was decreased by half in the group A+M compared to the group C (*p = 0.064) for sows in parities 2-5, the group A being intermediate (Figure 1). This reduction was also observed for gilts.

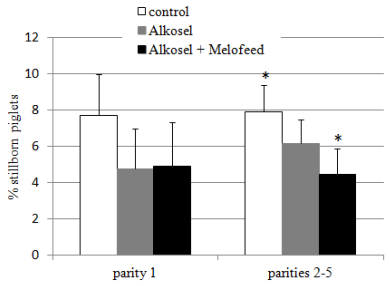

Figure 1. Percentage of stillborn piglets.

In addition, the combination A+M decreased the percentage of sows having 2 stillborn piglets or more, compared to group C (Table 1). The group A had intermediate values between groups C and A+M.

Table 1. Percentage of sows having 2 stillborn piglets or more.

	parity 1			parities 2-5		
	C	A	A+M	C	A	A+M
2 stillborn or more	**22%**	16%	**6%**	**35%**	23%	**16%**

The combination A+M also increased the percentage of sows having less stillborn piglets than the expected value given by the equation:
% of stillborn = 0.87 × litter size – 4.04.

Conclusions and Discussion

The antioxidant supplementation with SOD (primary antioxidant) and organic selenium (secondary antioxidant) proved to be beneficial and synergistic to decrease piglets mortality rate for sows in parities 1 to 5. A further step of this trial will be to analyze the effects of this supplementation on the following parity.

Acknowledgments

The authors thank the farmer (La Fennetrie, 37500 Marçay), Dominique Baudry and Thibault Le Treut for their participation in this trial.

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It influences of the supply of VIUSID vet powder on the hematologic parameters of the pigs

I Calero-Herrera[1], JC Rodrígez-Fernández[1], V Mendez-García[1], L Suarez[1], K Peña[1], RJ Marín[2], Y Bernal[1,2], J Gómez[3]
[1]*Departamento de Medicina Veterinaria y Zootecnia, Universidad de Sancti Spíritus, Cuba,* [2]*Centro Genético Porcino "Cabaiguan", Cuba,* [3]*Instituto Provincial de Medicina Veterinaria, Sancti Spíritus, Cuba.* ibrahin@uniss.edu.cu

Introduction

The study of certain blood parameters in pigs allows us to understand its health status, they can be used as indicators of the level of well-being or of stress of the animals and by means of them you can evaluate the effect of food additives or other products. The research has developed to evaluate the influence of VIUSID® vet Powder on blood parameters of pigs.

Materials and Methods

Experiments were carried out on different categories as explain below:

1. 33 pigs were used of it feeds, 17 used as control and 16 under treatment. The treatment consisted on supplementation of 2g of VIUSID® vet Powder/kg of food during 52 days. Blood sampling to 22 pigs *(12 treated and 10 control)* after 40 days of treatment with *when they took 40 days consuming the preservative*

2. 67 pregnant sows were studied. They were grouped as follow: Group I: control (21 sows), Group II: feed with meal + VIUSID® vet Powder (1g VISUID®/kg of meal) from 15 days before the childbirth till 15 days post childbirth (21 sows), and Group III: idem to the previous one but with of 2 grams VISCUID®/kilogram of meal (25 sows). Blood samples from 48 sows (16 for treatment), between 21 and 26 days post childbirth were taken.

3. 64 sows (growing stage) of 97 days old, 26 control and 38 under treatment were used in this experiment. The treatment consisted on supplementation of 2g of VIUSID® vet Powder/kg of food during 70 days. Blood samples from 26 sows (14 control and 12 under treatment) were taken at the beginning and at the end of the experiment.

Statistical analysis to hematocrit, the plasma proteins at the beginning and at the end, the count of leukocytes and to the difference amongst proteins, at beginning of experiment were carried out through Test T for same variances, T test was also used for related samples and to evaluate the percentage of animals with leucocitosis the hypothesis Test was used for proportions.

Results

It has been showed that animals that received the product had a significantly bigger value ($p < 0,05$) for leucocites when compared to the control. Nevertheless no significant differences were found in the differential count of leukocytes, for segmented neutrophils and for the rest of the cells (Eosinophiles, Basophiles, Monocytes). Treatment groups showed a bigger quantity of lymphocytes which statistically differed ($p \leq 0,05$) of the controls.

Table 1. Blood values in the experiment with pigs in fathering (Media).

Variables (U/M)	Control	Treated	p =
Samples u.	10	12	
Hto ($x10^{-2}$ l/l)	41,20	38,92	0,15
Hb (g/dl)	12,36	11,68	0,15
Leukocytes. ($x10^6$./l)	11775,0	15416,6	0,05 *
Seg. Neut. ($x10^6$./l)	4481,6	6281,1	0,133
Lymphocytes ($x10^6$./l)	5969,8	8707,7	0,050 *
Other cél. ($x10^6$./l)	427,8	1323,5	0,298

In the experiment with the pregnant sows, all the studied variables showed values into the normal ranges for the species. Those values did not differ statistically to each other.

In the experiment with the sows in growing stage it was found that the animals that received the product significantly ($p < 0,05$) increased the hematocrit when compared to the control, the sows treated they increased their hematocrit in 20,38% respect to the values they had at the beginning of the experiment, the control sows showed a slightly increase of 10,82%. Plasmatic protein were increased lightly in those that received treatment while control sows suffered a statistically significant reduction ($p < 0,05$).

Conclusions and Discussion

The supply of VIUSID® vet Powder shows a significant influenced on blood parameters in fathering pigs and in replacement sows but not in pre-weaning sows.

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Impact of trace elements and vitamins supplementation in sows at weaning on weaning-to-estrus interval, fertility and prolificacy depending on their body condition

F Bouchet[1], P Pupin[2], C Chevance[1], A Lebret[1], J Metais[1], P Berton[1], V Normand[1]
[1]*Porc.Spective Groupe vétérinaire Chêne Vert Conseil, ZA de Gohélève, 56920 Noyal-Pontivy, France,* [2]*Synthèse Elevage, Rue Marie Curie, 35137 Pleumeleuc, France,* v.normand@chenevertconseil.com

Introduction

Vitamin requirements are often based on the levels needed to prevent the development of deficiencies rather than to optimize production performances *(*1).
The aim of this study was to test the impact of vitamins and trace elements supplementation (Istruvit®) at weaning on weaning-to-estrus interval (WEI), fertility and prolificacy, regarding body condition.

Materials and Methods

This was a comparative study conducted between May and November 2011 in a French herd of 270 sows.
On Monday before weaning (Wednesday), 120 sows were randomized in order to obtain 2 groups (C and T) equivalent regarding litter rank and backfat thickness (BFT) measurement. BFT were measured with a Renco Lean-Meater (Table 1).In each group, sows were allocated in 2 subgroups according to body condition (L: Lean or N: Normal):

- CL: Control batch with a BFT ≤ 12mm (n=23),
- CN: Control batch with a BFT ≥ 13mm (n=35),
- TL: Istruvit® batch, with a BFT ≤ 12mm (n=26),
- TN: Istruvit® batch, with a BFT ≥ 13mm (n=36),

The 62 sows of the test group (T=TL+TN) received Istruvit®: 40g per day for five days, starting on the day of BFT measurement. All the sows (C and T) received the same feeding program.

Table 1. Test and control groups are comparable (ANOVA)

		T=(TL+TN)	C=(CL+CN)	p	
Litter rank	Mean	3,19	3,69	-0,50	NS
	SD	2,46	2,32	0,13	NS
BFT weaning	Mean	13,43	13,25	0,18	NS
	SD	3,21	2,86	0,35	NS

Parameters studied
The WEI was calculated as the difference in hours, between the time of weaning and the time of the observation of the first signs of estrus (immobility) was first noticed by the farmer. For each sow, the fertility result and the number of total born and alive born piglets at the next parturition were recorded.
Statistical analyses
The effect of Istruvit® on WEI and on prolificacy was tested using ANOVA. The impact of Istruvit® on fertility was studied using the Khi² test.

Results

WEI results (Table 2):
The WEI average of lean sows in the CL group is longer than those on the sows of the other subgroups (not significant). The WEI average of the lean sows in the TL group is close to those in the CN and TN group (Not significant).
Fertility results (Table 2):
The difference between the subgroups is not significant.

Table 2. Fertility and WEI of the 4 subgroups

	BFT ≤ 12 mm		BFT ≥ 13mm	
	CL	TL	CN	TN
Number of sows	23	26	35	36
WEI (hours)	109,7	97,7	98,4	102,0
Gestating sows	22	23	32	31
Fertility rate	96%	88%	91%	86%

Prolificacy results (Figure 1):
The prolificacy of sows in the test group (15,48 total born) is statistically higher (p=0,046) than those in the control group (14,22 total born). Istruvit improves the prolificacy of sows whatever their body condition.

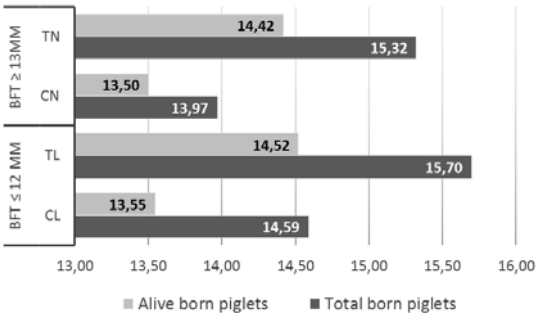

Figure 2. Detailed prolificacy of the subgroups

Conclusions and Discussion

The WEI is longer in lean animals, a phenomenon observed by Quesnel (2). This study demonstrates the benefit of vitamins and trace elements supplementation on sows around weaning, particularly in order to improve prolificacy. More sows might be needed to clarify the impact of Istruvit® on reproduction depending on body condition.

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Standardized ileal digestibility of amino acids from safflower meal measured in growing pigs

G Mariscal-Landín[1], ER Ramirez[1], VA Balderrama P[2], JC Baltazar V[2], JA Cuarón I[1]
[1]*Centro Nacional de Investigación en Fisiología Animal – INIFAP, km 1 Carretera a Colón, Ajuchitlán Querétaro, México. CP 76280,* [2]Estudiante de Maestría en Ciencias, Posgrado de Producción y Salud Animal-UNAM
mariscal.gerardo@inifap.gob.mx

Introduction

Safflower (*Carthamus tinctorius L.*) is an oilseed traditionally used as a source of stain, oil and protein (1). There is no information about its amino acids (AA) standardized ileal digestibility (SID). This lack of information and its high fiber content limit its inclusion in swine feeds. The aim of this work was to determine the AA SID of two Safflowers meals produced in Mexico.

Materials and Methods

Casein and two Safflower meals [Guadalajara (Gdl) and Morelia (Mor)] were used to produce three experimental diets (Table 1); one reference diet using casein as the sole protein source and two experimental diets using casein and one safflower meal as protein sources.

Table 1. Experimental diets

	Cas	Gdl	Mor
Casein	19.00	13.11	14.89
SFM-Gdl		20.00	
SFM-Mor			20.00
Maize starch	61.24	56.13	54.35
Fiber	9.00		
Others[1]	10.76	10.76	10.76

[1] Include: Maize oil, Sucrose, Salt, Calcium carbonate, Dicalcium phosphate, Salt, Vitamins, Minerals and Titanium.

Eight pigs weighing 35 kg were fitted with a T cannula at terminal ileum (2). The experimental diets exceeded NRC (3) requirements; titanium oxide was added at 2.5g/kg as an indigestible marker. Pigs were fed at 2.5 times their digestible energy (DE) requirement (3). The experimental periods lasted 7 d (5 d of adaptation and 2 d for collection). During the first period, four pigs consumed one of the experimental diets (casein-safflower diets), in the second the eight pigs consumed the reference diet. Apparent ileal digestibility (AID) and SID were calculated as recommended (4). The SID of safflowers meals was estimated as recommended (5). The data were analyzed as a complete random design (6).

Results

Table 1 shows the SID of both safflower meals; the values were similar between safflower meals (P>0.05). The average value of SID was of 81.5% and 86.9% for Guadalajara and Morelia respectively.

Table 2. Safflower, amino acids standardized ileal digestibility

	Gdl	Mor	EEM
Lysine	79.2	80.4	6.49
Arginine	84.1	94.0	4.69
Histidine	84.5	86.8	4.58
Leucine	86.6	88.4	4.54
Isoleucine	73.0	80.3	5.26
Valine	72.5	79.7	4.84
Phenylalanine	88.7	89.9	3.73
Threonine	83.2	95.6	7.31

Conclusions and Discussion

Safflower protein is deficient in lysine and marginal in threonine. The SID reported here is similar to that reported in roosters (7). The results show that it is possible to include safflower meal to swine diets as a protein and amino acids source.

Acknowledgments

To National Institute for Forestry, Crops and Livestock for the financial support.

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Effect of an anti-inflammatory feed additive based on plant extracts on the performance of lactating sows

F Astorga[1], M Martín[2]
[1]EMEAA Technical Manager, BEDSON Europe, Madrid, Spain, [2]European Natural Additives, Madrid, Spain,
federico.astorga@bedson-eu.com

Introduction

The dysgalactia postpartum syndrome (DPPS) is the leading cause of neonatal problems can mainly on first and second cycle sows. (1) The frequency of onset is usually between 15-20% of the sows in the herd, although other studies put the figure as high as 33% (2). The consequences for piglets are put at increased mortality, diarrhea and decreased weight gain.

In the usual treatment of this problem is strongly recommended the use of anti-imflammatories as symptomatic treatment. The flavonoids apigenin and hesperidin contained in some plant extracts behave as powerful anti-inflammatory agents specifically inhibiting CLOX2 and the release of IL-1 IL-6 and TNFα by the macrophages, and have a high antioxidant potential protecting tissues from the effects of ROS and RNS (3,4,5).

The aim of this study was to test the effect on piglets of the administration on sows' peri-partum feed of an additive based on this type of plant extracts.

Materials and Methods

Animals: 24 sows of different parity ($\bar{x}$= 3.66) divided into 2 groups (Treatment and Control) randomly distributed and 6 repetitions for a total of 144 sows. 1626 piglets were monitored.

Length of lactation: 21 days.

Treatment: 1.5 Kg of BEDSON® CL per MT of peri-partum feed (7 days before and 7 days after farrowing)

Weight: Piglets were weighed at farrowing and weaning on a scale Baxtran TQ60P (Capacity: 60 Kg Accuracy: 5 g).

Cross-fostering: adoptions of piglets were only allowed within each group.

Results

No differences were found on piglets weight at farrowing but statistically significant differences were found at weaning ($p<0.05$). Weights means are shown in Table 1 and 2. After standardization of the weight at 21 days of age, it was defined a difference of 748 g per piglet.

Mortality rates of the piglets during lactation had statistically significant differences ($p<0.05$) as shown in Table 3.

Conclusions and Discussion

BEDSON® CL could be a helpful feed additive for decreasing the symptomatology of DPPS and its effect on growth and survival of the piglets. The growth and survival of piglets seems to confirm that there is a reduction in the possible subclinical inflammation and an improvement in nutrient use by the sow for milk synthesis. More studies are needed to confirm these effects on other conditions.

Table 1. Weight Means of piglets at farrowing

Group	Mean	Standard deviation	Coefficient of variation
Control Group	1,418[a]	0,223	15,74%
BEDSON CL Group	1,399[a]	0,197	14,10%

(a) No statistically significant differences (p >0.05)

Table 2. Weight Means of piglets at weaning

Group	Mean	Standard deviation	Coefficient of variation
Control Group	5,684[a]	0,890	15,66%
BEDSON CL Group	6,461[b]	0,987	15,28%

(a,b) Statistically significant differences (p <0.05)

Table 3. Mortality rates of piglets during lactation

Group	Mean	Standard deviation	Coefficient of variation
Control Group	12,86%[a]	0,037	29,02%
BEDSON CL Group	8,49%[b]	0,022	26,05%

(a,b) Statistically significant differences (p <0.05)

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Nucleotides supplementation reduce intestinal villous atrophy in newly weaned piglets

C Camacho-Rea[1], G Villar[2], S Solorio[3], A García[3], L Solano[1], R Martínez[4], F Pérez-Gil[1]
[1]*Departamento de Nutrición Animal, INCMNSZ,* [2]*Apligén, S. A. de C. V.,*
[3]*Lesaffre Feed Aditives,* [4]*FMVZ-UNAM,* carmen.camachor@incmnsz.mx

Introduction

Nucleotides are not essential nutrients, however, in some conditions as gut injury, periods of rapid growth or stress situations nucleotides may be conditionally essential, and exogenous sources may confer biological benefits to tissues that undergo rapid turnover such as intestinal tissue (1). Weaning is accompanied by stress and low feed intake, which seems to be the main reason for the growth stasis after weaning and for morphologic and histologic changes of the small intestine (2), so that dietary nucleotides could reduce intestinal damage observed at weaning. The aim of this study was to evaluate the effect of nucleotides supplementation on the length of the villous and crypt depth in the jejunum of newly weaned piglets.

Materials and Methods

Sixty piglets weaned at 21 days of age were randomly assigned to one of three treatments. Treatment 1 or control diet (n=20) was a corn-soybean based diet. Treatment 2 (n=20) was control diet plus 1000 ppm of yeast extract with nucleotides, 5' IMP ,5'GMP at 2.6 % and 3.6 % respectively. Treatment 3 (n=20) was control diet plus 1000 ppm of a yeast extract with nucleotides, 5'IMP, 5'GMP at 5.0 % and 5.5 % respectively. Five piglets at 21 days of age were sacrificed and used as reference for histological analysis and at 28 days of age five piglets from each treatment were sacrificed. The small intestine was dissected and jejunum samples were taken for histologic analysis. Tissue sections were mounted on slides and stained with hematoxylin and eosin. Each slide was scanned and the height of 10 villous and crypt depth was measured using an optic microscope Motic B3 profesional. Data were analyzed using analysis of variance on a linear model and treatment means were compared based on a Tukey test.

Results

In this study, the height villous of reference piglets were 590± 25.3 µm and the crypt depth were 100 ± 5.4 µm. There were significant differences between treatments for the length of the intestinal villous ($P < 0.01$) and crypt depth ($P < 0.01$). Mean height villous in treatment 1, 2 and 3 were 211.65 ± 23.31µm, 289.91 ± 15.85 µm and 371.95 ± 16.21 µm respectively and they were significantly different from each other ($P < 0.05$). Mean crypt depth in treatments 1, 2 and 3 were 151.88±6.94 µm, 138.57±4.72µm and 115.54±4.83 µm respectively. Mean crypt depth in 3 was different from mean of treatments 1 and 2 ($P < 0.05$) and the average of the two latter were not different from each other ($P > 0.05$).

Conclusions and Discussion

It is known that the low feed intake immediately after weaning is responsible for villous atrophy and reduced growth rate in newly weaned (2). In the present study all animals seemed healthy throughout the experiment and the histological changes that commonly occur in the small intestine immediately after weaning was observed. However, the nucleotide supplementation had a beneficial effect since villous atrophy was reduced compared with the piglets from control diet. In addition, crypt depth was less in piglets fed with nucleotides which indicate that there was a less mitogenic activity in the crypt. This result was similar to the findings of Martinez-Puig (3) who showed a positive effect of yeast extract on villous height in piglets weaned at 21 days of age. In conclusion, the findings from the work reported in this study indicate that dietary nucleotides may be beneficial during the post weaning period in piglets where it is expected that they have a high tissue requirement for nucleotides and where dietary supply of nucleotides is reduced compared to the supply from sow milk, for this reason dietary nucleotides may benefit the intestinal health.

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Effect of an anxiolytic feed additive based on plant extracts on the performance of weaned piglets

F Astorga [1], M Martín[2]
[1]EMEAA Technical Manager, BEDSON Europe, Madrid, Spain, [2]European Natural Additives, Madrid, Spain,
federico.astorga@bedson-eu.com

Introduction

After mixing animals at different moments of the production cycle (transition, fattening, gestation), pigs express some aggressiveness due to the necessity to establish a certain hierarchy (1,2).
This aggressiveness involves injuries and wounds that easily complicate in piglets with low immunity, mainly from first cycle sows.
Extract of valerian root (3) and passion flower (4) have demonstrated some anxiolytic activity due to the effect in GABA receptors of the neurons.
The aim of this study was to test the effect on piglets of an additive (BEDSON® EX) based on this type of plant extracts in the reduction of fights and improving production parameters.

Materials and Methods

Animals: 445 piglets, divided into two groups of 229 and 216 (Group CONTROL and TREATMENT Group), randomly distributed in 32 pens in 3 different rooms.
Length of nursery: 6 weeks.
Treatment: 1 Kg of BEDSON® EX per MT of pre-starter feed (7 days before and 7 days after weaning)
Weight: Piglets were weighed at weaning and at the end of nursery on a scale Baxtran TQ60P (Capacity: 60 Kg Accuracy: 5 g).
Fights and activity: behavior of the piglets after weaning was recorded with cameras Fujifilm FinepixF500EXR.

Results

The number of fights was significantly **reduced by 74.86%.** The rest period was increased by 20.01% for the treated group.
No differences were found on piglets weight at weaning but statistically significant differences were found at the end of nursery ($p<0.05$). Weights means are shown in Table 1 and 2. Mortality rates of the piglets during nursery had statistically significant differences ($p<0.05$) as shown in Table 3.
The difference in feed intake and growth during the first week post-weaning was decisive in the results.

Conclusions and Discussion

The increasing selection pressure towards more rustic piglets seems to have brought with it greater aggressiveness. The use of an additive based on plants with anxiolytic effect reduced the number of conflicts and improved growth performance. The advantages related to the use of this additive, in this test, are related to the production of more pigs, heavier pigs, and less spending on medication and working time to cater secondary infections of the skin lesions.

Table 1. Weight Means of piglets at weaning

Group	Mean	Standard deviation	Coefficient of variation
Control Group	5,297 [a]	0,323	6,09%
BEDSON EX Group	5,328 [a]	0,351	6,59%

(a) Superscripts indicate no statistically significant differences within main effect ($p>0.05$)

Table 2. Weight Means of piglets at end of nursery

Group	Mean	Standard deviation	Coefficient of variation
Control Group	16,086 [a]	1,761	10,95%
BEDSON EX Group	19,076 [b]	1,826	9,57%

(a,b) Superscripts indicate statistically significant differences within main effect ($p<0.05$)

Table 3. Mortality rates of piglets during nursery

Group	Mean	Standard deviation	Coefficient of variation
Control Group	7,73 [a]	0,048	48,1%
BEDSON EX Group	3,63 [b]	0,037	132,8%

(a,b) Superscripts indicate statistically significant differences within main effect ($p<0.05$)

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Effect of immunological castration (Improvest®) on the growth performance of finishing pigs

CL Puls[1], M Ellis[1], FK McKeith[1], M Mellencamp[2], AL Schroeder[2]
[1]*Department of Animal Sciences, University of Illinois, Urbana, IL,* [2]Zoetis, Florham Park, NJ
mellis7@illinois.edu

Introduction

Intact males have been shown to have greater feed efficiency and to produce leaner carcasses than physically-castrated barrows (1,2). However, intact males are not commonly used for pork production because of the risk of boar taint, the off-odor when pork from intact males is cooked (3). Improvest® (*gonadotropin releasing factor analog- diphtheria toxoid conjugate*; Zoetis) is an immunological product administered in 2 doses, with the second dose effectively castrating the pig. This product allows swine producers to take advantage of the increased growth performance of intact males while reducing the risk of boar taint. Limited research has been carried out on the impact of feeding ractopamine (RAC) to immunological castrates. Two studies were conducted to evaluate the growth performance of immunological castrates (IC) compared to intact males (IM; Study 1 only), physically-castrated barrows (PC) and gilts (G) and gender responses to feeding RAC (Study 2 only).

Materials and Methods

The studies were carried out for 8 weeks from 16 (~65 kg BW) to 24 (~130 kg BW) weeks of age involving 340 pigs. Improvest was given in two 2-mL doses administered at the start of study and 4 weeks later, respectively. Study 1 was carried out as a RCBD with 4 treatments: 1) IC, 2) IM, 3) PC, and 4) G with 10 groups/sex. Study 2 was carried out as a RCBD with a 3 × 3 factorial arrangement of treatments: 1) Sex (IC, PC, G) and 2) RAC inclusion level (0, 5, 7.5 ppm) with 5 groups/Sex × RAC subclass. In Study 2, RAC was fed for the final 21 days of the study period. Pigs were housed in groups of 4 at a floor space of 1.18 m^2/pig. Diets in both studies were formulated to meet nutrient requirements of intact males (4). Pigs were weighed at the start, wk 4 of study, and end of study. All feed additions and feed remaining in the feeder at the time of pig weighing were recorded. Data were analyzed using the PROC MIXED procedure of SAS (Cary, NC, USA). Differences between means were considered different at $P \leq 0.05$.

Results

The LSMEANS for the effects of sex on overall growth performance for the 2 studies are presented in Table 1. In Study 2, there were no sex by RAC level treatment interactions ($P > 0.05$). Feeding RAC at 5 or 7.5 ppm for 21 d had no effect ($P > 0.05$) on feed intake but increased growth rate (ADG = 1066, 1294, and 1222 g/day for the 0, 5, and 7.5 ppm RAC levels, respectively) and feed efficiency (G:F = 0.303, 0.364, and 0.354 kg:kg, respectively) compared to the control (0 ppm).

Table 1. LSMEANS for the effects of sex on overall (8-wk period) growth performance of pigs

	Sex				
	IC	IM	PC	G	SEM
Study 1					
ADG, g	1151[a]	1087[b]	1033[b]	965[c]	22.5
ADFI, kg	3.08[a]	2.70[b]	3.05[a]	2.70[b]	0.063
G:F, kg:kg	0.375[b]	0.403[a]	0.338[d]	0.358[c]	0.0056
Study 2					
ADG, g	1209[a]	-	1076[b]	1024[c]	18.2
ADFI, kg	3.07[a]	-	3.18[a]	2.71[b]	0.054
G:F, kg:kg	0.394[a]	-	0.338[c]	0.379[b]	0.0072

[a,b,c,d]Means with different superscripts are different ($P \leq 0.05$).

Conclusions and Discussion

The results of these studies are in agreement with previous research, which has shown that after the second Improvest dose IC grow faster compared to PC and G (5,6) and also have improved feed efficiency compared to G and PC (2,6).

The lack of Sex by RAC treatment interactions in Study 2 suggest that the advantages of feeding RAC will be similar in IC as in other sexes, which is in line with previous research (7).

The results of these two studies highlight the potential of IC compared to PC to improve feed efficiency during the finishing phase and reduce production costs.

Acknowledgments

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Improve profitability and welfare at weaning and regrouping piglets with an allostatic modulator

ME Rubio-García[1]; S Valdez[2]; AJ Ibarra[3]
[1]*Morub, S.C.* [2]*Independent Advisory*, [3]*ATISA*, saval_29@yahoo.com.mx

Introduction

Weaning and regrouping piglets according to their size is a common practice, which is done to maintain constant flow of uniform piglet litters. Those stressful managements trigger fights and nervousness among piglets and affect their welfare. However, this behavior is conducted in order to establish the hierarchy. Negative effects such as: skin damage (mainly ears, tail and back), immunosuppression, low feed intake and weight loss develop [(1,2)].Welfare pig´s is very important, due to the economic impact that involves animal stress, which increases allostatic load in order to maintain homeostasis [(3,4)]. The use of an allostatic modulator (AM) (combination with 50g of AA, 62.5g of ASA and 251 mEq of Cl, Na and K per L) can minimize animal allostatic load and prevent illness, lower productivity and piglets die. The aim of this study was to reduce weight loss in weaned and regrouped piglets with an AM.

Materials and Methods

We conducted the study in a commercial farm located in Colima, Mexico. The area has humid-warm weather, with 36 °C average annual temperature and RH from 50 to 70%. The study was conducted 10 months from May to March. We used a total of 20,795 hybrid piglets divided in 21 replicas. Piglets were weaned at 19-21 days old, and regrouped according their size. At 77 days old they were relocated in a new feedlot until 152 days old. Feed and water were provided *at libitum*. The experimental arrangement was conceived using 2 treatments (T1: with an AM and T2: Control without AM). The AM was given at a dose of 1 g / L of drinking water after weaning and regrouping piglets for 5 days after these managements. Collected data were: Mortality, downgrade pigs, weight gain and feed conversion. Obtained data was analyzed by T-test, and the difference among groups was considered significant at $P \leq 0.05$ (STATISTIX 9.0, Analytical Software. Tallahassee, FL, USA).

Results and Discussion

Early weaned improve pig production by reducing fights and injuries[(2)]. Results in our study with early weaning at 20 days old showed lower mortality (P=0.0027) in piglets with AM. In addition, when pigs were relocated in a new feedlot the use of AM showed less downgrade pigs (P=0.049). At the end of growing period, weight gain was 2.719 Kg more for groups with AM (P=0.0002), without affecting feed conversion (P=0.14) (Table 1). This results show that AM reduces allostatic load, and allows better pigs development, at any age of stressful managements. Welfare in pigs reduces allostatic load without compromises their profitability.

Table 1. Performance parameters of pigs with an allostatic modulator via drinking water for 5 days after managements.

Parameters	AM	Control	P
No. of dead piglets	200[b]	324[a]	0.0007
% Mortality	2.02b[b]	2.97[a]	0.0027
No. of downgrade pigs	95[b]	178[a]	0.049
% Mortality + % Downgrade pigs	2.98[b]	4.6[a]	0.0011
Mean weight gain (Kg/pig)	97.19[b]	94.47[a]	0.0002
Feed conversion	2.8[a]	2.78[a]	0.14

a,b Means followed by different superscript letters differ significantly.
AM= Allostatic modulator

Conclusion

The use of AM reduce weight loss in weaned and regrouped piglets

Acknowledgments

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Impact in swine production costs generated by inmunization with recombinant vaccine candidates against *Leptospira*

JC Fernández [1], W Jirón [2], N Batista [3], JF Infante[3]
[1]*National Center of Biological Safety, Playa, Havana. Cuba.* [2]*Veterinary Medicine School, León, Nicaragua.* [3] *Finlay Institute. La Lisa. Havana. Cuba,* julioc@orasen.co.cu

Introduction

Leptospirosis is consider an infection disease very contagious caused by spirochetas of generus Leptospira, this disease affect negatively the swine production: economically, socially and veterinary health. The main economy lost is focus in reproductive and productive field beside it is considering a zoonotic disease lethal for human been[1]. The control and prevention of it is based in a strategy that includes use of antibiotics, vaccines and zootechnical handle[2]. The outbreack of this disease in a production zone increase costs associated with swine products and derivates, because therapeutic control measures (chosen many times instead of immunization for avoid the costs of its) are apply to late due difficult diagnosis of the disease furthermore high antibiotics prices and finally the high concentrations of antibiotics in products for commercialization and microbe resistant issues. On the other hand, traditional prophylactic control, even more effective face some problems as limited protection against serovars no include in the vaccine and high costs of immunization in big swine population[3].This study evaluates the impact in swine production costs generated by immunization with effective recombinant vaccine candidates that include protector serovars antigens for swine apply via oral and intramuscular and compare costs of this experience with therapeutics treatments. Also assess production costs and immunizations of new vaccine formulations and compares with traditional vaccine.

Materials and Methods

The assay was conducted in four farms in a zone with reports of leptospirosis but before immunization no antibodies against *Leptospira* were detected. Animals were distributed randomly in four experimental groups: control group no immunized (A), animals immunized with traditional vaccine (B), animals immunized with novel recombinant vaccine via intramuscular (C) and animals immunized with novel recombinant vaccine via oral (D). For all groups were evaluated: weight, percent of abortion, amount of animals treated for leptospirosis, cost of immunization and production.

Results

The results obtained demonstrate that low production cost was related with groups immunized wit novel recombinant vaccine via oral and intramuscular without statistical differences between them, also the low immunization cost was liked to animals immunized via oral with recombinant vaccine and production costs of these novel vaccine are not different statistically and more effective than traditional vaccines. The major gain of weight was observed in groups C and D, with marked differences respect group A. In 98 % of all dead animals were detected leptospiras in organs culture. The group A was less productive than others due to high food consume and treatments with antibiotics and other drugs against leptospirosis. In all populations was observed that in non-immunized groups reproductive females and newborn were age groups more susceptible.

Conclusions and Discussion

Analysis of the results presented demonstrate that lower production cost were obtained with group C and D, and this last one group also showed the best economy parameters due to low immunization cost related with oral vaccines. Another important finding was differences between groups B and C, D respectively; evidence that in the zone circulates leptospira serovars no includes in traditional vaccine, demonstrating the importance of apply vaccines of new generations against leptospirosis for better economic results in swine production.

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Survey in claw lesions of sows in Korea

B Kim, JY Jung, SM Kim, BE Park, JH Jo, JH Han
College of Veterinary Medicine and Institute of Veterinary Science, Kangwon National University, Chuncheon, Gangwon, Republic of Korea, chess55@nate.com

Introduction

The lameness is the second reason that sows are removed from swine herds. According to a survey, 6~35% of sows are removed because of the lameness and by the report of the United States Department of Agriculture(USDA), the economic loss due to lameness in the US swine herds was over $24 million. In addition, the lameness is an index of animal welfare quality protocol in Europe.

Claw lesions is one of major cause of lameness in sows. As factors of claw lesions, there are bacterial infection, environment, nutritional statues, genetic factors and others; fractures, laminitis, osteomalacia, trauma and porcine stress syndrome(PSS). The surface of the floor and the physical properties can influence on the claw lesions and there is a report that there is a relationship between structure of the farm and incidene rate of the claw lesions.

This survey was performed to investigate prevalence of sow claw lesions in Korean swine herds according to crate, breed and parity.

Materials and Methods

A total number of 684 sows composed of 3 breeds (F1, YY, LL) from the 8 farms were used for investigating prevalence of sow claw lesions. The parity is devided into 1~2, 3~4, and 5~8. To find out the relationship between the claw lesions and crate, 415 sows were raised in plastic crate and 269 sows were raised in tribar (Table 1). The lesions of claw were classified into wall cracks of horizontal (CWH), wall cracks of vertical (CWV), length of toes (T) and length of dew claws(DC). Each lesion was scored as 0, 1, 2, 3 (Score 0; no lesions, Score 1; small, superficial cracks or lesions in epidermis, Score 2; serious lesion in the epidermis or deep lesions extended into the corium, Score 3; serious and deep cracks extended into corium or subcutis.) according to the severity of the lesion.

Results

Incidence rate of claw lesions in sows raised on punched plastic crate was higher than that of sows raised on tribar crate. Groups of hybrid and high parity showed high prevalence of claw lesions than groups of inbreed and low parity respectively (Fig 1).

Table1. Experimental design according to crate, breed, and parity

Farrowing crate	Punched plastic		Tribar		Total
Parity	F1	YY	YY	LL	
1~2	72	30	60	34	196
3~4	155	37	66	31	289
5~8	99	22	48	30	199
total	326	89	174	95	684

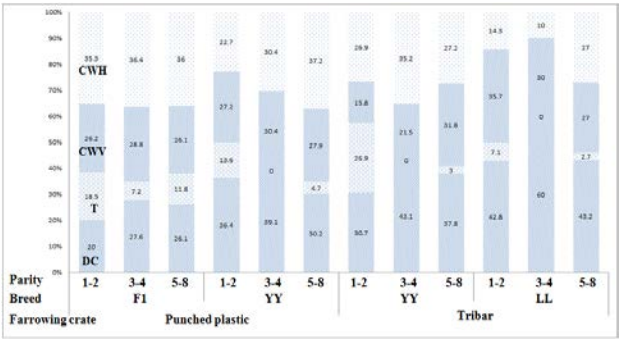

Figure 1. Relationship between claw lesions and crate, breed and parity (CWH, Wall cracks of horizontal; CWV, Wall cracks of vertical; T, Length of toes; DC, Length of dew claws.)

Conclusions

These results may be elementary data useful in establishment of preventive measures for swine herds to reduce economic loss resulted from lameness with craw lesions.

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Gonadotropin improves estrus in late-puberty replacement gilts in Thailand

A Roongsitthichai[1], P Tummaruk[2], N Am-in[2]
[1]*Department of Veterinary Clinical Science, Faculty of Veterinary Sciences, Mahasarakham University, Mahasarakham 44000, Thailand,* [2]*Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand,* Nutthee.a@chula.ac.th

Introduction

Puberty in gilts is regarded when they expressed first standing estrus and first ovulated (1). The replacement gilts should be in first estrus before 200 days of age (2). Anestrus can be delivered from several factors. One of the possible reasons is low or insufficient endogenous gonadotropin (3), contributing to late puberty in pigs. A number of studies have attempted to stimulate estrus in replacement gilts. A previous study reported that an injection of gonadotropin helps decrease days to estrus after weaning in first and second litters (3). Comprehensive studies on improving estrus in late-puberty gilts were meager. Consequently, the purpose of the current study was to examine the capability of gonadotropin in stimulating estrus in late-puberty gilts raised in Thailand.

Materials and Methods

In total, 136 gilts which did not express first observed estrus within 200 days of age and weight ≥120 kg were included. They were accommodated in an open housing system in eastern Thailand. Due to the administration of gonadotropin (Fertipig®, CEVA Animal Health, Thailand), they were classified into control (n=58) and treatment (n=78) groups. After the injection for 2 days, estrus detection by back pressure test, together with boar contact was conducted. Those expressed estrus signs were recorded and examined the follicle characteristics by ultrasonography (Aloka SSD-500V, Tokyo, Japan) in order to investigate dominant follicles. Nonetheless, those did not show estrus signs within 14 days after treatment would be removed to the slaughterhouse so as to scrutinize ovaries and uteri. Recovery rate of the replacement gilts and farrowing rate were compared between groups by chi-square and total born piglets, and piglets' birth weight were compared between groups by Student's t-test (SAS 9.3, SAS Institute, Cary NC).

Results

Reproductive data of the gilts with late puberty are displayed in Table 1. The late-puberty gilts treated with Fertipig® could express estrus signs higher than those in control group significantly (92.31 vs 25.86%, $P<0.05$) (Fig 1). In addition, the gilts in treatment group significantly possessed higher farrowing rate than those in control group (80.7 vs 53.3%, $P<0.05$) (Table 1).

Conclusions and Discussion

Gonadotropin was able to stimulate estrus in the gilts with delayed puberty. An abnormality at Hypothalamic-pituitary axis, producing low GnRH, was one of the problems causing late puberty in pigs (4). According to Fertipig® was composed of 400 IU eCG and 200 IU hCG, it could stimulate estrus in late-puberty gilts in Thailand.

Table 1. Reproductive data of the replacement gilts between treatment (Fertipig®) and control groups from 136 gilts

Parameters	Fertipig® (n=78)	Control (n=58)
Age (d)	209.9±5.4[a]	207.6±5.2[a]
Body weight (kg)	124.8±2.5[a]	125.0±2.8[a]
Backfat (mm)	17.3±1.8[a]	17.4±1.8[a]
Farrowing rate (%)	80.7[a]	53.3[b]
Total born (piglets)	10.1±1.5[a]	9.5±0.9[a]
Birth weight (kg)	1.2±0.2[a]	1.1±0.2[a]

(a, b) Superscripts demonstrate statistical significance ($P<0.05$)

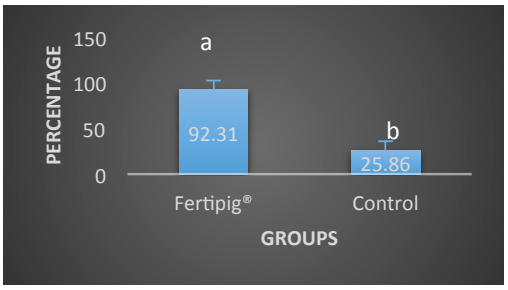

Figure 1. Recovery rate of the replacement gilts between treatment and control groups.
(a, b) Superscripts indicate statistical significance ($P<0.05$)

Acknowledgments

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High sterilization material MaSSC® for the reduction of the odor and microorganisms from pig houses

S Sato[1], R Uemura[1], Y Sasaki[2], M Sueyoshi[3], K Yamamoto[4], N Yakiyama[4], S Sakaguchi[4], H Yoshinaga[4]
[1]*Department of Veterinary Medicine,* [2]*Promotion of Tenure Track,* [3]*Center for Animal Disease Control, University of Miyazaki, Miyazaki 889-2192,* [4]*Technology Development Center, FUJICO CO., LTD., Kitakyushu 804-0054, Japan.* a0d802u@cc.miyazaki-u.ac.jp

Introduction

Odor emission from pig production is an increasing problem as pig houses tend to be larger and the production is increasingly intensified (1). In addition the neighbouring communities are growing closer to the farms in Japan.

The MaSSC® is a photocatalyst product with a high environmental purification function using the thermal spraying method. The photocatalyst generates pairs of electrons and holes that yield reactive oxygen species (ROS) such as hydroxyl radicals (·OH) and superoxide anions ($O2^-$) that act as biocides. This product has been used to reduce the number of bacteria in the restroom by antibacterial floor tiles, and to reduce the amount of ammonia concentration. This study was performed to investigate if the MaSSC® products can be a realistic technology for treating odors caused by livestock production.

Materials and Methods

Ten piglets were lodged in each unit with an initial age of 25 days. The experiments were conducted in two experimental pig houses at the University of Miyazaki in Japan (Fig. 1). One unit (5 piglets) was used as the control, whereas the another unit (5 piglets) was installed with MaSSC® products including a MaSSC solar reactor, zeolite board, air purification equipment MC-T and an inlet port unit. Counts of airborne bacteria and particulate matter (PM) gas concentrations were continuously monitored in the two units for 16 days. The number of airborne bacteria and PM in three size ranges (0.5, 1.0, and > 3.0 µm) were measured. An instantaneous Microbial Detection (Azbil BioVigilant, Inc, Tucson, Arizona) was used for airborne bacterial and PM sampling. Selected odorants were measured by Infrared Photoacoustic Detector (IPD, multi gas monitor type 1412, INNOVA, Copenhagen, Denmark). A wipe test on the floor was performed 6 times for measurement of the adherence bacterium. Gas chromatography was performed 3 times for measurement of short chain fatty acids (normal butyric acid, isovaleric acid).

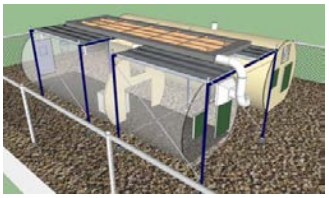

Figure 1. The experimental pig houses

Results

The average concentrations of NH_3, CH_3CHO and CH_3COOH in the control unit house were 8.80, 0.21 and 0.52 ppm, respectively, and in the experimental unit were 1.24, 0.04 and 0.14 ppm, respectively (Fig. 2). The average number of airborne bacteria and PM in the range of 0.5 µm in the control unit were 468 and 5844, respectively, and in the experimental unit were 163 and 2979, respectively. The average number of airborne bacteria and PM in the range of 1.0 µm in the control unit were 782 and 1121, respectively, and in the experimental unit were 70 and 156, respectively. The average of the amount of floor-adhesion bacteria (log_{10}CFU/ml) in the control unit was 3.95, and the experimental unit was 2.45. The average of the normal butyric acid in the control unit was 13.59 ppm, and the experimental unit was 4.02 ppm.

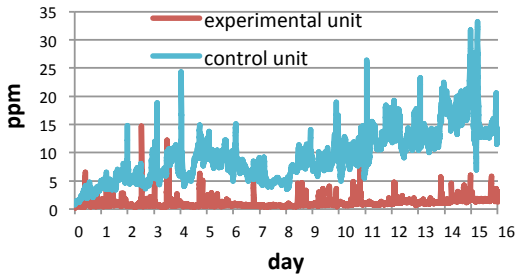

Figure 2. Concentration of NH_3 in the two units during the 16 days

Conclusions and Discussion

The gas concentration and the number of airborne bacteria and PM were reduced in the experimental unit house compared to the control unit.

In this study, we used a MaSSC device set (solar reactor, zeolite board, air purification equipment MC-T, inlet port unit). We consider which combinations of devices or which single device is the most effective.

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Factors associated with colostrum intake in neonatal piglets

M Nuntapaitoon, A Choonnasard, N Prayoonwiwat, P Wuttiwongtanakorn, S Vichitvanichpong, T Vetchapitak P Tummaruk
Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Pathumwan, Bangkok, 10330, Thailand, Padet.T@chula.ac.th

Introduction

Colostrum provides energy source and passive immunity to the neonatal piglets. Inadequate colostrum intake reduces body weight gain and increases pre-weaning mortality (1,2). The colostrum production depends on both the sow ability to produce colostrum and the ability of the piglet to stimulate the teat (3). The present study investigates factors associated with colostrum intake in neonatal piglets under field conditions in Thailand.

Materials and Methods

The present study was performed in a commercial swine herd in the eastern part of Thailand in January 2014. A total of 95 neonatal piglets were investigated. The sows were kept in a conventional open-house system. The sows were moved to the farrowing pens about one week before the expected farrowing date. Farrowing process and the piglet's birth weight were carefully determined. Farrowing intervention was performed if necessary. Birth order and the time elapsed between piglets delivering were monitored. Bodyweight of the piglets was measured at about 24 h after farrowing by using electronic balance **(**Universal Weight Enterprise Co. Ltd., New Taipei, Taiwan). The body weight gain of the piglet during the first 24 h period of life was calculated. An individual colostrum intake of the piglets was calculated (4): Colostrum intake (gram) = -217.4+(0.217*t) + (1,861,019*BW2/t)+BW*(54.8-1,861,019/t)*((0.9985-3.7*10^{-4}*tFS)+ (6.1*10^{-7}*tFS^2)); where BW=birthweight (kg), BW2=bodyweight at the second weighing (kg), t= time elapsed between the first and the second weighting (min), and tFS = the interval between birth and first sucking (min). The statistical analyses were carried out by SAS. Pearson's correlation was used to analyze the association between the colostrum intake and all possible related factors, i.e., farrowing interval, birth weight, body weight gain, birth order, total piglet born per litter (TB) and piglets born alive per litter (BA). $P<0.05$ was regarded to be statistically significant.

Results

Mean colostrum intake of the piglets was 245±167 grams (range 0-595). Factors associated with colostrum intake are presented in Table 1. The major factors associated with colostrum intake in neonatal piglets included parity number, birth order, time elapse between piglets, birth order, body weight at 24 h after birth, TB, BA and body weight gain ($P<0.05$). Birth weight and the total farrowing duration were not associated with colostrum intake ($P>0.05$).

Table 1. Factors associated with colostrum intake in neonatal piglets (n=95)

Variables	Correlation coefficient (r)	*P* value
Parity number	-0.551	<0.001
Birth order	-0.296	0.004
Total born	-0.347	<0.001
Born alive	-0.310	0.002
Time elapsed between piglets delivering	0.381	<0.001
Body weight gain	0.988	<0.001
Body weight at 24 h	0.503	<0.001
Birth weight	0.132	0.214
Farrowing interval	0.041	0.691

Conclusions and Discussion

The present study demonstrated that colostrum intake in neonatal piglets positively correlated with time elapsed between piglets delivering and bodyweight at 24 h of life, but negatively correlated with sow's parity number, birth order, TB and BA. This is in accordance with earlier studies (2,3). In addition, the present study found that piglets born earlier had a higher colostrum intake than those born later. Likewise, piglets from the larger litter had a lower colostrum intake than those from the smaller litter. The reason might be due to that piglets born later as well as those in the large litter have had a higher competition to obtain the teat than those in a smaller litter or those born earlier. Time between each piglets delivering also positively associated with colostrum intake. This might be due to that piglet care might be better when the time was extended. This reflected the importance of farrowing supervision under field conditions. In could be concluded that, to enhance colostrum intake in neonatal piglets, special care of the new born piglet be focused on those born with old sows, later-born piglets, and those in the large litter.

Acknowledgments

National Research Council of Thailand 2014

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Effect of induced parturition on the incidences of umbilical rupture, blood oxygen saturation, blood glucose concentration and colostrum intake in neonatal piglets

P Tummaruk, M Nuntapaitoon, A Choonnasard, N Prayoonwiwat, P Wuttiwongtanakorn, S Vichitvanichpong, T Vetchapitak

[1]*Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Pathumwan, Bangkok, 10330, Thailand,* Padet.T@chula.ac.th

Introduction

The induction of parturition in pig is generally conducted by prostaglandins F2α administration. The practical purpose of induce parturition is to increase the synchrony of farrowing facilitating farrowing supervision. Nevertheless, the induction of parturition sometime increases the risk of piglet mortality (1). The present study aims to investigate the effect of induced parturition on the incidences of umbilical rupture, blood oxygen saturation, blood glucose concentration and colostrum intake in newborn piglets under field conditions.

Materials and Methods

The present study was performed in a commercial swine herd in Thailand in January 2014. A total of 95 piglets were investigated. The sows were kept in individual crate in a conventional open-house system. The sows were moved to the farrowing pens about one week before expected parturition. The sows were randomly assigned into 2 groups: control and induced parturition. The induction of parturition was carried out by administration of 1 ml (87.5 µg) of cloprostenol intravulvosubmucosa (Planate®, MSD) on the due date of parturition. Farrowing process was carefully supervised. Farrowing intervention was performed if necessary. Birth weight, farrowing interval, the occurrence of umbilical rupture, blood oxygen saturation, blood glucose concentration and body weight of the piglets at 24 h after birth were determined. An individual colostrum intake of the piglets was calculated according to a previous study (2). The statistical analyses were carried out by SAS. Student's *t* test were used to analyze the effect of induced parturition on blood oxygen saturation, blood glucose concentration, farrowing interval, birth weight, total number of piglet born per litter (TB) and number of piglets born alive per litter (BA) and colostrum intake. Chi-squared analysis was used to analyze the effect of induced parturition on the evidence of farrowing intervention and umbilical rupture. $P<0.05$ was regarded to be statistically significant.

Results

Reproductive parameters and colostrum intake in newborn piglets after induced parturition compared with normal parturition are shown in Table 1. The incidence of farrowing intervention and umbilical rupture was higher in the piglets born from sows induced parturition ($P<0.05$). Moreover, piglets born from sows induced parturition had a lower blood glucose concentration both at birth and at 24 after birth. Nevertheless, the induction of parturition did not influence the colostrum intake of newborn piglets (Table 1).

Table 1. Reproductive parameters and colostrum intake in newborn piglets (mean ± SEM) after induced parturition (treatment) compared with normal parturition

Variables	Control (n=37)	Treatment (n=58)
Parity number	4.2±0.3[a]	3.7±0.1[a]
Total born	10.8±0.4[a]	15.7±0.5[b]
Born alive	10.1±0.4[a]	14.7±0.4[b]
Farrowing interval (h)	162±11.0[a]	211±10.9[b]
Birth weight (gram)	1.42±0.04[a]	1.61±0.04[b]
Farrowing intervention (%)	3/37 (8.1%)[a]	20/58 (34.5%)[b]
Umbilical rupture (%)	1/35 (2.9%)[a]	9/50 (18.0%)[b]
Blood oxygen saturation (%)	92.5±1.5[a]	89.3±1.6[a]
Blood glucose at birth (mg/dl)	53.9±2.0[a]	45.7±1.6[b]
Blood glucose at 24 h (mg/dl)	112.7±6.9[a]	95.7±3.8[b]
Colostrum intake (gram)	233±28[a]	252±21[a]

[a,b] different superscript within row differ significantly

Conclusions and Discussion

Induced parturition is a management tool that can reduce piglet mortality (1). In the present study, the induction of parturition increased the incidence of umbilical rupture and decrease blood glucose concentrations. This indicates the importance of farrowing supervision in the sows induced parturition. It could be concluded that induction of parturition increase the incidence of farrowing intervention and reduced blood glucose concentration but did not reduce colostrum intake of newborn piglets. Farrowing supervision was recommended in sow induced parturition.

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National Research Council of Thailand 2014

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Economics effects of PRRSV in breeding herds and growing-pig herds

J Amador, MA Trujillo, JI Sánchez, M Robles, J Garcia, J Nava
Department of Medicine and Husbandry of Pigs. Faculty of Veterinary Medicine.
National Autonomous University of México, mvzjovaniac@unam.mx

Introduction

In Mexico, it has been estimated the economic impact of an outbreak of PRRSV in farms of full cycle.

At the International Symposium of PRRSV: yesterday, today and tomorrow, CU, Mexico, 2012; Alberto Herrera believes that this disease in Mexico could be costing between US $80 and 120 million per year for the pork industry, provided that the economic impact of an acute outbreak of PRRS costs between US $208 and US $292 per sow per year, in the production line and when there are persistent infections it ranges from US $5 per pig for slaughter up to US $12.50 in acute cases. In the same forum Leonardo Perez, estimates that economic impact start at US $ 9.72 until US $ 21.08 per pig and US $ 185.4 to US $ 421.6 per female per year.

The total cost of productivity losses due to PRRSV in the US national breeding and growing-pig herd was estimated at US $664 million annually.(1)

Materials and Methods

The study was based on information from 650 sows in production of farms of full cycle in the center of México with continuous flow production, production and economic records of the year 2009 (first period) and 2010 (second period), advice on feed production, genetics specialized in pig production, use of artificial insemination and weaning between 19 and 23 days and advice in pig production.

Treatments for comparison economical parameters of breedind área and the production line:

- Group I: Animals free of PRRSV in the first period with subsequent infection
- Group II: Animals free of PRRSV in the first period.
- Group III: Animals free of PRRSV in the second period.
- Group IV: Animals with PRRSV in the second evaluation period.

Reproductive, productive and economic parameters were compared with analysis of variance to check statistically significant differences between means. To find out which groups are statistically different, an additional Tukey-kramer test was performed.

Results

The values obtained in the cost per kilogram and costs per sow per year are shown in Table 1.

Table 1. Values of cost per kilogram and cost per sow per year.

Variable	Group I	Group II	Group III	Group IV
Cost/ kilogram	US $1.62 ab	US $1.55 ab	US $1.36 a	US $1.85 b
Cost/ sow/ year	US $249.62 a	US $179.12 b	US $185.04 b	US $244.54 a

The averages of the groups sharing literal, show no significant statistical difference($p<0.05$).

Conclusions and Discussion

The results of the production costs presented in this study, only coincide with the upper range reported by Neumann, the analysis of this indicator increased between $ 242.97 and $ 523.32 pesos per pig of 89 kilograms. The cost per sow in the group IV showed an increase of $ 713.98 pesos compared with group III, is the same range reported by Neumann et al (2005) and Nieuwenhuis et al. (2012) however smaller than the rest of the reports made in the nineties by various authors and Herrera y Pérez in 2012. The most contrasting differences were found in the percentage of fertility, where significant difference was obtained compared with the control groups and group of sows without the PRRSV, 73.21%, 82.87% and 83.58% respectively. The pre-weaning mortality rate was increased in the group of infected sows compared to the control, with 6.54%. The average feed conversion was lower in the control animals compared to those infected animals with 3.37% and 4.81, respectively.

The group of infected sows with PRRSV showed increased production cost compared to the control groups and the group of sows without the PRRSV. Finally we can say that we need to work hard in this field of work because there are very few publications Mexico and in the World.

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Production costs and profitability of metropolitan small-scale swine farms in Mexico City

[1]A Mercadillo S, [1]N Losada E, [1]R Martínez G, [1]M Haro T.
[1]*Departamento de Medicina y Zootecnia de Cerdos. Facultad de Medicina Veterinaria y Zootecnia, UNAM.*
alemersie@hotmail.com

Introduction

Small-scale swine production in Mexico embodies between 20 and 30% of the gross national product with an average inventory of 40%.[1] Backyard production models lack a financial production control due to the inherent difficulties of this system which in some instances combines the family housing, the women, elders or even the owner's labor, with the animal inhabitance. These are not considered in the labor market and therefore there is no notion of their input cost. Likewise, the use of byproducts from the food industry and the house waste do not have a definite market value. Under these circumstances the economic analysis to evaluate the small-scale or backyard farms productivity system is quite limited or even absent. The objective of the present study was to evaluate economic returns of small-scale farms and determine the impact of different input costs. [2, 3]

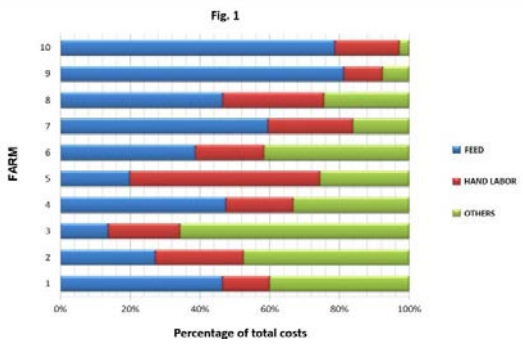
Materials and Methods

Ten swine production units were chosen from several urban sites of Mexico City and suburban areas of the State of Mexico. The production units included volunteer, independent producers, and producers affiliated to regional livestock developing programs. The farms had a person in charge of the unities management and included an animal range of 2 to 300 animals from breeding, growing/finishing or integrated cycle units. A production unit report was requested for a minimum of 12 months. All production units were characterized in a cross-sectional study with a static survey.[4]The economic aspects were examined with a quantitative analysis of the production records. Likewise, an accounting analysis was performed for the fixed and variable production costs.

Results

Eighty percent of the farms were found profitable with an average of 30.7%, and a range of 2 to 88%. Two farms had a loss of 12 and 17% respectively. Feed was the highest production cost with an average of 39.19% and a range of 8.9 to 81.95% (Fig. 1). In two of the growing-finishing farms the purchase of piglets was the highest production cost with 32.98 and 87.05%. In 70% of the farms, hand labor was the second highest cost with an average of 18.2% and a range of 8.97 to 29.04%. Only 2 of the farms reported the costs of veterinarian fees representing the 3.95 and 0.02% of the total costs.

Fig. 1



Conclusions and Discussion

It can be acknowledged from this study that most of the farms studied are profitable. Even though feed represents the highest cost production, such percentage varies depending on the feed ingredients used. Hand labor cost is also very variable. Income in piglet farms is very low according to the investment. Therefore, breeding and growing their own pigs is recommended to small-scale swine producers.

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Preweaning growth rate of piglets and its relation to body surface temperature measured by infrared thermography

Y Sasaki[1], K Furusho[2], R Uemura[2], M Sueyoshi[2,3]
[1]*Promotion of Tenure Track,* [2]*Department of Veterinary Medicine,* [3]*Center for Animal Disease Control, University of Miyazaki, Miyazaki 889-2191, Japan,* ysksk@cc.miyazaki-u.ac.jp

Introduction

Infrared thermography is a non-invasive technique for recording body temperature without touching the animal (1). In field conditions, it is difficult to restrain and handle piglets. Thus, a body surface temperature measured by infrared thermography would be useful for detection of growth retardation or disease problems. However, the reliability of infrared thermography for monitoring body weight and growth rate of piglets during lactation, and the early prediction of piglets with growth retardation, have not been assessed. Thus, the aim of the present study was to quantify the body surface temperature of suckling piglets in the eye, base of ear, back and anus, and to determine the association between body surface temperature and body weight, growth rate and age of the piglets during lactation.

Materials and Methods

Six sows with 11–13 suckling piglets were randomly selected at day 4 after farrowing, to give a total of 72 piglets. On the farm, cross-fostering of piglets from large litters to smaller litters was performed within the first 2 days after farrowing to equalize the number of suckling piglets. Experiments were performed from day 4 of lactation to day 24. Piglets were identified by an ear tag, and were weighed every second day from day 4. Growth rate was measured by weight gain per 2 days as calculated by subtracting body weight of piglets at the day from the weight 2 days prior.

A handheld infrared camera (Thermo Shot F30, NEC Avio Infrared Technologies Co., Ltd., Tokyo, Japan) was used to collect thermal images from the eye, base of ear, back and anus from each piglet every second day from day 4. Each measurement was performed at 15:00 to minimize circadian variation. To avoid confounding effects of the heat lamp, piglets were kept outside of the heating area for 5 minutes before observation. An observer held piglets during the measurement. The camera was used in a fixed position and all images were captured at a distance of approximately 1.0 m from the subject. Termal images were analyzed with InfReC Analyzer NS9500 Lite (NEC Avio Infrared Technologies Co., Ltd.) for determination of body surface temperature (°C) in each region. Statistical analyses were performed using SAS version 9.3 (SAS Inst. Inc., Cary, NC, USA).

Results

During the observation, the average body weight of 72 piglets from day 4–24 after birth increased from 2.0±0.05 to 5.3±0.14 kg (P<0.05). The average growth rate was 0.32±0.01 kg. The overall mean body surface temperature in the eye, base of ear, back and anus were 35.5±0.04, 39.5±0.03, 37.9±0.03 and 38.6±0.03°C, respectively. Body surface temperature was associated with age of piglet and growth rate (P<0.05), but not with body weight. Piglets at day 14 had the highest body surface temperature in all regions (P<0.05). Piglets at day 24 had a lower body surface temperature than at days 4–18 (P<0.05). There was also a significant interaction between the age of piglets and growth rate for the body surface temperature in all regions (P<0.05). Body surface temperature increased as the growth rate increased only at day 12 and day 14 after birth (P<0.05).

Conclusions and Discussion

We quantified body surface temperature of suckling piglets daily by infrared thermography. Piglets with low body surface temperature had a lower growth rate at days 12 and 14. This relationship during a limited period of lactation may be caused by the vaccinations performed at day 10–14, as external stress can lower activity and decrease feed intake and daily weight gain (2). These findings indicate that body surface temperature can be used as an indicator of piglet health. Extra attention should be given to piglets that exhibit decreased or maintained body weight after vaccination.

In contrast to the relationship between body surface temperature and growth rate, the body weight was not related to body surface temperature. This finding indicates that it is difficult to find litter variation of piglet weight using infrared thermography. Overall, these data suggest that body surface temperature may reflect current health status, but not be related to the body weight.

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Use of heart girth to measure live body weight in pigs

F Voisin, E Pagot, A Trotel, M Dia, B Delaporte
Zoopole Developpement, CTPA, Ploufragan, France
florian.voisin@zoopole.asso.fr

Introduction

Animal weight prediction using body measurements is used in various species to compensate for the absence of a weighing scale. However, precise weight evaluation in pigs might be useful for farm management. Thoracic circumference is more precise than visual estimation1,2,3, and it is also simpler than the use of multiple body measurements1,3,4. The aim of this study is to provide a recent reference for weight measure in fattening pigs using heart girth.

Materials and Methods

In 5 French farms, 557 cross-bred pigs were individually identified and weighed between entry into fattening and the end of the fattening period. Their heart girth (HG) was measured, using a tape placed behind the elbow tips (Fig. 1). The relation between body weight (BW) and girth measure was then addressed using standard least squares regression, with calculation of the coefficient of determination (R^2).

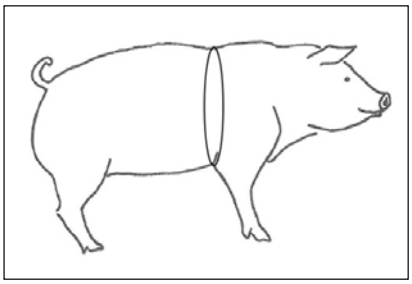

Figure 1: Heart girth measure

Results

Individual data are summarized in table 1. On the whole sample, the non linear regression between BW and HG results in Equation 1(R^2=0,958). The gender distinction leads to Equations 2 (R^2=0,961) and 3 (R^2=0,955).

Table 1. Heart girth (cm) and Body weight (kg) by Gender

	Total		Castrate		Female	
N	557		271		286	
Data	BW	HG	BW	HG	BW	HG
Mean	82.3	91.8	82.2	91.5	82.4	92.1
Min	18.4	55	19.2	55	18.4	57
Max	131.4	112	131.4	112	122.2	112

Equation 1 BW = 0,008 HG^2 + 0,577 HG – 40,474
Equation 2 BW (Castrates (C)) = 0,007 HG^2 +0,712 HG – 45,613
Equation 3 BW (Females (F)) = 0,009 HG^2 + 0,506 HG – 37,940

The equations were used to generate table 2, predicting body weight from girth measure.

Table 2. HG-BW abacus

HG	BW			HG	BW		
	C	F	C or F		C	F	C or F
55	15	17	15	101	98	105	99
60	22	25	23	102	100	107	102
65	30	33	31	103	102	110	104
70	39	42	39	104	104	112	106
75	47	51	48	105	106	114	108
80	56	60	57	106	109	117	111
85	65	70	66	107	111	119	113
90	75	81	76	108	113	122	115
95	85	91	87	109	115	124	117
100	96	103	97	110	117	127	120

Conclusions and Discussion

The amount of data is important. All 3 equations lead to a 96% prediction of the body weight. The R^2 obtained are in line with previous work[4]. This confirms the reliability of girth measurement as a costless and more reliable tool to predict body weight than visual estimation which depends upon the observer.
The table above can be used in different ways, among which :
- sorting the pigs at slaughter weight for income maximization
- feeding management for cost control
- medication dosing for reduction of the risk of resistance developpement

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P.225

Effect of Improvac® vaccine on carcass cutting yield in male pigs under field conditions in Thailand

K Sangvixienkit[1], K Lertphitak[1], J Thongkaew[1]
[1]*Zoetis (Thailand) Limited,* kanyarat.lertphitak@zoetis.com

Introduction

In Thailand carcass quality is an important factor for selling and buying, there are 5 parts of carcass that are easier to sell and result in a higher income to the seller. These cuts include the loin, fillet, ham, foreleg and belly. There is a strong trend globally for pork consumers to buy and consume more lean meat for health. Therefore pig producers must continually strive to improve their pork carcass quality. Non-castrated boars typically have less fat and a greater proportion of lean muscle tissue than physically castrated pigs (2). Improvac (Zoetis, Madison, NJ) is a vaccine used for the immunological castration of male pigs. The 2nd dose of Improvac is timed to occur in the late finishing phase, and allows producers to maximize the production and carcass benefits of raising entire boars, and yet provide sufficient time for any boar taint compounds present in the body to be depleted. Several authors have recorded a reduction in fat and/or an increase in muscle in Improvac vaccinated pigs compared to physically castrated pigs (4, 5). The purpose of this trial was to compare the carcass cutting yield between Improvac vaccinated pigs and surgically castrated pigs under field conditions in Thailand.

Materiasl and Methods

The pigs for this trial were selected at random from another on-farm growth performance trial. Ten pigs were selected at random, 5 castrates and 5 Improvac vaccinated males. The Improvac vaccinated pigs were given 2 mL by subcutaneous injection in the neck at 15 and 19 weeks of age as per label instructions. The pigs in both treatments were fed the same diets and kept under the same conditions. At 24 weeks of age the pigs were slaughtered and cut by the same people and same method in a commercial slaughterhouse. One side of each carcass was dissected into the 5 key parts; loin, fillet, ham, foreleg and belly. The skin, bone and fat were removed and the lean meat weighed. The average carcass weight was not significantly different between the 2 groups and ranged between 102 to 106 kg across the groups. Difference between treatments was assessed with the Student's t-test.

Results

The results of the carcass dissection are shown in Table 1. For 4 of the 5 primal cuts the average weight was higher significantly higher ($P<0.05$) in the Improvac treated pigs than in the castrated pigs. Combined overall there was an additional 1.94 kg of lean meat in the ½ carcass of the Improvac pigs compared to the surgical castrates ($p<0.05$).

Table 1. Average primal cut weights (kg) for the 5 major primal cuts of value in Thailand. Skin, bone and fat removed.

	Improvac	Castrates	Diff	P
Loin	6.60	6.68	-0.08	0.365
Fillet	0.48	0.41	+0.07	<0.001
Ham	8.24	7.33	+0.91	<0.001
Picnic shoulder	4.16	3.59	+0.57	<0.001
Belly	6.16	5.68	+0.48	0.026
Total	25.63	23.69	+1.94	0.013

Conclusions and Discussion

The results of this trial confirm the findings from several other countries, namely that the carcasses from Improvac vaccinated pigs have a significantly higher cutting yield than carcasses from surgical castrates (1, 4, 5). The full carcass from Improvac vaccinated pigs contained almost 4 kg of extra lean meat when compare with castrate carcasses. The increased lean meat content in the Improvac pigs is also reflected in a higher carcass grade applied at slaughter (4). The results demonstrate that Improvac is a choice that is able to improve carcass quality and profitability.

Acknowledgement

Thank you to the farm owner and managers of Donsala farm, who worked and shared their data with us.

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Design of a genetic improvement program "on demand": Covering the needs of every costumer

A Muñoz[1, 2], G Ramis[1], MD Garrido[3], JM Herrero-Medrano[4], G Usero[2], J Corchero[5], JJ Quereda[1], L Calvo-Adiego[6], AI Rodríguez[6], FJ Pallarés[7], MB Linares[3], E Hanenberg[4]

[1]Dep. Producción Animal. Universidad de Murcia. Spain, [2] TOPIGS Ibérica, Spain, [3] Dep Tecnología de los Alimentos. Universidad de Murcia, Spain. [4] TOPIGS Research center IPG B.V., The Netherlands, [5]TOPIGS International, The Netherlands, [6]Dep. I+D+i Incarlopsa, Spain, [7]Dep. Anatomía y Anatomía Patológica Comparadas. Universidad de Murcia, Spain, antmunoz@um.es´

Introduction

The genetic improvement has been deeply focused on reproductive and productive performances over the last 50 years. However, the consumers have increased their level of demand regarding meat quality. So, more and more is necessary to develop genetic improvement programs "*on demand*" of the producer, concerned to cover the consumers requirements. TOPIGS has developed a program on demand focused on meat quality together with zootechnical performances in a Duroc line.

Materials and Methods

The program was based on 1) accurate data collection of premortem parameters regarding finishing performances, and meat performances such as intramuscular fat by ALOKA, backfat thickness and loin deep. Postmortem lean content by autoFOM was assessed and a wide range of analytical data were recorded regarding fat content, texture, marbling, color, etc. On cured ham samples were analyzed traits such as color, texture, odor, juiciness, etc 2) Genetic analysis to estimate the heritability and the phenotypic and genetic correlations by quantitative approaches, 3) Meat quality analysis, including fresh meat (loin and ham) and cured ham 4) Sensorial analysis of the cured ham to estimate genetic parameters and 4) genome-wide association study (GWAS) using 60K SNPs array to calculate genetic parameters and to establish relation between fresh and cured meat quality and genetic.

The meat quality analyses were performed in the Dep. of Food Technology at the University of Murcia. The Illumina's 60K SNPs chips were analyzed at GeneSeek (USA) after DNA isolation from each animal. The relation between SNPs markers and productive traits was establishing using a Bayesian variable selection model. Calculations for genetic parameters estimation were done using a classical approach by means of the software package ASREML.

Results

Finally, productive data from 1,590 slaughtered animals included in 30 different slaughtered batches were collected. Carcass classification was obtained from 1,292, meat quality analyses were performed on 249 samples and 60K SNPs data were obtained for 244 animals.

As regards heritability estimation, the use of information from 60K SNPs for this purpose showed better results, in terms of standard deviation decrease, than the classical quantitative approach, even using a small population size.

The sensorial study and the analytical data showed a good correlation for most of traits related to appearance, taste, texture and acceptance.

The GWAS allow identifying eight QTL regions (explaining >2% of σ_g^2) for traits such as Marbling, Ultrasound back fat, Ultrasound Intramuscular fat, Intramuscular fat, and daily gain during test. Talking about cured, seven QTL regions for Total acceptance, Intramuscular fat, Mellowness and Hardness for cured ham traits were identified.

Conclusions and Discussion

The use of information in vivo related to Intramuscular fat obtained by ALOKA would allow to predict the real intramuscular fat content in carcasses and the heritability of the trait will allow to implement this technic in improvement programs. On the opposite the information of autoFOM post mortem did not supply additional useful information. The GWAS has given a lot of information related to cured ham traits that will help in marked assisted selection (MAS) for lines focused on ham production. The correlation among sensorial traits and analytic data has been medium-high. This information will be used to improve the product on the basis of consumer's acceptance. The use of sensorial panels, together with molecular analysis is a key point in the design of genetic improvement programs *on demand*.

Acknowledgments

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Opportunities to improve transport losses: Efficacy of trailer bedding and boarding levels

JJ McGlone[1], AK Johnson[2]
[1]*Laboratory of Animal Behavior, Physiology and Welfare, Texas Tech University, Lubbock, TX,* [2]*Department of Animal Sciences, Iowa State University, Ames, IA,* john.mcglone@ttu.edu (www.labpw.ttu.edu)

Introduction

Over 100 million pigs go to market in the USA yearly. Transport losses are most commonly in the form of dead on arrival (DOA), and non-ambulatory (NA) pigs. Based on a sample of 2.7 million commercial pigs, the rate of DOA in the USA was 0.2% and the rate of NA pigs was 0.22 % (1). Total transport losses (D&D) which is the summation of DOA and NA exceed 0.22% year-round in the USA. One factor that has been incompletely evaluated as a cause of transport losses is the configuration of trailers pulled by semi tractors (trucks).

The objective of this paper is to summarize a series of studies that were conducted over all seasons over the past three years to evaluate trailer bedding and boarding levels.

Materials and Methods

Typical pig trailers in this study were trailers that measured about 16 m long by 2.6 m wide with two decks. Decks were in a "pot belly" configuration (the compartment between the wheels was lower in elevation than the compartments over the wheels).

In Study 1, three data sets were generated when trailers had varying amounts of bedding (1 to 12 bales of bedding; each bale 0.3 m^3 of wood shavings) in each season applied to them. In the winter, we examined 6 or 12 bales/trailer while trailers. In the mild season (spring and fall) we evaluated 3, 6 and 12 bales/trailer and in the summer 3, 5, 7, and 9 bales were evaluated. Boarding levels followed the Transport Quality Assurance Program guidelines (2). In Study 2, bedding was held constant by covariate (range = 1 to 10 bales/trailer) while the boarding levels were varied over a range of seasons and air temperatures. In both studies, investigators collected data on 1,005 trailers (the experimental unit) containing 158,688 pigs.

Results

Transport losses did not change in the winter when either 6 or 12 bales were applied per trailer (Table 1). In the Spring/Fall and Summer, transport losses did not vary significantly with bedding ranging from 3 to 12 bales/trailer (Table 1). However, we observed a tendency for more transport losses in the summer when heavier (7 or more bales) were used per trailer.

When bedding was held constant by covariate, the boarding levels did not impact transport losses during mild air temperatures (5.1 to over 15 C; Table 2). However, during cold weather (< 5 C), transport losses increased when less than 30% boarding was used. This loss was primarily in DOA pigs (60% of D&D) in cold weather.

Conclusions and Discussion

Overusing bedding with no associated benefit has an economic cost. During all seasons, pigs with less bedding had similar transport losses as did pigs with more bedding. Trailers are typically fully open in warm weather (0 % boarding) and nearly fully closed in very cold weather. Changing the boarding levels from 25 to 75% closed had little impact on pig transport losses from 5.1 to 15 C. When air temperature was below 5 oC, having less than 30% boarding caused an increase in transport losses and should be avoided.

Data from this study can serve as a basis to make objective decisions about bedding and boarding levels used on trailers to improve pig welfare and economics.

Table 1. Rates of transport losses (D&D) with varying bedding (Study 1). N = 710 trailers with 112,078 pigs.

Season	No. pigs	Bales/trailer			
		3	5-6	7-12[4]	*P*-value
Winter[1]	28,503	--	0.11	0.18	NS
Spring/Fall[2]	44,900	0.20	0.22	0.09	NS
Summer[3]	38,675	0.17	0.42	0.50	0.07

[1] 7.8 C average air temperature
[2] 12.1 C average air temperature
[3] 22.4 C average air temperature
[4] 6 and 12 bales evaluated in Winter; 3, 6 and 12 bales evaluated in Spring/Fall and 3, 5, 7, and 9 bales were evaluated in Summer.

Table 2. Rates of total transport losses (D&D) and trailer boarding (% closed). N = 295 trailers and 46,610 pigs. Study 2.

Boarding	Temperature Bins (oC)			
	< 5	5.1-10	10.1-15	< 15
< 30%	0.94	0.56	0.99	0.30
31-60%	0.25	0.47	0.66	0.45
> 60%	021	0.32	0.38	0.30
P-value	< 0.05	NS	NS	NS

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An analysis of piglet birth weight in relation to litter size from piglets born from sows that were housed in group gestation with Electronic Sow Feeding Stations (ESFS)

R Galofre[1], R Segundo[1], J Sanmartin[1], C Martinez[2], X Miranda[4], P Escribano[1]
[1]*Veterinarian practitioner, MSc.* [2]*Veterinarian practitioneer,*[4]*Agronomic Engineer,*
Optimal Pork Production Pig Consultant Group. Lerida, Spain. r.segundo@oppgroup.com

Introduction

There is some published evidence that suggests that litters from group gestation sows yield heavier piglets at birth than sows that gestated in crates[1]. However, documentation is scarce, regarding statistical aspects of these litters.

Materials and Methods

At Albesa-Ramadera 3,200 sow, Site 1 farm, in Catalonia, Spain, litter weight of piglets born alive (PBA) was recorded at birth and correlated to litter size, between January 2011 and August 2013.

This farm is a commercial integration that utilizes Selección Batallé Genetics in their sow and boar line.

The farm has large group gestation (160 sows per group) and utilizes Electronic Sow Feeding Stations (ESFS). (Compident 7®,Schauer Agrotronic GmbH)

To reduce variation, and to adapt to the available parities present on the farm at that moment of the study, only second parity litters were recorded in this study. Piglets. Born dead, were not weighed with the litter or considered in the analysis.

Litters from gestations under 110 days and litters with less than 7 piglets or more than 15 were also excluded from this study, for considering they were too few to be statistically valid. All reasons for exclusion summarized less than 13%.

A total of 947 litters with 13.152 piglets were included in the analysis.

Litters were grouped by average number of piglets born alive, and the average weight of each group recorded.

A regression analysis was applied to evaluate the correlation and data consistency between litter sizes.

All data was recorded electronically by use of PDA´s and introduced into the Farm´s Mother® software platform.

Results

N° of Farrowing recorded	947
Total piglets born	13152
Total piglets born alive	11.926
N° of piglets born dead	1226
Av, Total born/sow	13,88
Av, Total born alive /sow	12,59
Av. Total born dead/sow	1,29
Av.Litter weight born alive	20,39
Av. Piglet weight	1,62

Piglets Born Alive	Average Weight (kg)
7	1,99
8	1,81
9	1,78
10	1,69
11	1,71
12	1,67
13	1,58
14	1,58
15	1,47

Conclusions

Although it was not possible to establish an on farm comparison of piglets birth weights born form conventional systems, litter weight from group gestating sows on ESFS, throughout the whole range of litter sizes was considered to be, very good.

The regression analysis shows a linear trend. For every extra piglet born over 7 piglets, the average weight of the piglets decreases 50 gr.

Discussion

The possibility to follow with high accuracy the ideal feed curve for every individual sow throughout gestation, as well as, allowing the sows to exercise during gestation, and a lower stress level associated to large group gestation may contribute to higher litter weights.

Although a "genetic effect" on the piglet weight, could not be discarded in this study, further research is recommended to fully understand the relative contribution of exercise, precision nutrition, stress and genetics on the litter weight at birth.

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The use of bedding in ramp to reduce slipping and falling while loading weaned pigs

A Garcia[1], A Sapkota[2], J McGlone[1]
[1]*Laboratory of Animal Behavior, Physiology and Welfare, Texas Tech University, Lubbock, TX,*
[2]*Department of Animal and Food Sciences,* arlene.garcia@ttu.edu

Introduction

The use of non-slip surfaces during loading and unloading of pigs play an important role in animal welfare and economics of the pork industry. Few studies exist on the effects of loading and unloading weaning pigs for transportation. Currently, the guidelines (Trucker Quality Assurance, TQA, 3) available only suggest the use of ramps below 20° to load and unload pigs. The objective of this study was to investigate the effects on welfare of weaned pigs being loaded or unloaded over various ramp slopes, bedding material, and moisture levels across two seasons.

Materials and Methods

Three ramp angles (0, 10 or, 20 degrees), 5 bedding materials (none, sand, feed, wood shavings or wheat straw hay), and 2 moistures (dry or wet bedding; >50% moisture) over 2 seasons (>23.9°C summer, <23.9°C winter) were assessed for slips/falls/vocalizations on weaning barrows and gilts for a total of 60 treatments. Pigs were put in units of 20 pigs per group. Five, 20-pig replications of weaned pigs (5 replications X 20 pigs/replicate = 100 pigs X 60 treatments = 6,000 weaned pigs) were conducted. The ramp was a metallic solid-sided chute, 0.8 m wide with cleats 0.3 m apart. "Score" was calculated by the sum of slips, falls, and vocalizations.

Results

With the exception of using feed as a bedding, all beddings provided some protection against slips, falls, and vocalizations (P= 0.0001); score levels were lower for bedding and conditions that provided the most benefit. Providing bedding reduced scores regardless of whether the bedding was dry or wet (Fig. 1). Scores increased as the slope increased (Fig. 2). The total time it took to load and unload the weaning pigs at a 20 degree slope increased as the score increased. Total time was lowest for hay, compared to all other bedding (Table 1).

Conclusions and Discussion

Provision of bedding, other than feed, at slopes greater than zero, decreased slips, falls and vocalizations. As the ramp slopes increased above 0 degrees slips, falls, and vocalizations increases significantly, especially if no bedding was provided. Feed was not acceptable bedding, but sand, sawdust and hay prevented slips, falls and vocalizations even at the 20 degree slope.

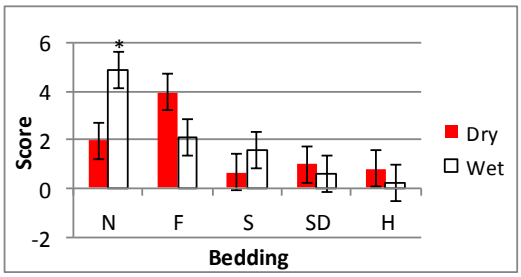

Figure 1. Score of slips/falls/vocalizations with wet or dry bedding. Dry bedding is represented by solid bars and wet bedding with open bars. * differed, P < 0.05.

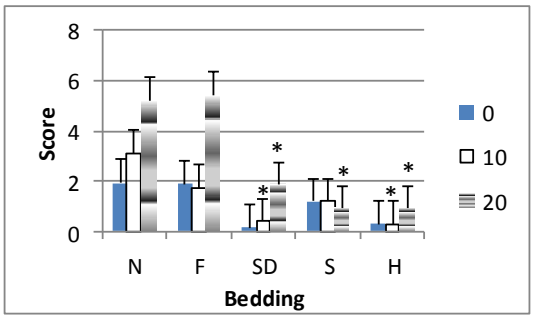

Figure 2. Score of slips/falls/vocalizations with different beddings, at different slopes. Least square means accompanied by * different at P < 0.05. N=5,847.

Table 1. Least square means for score and total time for beddings accompanied by P-values. [a,b] P < 0.05.

Bedding	20° Score	Time, s	*P*-value
Nothing (N)	5.25[a]	126.0[a]	NS
Feed (F)	5.45[a]	126.6[a]	NS
Sawdust (SD)	1.90[b]	121.5[a]	NS
Sand (S)	0.95[b]	101.5[ab]	NS
Hay (H)	0.95[b]	90.1[b]	0.02

Acknowledgments

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Comparison of two anesthetic techniques (Azaperone-Propofol and Azaperone-Metomidate) on the castration of sows under field conditions

A Gómez[1], L Palma[1], M Lara[1], J Ibancovichi[1]

[1] *Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Instituto Literario 100 Ote, Toluca, 50000, México,* avgg_9@Yahoo.com

Introduction

In veterinary practice, general anesthesia or chemical restraint in swine is required for diagnostic and surgical procedures including hernia repair, cesarean, physical and radiographic examination. Several anesthetic agents have been used in pigs but there are different opinions about its use (3,4). Pigs are difficult to anesthetize animals under practical conditions. For this reason the drugs are administered intramuscularly to produce a degree of sedation are preferred so that the subsequent handling and venipuncture easier to carry (1.2). Pig anesthesia under field conditions requires the use of anesthetics and techniques that are practical and economical but do not require sophisticated or expensive equipment (5).

Materiasl and Methods

10 hybrid sows were used in growth with an average of 37 kg. Two groups (A and B), each group consisted of five sows were randomly formed. Each bristle group A was tranquilized with azaperone at a dose of 0.4 mg / kg body weight by intravenous route followed by application of a dose of propofol to 0.83 mg / kg body weight intravenously. Sows in group B were tranquilized with azaperone a dose of 0.4 mg / kg body weight intravenously followed by application of Metomidate at a dose of 2.5 mg / kg body weight intravenously. Both groups underwent ovariectomy on the left flank. The results of this study were subjected to a completely randomized design with 5 replicates per treatment. The averages of each variable were compared by the Duncan test.

Results

The averages for the induction time, recovery time, heart rate, respiratory rate and body temperature of anesthetized sows azaperone-propofol combination (AP) and azaperone-Metomidate (AM) are seen in Table 1.

Table 1. Averages induction time, recovery time, heart rate, respiratory rate and body temperature of anesthetized sows azaperone-propofol combination and azaperone-Metomidate

Type Anest	T. Ind.. min	T. Rec. min	F.C (L/min)	F. R. (R/min)	T. C. °C
A-P	3.2[a]	51[a]	72[a]	15[a]	38.1[a]
A-M	2.5[a]	78[b]	82[b]	14.1[a]	37.8[a]

(a,b) superscription indicate statistically significant differences ($p < 0.05$).

Conclusions and Discussion

In the present experiment an induction time of 3.2 minutes for the combination (AP) and a time of 2.5 minutes for the combination (AM) was found, no significant difference was observed ($p < 0.05$). The recovery time was greater for the combination than for AM AP combination ($p < 0.05$). From the results obtained and on the conditions under which this study was conducted it is concluded that the anesthetic combination AP can be used to perform minor surgeries such as castrations in growing pigs not involving deep sedation in pigs**.**

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A comparison of the behavior and productive performance of pigs fattened in an organic production systems

R Martínez[1], F Ramírez [1,], M Alonso [2], MA Herradora[1], G Ramírez [1]
[1] *Universidad Nacional Autónoma de México, Depto. de Medina y Zootecnia de Cerdos, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México.* [2] *Depto. de Producción Agrícola y Animal, Universidad Autónoma Metropolitana-X.* rmgamba@yahoo.com.mx

Introduction

In Mexico there have been some studies of breeding females for converting a conventional farm to an organic one (2); however, organic pig fattening has not been tried out where conditions in the areas of medical care, housing and food are different from conventional ones, therefore animal welfare and performance are unknown under these conditions. Thus, the objective of this study was to assess animal welfare and certain production variables under an organic system on small scale farms.

Materiasl and Methods

Eighty 7-week-old hybrid (14 kg) pigs were used; 40 of them (20 castrated males and 20 females), came from lactating sows in organic systems and the remaining 40 (20 castrated males and 20 females) were from the weaning area of an intensive system. Pigs were divided into two groups of 40 pigs of both genders and both origins in order to form organic pens (OP) and conventional pens (CP). Each pen was divided into two in order to offer an organic diet and a conventional diet. Two OPs were used each one 45m^2 with cemented floor area and a 70m^2 outdoor sand floor area. Each one housed 20 pigs (10 castrated males and 10 females of both origins), the space per animal were 1.2m^2 in covered areas plus 0.8m^2 in outdoor (4). The CPs were eight Danish style pens (1.0m^2 per pig), with five pigs per pen. An organic diet (OD) was prepared (4) and a control diet which was the commercial diet (CD) used on the farm. Both were offered twice a day according age and weight on a four-phase feeding program lasting 30 days (0-30, 30-60, 60-90 and 90-120) Behavioral sampling was carried out in lapses of 5 m on each of the 80 pigs in order to gather 160 h of records from a single observer between of 10-12 a.m. (Welfare-Quality, 2009). Each of the pens was evaluated with respect to the degree of moisture of the floors using a floor diagram (1). The degree of cleanliness was evaluated on a weekly basis (5). In the tegumentary evaluation were used the criteria of Koning (3). For all pigs were obtained daily weight gain (ADG), and the feed conversion ratio (FCR). For animal welfare variables, the non-parametric X^2 test was used, employing the Wilcoxon Test. In the production variables, analysis by Student *T* test was used in order to compare animals in both systems.

Results

There was a greater expression of exploratory, rooting, play and physical activity type behaviors in OP than in CP. The presence of idle behavior was greater in the CP than in the OP ($P < 0.05$); the effect of the time on physical activity, exploration and rooting behavior was no different. ($P > 0.05$); physical activity and exploratory type behaviors were observed at a higher frequency in females ($P < 0.05$); idle behavior and play were performed more by males ($P < 0.05$); gender had no effect on rooting ($P > 0.05$). The OP exhibited a higher percentage of moisture than the CP, 18.9±0.6% vs. 9.4±0.6%, respectively ($P < 0.01$). The lesions from the tegumentary evaluation were classified as mild; minor incidences of lacerations were witnessed in the cranial and media regions and the thoracic limbs in the OP ($P < 0.05$); locomotive disorders were not recorded in any of the animals tested.

No difference between the types of pen were found (100.3±2.1Kg in OP *vs.* 102.4±2.3Kg in CP; $P = 0.5102$). The total ADG was 751.2±1.4g and neither were differences found between the treatments by pen (0.762±0.02Kg in OP vs. 0.797±0.02Kg in CP; $P<0.05$). No differences were found for FCR (2.59±0.05 in OP and 2.45±0.05 in CP; $P<0.05$). The mortality rate was 9.38% in the CP vs. 0% in the OP.

Conclusion and Discussion

No differences were found in the production parameters of the two types of production; although in the organic type system there was evidence to show better animal welfare conditions. Under organic conditions pigs were allowed to express normal behavior typical of the species, which lead to the presence of minor lesions, good handling of the housing areas and a good productive performance, demonstrating superior animal welfare.

Acknowledgements

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The internal environment of sows during the reproduction cycle and in fattening pigs before and after slaughtering

G Kováč, Cs Tóthová, O Nagy, P Turek, J Novotný, M Pribula, T Vozár
Clinic for Ruminants and Swine, University of Veterinary Medicine and Pharmacy, Košice, Slovak Republic
kovac@uvm.sk

Introduction

The acute phase proteins (APPs) are a group of blood proteins that change in animals subjected to external or internal challenges, such as infection, inflammation, trauma, stress (1,2,4,5). They can be used as general markers of health, welfare, immunological stress, in the diagnosis of diseases, as well as for meat inspection. The objective of this study was to evaluate the concentrations of main APPs and some other variables in sows, and in fattening pigs before and after slaughtering.

Materials and Methods

Into the evaluation of APPs in various stages of reproduction cycle we included 24 clinically healthy sows, which were crossbreeds of Large white and Landrace. Blood samples were collected: I. – 1 month before parturition, II. – 1 week before parturition, III. – 1 week after parturition, IV. – 1 week after weaning. Blood samples were analyzed for selected APPs – haptoglobin (Hp, mg/ml) and C-reactive protein (CRP, µg/ml), glucose (Glu, mmol/l), total cholesterol (TCH, mmol/l) and total lipids (TL, g/l). The evaluation of the effect of stress at slaughtering was carried out on 7 apparently healthy Slovak white breed of pigs from a finishing unit. Blood samples were analyzed for Hp, CRP, total proteins (TP, g/l), Glu, lactate (Lac, mmol/l), creatinine-kinase (CPK, ukat/l), calcium (Ca, mmol/l), magnesium (Mg, mmol/l), phosphorus (P, mmol/l), sodium (Na, mmol/l), and potassium (K, mmol/l). Hp and CRP determined by commercial ELISA kits. Glu, TCH, Lac, TP, CPK, and P were assessed using commercial diagnostic kits on automatic biochemical analyzer Alize. TL were analyzed using commercial diagnostic kits by spectrophotometric method. The concentrations of Ca, Mg, Na and K were measured by atomic absorption spectrophotometric method. The significance of differences in values was evaluated by ANOVA-test, Student´s paired test, and Wilcoxon-test.

Results and Discussion

The results of the determination of evaluated variables in various stages of reproduction cycle in sows are summarized in Table 1. The data measured in fattening pigs before and after slaughtering are shown in Table 2.
The obtained results suggest that the reproductive state influences the production of APPs and indicate that around parturition there are also in sows important changes in the concentrations of some APPs as was found early (3). In fattening pigs we recorded a marked effect of stress and path. lesions on the values of Hp and some other biochemical variables, similarly to paper 1.

Table 1. Concentrations of evaluated variables in blood serum of sows during the reproduction cycle

VARIABLE		SAMPLE COLLECTION I	II	III	IV	$p <$
Hp	x	$1.3^{a,b}$	2.0^{a}	2.2^{b}	1.5	0.05
	±sd	0.5	1.0	0.6	0.4	
CRP	x	$16.3^{a,b}$	20.0^{a}	$41.3^{b,A}$	13.7^{A}	0.01
	±sd	9.4	12.1	39.8	11.3	
Glu	x	4.71	4.89	5.23^{a}	4.47^{a}	n.s.
	±sd	1.50	0.80	0.75	1.13	
TCH	x	2.18	2.19^{A}	$1.84^{A,a}$	2.11^{a}	0.05
	±sd	0.33	0.27	0.34	0.31	
TL	x	3.34	3.85	3.29	3.90	n.s.
	±sd	0.72	0.93	0.94	0.93	

The same indexes in lines mean significance of differences in values between the groups: a, b – $p < 0.05$; A – $p < 0.01$

Table 2. Concentrations of evaluated variables in blood of fattening pigs before and after slaughtering (x±sd)

VARIABLE	SAMPLE COLLECTION before	after	$p <$
Hp	1.52 ± 0.78	2.11 ± 0.65	n.s.
CRP	127.80 ± 34.58	80.44 ±45.13	n.s.
TP	70.74 ± 3.01	73.41 ± 1.85	0.05
Lac	3.54 ± 1.96	3.96 ± 1.55	n.s.
CPK	12.18 ± 3.59	38.21 ± 10.66	0.05
Ca	2.56 ± 0.24	2.73 ± 0.09	n.s.
Mg	0.77 ± 0.03	0.79 ± 0.07	n.s.
P	2.47 ± 0.20	2.44 ± 0.18	n.s.
Na	134.7 ± 8.12	149.9 ± 1.95	0.05
K	4.64 ± 0.60	6.51 ± 1.03	0.05

Conclusions

On the basis of the literature review and our results is possible to applied APPs with selected internal markers for the control health status of swine (age, reproduction, production stage etc.).

Acknowledgments

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Surface temperature change on sows under forced ventilation during farrowing

RG Garcia[1], IA Nääs[1], DE Graciano[1], FR Caldara[1]
[1]*College of Agrarian Sciences, Department of Animal Science, Dourados, Brazil* rodrigogarcia@ufgd.edu.br

Introduction

When lactating sows are heat stressed their evaporative loss increases, and it depends on the relative humidity of the rearing environment. In subtropical countries, evaporative heat is lost by the respiratory tract (4). Evaporative cooling is an adiabatic process which has been mainly used in swine production in order to cool the housing (1, 2). Infrared thermal imaging has been used to estimate surface temperature of different species, including swine (3).

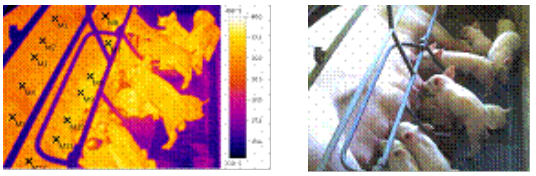
The aim through this work to assess the surface temperature of lactating sows, with two forms of ventilation: T_1, natural ventilation system and T_2, and adiabatic cooling system.

Materials and Methods

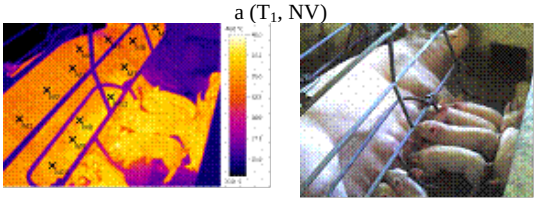
Data collection was conducted in a commercial farm, located in South Western Brazil, latitude 22º 37' 59" S and longitude 47º03'W. Twenty lactating sows were randomly selected. Ten were reared under natural ventilation (T_1) and 10 in pens using cooled ventilation (T_2). The adiabatic cooling was on when ambient temperature was above 23ºC. Skin temperatures were recorded using the Thermographic camera Testo®. Surface temperatures were collected during the morning and afternoon, in five days randomly chosen according to the treatments. The average of 12 selected points in the surface temperature profile recorded in each animal was calculated, and the Student's t test was applied.

Results

Figure 1 (a) shows the marked sections in the sows' skin surface temperature in T_1 and T_2.



a (T_1, NV)



b (T_2, AC)

Figure 1. Images of the lactating sow according to the environmental condition natural ventilation (a) and adiabatic cooling system (b).

Results of lactating sows' surface temperature are shown in Table 1.

Table 1. Mean lactating sows' surface temperature during the morning and afternoon exposed to the two treatments T_1 and T_2

	SURFACE TEMPERATURE *PER* TREATMENT	
Period	T_1	T_2
Morning	31.68 ± 1.73*	31.94 ± 8.78*
Afternoon	36.51 ± 1.51a	34.42 ± 1.25b

*Non-significant (p >0.05)
(a, b) Superscripts indicate statistically significant differences within main effect ($p \leq 0.05$)

Conclusions and Discussion

Sows' skin surface temperature presented a decrease in the afternoon in T_2, probably due to the decrease in the surface blood flow as the cold air ventilated over the sows' body. The difference between treatments in the skin surface temperatures was 2°C. In T_1, the variation in the sows' surface temperature was 5°C between the morning and afternoon (Table 2).

Temperature fluctuation during the lactating sows' rearing is suggested to be below 8°C (1, 2), and thermal neutral surface temperature is 34 ± 1 °C (2). Using adiabatic cooling the sows' skin temperature was reduced from 36.51± 1.51°C to 34.42 ± 1.25°C during the hottest time of the day (afternoon).

The use of adiabatic cooling was effective in reducing the sows' surface temperature minimizing the heat stress as thermal images showed.

Acknowledgments

Graduate Program of Animal Science-UFGD and CAPES, PVNS.

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Identification of joint swelling in pigs using infrared thermography

IA Nääs[1], DE Graciano[1], RG Garcia[1], FR Caldara[1]
[1]*College of Agrarian Sciences, Department of Animal Science, Dourados, Brazil* irenilza@gmail.com

Introduction

Joint arthritis can be found in swine of all ages generate additional expenses during production, and also it impairs animal welfare. It is a source of carcass condemnations at slaughter houses worldwide (4, 5). Lame animals may show weight loss and minor issues during rearing such as omphalitis, endocarditis and pneumonia (2, 6). Carcass condemnation index in Brazil due to arthritis has increased in last year from 0.5% in the 60's to 1.0% in the 90's (6). Visual identification of lame animals is difficult, and it lacks in accuracy.

This study aimed to evaluate the effectiveness identifying joint edema in swine using the infrared thermal image.

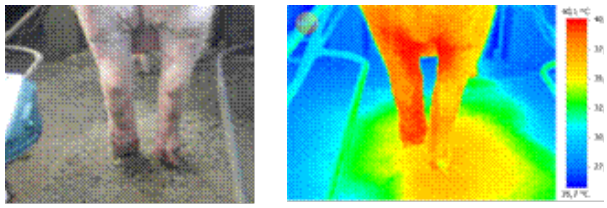
Materials and Methods

Data collection took place in a swine farm located in Center West of Brazil latitude 22°22'S and longitude 54°20' W. The climate characteristics were of sub tropical climate and annual dry bulb temperature of 20°C.

Five castrated pigs with a mean weight of 60 kg were randomly selected amongst those presenting slight lameness and joint edema hardly noticeable at a sight. Infrared images were taken using the the Thermographic camera Testo®. Ten points were selected in each limb, and the average of the values were calculated (1, 3). A histogram of the skin temperature was drawn, and the values of maximum, minimum and mean were found using the software Testo IRSoft®. Mean values were tested using the t-Student test.

Results

Figure 1 (b) shows the profile of the skin temperature in swine n. 5 with a joint edema. It is the result of a selected pig (number 5).



a b

Figure 1. Image of the back of the animal (a) and the infrared image of the same animal (b).

In Table 1, it is shown the skin temperature of the five pigs used in the study, which present joint swelling in one of the back limbs, and this issue was not identified visually.

Table 1. Skin surface temperature of the animals' rear leg with and without edema.

	SURFACE TEMPERATURE (°C)	
Animal	With joint edema	Without joint edema
1	35.8	31.6
2	34.2	32.9
3	34.6	33.7
4	34.5	32.9
5	38.7	38.8
Mean	35.5[a]	33.5[b]

(a, b) Superscripts indicate statistically significant differences within main effect ($p \leq 0.05$)

Discussion and Conclusion

The mean values of skin surface temperature were lower ($p \leq 0.05$) in the sound limb than in the leg with joint edema (Table 1). The difference was of approximately 2.0 °C and it helped the identification of the joint inflammation, for example in animal unit 5.

Infrared thermography has been used to detect slight difference in skin temperature that may not be seen under normal circumstances (1, 2, 4). In the present study, the infrared images were useful to identify joint lesions in pigs (5).

The use of infrared thermography showed potential for identifying lameness in pre slaughter conditions.

Acknowledgments

Graduate Program of Animal Science-UFGD and CAPES, PVNS.

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In vitro Pleuromutilin and Macrolide MICs and MBCs compared against European field isolates of *A. pleuropneumoniae*

M Vallé[2], I Morrisey[3], E Genet[3], S Hawser[3], U Klein[1]
[1]*Novartis Animal Health Inc., Basel Switzerland,* ulrich.klein@novartis.com, [2]*AM Consultant, St Bénigne France,* mvalle.amconsultant@sfr.fr, [3]*IHMA Europe Sàrl, Epalinges Switzerland,* imorrissey@ihmainc.com

Introduction

Actinobacillus pleuropneumoniae (*App*) is the cause of acute, sub-acute and chronic respiratory infections in pigs. Pleuromutilins (Tiamulin, Valnemulin) and macrolides (Tylosin, Tulathromycin) are currently used for the treatment of *App* infections in pig production. It is difficult to correlate *in vitro* activities of these compounds with the undeniable *in vivo* activity for these molecules[1]. The aim of this *in vitro* study is to compare pleuromutilins and macrolides by the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). Field *App* strains isolated from clinical cases of pig respiratory infection in different European countries were used for the investigations.

Materials and Methods

Bacteria strains: 10 field *App* isolates from European clinical submissions of pig respiratory infections (BE, DK, FR, GE, NL, SP, UK) in 2009 or 2010 were tested. All isolates were from pre-treatment samples and were selected according their MIC distributions and previous determinations[2,3].

Antimicrobial susceptibility tests: Tiamulin, valnemulin, tylosin and tulathromycin MICs were determined by broth microdilution in veterinary fastidious medium (CLSI standard method[4]) and by agar dilution (enriched chocolate Mueller-Hinton agar).

MBCs were determined according to the broth microdilution CLSI standard method[5].

Antimicrobial agents used: tiamulin hydrogen fumarate (Novartis AG, Basel, Switzerland), valnemulin hydrochloride (Novartis AG, Basel, Switzerland), tylosin tartrate (Sigma Sigma-Aldrich Co. LLC.) and tulathromycin (Pfizer Draxxin injectable solution).

Results

The MICs determined by broth microdilution confirmed previously generated MIC results for all tested drugs at ± 1 dilution: Valnemulin MICs were between 2 and 8 µg/mL, tiamulin and tulathromycin MICs were between 8 and 16µg/mL and tylosin MICs were between 32 and 64µg/mL. MICs determined by agar were found to be considerably higher for all test compounds: between 2x to 8x for tiamulin and valnemulin up to 8x, 16x for tulathromycin and between 2x to 4x for tylosin. MBCs of the test compounds were the same or one dilution higher than the MICs (MBC=MIC or 2xMIC). Exceptions were found for one *App* strain where the tylosin and tiamulin MBC was 4x the MIC (MBC=4xMIC). The results verify the same type of activity for all tested compounds and demonstrate bactericidal activity against *App* at a concentration equal or close to the MIC. The tiamulin-specific results are in agreement with the kill curve determinations presented in another IPVS communication[6].

Conclusions and Discussion

The MIC results on *App* a fastidious germ showed that the CLSI broth dilution method was as appropriate for pleuromutilins as for macrolides and confirmed the unsuitability of the agar dilution methodology for *App*.

The MBC/MIC ratio was 1 or 2 for the test compounds confirming tiamulin MBCs previously obtained in veterinary fastidious medium[1].

This low ratio indicated a bactericidal effect for all drugs tested at around the MIC as previously described for tulathromycin and tilmicosin[8] and also described for tiamulin[6,9,10]. This bactericidal activity of tiamulin against *App* was not inhibited, as seen before in serum[1] and showed a bacteriostatic activity at sub-MIC[6].

Denagard® is known to have an undeniable efficacy for respiratory pig infections even if the tiamulin MIC is around 8µg/mL[6]. Consequently, the tiamulin *in vitro* MIC, generally considered as high for *App* (an extracellular pathogen), is not linked to the plasma concentration in the animal body[10].

It is evident that to understand the clinical efficacy of this type of compound some other mechanism(s) must be further evaluated to find the appropriate PK/PD approach for dose justification.

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Duration of efficacy of tulathromycin injectable solution (DRAXXIN®) against *A. pleuropneumoniae* serotype 2 using an experimental challenge model

S Tanaka[1], T Furuya[1], T Tamada[1,] T Horii[1], K Utsumi[2], K Yamada[2], JRD Allison[3]
[1]*Zoetis Japan*, [2]*Kyodoken Institute*, [3]*Zoetis, Madison, NJ.* jim.allison@zoetis.com

Introduction

It has been already reported, in the US, that the duration of efficacy of tulathromycin (Draxxin®, Zoetis) (DRX) against *Actinobacillus pleuropneumoniae* (App) serotype 5 is 9 days (1). In Japan, App serotype 1, 2 and 5 are common, and in particular, serotype 2 has shown a high prevalence. Not only is it one of the important pathogens to cause Porcine Respiratory Disease Complex (PRDC), but it is one of critical factors hindering economic productivity. Consequently, the objective of this study was to determine the duration of efficacy of tulathromycin against App serotype 2 in a challenge model.

Materials and Methods

In this study, 20 pigs of the same age and weighing 17-21 kg were randomly allocated to 4 treatments. Before starting the pigs were not administered any App vaccines and were negative for App Complement Fixation antibody. In addition, another pig, from the same litters, was necropsied to confirm freedom from App - through the bacteria isolation from lungs as well as the lesions. Before challenge none of the pigs showed any clinical signs for respiratory diseases. Pigs in treatments 1 to 3 were injected with 2.5 mg of DRX per kg body weight at 9 (T01), 7 (T02) or 5 days (T03) before challenge with App; T04 was an untreated challenge group. For the challenge the pigs were inoculated in the bilateral nasal cavities with App2-cultured solution (1.0 X 10^6 CFU/mL/head). All pigs were euthanized 7 days after challenge and necropsies performed to examine lung lesions and isolate App. Clinical signs were monitored daily from 9 days before challenge until 7 days post challenge. Clinical signs were scored as; respiratory condition (0-4), coughing (0-3), vitality (0-3), appetite (0-4) and body temperature (0-4). Clinicians were blinded to treatment. Differences were tested using a Tukey-Kramer test for weight gain, and a Steel-Dwass test for clinical signs and lung lesion scores. This study was conducted in compliance with the Japanese regulatory guidelines for the ethical use of animals and animal welfare.

Results

In terms of clinical signs of respiratory disease, there were no significant differences among the 4 groups until after challenge. After challenge, the control pigs showed a typical bacterial pneumonia with a fever and an abdominal respiration. Pigs in T01, T02 and T03 showed from mild to moderate clinical signs. All pigs in the 3 DRX treatments showed an improvement in clinical signs compared to the controls 7 days after challenge (Figure 1). For seven days after challenge the average weight gains (AWG) in treatments 1 – 3 were higher than in T04. In particular, there was a significant difference between T03 and T04 (Table 1).

Table 1. Average weight gains (AWG) following challenge with App. Different superscript indicates statistical significance ($p<0.05$).

	T01	T02	T03	T04
AWG (kg)	$2.4^{a, b}$	$3.7^{a, b}$	4.0^{a}	0.7^{b}

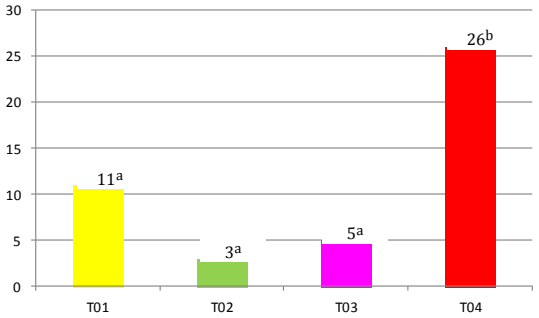

Figure 1. Comparison of the total clinical score per treatment at 7 days after inoculating App2 into the nasal cavities. Different superscript indicates significant difference ($p<0.05$).

At necropsy, although App2 was isolated from all pigs in all 4 groups, the lung lesions in T01, T02 and T03 showed lower scores compared with T04. Particularly, T03 was significantly lower than T04.

Conclusions and Discussion

The results demonstrate that even if administered before infection with App2, a dose of tulathromycin at 2.5mg per kg body weight was efficacious for the control of clinical disease, and the clinical efficacy lasted up to at least 9 days. Furthermore, there were no observed injection site reactions or other adverse events during the study. It was confirmed, therefore, that tulathromycin injectable solution (Draxxin®) was a highly safe and efficacious product for clinical use.

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Pharmacokinetics of Tildipirosin in nasal and oral fluids in weaner pigs after treatment with Zuprevo®

J Kauffold[1], HP Knöppel[2]
[1]*Faculty of Veterinary Medicine, University of Leipzig,Germany;* [2]*MSD Animal Health, Munich, Germany;*
kauffold@vetmed.uni-leipzig.de

Introduction

Tildipirosin (TP) is a newly developed antibiotic drug belonging to the group of macrolides and sold as Zuprevo ® by MSD/Intervet. TP has been shown to be effective against the most common pathogens causing pneumonia in swine, i.e. *Actinobacillus pleuropneumoniae (APP), Haemophilus parasuis (HP), Bordetella bronchiseptica (BB) and Pasteurella multocida (PM).* Pharmacokinetics of TP has been studied in lung tissue and bronchial secretions showing concentrations of TP above MIC90 between 1 to 14 days. The objective of this study was to investigate nasal and oral fluids for the pharmacokinetic of TP.

Materials and Methods

Study was performed in the University of Leipzig research and teaching farm. A total of 9 weaner pigs of an age of 11 weeks and average weight of 25-30 kgwere involved. Pigs were confined together in a pen of 9m^2 size. They were offered dry feed and water ad libitum. Animals were slightly sick, as they had some sneezing and occasional coughing, as well as slightly leaky noises. On day 0, animals were treated with TP IM in an amount according to their body weight. Animals had then nasal and oral fluid swabs collected 4 h and on days 1, 5, 10, 15 and 20 post treatment. Swabs were weighed prior to, and after sampling in order to calculate the amount of fluid that was absorbed by the swabs. Analysis of Tildipirosin was done by HPLC and MS

Results

Tildipirosin concentrations were measured in both nasal and oral fluids. Concentrations were higher in nasal than in oral fluids. Highest concentrations were observed at 4 h post treatment, then gradually declined to very low levels on day 5 in oral fluids, with a much slower and less substantial decline in nasal fluids. Concentrations above MIC90 in nasal fluids lasted for 24 h for *BB* (MIC90: 4 µg/ml), 5 days for *APP* (2 µg/ml), and for PM as well as HP (both 1 µg/ml) for 10 days. In oral fluids, TP was only detected 24 h for all four bacteria.

Conclusions and Discussion

This study has shown that Tildipirosin is secreted into nasal and oral fluids (Figure 1). The concentrations peaked a few hours post treatment and then gradually declined in both sample locations. The pharmacokinetics observed with nasal fluids greatly resembles that of lung tissue and also bronchial fluids[1,2]. Most importantly is the fact that, in the nose, concentrations above MIC90 lasted between 1 to 10 days ensuring that, an antibiotic "first defense line" is in place. In addition or alternatively, it may help clearing the nose of bacteria potentially resulting in a decrease of bacterial exhaust and transmission to sentinel pigs. In conclusion, the study has shown that TP is secreted into oral, but most importantly nasal secretions which additionally may help control pneumonia due to bacterial infections in pigs.

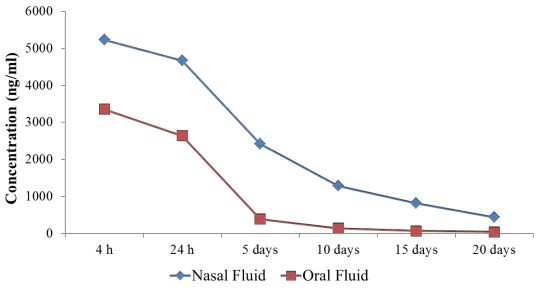

Figure 1. Concentrations of Tildipirosin in nasal and oral fluids 4 h to 20 days after treatment in weaner pigs (n = 9).

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P.240

MICs and MPCs of selected antimicrobials for *E. coli* and *P. multocida* isolates from pigs in the Czech Republic from 2008 to 2012

K Nedbalcova, Z Kucerova, P Alexa, K Nechvatalova
Veterinary Research Institute, Brno, Czech Republic, nechvatalova@vri.cz

Introduction

The effective antimicrobial therapy is compromised by the occurrence and spread of bacterial resistances detected worldwide. The effort to preserve the effectiveness of antimicrobials in human and veterinary medicine has been very intensive lately. The antimicrobials are used for therapy on the basis of the dosage strategy for antimicrobials according to their pharmacokinetic (PK) and pharmacodynamic (PD) parameters and determination of minimum inhibitory concentration (MIC) for the pathogen. A new concept in testing of antimicrobial resistance with the potential to reduce bacterial resistance is dosing of antimicrobials based on their PK/PD parameters and determination of the mutant prevention concentration (MPC) for the pathogen. The MPC is a parameter of sensitivity designed for a more accurate assessment of the potential of selection of a resistant mutant to antimicrobial substances than traditional methods for the determination of sensitivity. The difference between the MIC and the MPC is called the mutant selection window (MSW). When this window is wider and the difference between the MIC and MPC for the bacteria is greater, the risk of selection of resistant strains is also greater (2). In this study, we compared the MIC and MPC of frequently used antimicrobials for selected animal pathogens (*Escherichia coli* and *Pasteurella multocida*) isolated from clinical cases of porcine enteric and respiratory infections in the Czech Republic over the period 2008 - 2012.

Materials and Methods

MIC and MPC values for enrofloxacin, florfenicol, and tulathromycin were determined by a dilution micromethod, standardized according to the Clinical and Laboratory Standards Institute (CLSI) and the previously published studies (1, 4), using commercial kits (Trek Diagnostics Systems Inc., England; Trios, Czech Republic) in a group of 80 *E. coli* and 80 *P. multocida* isolates. MIC values were defined as the lowest antibiotic concentration that inhibited visible bacterial growth of culture with the density of 10^5 CFU/ml. The MPC was determined as the lowest concentration of an antimicrobial agent inhibiting visible growth of bacterial culture (the growth of resistant mutants) with density of inoculum $\geq 10^9$ CFU/ml. Calculated values MIC_{90} (MPC_{90}) were the lowest concentrations (mg/L) inhibiting growth of 90% of isolates in bacterial culture with appropriate density. Breakpoints of resistances were defined according to the CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST). MSW was determined by the difference between MPC_{90} and MIC_{90}.

Results

Based on the CLSI method for MIC determination the majority of the tested isolates were susceptible to the tested antimicrobial substances, with the exception of *E. coli* isolates which were mostly intermediately susceptible or resistant to florfenicol. The MPC values were found to be above the sensitivity/resistance breakpoints in the overwhelm- ing majority of cases.
The ratio of MIC_{90}/MPC_{90} which limited MSW was ≤0.12/2 mg/L for enrofloxacin, 0.5/≥64 mg/L for florfenicol and 4/≥128 mg/L for tulathromycin in porcine *P. multocida* isolates, 0.5/16 mg/L for enrofloxacin, ≥64/≥64 mg/L for florfenicol and 8/≥128 mg/L for tulathromycin in porcine *E. coli* isolates.

Conclusions and Discussion

MPC mostly better corresponds to the concentration of bacteria in the blood or in the target organ during acute infection of animals (and people) than MIC determination for inoculum with a density of 10^5 CFU/ml. Determination of the MPC and the assessment of PK/PD parameters of antimicrobials could be a new promising strategy of antimicrobial therapy contributing to a decrease in bacterial resistance. The correct dosage of antimicrobials resulting in serum concentrations equal to or higher than MPC (where it is possible) can help to reduce selection of resistant bacterial subpopulations (2, 3).

Acknowledgments

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Brachyspira spp identified in fattening pigs in Argentina

AI Carranza[1*], M Flores León[1], J Parada[1,2], PJ Tamiozzo[1,2], P Camacho[1], G Di Cola[1], JJ Busso[1], A Ambrogi[1]
[1]*Depto Patología Animal, Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto,*
[2] *CONICET. República Argentina,* acarranza@ayv.unrc.edu.ar

Introduction

Different *Brachyspira* (*B.*) species may be present in growing and finishing pigs, where *B. pilosicoli* and *B. hyodysenteriae* are considered the most pathogenic species, producing porcine intestinal spirochetosis (PIS) and swine dysentery (SD), respectively (1). Others, as *B. innocens*, *B. murdochii* and *B. intermedia*, are considered as no pathogenic. Nevertheless, recent investigations have proposed that they may produce several degrees of colitis and cause productive losses (3). Few studies about the prevalence of herds infected by different species of *B.* are available in the literature (5). Therefore, the aim of this study was to identify the species of *Brachyspira* in fattening pigs of confined farms in Argentina.

Materiasl and Methods

The study conducted in seven provinces of the major pig production area in the country, where 52 farrow-to-finish farms with more than 200 sows were selected and sampled. In each farm, fecal samples from 30 pigs of 22 weeks old, randomly selected, were taken. The samples collected from the rectum in individual plastic bags were refrigerated (4-8°C) until processed before 48 hours. Feces were plated on 7% horse blood agar containing colistin (25 mg/l), vancomycin (25 mg/l) and spectinomycin (400 mg/l), and incubated at 42°C for 7 days, under anaerobic conditions (AnaeroGen, Oxoid). When a fine film of growth and strong or weak β hemolysis was observed, a Gram stained smear was made. Any sample with spirochaetal forms were cultivated onto trypticase soy agar supplemented with 5% sheep blood, many times as necessary to purified. *B.* species were identified by biochemical test according to Hommez *et. al*. (2), using Rosco® tablets or by PCR–RFLP according to Rohde *et. al.* (4).

Results

Spirochaetes were found in the feces of pigs from 41 farms (78.8%). A β hemolysis zone and/or film of growth were observed in 270 (18%) out of
1496 fecal samples tested. Sixty-two strains of *B.* were identified by biochemical test, and 22 by PCR-RFLP (Table 1). A mixed infection by *B. hyodysenteriae*, *B. innocens and B. murdochii* was identified in a farm. A combination of *B. innocens* and *B. pilosicoli* was found in a farm, and of *B. innocens* and *B. murdochii* in 3 farms.

Table 1. Total strains, number of positive farms and prevalence of *Brachyspira* species found.

Species	Total strains	Farms +	Prevalence (%)
B. hyodysenteriae	**5**	**1**	**1.9**
B. pilosicoli	**4**	**2**	**3.8**
B. innocens	**39**	**17**	**32.7**
B. murdochii	**36**	**16**	**30.8**
Not identified	**186**	**38**	

Conclusions and Discussion

Different species of *Brachyspira* were identified in fattening pigs of Argentinian farms in the present study. Similar results were reported previously in Denmark (5), where *B. innocens* and *B. hyodysenteriae* were found in 34.2 and 2.5% of the farms, respectively. Despite the high prevalence of *B. innocens* and *B. murdochii* found, to date, little is known about their impact in the local swine production. But, according to Jensen & Boye (3), they may have a certain degree of pathogenicity, either alone or with others pathogens, and produce mild clinical or subclinical presentations. Furthermore, according to Hampson (1) infection or colonization with one or more of the nonpathogenic B. species, as was found in the present study, may complicate diagnosis of SD and/or PIS.
Further studies should be conducted to accurately evaluate the importance of *B. innocens* or *B. murdochii* infections in pigs, species that are widely spread in the local and world swine production.

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Occurrence of toxin gene *cpb2* in *C. perfringens* strains isolated from suckling piglets with and without diarrhea

A Dors[1], E Czyżewska[1], M Pomorska-Mól[1], A Nowak[1], S Zębek[1], D Borowska[1], Z Pejsak[1]
[1] *Department of Swine Diseases, National Veterinary Research Institute, Pulawy, Poland,*
arkadiusz.dors@piwet.pulawy.pl

Introduction

Clostridium perfringens is a Gram-positive, spore-forming bacterium that can cause a variety of toxic-specific lesions and gastrointestinal diseases in pigs. Some studies have reported an association between the presence of the *C. perfringens* β2-toxin gene (*cpb2*) and type A-associated diarrheal illness of neonatal piglets compared with healthy ones (1, 5). Previous studies confirmed the occurrence of active genes encoding α (*cpa*) and β2 (*cpb2*) toxins in *C. perfringens* strains isolated from healthy pigs in Poland (3, 4). The aim of this study was to assess the prevalence of *cpb2*-positive *C. perfringens* in piglets with diarrhea compared with unaffected suckling pigs and to determine its association with neonatal diarrhea in pigs.

Materials and Methods

A total of 379 pooled samples of faeces/litter from 1 to 6 litters from each herd were collected during a period 2011-2013 from suckling piglets (3-28 days of age) in 70 polish swine herds. Eighty-one samples have been collected from litters with diarrhea and 298 from litters without diarrhea. The samples were cultured on Columbia agar plates for *C. perfringens*. Typical colonies surrounded with characteristic hemolysis zone were observed after an overnight incubation at 41°C. Crude whole single colony lysate was used in multiplex PCR assays targeting virulence toxin genes *cpa, cpb* (β-toxin gene) and *cpb2*. Differences in prevalence of *cpb2* between litters with and without diarrhea were determined by chi-squared test (statistically significance at $p < 0.05$).

Results

Among 70 examined swine herds *C. perfringens* have not been found only in 7 herds (10%). Isolates carrying only *cpa* gene were found in 47.1% of herds. Only in 1.4% of herds *cpa* and *cpb* positive isolates were found. In 75.7% of herds isolates carrying *cpa* and *cpb2* have been found. Among 379 tested samples 224 have been positive to *C. perfringens*. Percent of *C. perfringens* positive samples in pigs with and without diarrhea is shown in Table 1. Occurrence of *cpb2* gene in *C. perfringens* isolates is shown in Table 2. There were no statistically significant differences between *cpb2* gene occurrence in samples from litters with and without diarrhea.

Table 1. The number and frequency of *C. perfringens* positive samples in pigs with and without diarrhea.

Samples from litters	Positive	
	number	%
With diarrhea (n = 81)	34	42
Without diarrhea (n = 298)	190	64

Table 2. Prevalence of toxin genes detected by PCR among *C. perfringens* strains isolated from pigs with and without diarrhea.

Samples from litters	No. (%) of isolates			
	total	*cpa*	*cpa+cpb*	*cpa+cpb2*
With diarrhea	34	13 (38%)	0 (0%)	21 (62%)
Without diarrhea	190	56 (29%)	1 (1%)	133 (70%)

Conclusions and Discussion

This study shows lack of association between the presence of *cpb2*-positive *C. perfringens* and neonatal diarrhea of suckling piglets. Present results are in opposite to the other studies (1, 5) but similar to results presented by Farzan et al. (2). The role of the *cpb2* in pigs enteritis is unclear and requires more precisely designed studies to address the hypothesis of an association between *cpb2* and diarrheal illness in neonatal piglets.

Acknowledgments

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Impact of the use of ceftiofur on the emergence of *E. coli* resistant to cephalosporins in four conventional pig farms

K Cameron-Veas[1], MA Moreno[2] L Garcia-Migura[1], L Fraile[3]
[1]*Centre de Reserca en SanitatAnimal (CReSA), Barcelona, Spain.* [2]*Centro de Vigilancia Sanitaria Veterinaria, Universidad Complutense de Madrid, Spain.*[3]*Animal Production Department, Lleida, Spain.*
lorenzo.fraile@prodan.udl.cat

Introduction

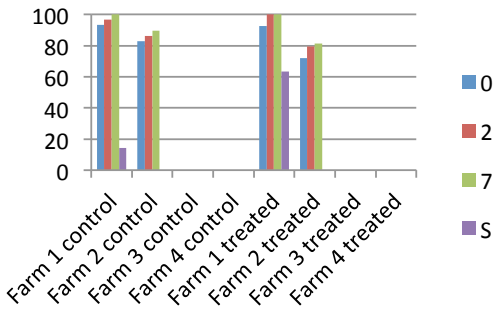
The use of ceftiofur is licensed for treatment of systemic bacterial infections in pig production. The worrisome of cephalosporin resistant (CR) *Escherichia coli* entering the food chain have raised the debate on the use of third and four generation cephalosporins for animal husbandry (Jorgensen et al., 2007). The aim of this study was to evaluate if the treatment with ceftiofur is a risk factor associated with the emergence of CR *E. coli* during the rearing period in four conventional farms, and assess if the farm is a reservoir of resistant bacteria that can enter the food chain.

Materials and Methods

This study was carried out in four farms belonging to a large farm integration system in Spain. In each farm, a total of 70 seven-day-old piglets were divided in two groups; control (n=30) and group treated (n=40) with ceftiofur (Naxcel®, Zoetis). Animals were fed under a standard nutritional program set by the company that included the use of amoxicillin, apramycin, tiamulin and oxytetracicline in a prophylactic way during the nursery period (21-70 days of age). Fecal swabs were taken from piglets before treatment with ceftiofur (aprox. 7 days-old), 48 hours and 7 days post-treatment. A final sample was performed before the animals departed to the slaughterhouse (180 days of life). Samples were plated in MacConkey agar supplemented with ceftriaxone (1mg/ml), and *E. coli* was selected based on colony morphology.

Results

The occurrence of CR *E. coli* before treatment with ceftiofur was extremely variable between farms (figure 1). Furthermore, they could not be recovered in two of the four farms. The highest percentage of samples positive for CR *E. coli* was obtained 48 hours post-treatment within the treated group. This value was approximately 8 per cent higher than the pre-treatment value. By the finishing time, all animals were negative for CR *E. coli* in three out of the four farms.



S= Slaughterhouse

Figure 1. Occurrence of CR *E. coli* in four conventional pig farms after ceftiofur treatment (days).

Conclusions and Discussion

CR *E. coli* were detected in seven-day-old piglets before any treatment was administered. Additional epidemiological studies to explain the high variability observed in the occurrence of these resistant bacteria before applying any antibiotic treatment are required. The ceftiofur treatment provided a window for detecting the presence of CR *E. coli* during the course of treatment; however, the use of ceftiofur did not pose enough selective pressure to select for long-term resistant organisms. The occurrence of CR *E. coli* decreased with the age of the animals in all the studied farms. In any case, the presence of CR *E. coli* in slaughtered pigs was only observed in one out of the four studied farms. These results suggest that control measures to reduce the prevalence of CR *E. coli* should be applied in a case by case situation.

Acknowledgments

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Antimicrobial susceptibility in *E. coli* isolated in edema disease episodes

D Vio, S Deotto, M Ustulin, T Di Giusto, G Conedera, M Cocchi
Istituto Zooprofilattico Sperimentale delle Venezie, SCT Friuli Venezia Giulia, Cordenons, Italy, dvio@izsvenezie.it

Introduction

Antimicrobial resistance (AMR) represents one of the major problems concerning health in both human and veterinary medicine, worldwide. Some resistant bacteria of animal origin can be an important source of human exposure, entering the food chain via meat products, e.g. (2). Foodborne enteric pathogens are considered to be more resistant to antibiotics than other bacteria (4). Among them, strains of *Escherichia* (*E.*) *coli* play an important role as it can be a reservoir of antibiotic resistant genes (2). In the swine industry, *E. coli* is one of the main pathogens: it can cause edema disease, post-weaning diarrhea and colibacillosis. In the last few years, AMR is described as on the rise for these isolates (2). Moreover, in human medicine infections caused by multiresistant Gram negative bacteria are increasing worldwide. Therefore, the aim of this study was to evaluate the antimicrobial resistance pattern in *E. coli* strains isolated from pigs affected by Edema Disease.

Materials and Methods

65 strains isolated from pigs presenting edema disease, belonging to 21 farms and isolated in the period 2006-2012 were chosen.

Antimicrobial susceptibility. The minimum inhibitory concentration (MIC) of gentamicin, enrofloxacin, oxytetracycline, chlortetracycline, and sulfamethoxazole/trimethoprim (SXT) was evaluated by broth microdilution using Sensititre plates (Trek Diagnostic, Cleveland, Ohio, USA).

Moreover, the presence of the extended spectrum beta-lactamase (ESBL) was evaluated in accordance with CLSI guidelines.

The test and the classification of the strains were carried out in accordance with the CLSI guidelines.

Results

The MIC results are summarized in table 1.

Table 1. Results of MIC

	No of strains (percentage)		
Antibiotic	S	I	R
Oxytetracycline	4 (6.1)	2 (3.1)	59 (90.8)
Gentamicin	45 (69.2)	3 (4.6)	17 (26.1)
Clortetracycline	6 (9.2)	0	59 (90.8)
Enrofloxacin	56 (86.1)	5 (7.7)	4 (6.1)
SXT	21 (32.3)	0	44 (67.7)

ESBL. No strains are positive for ESBL.

Conclusions and Discussion

As shown in table 1, more than 90 % of the tested E. coli strains were resistant to oxytetracycline and chlortetracycline.

As reported, in *E. coli* the resistance to tetracyclines is frequent. Different Authors referred high tetracycline resistance in *E. coli* with value ranging from 33% to 90%. Among others molecules, high level of resistance was found for enrofloxacin (86.1%) and for gentamicin (69.2%). The resistance to gentamicin is in agreement with the study of Mathew (3), but it is not in accordance with the data reported from other authors, for whom the percentage is low (1). In this study the resistance to enrofloxacin is high. Malik et al (2) describe a lack of resistance to this molecule in *E. coli* isolated from pigs. Percentage of resistance to SXT is high among our strains. The MIC value was impossible to evaluate, accurately (MIC≥2/38). For the purpose of this study, Kirby-Bauer assay was performed according to CLSI guidelines in order to classify the strains.

No EBSL strains were found. In *E. coli* strains to monitor the presence of the enzymes capable of the hydrolysation of different cephalosporin, a phenotypic assay was performed. This test is easy to do, but is not able to distinguish among the involved enzymes. Moreover, using the phenotypic approach, the low-level resistance can be undetected. The detection of ESBL-producing bacteria represents a crucial step in a diagnostic laboratory. Bacteria isolated from animals can be a reservoir of antibiotic resistance and so, they can be a source for human beings. For this reason, the monitoring (phenotypic and molecular methods) of antibiotic resistance in veterinary medicine is noteworthy. The results of this study show a high level of resistance to tetracyclines and to SXT in *E. coli* strains isolated from pigs. This implies the need to have routine surveillance studies in order to perform appropriate therapy on farms. Moreover the adoption of proper strategies can be useful in preventing the development and the diffusion of the resistance in this pathogen and in the commensal strains.

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Urinary tract infections in sows in Malaysia

A de Quatrebarbes[1], C Pommellet[1], CY Tee[2], LP Ong[3],
[1]Laboratoires Coophavet - MERIAL, [2]Rhone Ma Malaysia Sdn Bhd, [3]VetFood AgroDiagnostic,
francois.joisel@merial.com

Introduction

In Malaysia, the number of urinary tract infections (UTI) in sows is probably underestimated, as humidity and heat are important favoring factors (1). This study aimed to assess the prevalence of UTI, to determine their causative agents and the possible impact on health status and performance parameters.

Materials and Methods

Three Malaysian operations of respectively 1500 (A), 2000 (B) and 3000 (C) sows were included in the survey. Urine was sampled from late gestation sows at midstream from natural urination in sterile 50 mL polyethylene containers. All sows which could be sampled were included.

In order to detect the UTI two methods were implemented: first nitrite detection in fresh urine, which is consider pathologic (2), second bacterial identification and concentration in the fresh urine. Nitrite detection was performed using nitrite strip test 5300 Combur® 9 strip (Roche, Basel, Switzerland). Bacterial culture was performed following culture on a Dip Slide from Oxoid (Thermo Fisher Scientific, Waltham, MA, USA) with Mac Conkey Agar No. 3 medium and CLED Medium. The positivity threshold for urine infection based on bacterial count was set to 10^5colonies/mL (B+). Bacteria genus identification was performed only in these positive samples.

Results and Discussion

UTI detection results and bacteria identification are summarized in Tables 1 & 2.

Table 1. Chemical and bacteriological positivity for UTI in urine samples collected in 3 different Malaysian farms:

Farm	Total	Nitrite+		Nitrite -		%	%
		B+	B-	B+	B-	Nitrite+	B+
A	40	8	2	8	22	25%	40%
B	30	7	1	5	22	27%	40%
C	78	9	3	11	55	15%	26%
Total	148	24	6	24	94	20%	32%

Nitrite+/Nitrite - : positive/negative samples assessed by nitrite strip; B+/B-: positive/negative samples following bacterial culture.

Thirty-two percent of the sows (48/148) were positive for bacteriological analysis with a prevalence within the tested farms ranging from 26% to 40%.

The nitrite test allowed detecting only 56% of the total UTI. Indeed, this test does not detect UTI caused by non-nitrite productive bacteria (3) and the production of nitrite needs the urine to stay at least 2-4h hours in the bladder before miction. Consequently, this rate may be increased by sampling the first urine in the morning.

Table 2. Bacteria identification

Identification	Number of samples
E.coli	40
E.coli+Proteus	1
E.coli+Pseudomonas	1
E.coli+Bacillus	2
Acinetobacter	1
Klebsiella	1
Unidentified colony	2

Most of bacteria identified were *E.coli*, in few cases (9%) associated with other bacteria. All are germs commonly responsible of UTI in Europe (4).

Correlation with reproduction parameters

No statistically relevant correlations were found, probably because of the low number of sampled sows in each farm. If we consider all the sows from the 3 farms, the nitrite and bacteriology positive ones tend to have more stillborn than negative ones (p=0.082) and less live born piglets .

Conclusions

In conclusion, the study showed that there are UTI in Malaysia and that *E. coli* is involved in most of the cases. In addition, this study tended to show a correlation between UTI and the number of stillborn piglets and live born piglets.

Urinary tract infections are an underestimated problem in Malaysia. A systematic detection with nitrites strip would be an easy and fast way to check the status of Malaysian farms. When more than 20% of the heard is affected, a systematic treatment should be considered (5). In Malaysia the daily bathing of the sows in open houses farming is a common practice. Farmers use it to cool down the animals and wash the pens, leaving them on a constant wet flooring. The impact of such practice on the urinary tract health has not yet been measured but might probably have negative consequences.

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Exposure to the antibiotic avilamycin inhibits *E. coli* fimbriae and attachment

M Rostagno[1], G Pelger[1]
[1]*Elanco Animal Health, Greenfield, IN,* rostagno_marcos@elanco.com

Introduction

Escherichia coli is one of the most important causes of post-weaning diarrhea in pigs. Enterotoxigenic *Escherichia coli* (ETEC) is a major cause of post-weaning colibacillosis (PWC) in pigs, and one of the most economically important diseases for the swine industry worldwide (3). The ETEC strains produce five different adhesins, all of which are fimbriae (or pili) including K88 (F4), K99 (F5), 987P (F6), F41 (F7), and F18. The isolates implicated in PWC most frequently produce either K88 (F4) or F18 fimbrae (3,4). Fimbriae target specific receptors on the intestinal brush border, enabling ETEC to colonize the intestinal lining and secrete toxins, which cause diarrhea. It has been reported that avilamycin, an oligosaccharide antibiotic commonly administered in the feed, can improve the intestinal environment of the weaned pig, as indicated by increased gut microbial diversity, feed efficiency, and growth rates (1,6). However, the mechanism by which avilamycin may prevent PWD is not well known. There is evidence that avilamycin has an effect on *E. coli* in the swine gastrointestinal tract (1,2,5), but it is unclear whether the mechanism involves reduction of the pathogen as commonly believed for antibiotic feed additives, or other type of effect. Therefore, this *in vitro* study was conducted with the objective of determining the effect of avilamycin on *E. coli*.

Materials and Methods

A collection of *E. coli* strains was selected for testing the MIC against the antibiotic avilamycin, including: three K88 (F4) strains, two K99 (F5) strains, and an additional strain with no fimbriae (*E. coli* ATCC 25922). Mueller Hinton broth (MHB) was used for this study. *E. coli* strains with K88 (F4) fimbriae, and with no fimbriae (ATCC 25922) were observed in phase contrast and electron microscopy after exposure to avilamycin. A negative control treatment containing no avilamycin was also included. *E. coli* with K88 (F4) fimbriae, and *E. coli* with no fimbriae were also tested for attachment to brush border membranes. MHB containing different concentrations of avilamycin were prepared, and inoculated with the *E. coli* strains. Following incubation, aliquots of each dilution were added to a suspension of the brush border membranes in a multi-well microplate, and evaluated within one hour at room temperature through electron and phase contrast microscopy.

Results

The avilamycin MIC of all *E. coli* isolates was determined to be >512µg/ml. An assessment of the presence, quantity and/or quality of fimbriae on the *E. coli* strains indicated that avilamycin at concentrations of 4, 8 and 16 µg/ml resulted in a reduction of the number of fimbriae and/or caused damage to it as compared to the control group (0 µg/ml avilamycin). Exposure of the *E. coli* strains to avilamycin for a longer period of time (8 h versus 24 h) did not change the effect on the fimbriae. As expected, for the *E. coli* strain with no fimbriae, no clumping was observed when exposed to either, 0, 8 or 16 µg/ml avilamycin. However, clumping was observed for *E. coli* K88 (F4) exposed to 0, 8 and 16 µg/ml avilamycin, but not to 32 µg/ml avilamycin. The clumping macroscopically observed was also observed microscopically by phase contrast microscopy. There was attachment of the *E. coli* K88 (F4) to the brush border membranes, when grown in the presence of 0, 8 or 16 µg/ml avilamycin, but little or no attachment when exposed to 32 µg/ml avilamycin. The *E. coli* with no fimbriae did not show attachment when grown in 0, 8, 16 or 32 µg/ml avilamycin. The *E. coli* K88 (F4) attachment to the brush border vesicles was abundant without the presence of avilamycin and there was markedly less attachment in the presence of 32 µg/ml avilamycin.

Conclusions and Discussion

In conclusion, this study provided new evidence on how avilamycin may control PWC. It is commonly believed that antibiotic feed additives prevent enteric disease by reducing pathogenic populations. However, this study demonstrates a unique mechanism of action for the antibiotic avilamycin, which inhibited fimbriae formation by K88 (F4) positive strains of *E. coli*, impairing attachment to brush border vesicles. This evidence supports the application of avilamycin in the control of PWC.

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The method of piglets' post – weaning *E. coli* diarrhea treatment

IN Zhirkov
World Academy for Animal Husbandry, Volgograd, Russia zhircov@gmail.com

Introduction

The goal of these trials was to create the optimal scheme of coli diarrhea treatment weaned piglets using ecologically pure and cheap medicine.

One of the main causes of this disease is achlorhydria which destroys the organism natural barrier against environmental micro flora ingested with the feed. Moreover, weaning, absence of "milk defense", forming new groups of animals provoke the so called post-weaning stress which results in activation of sympathetico-adrenal system and achlorhydria. That is why we decided to find the substance which stimulates the luminal glands and normalizes gastric acid secretion and in that way to stop the colonization of small intestine with the environmental micro flora. This substance is very convenient to be sodium acetate.

Materials and Methods

Trials were conducted at the JS "Akhtubinets" swine–breeding farm during a mass coli diarrhea outbreak. Diagnosis was affirmed in the local veterinary laboratory. The strain of E. coli O141:K88 was identified in the intestine of dead piglets. Treatment was carried out with sodium acetate (JS "Khimprom"). Fifty piglets each were assigned to: control and experimental groups (CG and CC). Piglets were 40-45 days old and were kept in the adjacent pens. Feeding and maintenance conditions were equal for all animals. Experimental piglets were given 5 ml 3% aqueous solution of sodium acetate per os from a syringe cannula in 40 – 60 min before each prandial feeding for 10 days. Control piglets were treated with tylosine IM according to the manufacturer's instructions (the identified strain of E. coli was not acceptable to any antibiotics).

Results

Only one piglet died in the EG whereas in the CC – four and four animals continued to sick.

Quantity of the "diarrheic" days in EG was 26 that on 84,6% less, then in CC (48). Body weight gain in EG was 1,5±0,4 kg, in CC — 0,7±0,2 kg, i.e. on 114,3 % less ($P < 0,04$).

Conclusions and Discussion

The peroral use of 3% aqueous solution of sodium acetate is very effective and chip method of treatment diarrheic piglets. Antidiarrheic effect of this medicine may be assumed because of secretagogue properties of the acetic acid and its solts. Moreover as we have shown before (1) this substancies were the dramatic stimulants of piglets' growth. Another advantage of this group of drugs is their ecological safety for the animal body. Because the mode of action of aqueous solutions of salts of acetic acid is based on the stimulation of the body's own barriers, the adaptation of microorganisms to these drugs is not possible. Use of antimicrobial agents usually addictive pathogenic microflora. This implies further inefficiency antibiotic used.

Acknowledgments

The author is grateful to Dr. Sergey Volkhonsky for assistance in conducting experiments.

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A possibility of *E. coli* plasmid reducing following Flavomycin® administration in conventional pig farms

K Lugsomya[1], W Niyomtum[1], P Tummaruk[2], N Prapasarakul[1]
[1]*Department of Veterinary Microbiology,* [2]*Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand.* Email: Nuvee.p@chula.ac.th

Introduction

In modern pig industry, therapeutic use of antibiotics to control infectious disease is still commonly practiced. An increase of antibiotic resistant bacteria against antimicrobials have dramatically been emerging and directly impact on public health concerns (8). Moreover, not only pathogen is encountered by antibiotic use (3), but enteric commensal is also spontaneously adapted, resulting in risk of antibiotic resistance at farm-level (4). Resistant plasmid is a mobile genetic element possessing in *Enterobacteriaceae* that can be interchangeable between bacterial groups (6). Flavomycin® is a feed additive, belonging to the class of glycolipids and its active ingredient is flavophospholipol. It is mentioned on decreasing effect of plasmid transfer activity by conjugation interference, *in vitro* (9). The objective of the study was to evaluate the effect of Flavomycin® on types and frequency of plasmid replicons of *E. coli* derived from conventional pig farms with and without flavomycin in feed formula.

Materials and Methods

Study design; A total of 60 fecal samples were collected from healthy pigs comprising, 30 pigs without Flavomycin® in feed formula (control group; -) and 30 pigs with Flavomycin® (treated group; +). Each group was evenly distributed over different age groups (see table 1). All antibiotic formulation except Flavomycin® was exactly the same in the selected farms. Flavomycin® was given to piglets and growers at 8 ppm (100 g of Flavomycin® 80) as manufacturer recommended.

Sample collection and processing; At the same age, Fecal samples were taken from 5 pigs in control group and 5 pigs in treated group. At least six *E. coli* colonies per feces sample were selected from the high serial dilution (>10^6 CFU/g) and definitely identified on the routine technique for microbiology (5,6).

Identification of plasmids replicon typing; The 360 *E. coli* isolates (6 isolates x 60 pigs) were examined for the presence of 17 plasmid replicons using 5 multiplex and 2 simplex PCR (2). Amplicons were visualized on 1.5% Tris-acetate-EDTA agarose gels alongside a 1-kb ladder. The representative of amplicon were sequenced and compared to the National Center for Biotechnology Information database using BLAST.

Results and Discussion

The six homologous isolates from the same pig gave the similarity of replicon profiles. This confirmed validity of the selection criteria. In creeping pigs, no *E. coli* plasmid replicon was detected in all groups that might reflected relation between naïve bacteria and host with no antibiotic pressure. Eight of 17 replicons; *FIA, FIB, W, Y, P, FIC, A/C* and *T* were detected but at least 4 of 8 replicons were persisted in *E. coli* derived from nursery to finisher and breeders. In Flavomycin® treated group, the number of *FIA* and *W* decreases in most of observations except in grower. Distribution of *Y, P, FIC, A/C,* and *T* was variable among the observed periods, but the pigs supplemented with Flavomycin® showed a clear tendency in lower number of plasmids (Table1). Thus, reducing of the replicons associate with ESBLs was revealed in our study (1,4,8) that was consistent to low transferrable conjugative rate of *VanA* plasmid in VRE derived from Flavomycin® fed pig (7). This finding harmoniously confirmed Flavomycin® suppressing effects in dissemination of multi-resistant *E. coli* especially in fattening pigs (9).

Table 1. Comparison of plasmid replicons from 360 fecal *E. coli* isolated from pigs treated with and without Flavomycin®

Periods	Flavo-mycin	Plasmid replicon occurrence (percentage)							
		FIA	*FIB*	*W*	*Y*	*P*	*FIC*	*A/C*	*T*
Creeping (1-4 wks)	+	0	0	0	0	0	0	0	0
	-	0	0	0	0	0	0	0	0
Nursery (5-10 wks)	+	20	80	40	40	0	20	0	0
	-	40	60	100	40	0	40	60	0
Grower (11-12wks)	+	20	80	20	40	0	0	0	0
	-	20	80	20	40	0	0	60	0
Finisher (13 - 24 wks)	+	20	60	0	40	0	0	0	0
	-	40	60	40	40	20	40	0	0
Gestating sow	+	0	80	20	40	0	0	0	0
	-	40	40	40	40	0	0	0	60
Lactating sow	+	40	60	40	40	0	0	20	0
	-	60	20	60	60	0	0	60	0

Conclusion

Supplementing Flavomycin® to pigs possibly decreases the occurrence of resistant plasmid in porcine enteric *E. coli.*

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Piglet vaccination with Enterisol® Ileitis in a Subclinical *Lawsonia* positive Australian pig farm

B Lloyd[1], G Brooke[2], R Lising[2], M Howard[2]
[1]*Dr. Barry Lloyd Pty Ltd*, [2]*Boehringer Ingelheim Animal Health Pty Ltd, Australia*
merideth.howard@boehringer-ingelheim.com

Introduction

Pig production has continuously evolved, with husbandry practices and disease management becoming more intensive. Shortening the growth period from weaning to finishing calls for improved average daily gain (ADG) (1), with a healthy digestive system playing a major role. Ileitis, due to *Lawsonia intracellularis*, is a widespread enteric disease of pigs that has been well-documented to be a cause of global economic losses in the pig industry (2). Studies have shown vaccination with Enterisol® Ileitis can achieve improvements in growth rate, uniformity at sale, reduced mortality and improved feed efficiency (3,4). The following study was performed on an Australian privately owned piggery and explores production gains following administration of Enterisol® Ileitis in a subclinically affected *L. intracelularis* positive herd.

Materials and Methods

Prior to commencement of the study, serology for *L. intracellularis* was performed at an independent laboratory to determine herd challenge and optimal vaccination timing.

Four consecutive batches of 10 -14 day old piglets were included in the study - two batches were vaccinated with Enterisol® Ileitis and 2 batches remained as unvaccinated controls. Treatment order of the consecutive batches was - control, vaccinate, vaccinate, control. The vaccinated groups were orally drenched with Enterisol® Ileitis at 10 - 14 days of age when they routinely received Ingelvac CircoFLEX®. All pigs from vaccinated and control groups were individually weighed at 10 -14 days (vaccination), identified with Radio Frequency Identification Devices and individually reweighed at 18 weeks of age. Routine piglet vaccinations continued throughout the study and consisted of Ingelvac CircoFLEX® administered at 10 - 14 days of age and *M. hyopneumoniae* vaccination at weaning (3 weeks). All pigs received 44 ppm Lincomycin in feed from 3 to 12 weeks of age, and 50 ppm Kitasamycin constantly in feed from 12 weeks of age to market. Statistical analysis was performed using Statistica version 9.

Results

Pigs vaccinated with Enterisol® Ileitis performed significantly better than controls in both ADG and total gain (+28g and +3.47kg respectively, p<0.0001) (Table 1). Review of 18 week weights, shows that the vaccinated group had a higher frequency of heavier pigs and a lower frequency of lighter pigs compared to the control group (Figure 1).

Table 1. Weight and growth performance of vaccinated and control pigs

	Control	Vaccinated	Difference	p-value
Pig Numbers	780	663	117	N/A
2 wk Wt (kg)	5.26	5.21	0.05	0.388
18 wk Wt (kg)	66.19	69.66	3.47	< 0.0001
2wk-18wk Gain	60.93	64.45	3.52	< 0.0001
2wk-18wk ADG	525	553	28	< 0.0001

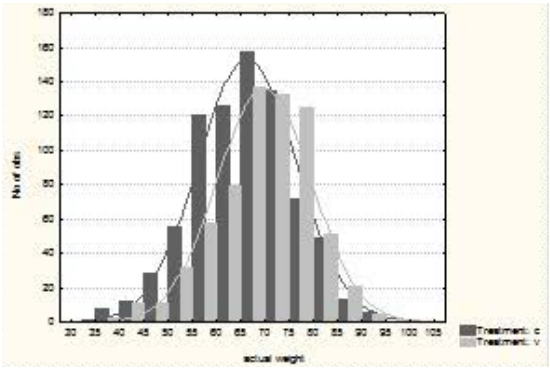

Figure 1. Distribution of 18 week weights of vaccinated and control pigs.

Conclusions and Discussion

Vaccination with Enterisol® Ileitis effectively improved ADG and weight gain, and produced heavier pigs in a subclinically affected herd.

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Gregory Stuart, Shaun Megson, Lisa Knobben, Boehringer Ingelheim Australia / New Zealand.

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Individual *L. intracellularis* serostatus is not a riskfactor for developing PHE

E Willems[1], N Wertenbroek[2]
[1]TOPIGS, Helvoirt, The Netherlands [2]Boehringer Ingelheim Vetmedica, Alkmaar, The Netherlands, eveline.willems@topigs.com

Introduction

Many pig herds are infected by the bacteria *Lawsonia intracellularis*. However the impact and clinical picture of this infection can differ enormously between herds. The acute form Porcine Hemorrhagic Enteropathy (PHE) can for instance occur in gilts 2-4 weeks after arrival on the commercial sow farm. An observation is that this happens more if the gilts come from a farm with a higher health status. Typically the PHE gilt cases would present with reduced appetite, palor, depression, black tarry faeces and high mortality. The objective of this field study was to investigate if the serological status of the gilts can be used as an indicator for likelihood of developing PHE. Our hypothesis was that seronegative animals being challenged with *Lawsonia intracellularis* have a higher risk to develop PHE than seropositive animals.

Materials and Methods

A multiplier (farm M) was selected with a known history of PHE cases at some of its clients (farm A, B and C), shortly after receiving the gilts. None of the pigs on these farms had been vaccinated against *Lawsonia intracellularis*. Farm M experienced no clinical problems caused by *Lawsonia intracellularis*. Farm M delivered 15, 20 and 15 gilts (study animals of around 7 months of age) to commercial sow farms A, B and C over a period of 14 days. All the clinically healthy gilts were bled one day prior to delivery (D-1) to the farms A, B and C. The serum samples were identified with the gilt ID number and stored at -70°C. All the gilts were monitored for clinical PHE on the commercial sow farms. In any case of a gilt developing clinical signs of PHE within 8 weeks after placement a blood sample was taken from the animal 2 weeks after the onset of the clinical signs. The blood sample was identified with the gilt ID number and stored at -70°C. For those gilts that did not show clinical signs of PHE, blood samples were taken 8 weeks after placement. All the blood samples were analyzed at the same time for Lawsonia antibodies (BioScreen Ileitis antibody ELISA, Hannover Germany).

Results

At the specific time frame of this trial only farm A experienced PHE in the study animals. On D12 after delivery 10 gilts stopped eating, showed signs of fatigue and had black faeces. On D13 one of these animals was found dead. Faecal samples of all tested gilts (9) were PCR positive for Lawsonia. The sick animals were treated with tylosine injection (Tylan, Elanco). On D26 from all animals that showed clinical signs blood samples were taken. On D56 all the other remaining animals were tested. In table 1 the percent inhibition (PI) results of the individual blood samples from the gilts on farm A.

Table 1. Individual PI results of gilts on day before (D-1) and on D +26 and +56 days after delivery.

animal ID	D -1	S/H *	D +26	D +56
9343	9,35	S	82,56	
9358	42,09	S	80,67	
9346	35,62	S	76,49	
9341	58,11	S	79,72	
9342	46,19	S	71,20	
9344	39,96	S	72,31	
9357	6,04	S	85,64	
9339	41,93	S	71,05	
9338	18,50	S	72,07	
9359	11,87	H		66,63
9349	22,37	H		70,57
9350	35,07	H		62,76
9348	21,34	H		63,08
9347	32,86	H		45,01

* clinical Sick/ Healthy at farm A
PI <20 = negative; 20-30 = doubtful; >30 = positive

Conclusions and Discussion

Independent whether gilts showed clinical signs of PHE, they seroconverted in both groups (clinical sick/ healthy gilts), and gilts with already positive serology (PI>30) at multiplier site, still got clinical problems. PHE apparently can occur in animals already infected by Lawsonia with tissue damage some weeks/months before showing clinical signs. This data together with other data (1) indicate that not on animal level, but at batch level the proportion of infected animals can be an indicator for risk of PHE. What factors are responsible to provoke the acute PHE form in these already at least weeks earlier infected exposed animals is unknown. Quorum sensing can be the mechanism, triggered by known factors like feed changes, heat stress, and transportation. Under natural conditions previous infection does not give full protection against re-challenge with L. intracellularis. More investigation is needed to fully understand this devastating and economical important disease with for the farmer often emotional big impact.

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Serological profiles for porcine proliferative enteropathy in 3 farrow-to-finish pig farms in Taiwan

M-TChiou[1], C-N Lin[1], H Liu[1], C-H Yu[2], C-Y Yang[3], T-C Chang[1]
[1]*Department of Veterinary Medicine, National Pingtung University of Science and Technology,* [2]*Boehringer Ingelheim Taiwan Limited,* [3]*Division of Animal Medicine, Animal Technology Laboratories, Agricultural Technology Research Institute* mtchiou@mail.npust.edu.tw

Introduction

Porcine proliferative enteropathy (PPE), caused by *Lawsonia intracellularis*, has a considerable economic impact on pig production worldwide. PPE is characterized by diarrhea, progressive weight loss, weakness and increase of feed conversion rate typically affecting pigs during the growing and finish period. A serological survey revealed that 78% of pigs at the age of 3 month were seropositive in Taiwan during 2006 to 2012. The aim of this present study was to investigate the serological profiles for PPE between 3 to 27 weeks of age in 3 farrow-to-finish pig farms.

Materials and Methods

Three farrow-to-finish pig farms located at Central and Southern Taiwan with long-term diarrhea problems during the growing and finishing period were selected for this study. Blood samples from cranial vena cava were collected from 10 pigs randomly selected at each age group from 3 to 27 weeks of age by 3-week intervals in each farm. Serum was separated and placed into the vials after centrifuged. All the samples were stored at -20°C until use.

An Ileitis ELISA kit (bioScreen, Germany) was applied for the specific anti-*L. intracellularis* antibody detection. The methods and procedures of Ileitis ELISA were according to the manufacturer's instructions.

Results

A similar pattern for the seroprofile was exhibited in these 3 pig farms. The *L. intracellularis* seropositive rate of pigs was increasing from 15 weeks of age and reaching the highest positive rates at 24-27 weeks of age. Specific anti- *L. intracellularis* antibodies could not be detected at 3-12 weeks of age, then first seropositive animals were detected at 15 weeks of age and subsequently antibodies titers were marked elevated until the end of this study (Fig. 1).

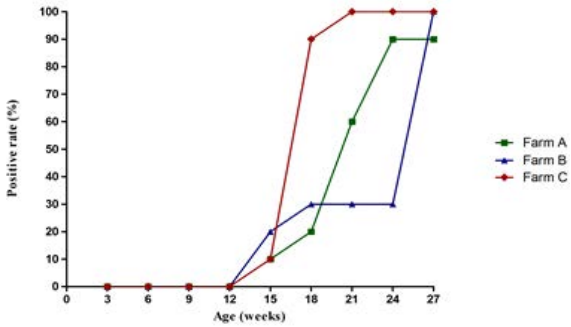

Figure 1. The *L. intracellularis* seropositive rate at different ages of pigs in 3 farms.

Conclusions and Discussion

Based on our data, PPE is highly prevalent in the farrow-to-finish pig farms, especially in growing and finish pigs. The data further indicates that pigs at 9-12 weeks of age and older were prone to infection with *L. intracellularis*. We speculate if pigs received PPE vaccination at weaning, it could provide adequate immune protection against *L. intracellularis* before 12 weeks of age.

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Field trial of a modified live vaccine against porcine proliferative enteropathy in Japan

T Morikoshi*[1], H Shimojima[2], A Wada[2], S Inagaki[1], K Karibe[1], T Shibuya[2]
[1] *Itochu Feed Mills;* [2]*BoehringerIngelheimVetmedica Japan*, asuka.koga.ext@boehringer-ingelheim.com

Introduction

Porcine proliferative enteropathy (PPE) is an enteric infectious disease caused by *Lawsonia intracellularis*. PPE appears in three forms, acute, chronic and subclinical. Rather than the rare acute form, which is characterized by severe hemorrhagic diarrhea and high mortality, the chronic and subclinical forms have been more of a concern, as they have a profound economic impact through considerable losses in daily gain and feed efficiency. The purpose of this study was to evaluate the effect of a live attenuated PPE vaccine (Enterisol® Ileitis) against losses associated with chronic and subclinical PPE.

Materials and Methods

The trial was conducted in a production system with 1138 breeding sows in the Aomori Prefecture, Japan. According to serology *L .intracellularis* infection occurred in this flow immediately after placement at finishing units at about 70 days of age. However no clinical signs for PPE were observed. About half of the finishing sites of the production system are run without use of antibiotics. In two of the sites where no antibiotics are used one batch of pigs vaccinated against ileitis was compared side-by-side with a non-vaccinated batch. Pigs vaccinated with Enterisol Ileitis at about 40 days of age via the drinking water using a proportioner. Individual weights and time to slaughter were recorded at slaughter per individual pigs. Based on overall weight gain and feed delivery feed conversion rate was calculated per treatment group and replicate. Pigs that were left on farm because they did not meet the minimum slaughter weight when the last regular pigs were shipped to slaughter were defined as runts.

Results

The vaccinated pigs showed numerically better performances in all parameters measured, and statistically significant in slaughter weight than the controls Table 1.

Table 1. Comparison of other parameters

	Average		
Parameter	Vaccinated	Non-vaccinated	Difference
Mortality (%)	3.63	3.65	-0.25%
Age (d)	176.41	177.32	-0.91
Runts (g)	8.7	10.4	- 1.7
Weight (kg)	111.85	109.38	2.48*
ADG (g)	640.84	619.46	21.37
FCR	2.53	2.66	0.13

*P<0.05

The feed conversion ratio (FCR) during fattening stage was improved by 0.11 and 0.15 in the first and second replicate comparing the vaccinated batches with the non-vaccinated batches. For the FCR from weaning to slaughter the improvements in FCR were estimated to be 0.08 and 0.11 for the first and second replicate.

Conclusions and Discussion

These results strongly indicated that the farm was negatively affected by chronic and subclinical PPE especially through losses in feed efficiency and weight gain and that the PPE vaccine had beneficial effects on production parameters. The improvement in FCR was more than sufficient to cover the vaccination cost, and these results are of particular significance in the face of increasing feed cost pressures. These results are in line with earlier studies on beneficial effect of vaccination on the market weight and ADG[1].

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M. hyopneumoniae prevalence in Belgian and Dutch pig herds using a tracheo-bronchial swab technique and eventual seasonal effects

F Vangroenweghe[1], D Maes[2], S Piepers[2], G Labarque[3]
[1]*Elanco Animal Health, BU Swine & Poultry, Anwerp, BELGIUM,* [2]*Swine Herd Health, Faculty of Veterinary Medicine, Ghent University, Merelbeke, BELGIUM, ,* [3]*Elanco Animal Health, R&D, Suresnes, FRANCE*
vangroenweghefr@elanco.com

Introduction

M. hyopneumoniae(M.hyo) – one of the main pathogens of the Porcine Respiratory Disease Complex (PRDC) – is still important in modern intensive swine farming in Europe (1). Diagnosis of mycoplasmal infections can be performed using different approaches: clinical signs, slaughterhouse checks of affected lungs, serological examinations of relevant animal groups or direct identification of the pathogen through polymerase chain reaction (PCR) techniques. Recently, a new sampling technique has been developed and validated for use in pigs, namely the tracheo-bronchial swab (TBS) technique (2). The aim of the present study was to obtain data on distribution of *M.hyo* infection throughout closed pig herds in Belgium and The Netherlands using the TBS technique during an entire year. Sampling was mainly focused on early diagnosis, since piglets can already be infected during suckling through the sow (3,4,5,6) and further spread of infection occurs after weaning (7,8).

Materials and Methods

Hundred and sixty-three closed pig farms were randomly selected through regular contacts with local veterinary practices. In every pig herd, at least 30 piglets were sampled in two age groups. The standard sampling protocol included 20 piglets at 3-5 weeks of age and 10-20 piglets in the 2nd half of the nursery stage (6-11 weeks of age). TBS were collected as described previously (2). The collected mucus was suspended into 1 mL of buffered saline solution and stored fresh until analysis. Real-time PCR (RT-PCR) analysis was performed according to the standard operating procedure of the laboratory (IVD GmbH, Hannover, Germany) (9) and PCR results were reported as negative or positive for the presence of *M.hyo*. The detection limit of the RT-PCR test was set at 300 DNA copies of *M.hyo* per mL of TBS suspension. Several weather data related to the specific sampling period were collected from a central weather station point in the Benelux. Statistical analysis was performed towards seasonal differences and effect of weather characteristics on *M.hyo* prevalence using using logistic regression models in SPSS 19.0 (SPSS, Inc. Headquarters, Chicago, Illinois, US).

Results

The overall prevalence of *M.hyo* at 3-5 weeks of age was 7.1%, increasing further to 10.9% at 6-11 weeks of age. The presence of *M.hyo* at the herd level at 3-5 weeks of age was significantly affected by the precipitation rate (β = -0.026; P = 0.03) during the week preceding the sampling. In older post-weaning piglets, the risk for a herd to be *M.hyo*-positive was significantly affected by the season (β = 0.65; P = 0.003). During autumn, an increased risk (OR = 1.98) for *M.hyo*-positivity could be observed, whereas during summer a decreased risk (OR = 9.8) was observed. The average outdoor temperature (β = 0.02; P = 0.007) during the week preceding the sampling was also related to *M.hyo*-positivity of the herd.

Conclusions and Discussion

In our study, the individual animal prevalence at 3-5 weeks of age was higher (7.1%) as compared to the study of Villarreal and coworkers (3) using nasal swabs (5.6%). This difference could be explained by the use of the more sensitive TBS technique. Furthermore, in the study of Villarreal et al., 2010 (3), only pig farms with typical clinical signs related to *M.hyo* were selected, whereas in our study, inclusion criteria did not require specific clinical respiratory problems. The results of the PCR testing of the TBS of the older piglets revealed an increasing prevalence of *M.hyo* during the post-weaning period, which is in accordance with other studies (7,8). The prevalence of *M.hyo* in Belgium and The Netherlands went up from 7.1% (3-5 weeks of age) to 10.9% (6-11 weeks of age). In accordance to Ségales et al. (10), effect of weather conditions could be observed on the prevalence of *M.hyo*.

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Economic impact of *M. hyopneumoniae* eliminations

PE Yeske

Swine Vet Center, P.A., 1608 S. Minnesota Ave, St. Peter, MN 56082 USA, pyeske@swinevetcenter.com

Introduction

Mycoplasma hyopneumoniae continues to be an economically important respiratory infection in swine. There are a number of different ways to eradicate Mycoplasma from herds. Adaptations to the Swiss method have also been successful at eradication. These would include herd closures allowing the herds to continue to farrow during the process[1, 2]. Vertical transmission studies demonstrate that transmission occurs up to 8 months post infection[3]. Herd closures have typically been 8-9 months duration and may or may not have off site breeding projects incorporated. These procedures have included a whole herd medication program at the end of the closure.

Another method is whole herd medication without herd closure using a long acting antibiotic such as Draxxin® (10% Tulathromycin, Pfizer), treating all pigs on site with an injection and repeating in 2 weeks[4]. The advantage of this approach is that the herd returns to negative status faster.

This study looked at economic advantage of *Mycoplasma hyopneumoniae* elimination.

Materials and Methods

- Identified 39 herds that had been through a *Mycoplasma hyopneumoniae* elimination procedure.
  - 27 herds used a herd closure and whole herd medication program.
  - 12 herds did a whole herd injection of medication program and no closure.
- All farms had negative replacement breeding stock available.
- Herd's mycoplasma status where monitored using: pigs in the finishing flow and blood testing just prior to marketing.
  - Clinical signs of Mycoplasma (coughing) in sow herds or finishing pigs.
  - Serology of pigs at the end of the finishing phase.
    - Using IDEXX test if vaccinated
- Data from a production system that tracks health status in finishing was used to model the economic outcomes of the elimination process.

Results

Herd closure and whole herd medication was 89% and 67% successful overall respectively. The average "survivability" of herds in the study was 47 and 34 months respectively as shown in Table 1.

Table 1. *Mycoplasma hyopneumoniae* elimination success in 39 farms by type:

	Herd Closure	Medication	Total
Number of Sows	71000	23000	94050
Number of Herds	27	12	39
Percent Negative at 1 year	96%	75%	90%
Percent Negative to date	89%	67%	83%
Average Months negative	47	34	43

Table 2. Difference in performance between *M. hyopneumoniae*(+) and (-) pigs

Per 1000 sows	Myco (+)	Myco (-)	Difference
Finishing Mortality	3.6%	2.2%	-1.4%
Finishing Culls (Underweight MKT)	2.4%	1.4%	-1.0%
Total Pigs Sold	25614	26281	666
Cost of Treatments	$ 0.63	$ 0.37	$ (0.26)
Finish ADG	1.76	1.87	$ 0.11
F/G	2.73	2.65	$ (0.08)

Table 3. Economic impact of *Mycoplasma hyopneumoniae* elimination.

Grow Finish Performance Opportunity		Per Pig
Treatments savings / Total	$ 7,280	$ 0.28
Total Dead Pigs	386	
Cost of Mortality	$ 73,783	$ 2.81
Reduced number of culls (head)	256	
Cull opportunity $	$ 24,455	$ 0.93
Cost of Performance		
Cost ADG	$ 38,137	$ 1.45
Cost F/G	$ 48,155	$ 1.83
Total Finisher	$ 191,810	$ 7.30
Whole Herd Opportunity Cost Impact		
Total (Finisher)	$ 191,810	$ 7.30
Cost per Sow		$ 191.81

Table 4. Return on Investment of *Mycoplasma hyopneumoniae* elimination.

Myco Elimination per sow Closure	$ 7.50
ROI to 1	26
Months to break even	0.47
Myco Elimination per sow Medication	$ 30.00
ROI to 1	6
Months to break even	1.88

Discussion

Mycoplasma hyopneumoniae elimination programs have had a good success rate with 89% of closures and 67% of medication programs being successful. Not only does production improve but so does profitability as demonstrated in the economic model and a good return on investment. Although there is always risk of failure the reward is good and success rates have been reasonable.

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Seroconversion after two different vaccination schemes against *M. hyopneumoniae* in two seronegative farms

A Pausenberger[1], T Frey[2], N Uebel[3], P Sanchez[4], G Gonzales Garcia[1], M Ritzmann[3], M Wendt[5]
[1]Elanco Animal Health, Bad Homburg, Germany, [2]Swine Health Service Baden-Wuerttemberg, Fellbach, Germany, [3] Clinic for Swine, Ludwig-Maximilians-University, Munich, Germany, [4]Elanco Animal Health,Alcobendas, Spain, [5] Clinic for Swine, Small Ruminants, Forensic Medicine and Ambulatory Service, University of Veterinary Medicine Hannover, Foundation, Germany, pausenberger_astrid@elanco.com

Introduction

Serological response to *M. hyopneumoniae(M.hyo)* infection or vaccination against *M.hyo* cannot be distinguished by usual test systems and is often hard to interpret due to great variation, especially in farms with low or no infectious pressure. The objective of this study was to display and compare serological responses after one shot and two shot vaccinations and unvaccinated control groups in two seronegative farms.

Materials and Methods

In two farms tested seronegative for *M.hyo* regularly, piglets were randomly allocated to different vaccination groups:
Farm A 100 piglets
-Group Aa (n=40) two shot on day 3 and 20
-Group Ab (n=40) one shot on day 20
-Group Ac (n=20) unvaccinated control
Farm B 80 piglets
-Group Ba (n=20) two shot on day 7 and 21
-Group Bb (n=20) one shot on day 7
-Group Bc (n=40) unvaccinated control
Blood samples were taken at:
-Farm A on days 2, 49, 77 of life
-Farm B on days 26, 75, 118 of life
Samples were tested by IDEXX ELISA with S/P <0.3 negative, 0.3-0.4 suspicious, >0.4 positive.
Data were statistically analyzed using the JMP® statistical software version 9.0.3 (SAS Institute Inc., Cary, NC, USA).

Results

Apart from 4 late positive results from the one shot group on farm B, S/P ratios of one shot-groups as well as of control groups remained under the cut-offs, whereas roughly half (56% resp. 65%) of the two shot-vaccinated piglets tested positive or suspicious after vaccination in at least one of the samples. No or only small differences were found in seroconversions between the unvaccinated control and the one shot-groups.
On farm A a significant statistical difference ($p<0.05$) was found on days 49 and 77 between the two shot-group on one hand and the one shot and control group on the other hand, which did not differ significantly (Table 1).

Table 1. Means and Standard Deviations (SD) of IDEXX ELISA S/P ratios (Farm A)

Farm A	Mean/(SD) day 2	Mean/(SD) day 49	Mean/(SD) day 77
Aa two shot	0.1196[a] (+/- 0.22) (n=40)	0.5106[b] (+/-0.34) (n=32)	0.3026[c] (+/-0.22) (n=21)
Ab one shot	0.0976[a] (+/- 0.08) (n=40)	0.0521[a] (+/-0.07) (n=34)	0.0629[a] (+/-0.06) (n=29)
Ac control group	0.0967[a] (+/-0.08) (n=20)	0.0270[a] (+/-0.06) (n=17)	0.0556[a] (+/-0.06) (n=12)

On Farm B a significant statistical difference ($p<0.05$) was found between the two shot and control group on days 75 and 118. Differences between two shot and one shot on day 75 as well as between one shot and control group on day 118 were also significant ($p<0.05$) (Table 2).

Table 2. Means and Standard Deviations (SD) of IDEXX ELISA S/P ratios (Farm B)

Farm B	Mean/(SD) day 26	Mean/(SD) day 75	Mean/(SD) day 118
Ba two shot	0.4035[a] (+/-0.16) (n=20)	0.1810[a] (+/-0.20) (n=20)	0.5625[a] (+/-0.36) (n=20)
Bb one shot	0.1280[b] (+/-0.08) (n=20)	0.0532[b] (+/-0.06) (n=19)	0.3456[a] (+/-0.36) (n=16)
Bc control group	0.0188[b] (+/-0.04) (n=40)	0.0121[b] (0.04) (n=39)	0.0700[b] (+/-0.12) (n=38)

Conclusions and Discussion

Nearly no measurable seroconversion could be detected after one shot vaccination. This does not implicate lack of efficacy as the level of seroconversion is not correlated to the level of protection (1). Seroconversion in farms so far negative for M.hyo and without any clinical symptoms could indicate that animals had received a two shot vaccination.

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Respiratory disease in finishers – comparisons of diagnostic tools

SS Jakobsen[1], CK Hjulsager[1], CS Christensen[2], P Lind[1], H Bak[2], LE Larsen[1]
[1]*National Veterinary Institute, Technical University of Denmark, Denmark;*[2] *Pig Research Centre, Danish Agriculture & Food Council, Kjellerup, Denmark.* [2]*Boehringer Ingelheim, Copenhagen, Denmark.* hanne.bak@boehringer-ingelheim.com

Introduction

Respiratory disease in pigs is a worldwide economic problem due to increased mortality, decreased growth rate, increased feed intake, costs for vaccines and antibiotics and increased work load (1, 3).

The disease has been termed Porcine Respiratory Disease Complex (PRDC) and is caused by a complex interplay between the animal, environmental factors and multiple pathogens of which Mycoplasma hyopneumoniae (M hyo), Porcine Circovirus 2 (PCV2), Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), Actinobacillus pleuropneumoniae (APP) and Swine Influenza Virus (SIV) play the major role in Denmark.

To apply the most appropriate measures to control, prevent and treat the disease, it is essential to identify the pathogens circulating in a given herd. The aim of this study was to investigate the correlation between post mortem findings at slaughter, clinical symptoms and the detection of respiratory pathogens using oral fluids (OF) and serology.

Materials and Methods

In 4 Danish herds (table 1), 8 batches of pigs were sampled every 2 weeks during the finishing period for OF, and at each sampling, a clinical index of cough (CI) was measured by the standards set by (2). Just before slaughter of each batch, 20 blood samples were obtained by random selection, and 20 pigs were randomly selected for post mortem examination.

OF was analyzed by PCR for detection of SIV, PRRSV, PCV2 and M hyo. Blood samples were analyzed for antibodies against M hyo using ELISA (National Veterinary Institute, Frederiksberg, Denmark). Statistical analysis was carried out using Microsoft Excel, with p=0.05 set as level of significance.

Table 1.Survey of the herds included in the study. For each herd, 2 batches were included.

ID	Type [a]	Pigs/ batch	% mort	ADG [b]	FCR [c]	Vac-cines
A	FF	180	2.8	861	3.21	Mhyo
B	FF	200	3.4	983	2.94	Mhyo
C	FIN	304	2.0	891	2.77	PCV2
D1	FIN	350	1.6	910	2.60	None
D2	FIN	250	1.7	958	2.58	None
Mean value Denmark			3.6	905	2.78	

[a] FF: Farrow to Finish, FIN Finishing herd. [b] Average Daily Gain (g/day). [C] Feed Conversion Rate, FE/kg gain

Results

There was a significant correlation (P=0.033) between number of M hyo antibody positive pigs at slaughter and peak index of cough during the finishing period, but a significant negative correlation (P=0.0002) between weeks from peak index of cough until slaughter and the percentage of pigs with Myco-plasma-like lesions at slaughter. Hence, a high number of M hyo antibody positive pigs results in a higher prevalence of lesions, but if coughing occurs early in the finishing period, the lesions heal off before slaughter.

The intensity of coughing as measured by CI has a significant correlation to lung tissue scars at slaughter, shown by a significant correlation (P=0.043) between peak index of cough during the finishing period and the percentage of pigs with lung tissue scars.

For pleurisy lesions, there was significant correlation (P=0.012) between time lapsed from peak levels of PCV2 until slaughter and the percentage of pigs with pleurisy at slaughter. Furthermore, there was a significant correlation (P=0.029) between weeks from peak index of cough until slaughter and the percentage of pigs with pleurisy at slaughter.

Conclusions and Discussion

Post mortem examinations are a valuable tool for gathering information on the respiratory health status in herds, but do not provide sufficient information on which pathogens that are circulating in the herd. Oral fluids is an easy non-invasive and reliable tool for diagnosing viruses, however if you want to know if M hyo is present it is better to obtain blood samples or to examine a piece of lung by PCR.

It need to be kept in mind that this study was conducted in herds that were seropositive for M hyo, and that the results may be different when applied on farms that are seronegative.

Acknowledgements

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Dynamics of *M. hyosynoviae* detection and clinical presentation

J Schwartz[1], L Bruner[2], B Evelsizer[2], B Konz[2], A Rovira[1], M Pieters[1]
[1]*University of Minnesota, College of Veterinary Medicine, St. Paul, MN,* [2]*Swine Vet Center,St. Peter, MN,* [3]*Novartis Animal Health, Greensboro, NC,* schwa851@umn.edu

Introduction

Mycoplasma hyosynoviae (*Mhs*) infection is known to cause arthritis and lameness in pigs[1]. Discomfort associated with arthritis compromises animals' welfare and costs producers in the form of increased antibiotic usage and losses close to market. However, little information is available regarding the epidemiology and diagnostics of this pathogen, despite an increased prevalence in recent years. Therefore, this investigation was proposed with the objectives to: 1) Evaluate *Mhs* detection dynamics across a naturally challenged flow within a production system. 2) Assess the correlation of diagnostic data and incidence of clinical signs.

Materials and Methods

A production system in Minnesota with a PRRSV positive flow and a history of *Mhs* challenges was chosen for this study. Sampled groups were: GDU at 0, 4, 8, 12 &16 weeks post placement; Sow farm, where 30 gilts/sows, 10 P0's, 10 P1/2, 10 P3+ and one piglet of their progeny were sampled. Piglets from the sow farm were serially tested at 0, 3, 5 & 7 weeks of age. Pigs at 10, 13, 16, 19 & 22 weeks of age within same flow were sampled cross-sectionally. Within each group, 10 oral fluid and 30 tonsil swabs were collected and a lameness evaluation was performed. Ten random pens were selected per sample group, from which 3 tonsil swabs and 1 oral fluids sample was collected. Swabbing was directed at lame pigs within a randomly selected pen. Clinical evaluation of gait was performed on pen and barn basis, and lameness was expressed as a function of severity and percentage of lame pigs. Pigs were assigned a lameness score from 0-4[2]. All samples were assayed by real-time PCR for *Mhs* at the UMN-VDL. The correlation coefficient between diagnostics and clinical presentation was evaluated by Pearson's analysis.

Results and Discussion

Results obtained from all age groups, sample types and clinical evaluation data are presented in Table 1. Sixty percent of sows tested positive, while their progeny were negative at 1-2 days of age, and 13% of the piglets tested positive at 3 weeks of age. The decline in % positive samples in week 5 could be the result of antibiotic treatment. Oral fluids yielded consistently lower Ct values and greater % positive samples (compared to tonsil swabs); suggesting it is a more sensitive tool for detection of *Mhs* colonization. Tonsil swab results were correlated with lameness score (R=0.82). Our results showed piglets were colonized prior to weaning, making it difficult to source *Mhs* negative piglets from a positive sow farm, and suggesting that colonization in the sow farm may play a role in the clinical presentation downstream. In this pig flow, week 16 GDU is operated as a continuous flow; causing potential exposure, shedding and propagation of the pathogen into the sow farm with the arrival of gilts. An increased % positive oral fluids and tonsil swabs at week 19, with a mild increase in clinical presentation suggests that surveillance and subsequent intervention may be a means of preventing severe clinical presentation in finishing.

Table 1. Percentage of positive pigs for *Mhs* in tonsil swabs and oral fluids by PCR and prevalence of clinical lameness at the time of sampling.

Sample Group		Tonsil Swabs	Oral Fluids	Lameness
GDU	Week 0	6.67	66.7	3.42
	Week 4	53.3	100	18
	Week 8	30	90	4.98
	Week 12	23.3	100	5.67
	Week 16	50		
Sow Farm	Dams	60		
	Newborns	0		
	Week 3	13.3		
Grow-Finish	Week 5	3.4	20	1.2
	Week 7	20	50	1.35
	Week 10	3.3	10	5.12
	Week 13	0	30	4.89
	Week 16	0	20	6.93
	Week 19	46.7	100	9.34
	Week 22	66.7	100	16.67

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Effect of *M. hyopneumoniae* lung lesion score on pig performance and carcass value

F Bravo de Laguna[1], A García[2], R Bautista[2], R Santamaria[3], M Jimenez[3], R Menjón[3]
[1]*Nutreco R&D,* [2]*Inga Food S.A,* [3]*MSD Animal Health*
ruth.menjon.ruiz@merck.com

Introduction

The main objective of the current study was to assess the efficacy of vaccination against M. hyopneumoniae, by evaluating the impact on pig performance, lung lesion score, and economical impact.

Materials and Methods

The present study was performed in a farm located in the region of Aragón (Spain) which tested positive for *M. hyopneumoniae.* In total, 1497 piglets started the trial with an initial body weight of (mean ± CI 95%) 5.95 ± 0.06 kg. Piglets were allocated in groups so that 50% of the animals of each group were vaccinated and the other 50% not. All the piglets were vaccinated against Circovirus in the first days of life. Piglets were vaccinated against *M. hyopneumoniae* with a single dose of either M+PAC® (MSD Animal Health), vaccine A, or control group. Following parameters were observed: individual body weight at weaning, at start and end of the fattening period; average daily gain (ADG); lung lesion score on a scale of 0 (absence of lesions) to 5 (very damaged), NS (very severe degree of pleuritis and pneumonia); economical impact of lung lesions (1). During the study period, the mean carcass price per kg was 1,742 €, with a 77% mean carcass performance. The Linear Method (GLM: program SPSS 15.0) was used for statistical analysis.

Results

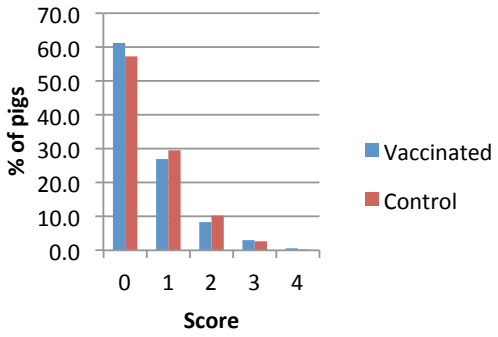

Figure 1. Percentage of pigs which every score, in total vaccinated pigs (both groups) vs control group

The lung lesion scores were significantly different (P<0.05) between M+PAC® and control treatment, with M+PAC® pigs having fewer lesions. Overall, 61.2% of all vaccinated animals (combined M+Pac and Vaccine A) had a score of 0, compared to 57.3% of the control animals and more control animals had a score of 1 or 2.Specifically, 64.0% of the M+PAC® animals, and 58.5% in Vaccine A animals had a score of 0.

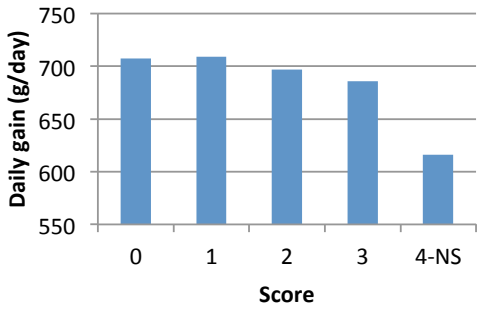

Figure 2. Relation between ADG and lung lesion score (all pigs)

Score 4 or NS (not scored) resulted in a decrease of 91 g/day in ADG compared to score 0 lesions. Based on this, the economical impact of this loss of performance was estimated. Compared to score 0 pigs, score 4 pigs would have a 12.3 kg lower slaughter weight and a 9.48 kg lower carcass weight; resulting in an average of 16.5 €/pig less per carcass.

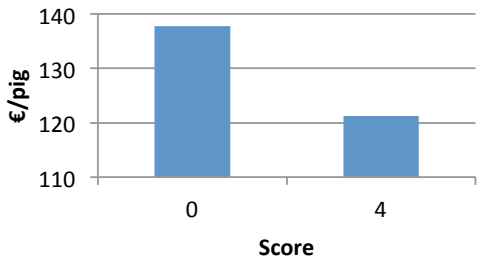

Figure 3. Estimation of carcass value of animals with a lung lesion score of 0 or 4.

Conclusions and Discussion

The effect of *M. hyopneumoniae* lung lesions on the average daily gain causes a considerable loss of carcass value. Therefore, implementation of *M. hyopneumoniae* vaccination programs is justified to improve return on investment.

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Study of the proteins in the supernatant of *M. hyopneumoniae* cultures with biological activity

J Camacho [1], E Hernández-Baumgarten[1], RE Miranda[2], F Sotres[1], R Alonso[2], H Lara[3], F Quezada[3], L Serrano[1], Trujillo D[1], González S[1] Ciprián A[1], Mendoza S[1]
[1]*Facultad de Estudios Superiores Cuautilán-UNAM;* [2]*Facultad de Medicina Veterinaria y Zootecnia-UNAM,* [3]*Laboratorios Avi-Mex, SA de CV. México.* jecm_iztla@yahoo.com.mx,

Introduction

Mycoplasma hyopneumoniae (*M. hyo*) is the etiological agent of enzootic pneumonia and it is considered a primary pathogen if the respiratory complex of pigs; a multifactor complex of chronic respiratory diseases with many unknown factors associated with important economic losses to the pig producers around the world (1,5). In recent times there are reports of numerous proteins from *M. hyo* with variable molecular weight ,(p7413, p7048, p6523, p4026, p467, p159, p102, p97, p89, p74, p72, p70, p65, p60,p54 p46, p42, p41, p36 kDa.), in the cell membrane, and, cytosol and it has been demonstrated that it is capable of secreting some proteins to the liquid culture medium. These proteins have different biological activities, some are adhesins, capable of causing cytopathic effect and/or immunologic activity (1,7,9*)*. For this reason it is essential to have more information over products that the microorganism may secrete into the medium to understand its biological activities and broaden the picture and better understand this etiologic agent.

Materials and Methods

The reference strain of *M. hyo* (strain J, NCTC 10110) was utilized through this study and was grown in Friis broth (3) The number of viable organisms was determined by a series of tenfold dilutions in Friis broth and the titer is expressed in color changing units (CCU). One 500 ml of Friis medium was inoculated with 10^5 CCU/5ml. A similar flask was left as control and were incubated in a shaker incubator at 37°C. From each culture an 8 ml simple was withdrawn on days 3,5,7,9,11,13,15,17,19 and 21 of growth. Each simple was centrifuged at 30,000g for 30 minutes. The supernatants were tested by SDS-PAGE as described by Laemli (1970) with a polyacrylamide gel gradient from 3 to 12%. The separated proteins were immune-transferred to nitrocellulose membranes (inmobilon-P Millipore) (8). The membranes were blocked with 5% skim milk and incubated for one hour with known positive serum diluted 1: 25. For one hour, washed and incubated with a conjugated anti-pig serum labeled with peroxidase, diluted 1:200 the color was developed with 4-chloro-1-naphtol.

Results

In the SDS-PAGE performed on both cultures, there were proteins detected. In the culture with the *M. hyo* complete a 97 KD protein was detected both in the complete microorganism as well as in the supernatant. The immunotransference was conducted with the supernatants and a 30kD band was observed from day 7 onward of bacterial growth.

Conclusions and Discussion

This datum is similar to the report by Assuncao *et al* 2005, only that they worked with whole cells. Okada (6) working with the supernatants produce an antiserum to these proteins, and also proving that the *M. hyo* secrets antigenic proteins in the growth medium. This work will be continued to detect some biological activities of these proteins.

Acknowledgements

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Development of a co-agglutination test for the serology of *M. hyopneumoniae* in farm pigs

L Serrano[1], E Hernández-Baumgarten[1], RE Miranda[2], F Sotres[1], R Robles[1], H Lara[3], F Quezada[3], J Camacho[1], D Trujillo[1], S González[1], A Ciprián[1], S Mendoza[1]

[1]*Facultad de Estudios Superiores Cuautilán-UNAM;* [2]*Facultad de Medicina Veterinaria y Zootecnia-UNAM,* [3]*Laboratorios Avi-Mex, SA de CV. México.* charbander@hotmail.com

Introduction

Mycoplasma hyopneumoniae is considered as the primary agent of the Enzootic chronic pig pneumonia (NE); characterized by a high morbidity and low mortality. Pigs of all ages are susceptible (Ross, 1984; Clarck, 1999; Ciprián *et. al*. 2005). Prevalence is high in in mid-finished pigs (between 14 and 20 weeks) (Sibila *et al*., 2009). In order to have a final diagnosis of this disease we need to combine clinical history macroscopic and microscopic lesions and isolation of the agent involved. There are no field tests for this disease, and our aim is to develop a simple and fast test for this purpose. (Ross, 1999; OIE, 2004).

Materials and Methods

The microorganism was propagated in Friis medium (reference strain SJ) and grown massively in order to have enough biomass for the trial. Afterward it was and the antigen was reacted to latex microparticles and the proper union between bacteria and latex particles was confirmed by electron microscopy. For determination of sensitivity and specificity, positive sera obtained from previous experiments were used. Finally, the results were compared with an ELISA test.

Results

The method for solid and liquid media and massive growth of the organism was successfully standardized for the *Mycoplasma hyopneumoniae* SJ strain.

The union of the antigen and the latex particles was standardized and the union between the two was measured by electron microscopy.

The trial was carried with reference antisera, with the positive serum giving a positive agglutination, and the negative serum a negative agglutination. The test is being run with the various sera to establish sensitivity and specificity.

Conclusions and Discussion

The diagnostic of *M. hyopneumoniae* from a simple coagglutination test can be a rapid and inexpensive that allows us to identify this microorganism in the farm thus helping us define the agents causing respiratory problems.

Acknowledgements

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Intradermal vaccination of piglets against *M. hyopneumoniae* with the needle-free IDAL device; clinical evaluation

L Beffort[1], M Ritzmann[1], K Fiebig[2], M Eddicks[1]
[1]*Clinic for Swine, Ludwig-Maximilians-University, Oberschleissheim, Germany,* [2]*MSD Animal Health, Unterschleissheim, Germany,* m.eddicks@lmu.de

Introduction

Mycoplasma hyopneumoniae (M hyo) is widespread in the pig population and leads to major economic losses due to reduced daily weight gain. Vaccination is an important strategy to control the clinical diseases associated with M hyo. Needle-free injection eliminates not only the risk of needle residues in the pork carcasses (1) but also reduces the haematogenous transmission of infectious diseases (2). The aim of the present study was to assess the safety and efficacy of the inactivated M hyo vaccine, Porcilis® M Hyo ID ONCE.

Materials and Methods

The study was carried out in a commercial 1000 sow farrow-to-finish farm in Germany. The piglets were allocated to 2 vaccine groups (VC1 (n = 138): Porcilis® M Hyo ID ONCE, IDAL; VC2 (n = 144): M+PAC®, i.m.) and 2 control groups (CC1 (n = 70): Diluvac forte, IDAL; CC2 (n = 68): Diluvac forte i.m.) receiving only adjuvant at 3 weeks of age. As safety parameters the diameter, the consistency of the local induration at the injection site and signs of inflammation such as redness and calor were assessed for seven days after vaccination. Bodyweight (BDW) and average daily weight gain (ADWG) were measured as efficacy parameters. For efficacy parameters the CC1 and CC2 were combined to one control group.

Results

In general, the observed injection site reactions (ISR) were of minor extent with a maximum diameter of 1.5 cm. The amount and quality (diameter and consistency) of injection site reactions were not significantly different between the animals within the 2 control groups. The amount of ISR was higher in the vaccinated groups compared to their respective controls ($p \leq 0.035$). The quality of the ISR concerning diameter and consistency was more severe in the animals from the VC1 compared to VC2 ($p = 0.001$ and $p < 0.001$ respectively). No systemic side effects were observed in any pigs after vaccination.

The mean body weight (BDW) at the end of finishing as well as the ADWG within the finishing period and over the entire study period was significantly higher for the animals of the vaccinated groups compared to the animals of the control. Within the animals of the vaccinated groups no significant differences concerning BDW and ADWG were observed (table 1).

Table 1. Performance parameters: ADWG (g/day) and BDW (kg).

	Period	VC1	VC2	CC
BDW	3.week	6.25	6.18	6.24
ADWG	3-11. week	434.82	438.12	422.69
BDW	11. week	30.16	30.28	29.49
ADWG	11-24. week	913.44[*1]	924.54[*2]	875.57[*3]
BDW	25. week	112.30[*4]	113.49[*5]	108.29[*6]
ADWG	3.-25. week	731.89[*7]	740.03[*8]	703.79[*9]

[*]1,[*]3; [*]2,[*]3 $p \leq 0.005$; [*]4,[*]6; [*]5,[*]6 $p \leq 0.005$; [*]7,[*]9; [*]8,[*]9 $p \leq 0.004$

Conclusion and Discussion

The study results support that the intradermal administration of Porcilis® M Hyo ID ONCE is efficacious by improving the performance parameters average daily weight gain and bodyweight at the end of finishing. There were no differences concerning the performance parameters between intramuscularly or intradermal vaccinated piglets. Furthermore it could be shown that the needle-free intradermal administration is safe for pigs at three weeks of age. With respect to the differences concerning the ISR between the vaccination groups these findings are probably due to differences in vaccine formulations and not due to the way of application as both control groups (no differences observed) received the same adjuvant.

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Seasonality effect and dynamics of *M. hyopneumoniae* infection

J Bringas[2], A Vidal[1] , J Jovellar[1] , P Nuñez[2], P Sánchez[2], I Huerta[2]
[1]*Vall Companys* ,[2]*Elanco Animal Health, Spain*, nunez_ulibarri_pedro@elanco.com

Introduction

Mycoplasma hyopneumoniae (M. hyo) is the primary pathogen of swine enzootic pneumonia (SEP), a chronic respiratory disease and one of the most widespread and costly diseases in intensive pig production systems (1,2). Nowadays, there is a lot of information on comparison of methods to evaluate the prevalence and extent of pneumonia lesions in lungs and its correlation as a reliable screening tests (6,7), moreover, another study (5) carried out by Pallares et al., in the south east of Spain, showed as well a strong relationship of macroscopic evaluation of the lungs with histopathological and inmunocytochemical diagnosis ($p<0.001$). The main objective of this study was to demonstrate the impact of seasonality on incidence and M.hyo like maximum lung lesions on the production system.

Materials and Methods

A total of 48.778 pigs were included on this study, all of them Non-Vaccinated, coming from the north-east of Spain, all farms were tested positive to M.hyo. The period of the study was from April 24th of 2012 to August 2nd of 2013, the lungs of fattened pigs were examined in the slaughterhouse and the severity of the lung lesions was evaluated using the 0 to 5 method as described previously (3), and all of them were scored by the same person, a highly trained veterinary to avoid differences. The experimental unit on this study is the fattening site. €. Data were statistically analyzed using the JMP® statistical software version 9.0.3 (SAS Institute Inc., Cary, NC, USA).

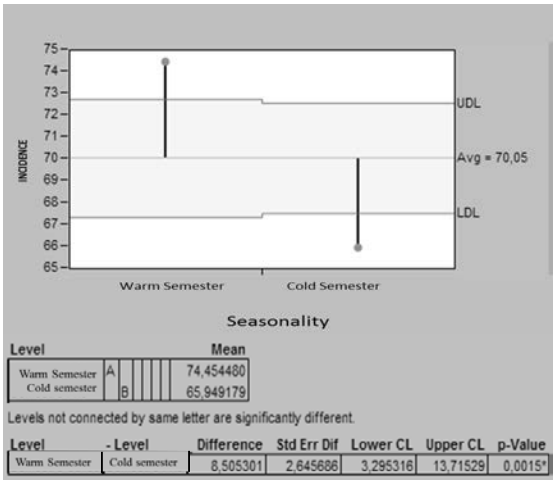

Figure 1. Incidence due to seasonal variation.

Results

Seasonality in respiratory diseases is something expected and accepted; but not described in many studies, M.hyo dinamycs are very variable and they can vary for several factors, such as production system, infection pressure on the farm, management and season (8). In this study if data was organized by warm (spring and summer) and cold (fall and winter) semesters, statistically differences were found depending on its incidence (figure 1) and on its maximum lung lesions score as shown on figure 2.

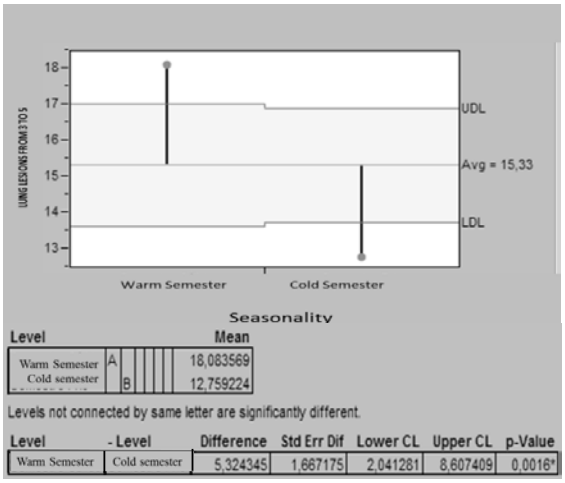

Figure 2. Seasonality due to maximum lung lesions.

Conclusions and Discussion

SEP incidence and maximum lung lesions are affected by seasons and statistical differences have been found between warm and cold seasons on mycoplasma dynamics. In this case, piglets that have been raised on cold weather they probably got higher incidences and maximum lung lesions than the ones that have been raised on warm weather.

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Relationship between pig performance and *M. hyo* like lung lesions score at slaughter influenced by its incidence

J Bringas[2], A Vidal[1] , J Jovellar[1] , P Nuñez[2], P Sánchez[2], I Huerta[2]
[1]*Vall Companys,* [2]*Elanco Animal Health, Spain,* nunez_ulibarri_pedro@elanco.com

Introduction

Mycoplasma hyopneumoniae (M. hyo) is the primary pathogen of swine enzootic pneumonia (SEP), a chronic respiratory disease and one of the most widespread and costly diseases in intensive pig production systems (1,2). Nowadays, there is a lot o
f information on comparison of methods to evaluate the prevalence and extent of pneumonia lesions in lungs and its correlation as a reliable screening tests (6,7), moreover, another study (5) carried out by Pallares et al., in the south east of Spain, showed as well a strong relationship of macroscopic evaluation of the lungs with histopathological and inmunocytochemical diagnosis ($p<0.001$). The main objective of this study was to establish the impact of M.hyo like lung lesions incidence due to *Mycoplasma hyopneumoniae* on pig performance on the production system.

Materials and Methods

A total of 48.778 pigs were included on this study, all of them Non-Vaccinated, coming from the north-east of Spain, all farms were tested positive to M.hyo. The period of the study was from April 24th of 2012 to August 2nd of 2013, the lungs of fattened pigs were examined in the slaughterhouse and the severity of the lung lesions was evaluated using the 0 to 5 method as described previously (3), and all of them were scored by the same person, a highly trained veterinary to avoid differences. The experimental unit on this study is the fattening site. The production parameters evaluated on this study were: ADG (Average Daily Gain), EFCR (Economical Feed Conversion Ratio), % Mortality and medication cost in €. Data were statistically analyzed using the JMP® statistical software version 9.0.3 (SAS Institute Inc., Cary, NC, USA).

Results

The prevalence of pneumonia ranges, depending on the batch, from 15% to 100%, with an overall prevalence of 69.9%. For each batch, an average of all the production parameters was calculated, as shown in table 1, Batches were classified in 4 different interval groups by quartiles: <55%, 56-76%, 77-88% and >88% of incidence. Different publications (2,4,8) has shown as well the negative correlation of the pneumonic lesions on the ADG, in this study between the highest prevalence and the lowest a statistically significant difference of 43 grams have been found. In terms of EFCR between the group of <55% and the >88% the difference was 50 grams, also statistically differences on mortality between the batches of lower than 55% of incidence and the batches with greater than 76% have been found. On medication cost the differences were significant between the batches with incidences fewer than 76% and the ones greater than 76%.

Table 1. Pig performance, according to the incidence intervals.[1]Levels NOT connected by the same letter are significantly different

Incidence intervals	ADG	Economical FCR	% Mortality	Medication cost
<55	687 [a]	2.51 [a]	2.41 [a]	1.93 [a]
55-76	671 [b]	2.54 [ab]	2.76 [ab]	1.98 [a]
76-88	662 [b]	2.52 [ab]	3.01 [b]	2.43 [b]
>88	645 [c]	2.56 [b]	3.31 [b]	2.77 [c]

Conclusions and Discussion

In this study the ADG and the medication cost have been the two parameters more affected by the M.hyo like lung lesions, although statistical differences have been found in all the production parameters evaluated. Going to slaughterhouse and knowing more our SEP prevalence and choosing the right decisions to control the incidences will be an strategic movement to get an optimal efficiency.

Acknowledgments

The authors are grateful to the slaughterhouse and the herd veterinarian for their collaboration.

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P.264

Different profiles of pathogenicity among eight isolates of *P. multocida* A on the experimental reproduction of pneumonia and pleuritis in pigs

JX de Oliveira Filho[1]; J Bassani[3]; J Lazaroto[4]; MAZ Morés[2]; R Rebelatto[2]; C Klein[2]; DESN de Barcellos[1]; N Morés[2]
[1]Department of Animal Medicine at UFRGS, Brazil; [2]Embrapa Swine and Poultry, Brazil; [3]Agro Veterinary Sciences Centre at UDESC, Brazil; [4]Department of Animal Science at CAV/UDESC, Brazil, david@ufrgs.br

Introduction

Pasteurella multocida (PmA) is one of the bacterial agents most commonly isolated from pneumonic lesions in pigs. However, its role as a primary agent of pneumonia remains unclear (2). The present study aimed to evaluate the clinical and pathological picture of the disease in specific pathogen-free pigs (SPF) challenged with eight different PmA strains.

Materials and Methods

The experiment complied with Ethical Principles in Animal Experimentation, and was approved by the Ethics Committee on Animal Experimentation (CEUA/CNPSA) (Protocol #005/2010). Sixty-four pigs of approximately 120 days of age, from an SPF herd free of PmA and D, *B. bronchiseptica*, *A. pleuropneumoniae* (App), *H. parasuis* (Hps), *M. hyopneumoniae* (Mhyo) and influenza A virus were equally distributed into eight groups **(G1 a G8)**. Each group was challenged with an isolate of PmA obtained from animals with lesions of pneumonia (A to H) assigned to six experimental groups (E): **E1**: G1-A e G2-B; **E2**: G3-C e G4-D; **E3**: G5-E; **E4**: G6-F; **E5**: G7-G; e **E6**: G8-H. Two control pigs were included in each experimental group (G0) and inoculated with sterile saline (total of 12 pigs). Each pig from groups G1 to G8 received 3.0 mL (1.5 mL/nostril) of the respective inoculum with 10^7 CFU/mL of PmA, administered by slow intranasal drip. All pigs were clinically evaluated twice daily (rectal body temperature - TR, dyspnoea and coughing), starting from the 3th day before inoculation until the 5th day post-inoculation (5dpi) when they were euthanized by electrocution, bled and necropsied. Several organs portion were collected to histopathological and bacteriological assays.

Results

There were not clinical changes in all animals in the pre-challenge period. Fever (TR $\geq$ 40 º C) and dyspnoea were the most frequent clinical sign presented by animals in the challenged groups, with the exception of G0, G6 and G8 that remained healthy. Three distinct patterns at necropsy were observed, associated or no: 1. fibrinonecrotic cranioventral bronchopneumonia-Bp (G1, G3, G7); **2.** Diffuse pleuritis associated or no with pericarditis and peritonitis (G3, G5, G7); **3.** Locally extensive necrosuppurative pleuropneumonia (G1, G2; G3, G4, G7). The severity of clinic pathological changes classified the PmA strains in: highly pathogenic (A, B, C e G); low pathogenicity (D e E); and not pathogenic (H e F). Septicemia occurred in several pigs of G1, G2, G3 e G7, characterized by septic microthrombi in liver and kidneys, with isolation of PmA. Additionally, two animals presented otitis interna with profuse isolation of PmA.

Conclusions and Discussion

Herein it was demonstrated that PmA may act as a primary agent of bronchopneumonia and polyserositis in pigs, evolving to septicaemia. Although PmA has been considered as a secondary agent of pneumonia in pigs. (2), animals challenged with eight different strains of PmA showed three different pathogenic profiles: highly pathogenic strains (A, B, C e G); low pathogenicity (D e E); and not pathogenic (H e F). Besides the classic lesions already described of cranioventral fibrinosupurative bronchopneumonia and pericarditis by PmA (3), lesions similar to those of Hps and App were also found (1). In Brazil, pneumonia is very prevalent in finishing pigs and is characterized by high mortality and lesions of septicemia, including necro-hemorrhagic pneumonic lesions. This study showed differences in pathogenicity between PmA strains isolated from outbreak in Brazil. Collectively, these results indicate the need of laboratorial assays to define the etiology of pneumonia in finishing pigs in the conditions of Brazilian herds.

Acknowledgments

Coordination of CAPES for the scholarship; Embrapa for funding the research project

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Heterologous protection of a Δ*fur* *P. multocida* bacterin in a co-infection with *M. hyopneumoniae* and *P. multocida*

M Sibila[1], M Nofrarías[1], S López-Soria[1], J Marca[2], S Campoy[3], M Llagostera[3], I Badiola[1,4], V Aragon[1,4]
[1]*Centre de Recerca en Sanitat Animal (CReSA), UAB-IRTA, Spain,* [2]*Aquilon CyL, León, Spain,* [3]*Department of Genetics and Microbiology, UAB, Spain,* [4]*Institut de Recerca i Tecnologia Agroalimentaries (IRTA), Spain,*
marina.sibila@cresa.uab.cat

Introduction

M. hyopneumoniae (Mhyo) and *Pasteurella multocida* (Pm) are frequently found at slaughter in the lung lesions of pigs with pneumonia. Both pathogens are involved in enzootic pneumonia, a chronic pulmonary disease. Mhyo seems to potentiate the lesions produced by Pm infection (1). Iron uptake is essential for *in vivo* survival of bacterial pathogens, including Pm. During infection, iron-uptake systems are expressed to guarantee the survival of the pathogen inside the host. This function is regulated by the repressor Fur. Mutations of the *fur* gene in bacteria produce a de-repression of genes involved in iron acquisition, which translates in an increased transcription and subsequent translation of iron-uptake proteins, some of which are located on the bacterial surface. These proteins are commonly conserved among the members of the same bacterial genus. Thus, a bacterine produced with a *fur* mutant of Pm would expose on its surface conserved iron-acquisition proteins, which are essential for survival, and could induce cross-protection against a challenge with a different strain of Pm.

Materials and Methods

Vaccination: Groups of 12 pigs were vaccinated (group T01) or not (group T02) with 10^{10} CFU of heat-inactivated Δ*fur P. multocida* PM1094 vaccine at 5 and 7 weeks of age.

Infection: Groups T01 and T02 were challenged with Pm, after 2 weeks of a previous infection with Mhyo (1). Mhyo infection was performed intratracheally in two consecutive days with 5 x 10^7 CCU in 5 mL of strain 232. Pm inoculation was performed by intratracheal inoculation of 10^7 CFU in 5 mL and intranasal inoculation of 10^6 CFU in 1.5 mL per animal of strain P-1344 (isolated from the lung of a pig with pneumonia).

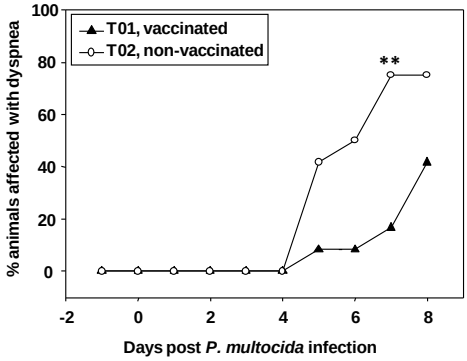
Clinical signs after challenge, with special emphasis on respiratory signs, were daily recorded. At necropsy, lung lesions were scored as described (3).

ELISA: serum samples were taken before and after vaccination and were tested in an in-house ELISA using outer membrane proteins (OMP) of the vaccine and the challenge Pm strains.

Results

Vaccinated animals presented a significant increase of antibodies against OMP from the Pm strain included in the vaccine and the Pm strain used in the challenge.

The combination of Mhyo and Pm (groups T01 and T02) did not produce severe disease that indicated the necessity of euthanasia, and therefore all infected animals were euthanized at the end of the study; i.e., 8 days after inoculation. When pigs from groups T01 and T02 were compared, no statistical differences were found in the majority of parameters analysed. Although lung lesions in the non-vaccinated animals were more severe than those found in the vaccinated animals, no statistical difference was detected. Bacterial isolation and body temperature were similar between both groups. The major difference between these two groups was observed in the lower percentage of animals with dyspnea found in the vaccinated group.



, p<0.001

Conclusions and Discussion

Cross-reaction with the Pm strain used in the challenge was detected by ELISA. The number of animals affected with dyspnea and the severity of this respiratory sign was reduced by vaccination with the Δ*fur P. multocida* bacterine. The challenge with Pm (preceded by Mhyo inoculation) was successful in reproducing disease, although a mild one.

The lack of statistical differences in the majority of the studied variables could be due to the low number of animals included in each group. Besides, an optimization of the combined infection model of Mhyo plus Pm (doses, strains, time to necropsy) may improve the evaluation of this or other vaccines.

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Control of salmonellosis in a wean to finish flow through the use of a modified live *Salmonella* vaccine

T Gillespie[1], M Inskeep[1],
[1]*Rensselaer Swine Services, Rensselaer, IN,* tom.gillespie@rssvet.com

Introduction

Salmonella typhimurium is a ubiquitous gram negative bacterium found in all vertebrates. This organism is a common cause of enterocolitis in swine and can have varied clinical signs, but diarrhea, inappetance and ill thrift are common. Vaccines have been utilized in control strategies. This paper describes the control of salmonellosis in wean- to- finish (WF) flow of pigs through the use of vaccine.

Materials and Methods

A 2,800 sow herd in the east-central United States had a chronic history of diarrhea occurring 2 weeks post-placement in the W/F barns. Historically, the diarrhea had a predictable time of onset and was susceptible to treatment with water soluble antibiotics. Diagnostics determined the definitive diagnosis of the colitis to be Salmonella typhimurium. Sporadic recovery of PRRSV, Influenza type A virus (IAV) and *Mycoplasma hyopneumoniae* were also present during this period of time in either the sow herd or finisher animals but was neither consistent nor definitive. A diarrhea also occurred sporadically in the sow farm, although a definitive diagnosis was not obtained.

A salmonella vaccination (Enterisol SC-54, Boehringer Ingelheim Vetmedica Inc, St. Joseph MO) program was initiated in August of 2012. All sows and boars were vaccinated in a mass vaccination approach at that time. The vaccine was initiated as a standard replacement gilt vaccination program. Piglets were initially vaccinated intranasally at the sow farm, although vaccination at arrival to the WF building began after six months into the vaccination program.

Results

The chronic diarrhea due to salmonella typhimurium colitis was eliminated immediately after the initiation of the vaccination program. The sow herd received a second mass vaccination 11 months later. There was a small numeric increase in W/F performance as seen in Table 1.

Table 1. Wean to finish performance parameters pre and post-vaccination with Enterisol SC-54.

	% Mortality	Average Daily Gain	Feed Effi-ciency	# of close-outs
Pre-vaccination	6.09%	1.46	2.65	33
Post-vaccination	5.50%	1.43	2.61	9

Conclusions and Discussion

To date, the vaccine has been safe in that there have been no adverse events recorded and has also been efficacious[1]. The program has resulted in the abatement of the chronic post weaning diarrhea as well as improved performance. Even though the performance numbers are not statistically significant, we point out that the small number of closeouts post vaccination has limited scientific scrutiny. Vaccination has safely resulted in the control of salmonellosis with stopping the clinical signs of diarrhea. Additional benefit was a reduction in the use of antibiotic therapies for starting pigs post weaning. When a net benefit approach to applied economics was used, it was found that a savings of $0.58 (USD) per animal was achieved in reduced antibiotic costs. Additional savings in labor, overhead costs, less culls added $0.18 per animal, for a total of $0.76. Vaccine cost $0.52 per dose; therefore, net benefit per animal of $0.24 or a positive $240 per every 1,000 head placed was attained.

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Effects of anti-*Salmonella* bacteriophage therapy on *Salmonella* infection in weaning pigs

H-S Cho[1], B-S Kim[1], S-Y Chon[2], S-H Kang[2], S-I Yoo[3], D-K Choi[3], B-Y Jeong[4], W-I Kim[1]
[1]*College of Veterinary Medicine, Chonbuk National University, Jeonju, Korea,* [2]*Intron Biotechnology, Seongnam, Korea,* [3]*CTCbio, Hwaseong, Korea,* [4]*Animal and Plant Quarantine Agency, Anyang, Korea,* kwi0621@jbnu.ac.kr

Introduction

Salmonella has been one of the major pathogens causing tremendous economic loss to the Korean swine industry. Due to the new law that prohibits the use of antibiotics in animal feeds in Korea, development of antimicrobial alternatives became necessary. Among various alternatives, bacteriophages had received great attention as a possible alternative for antibiotics in livestock industry because it has been demonstrated that bacteriophages are non-hazardous self-replicating agent that can infect and multiply in bacteria to prevent bacterial diseases in previous studies (1, 2). Therefore, it was hypothesized that treatment of pigs infected with salmonella with anti-salmonella bacteriophages can enhance growth performance and salmonella resistance and the effect of bacteriophages on salmonella infection in weaned pigs was evaluated in the current study.

Materials and Methods

Four-week-old, 20 pigs were purchased and divided into 4 groups (Table 1). Phage control (PC) group, Pre-treatment (PT) group, and Pre- and post-treatment (PPT) group were fed with 5 ml of salmonella-specific bacteriophage mixture (SEP-1, SGP-1, STP-1, STP-2) for 3 days before challenge. PT, PPT and challenge control (CC) groups were challenged with *Salmonella Typhimurium* at 10^8 CFU/ml except for the PC group (No salmonella challenge). Then PC and PPT groups were fed with 5 ml of the bacteriophage mixture for another 3 days after challenge. Fecal swaps were taken from all of the pigs in the groups on a daily basis and tested for bacterial shedding. Serum samples were collected on a weekly basis and tested for the distribution of bacteriophage and antibody production. The weight of the pigs was also measured on a weekly basis. All of the pigs were sacrificed for pathological evaluation at 2 weeks after challenge.

Table 1. The design of animal study

Group	Pre- treatment (1-3d)	Challenge (4d)	Post- treatment (5-7d)
Phage Control **(PC)**	Phage	None	Phage
Pre- and post-treatment **(PPT)**	Phage	Salmonella	Phage
Pre-**T**reatment **(PT)**	Phage	Salmonella	None
Challenge control **(CC)**	None	Salmonella	None

Results

Pigs treated with bacteriophage (PT and PPT) showed a higher weight gain after challenge with salmonella as compared with CC group. PCR results indicated that salmonella shedding in feces was significantly higher in pigs treated with bacteriophage (PT and PPT groups) until 4 dpc when compared with CC group. However, the levels of salmonella shedding were lower at 5 dpc and ceased at 7 dpc, which was earlier than CC group.

Conclusions and Discussion

In general, pigs treated with anti-salmonella bacteriophage showed lower levels of clinical signs and bacterial shedding in feces and higher levels of weight gains after challenge with salmonella. Though there was no significant negative effect observed in PC group, the safety concern related to the continual use of bacteriophage for disease control will be evaluated by determining distribution of residual bacteriophage and disruption of commensal microbial populations in pig intestines.

Acknowledgement

This research was supported by Technology Development Program (Project No. 1121314) for Bio-industry, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.

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Attenuated *S. typhimurium ΔZnuABC* is protective against salmonellosis in piglets

J Ruggeri[1], M Pesciaroli[2], B Gaetarelli[1], FE Scaglione[3], P Pregel[3], S Ammendola[4], A Battistoni[4], E Bollo[3], GL Alborali[1], P Pasquali[2]

[1]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, 25124 Brescia, Italy;* [2]*FAO Reference Centre for Veterinary Public Health, Department of Veterinary Public Health and Food Safety, Istituto Superiore di Sanità, 00161 Rome;* [3]*Department of Veterinary Sciences, Università degli Studi di Torino, 10095 Torino, Italy;* [4]*Department of Biology, Università di Roma Tor Vergata, 00133 Rome, Italy,* giovanni.alborali@izsler.it

Introduction

Salmonella enterica serovar Typhimurium (*S.*Typhimurium) is a problem for human health and for livestock productions [1, 2].

Many actions, such as bio-security programs, are applied to reduce *Salmonella* spp. prevalence and the use of vaccines could represent a complementary solution to minimize the spread in pigs [3, 4]. Recently, we produced a mutant strain of *S.* Typhimurium protective either in mouse or pig models of salmonellosis [5-9].

Here, we have assessed if *S.*Typhimurium Δ*znuABC*, parenterally administered, is also protective in piglets. In addition we used a different challenge model to simulate a natural infection.

Materials and Methods

Twenty-five weaned piglets were divided into 4 groups. Group A (5 piglets) was intramuscularly vaccinated with 10^4 CFU of *S.* Typhimurium strain *ΔznuABC;* groups B (5 piglets) and C (4 piglets) were intragastrically vaccinated by gavage with $5x10^7$ and $5x10^5$ CFU of *S.* Typhimurium strain *ΔznuABC*, group D (11 piglets) was control group. Feces were taken at 1, 2, 7, 14 and 21 days after vaccination.

Six weeks after vaccination, 5 naïve piglets of group D were challenged with $4x10^8$ CFU wild type *S.*Typhimurium and all groups were allocated in the same barn for two weeks, to favor contact among uninfected and shedder animals. Feces were taken once a week until animal sacrifice, to estimate the spread of the microorganism. Piglets were euthanized four weeks after contact and samples of ileocecal lymph nodes, ileum, caecum, and colon were collected to be submitted to microbiological and histological analyses. Microbiological analyses were conducted according to ISO 6579:2002.

Results

Attenuated *S.*Typhimurium *ΔznuABC* is not eliminated after parenteral administration but eliminated after oral administration for a limited period of time.

The attenuated strain reduces fecal shedding, organs lesion and colonization of wild type *S.* Typhimurium. Macroscopical and histological analyses evidenced that group B and C showed ileum wall thickening and reactivity of ileocecal lymph nodes. Group A showed an intermediate degree of reaction, mainly characterized by ileum wall thickening. Lastly, group B didn't show any reactions.

Histologically, group D showed the worse lesions in all the examined intestinal tracts. Villi conglutination and necrosis were associated to vasal congestion and lymph nodes depletion. Group C, being the most affected of the vaccinated groups, showed microscopical lesions similar to that observed in group D. Group A and B seemed to show a milder degree of lymphocytic and eosinophilic inflammation.

Conclusions and Discussion

Pigs orally or intramuscularly vaccinated with a *S.*Typhimurium *ΔznuABC* mutant strain are protected against infection with a virulent *S.*Typhimurium strain.

Vaccination is a method suggested to decrease the prevalence of *S.* Typhimurium in swine farms and to prevent its dissemination through the pork production chain.

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Retrospective study and antimicrobial susceptibilities of *S. enterica* serovar choleraesuis isolated from swine salmonellosis outbreaks during 2013 in Brazil

FA Vannucci, G Oliveira, MR Henriques, KCP Reis, LEM Bouillet, WV Guimaraes, DL Santos, LF Santos, JL Santos
Microvet – Microbiologia Veterinaria Especial, Vicosa, MG, Brazil, fvannucci@microvet.com.br

Introduction

Septicemic disease caused by *Salmonella enterica* sorovar Choleraesuis has been well characterized in pigs worldwide (1). In Brazil, until 2012, there were no information about the distribution and the importance of this disease in the swine industry. At beginning of 2013, an increasing number of outbreaks were detect in four States (Minas Gerais, Paraná, Santa Catarina e Rio Grande do Sul). Although subclinically infected pigs are considered the most common source of new infections (1), the source for these outbreaks still have to be elucidated. The objectives of this study was to characterize the clinical, pathological and microbiological features of swine Salmonellosis identified in 2013 in Brazil and to determine the *in vitro* susceptibilities of 16 *S.* Choleraesuis isolates against commonly used antimicrobials.

Materials and Methods

During 2013, over 60 *S.* Choleraesuis isolates were obtained from outbreaks of swine salmonellosis in 16 different systems distributed in four States of Brazil (Minas Gerais/n=12, Parana/n=1, Santa Catarina/n=1 e Rio Grande do Sul/n=2). Production phase, clinical signs, lesions and organs of *S.* Choleraesuis isolation was recorded.

One isolate from each system, totalizing 16 strains, were selected to determine the *in vitro* susceptibilities against commonly used antimicrobials using Kirby-Bauer disk diffusion method (2). A panel of 11 discs was used in different concentrations according to the manufacturer: amoxicillin (10µg), ampicillin (30µg)/colistin (15µg), ciprofloxacin (5µg), doxycycline (30µg), enrofloxacin (5µg), florfenicol (30µg), gentamycin (10µg), lincomycin/spectinomycin (109µg), neomycin (30µg), norfloxacin (10µg) and tetracycline (30µg). Each isolate was categorized as susceptible, intermediate and resistant for each antimicrobial according to the standard procedures established by the Clinical Laboratory Standards Institute (CLSI) (3).

Results

The age range of affected animals was between 28 and 121 days. Nine out of the 16 systems experienced outbreaks in the nursery phase (21-60 days), four in the grower (60-110 days) and three in the finisher (>110 days). Coughing (50%) and wasting or ill thrift (37.5%) were the clinical signs more frequently associated with the outbreaks followed by fever, dyspnea, cyanosis of extremities (especially ears) and diarrhea.

The histopathological lesions most commonly observed in *S.* Choleraesuis outbreaks were acute interstitial pneumonia with presence of fibrin thrombi in interalveolar capillaries and necrotic hepatitis associated with the presence of paratyphoid nodules. *S.* Choleraesuis was most frequently isolated from lungs (94%), spleen (62%) and liver (56%).

The results for the *in vitro* susceptibilities are summarized in Figure 1.

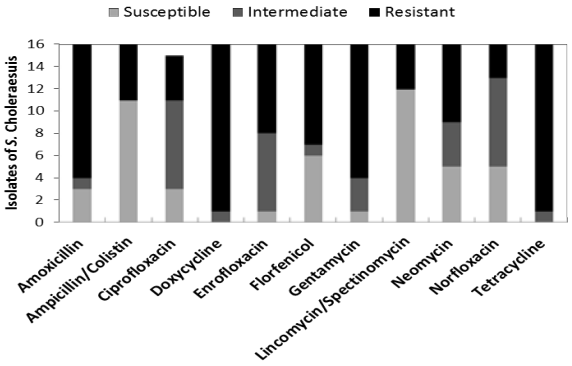

Figure 1. *In vitro* susceptibilities of 16 *S.* Choleraesuis isolates against 11 antimicrobials categorized according to the CLSI.

Conclusions and Discussion

The results showed that *S. enterica* sorovar Choleraesuis causing septicemic disease is distributed in the four States of Brazil which represent the most important regions of swine production. Respiratory and circulatory disorders have been the main characteristics of these outbreaks. The patterns of *in vitro* susceptibility against a panel of antimicrobials corroborate with previous reports showing high frequency of resistance for tetracycline derivatives (4). In contrast, 75% of the isolates were susceptible to lincomycin/spectinomycin.

Acknowledgments

All colleagues and colaborators from the Microvet

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P.270

Comparison of four commercial transport media and a swab without transport medium for the survival of *S. hyicus*

KL Takeuti[1], H Jacobi[1], ML Bernardi[1], DESN Barcellos[1]
[1]*Setor de Suínos, Veterinary Faculty, UFRGS, Porto Alegre, Brazil,* karinelt87@yahoo.com.br

Introduction

Four commercial transport media (Amies, Amies with charcoal, Cary Blair and Stuart) and swabs without transport medium were assessed regarding their capacity to preserve *S. hyicus* for periods of up to 10 days at two different temperatures (room temperature [20-25ºC] and refrigerated [4-8ºC]). We did not find in the literature information on the effect of temperature and transport media for the survival of this bacteria.

Materials and Methods

The Roll-Plate Method was used with a reference strain of *S. hyicus* (ATCC 11249), following recommendation of NCCLS (1). Incubation by 10 days in two temperatures was used and after that, swabs were plated in Tween 80 Agar, counting bacterial colonies at each 24 hours. The survival of *S. hyicus* for periods of up to 10 days were compared among the types of swabs in each temperature and among temperatures inside each type of swab using the non-parametric test Kruskal-Wallis (SAS® 9.1.3, Cary, NC, USA).

Results

The results are shown in Table 1. Samples held in all transport media showed significantly better performance ($P \leq 0.05$) in refrigeration. Storage in refrigeration in Amies medium performed better than all other transport media and swabs ($P \leq 0.05$). In room temperature, Amies medium and swabs without transport medium worked similarly ($P > 0.05$), and presented the bests results in this temperature. Additionally, refrigerated Amies medium showed high performance for up to nine storage days and swabs without transport medium for up to three days.

Conclusions and Discussion

The high performance in refrigerated Amies indicated that this is the most suitable medium for shipping of *S. hyicus*. However, considering *S. hyicus* resistance to desiccation, easiness of transportation and low cost, swabs without transport medium could also be indicated, when stored at room temperature. This form of shipment could be viable when the time predicted to transport to the laboratory would be smaller than 48 hours, since this form of preservation showed survival for up to 72 hours.

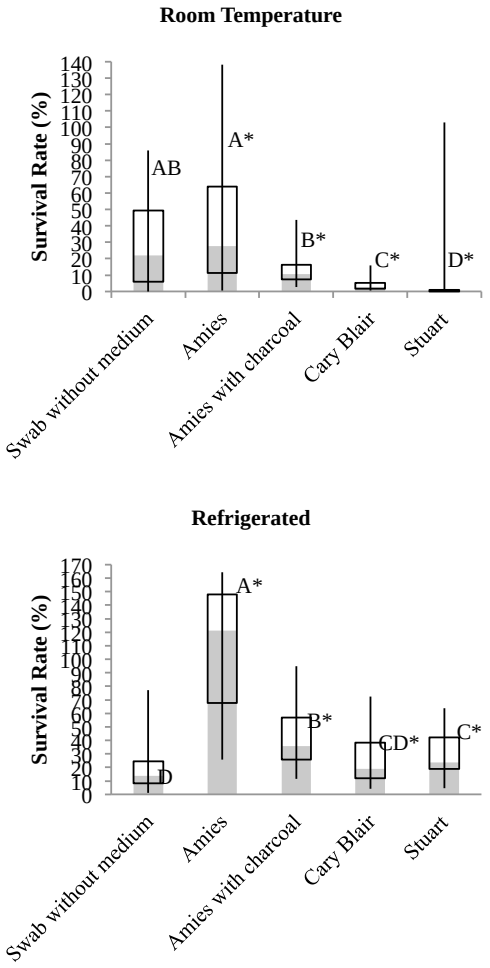

Figure 1. Survival of *S. hyicus* for periods of up to 10 days at two different temperatures. The boxes show minimum, first quartile, third quartile and maximum values. The superior limit of the grey bar represents the value of median. Different letters indicate statistical differences among the types of swabs in each temperature ($P \leq 0,05$). Asterisks indicate statistical differences among temperatures inside each type of swab ($P \leq 0,05$).

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Isolation and characterization of strains of *S. suis* in swine farms in Mexico

A Romero-Flores[1], S Mendoza[1], R Carreón[2], E Hernández-Baumgarten[1], R Galvan[2], R Alonso[2], A Ciprian[1], B Montoya[1], O Cuahutle[1], S González[1] and M Gottschalk[2].

[1]*Facultad de Estudios Superiores Cuautilán-UNAM;* [2]*Facultad de Medicina Veterinaria y Zootecnia-UNAM,* [3]*College of Veterinary Medicine, University of Montreal, Canada.* *Grants: PAPIIT ITE218711-3 and CONS-23*
ariannaunam@gmail.com CONACYT scholarship No. 492133 and *PAEP (UNAM.)*

Introduction

Streptococcus suis (*S. suis*) is an important pathogen of swine. It is a well-known fact that most pigs are carrier of multiple serotypes of *S. suis* in the upper respiratory tract. It can also be a cause meningitis, arthritis and septicemia. It affects the production parameters of pigs causing important economic losses in farms. It is also a zoonotic disease affecting people that work in close contact with pigs (1, 4). We characterized the strains of *S. suis* isolated from farm employees and pigs by means of molecular techniques (PCR, and serotyping) in order to determine which of the 35 serotypes of the existing 35 affected animals and were present in personnel in Mexico (4).

Materials and Methods

We established three groups, from the first one the tonsils of clinically healthy animals were obtained (n=85); the samples of the second group consisted of internal organs from clinically ill animals (n=77) and those of the third group were from pharyngeal secretions taken from farm personnel (n=60). The samples were cultured in 5% blood-agar. The α-hemolytic colonies were selected. A PCR for the *gdh* gene was conducted to determine that we were dealing with *S. suis* (2), and the positive colonies were genotyped to identify the serotype (3).

Results

In the group of healthy pigs, all samples were negative for *S. suis*. Similar results were obtained with the samples from the farm workers. In the group of ill pigs, 6 samples were found positive for *S. suis.* From these samples, one beloged to the serotype 1/2, two samples to serotype 2, one sample to serotype 3 and two samples were positive to serotype 7.

Conclusions and Discussion

The fact that none of the workers in the study group resulted positive seems to indicate that either the workers are not carrier of this pathogen or that the method used lacks sensitivity. Since it is known that *S. suis* is zoonotic, the failure to detect it, seems to indicate that a single sample from each person may not be sufficient to detect occasional presence of bacteria. We may need to use repeated sampling of each person, or more sensitive techniques. The human samples represented both clinically healthy and *S. suis* affected farms. Concerning the 85 samples in the clinically healthy farms that were negative, suggest that the prevalence of *S. suis* in healthy carriers is low. Again, this may also be the consequence of a low sensitive technique used. Most publications indicate a high level of infection in clinically healthy pigs, since *S. suis* is a normal inhabitant of the upper respiratory tract of pigs. *S. suis* could be isolated, on the other hand, from sick animals, with the presence of serotypes 1/2, 2, 3, and 7. This is the first time that serotypes other than serotype 2 are reported in Mexico.

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Economic analysis of the sow's repleacement until 3rd parity

F Salvini, G Guadagnini, M Bresaola
PigVet, Brescia Italy
salvinifrancesco@gmail.com

Introduction

The economic and productive parameters are the most common and the easier parameters to analyse in a pig farm. It's simple to record the number of weaned piglets or the cost of an unproductive day but in a financial statement there a some smaller parameters that are difficult to calculate and to consider their real economic impact. One of these parameters is the percentage of sows that replaced before the third parity.
The aim of this work is to consider these parameters and try to establish the economic impact of the "genetic cost" on the financial statement.

Material and method

In the study we compared two farms, located in the north of Italy, that breed 550 sows and that have comparable productive and sanitary parameters.
The farms manage the replacement in the same way: they buy PRRS negative gilts at 6 kg of body weight but from two different genetic companies. The gilts are located in a quarantine rooms for acclimatising, growing and vaccination plan.
The "genetic cost" for every served gilt was 100 € and it was the same for both the farms.
We tried to divide this cost for the number of weaned piglets in the reproductive life of the sow as it's shown in table 1.

Table 1. Incidence of "genetic cost" based on the number of weaned pigs

PARITY	WEANED PIGLETS	GENETIC COST FOR WEANED PIGS (€)	RESIDUAL VALUE
0	0	100	100
1	10	10	86
2	20	5	72
3	30	3,3	58
4	40	2,5	44
5	50	2	30
6	60	1,6	16
7	70	1,4	0

Analysisng the data on the table, we consider the genetic cost divided for the production of weaned piglets.
The genetic cost if you lose a sow after the first parity is divided on ten pigs weaned so they have 10 € of genetic cost each, instead if you eliminate a sow after 7th parity the genetic cost is divided on 70 piglets weaned, so they reach 1,4 € each of genetic cost.

Results and Discussion

The analysis of the productive data at the end of 2011 showed that the farm B had lost more sows before the 3rd parity. Farm A eliminated 52 sows before 3rd parity that are the 22,2 of the total number of eliminating sows. Instead farm B eliminated 105 sows before 3rd parity that are 37,5% of the total number. Farm B had to replace more than farm A with an important impact on the financial statement.

Table 2. Number and percentage of eliminated sows divided for parity

Parity	Farm A			Farm B		
	N° EL	% EL	%	N° EL	% EL	%
1	26	11,1		56	20	
2	13	5,5	22,2	25	8,9	37,5
3	13	5,5		24	8,6	
4	9	3,9		11	3,9	
5	5	2,2	24,9	16	5,7	27,5
6	20	8,6		22	7,9	
7	24	10,2		18	10	
>7	124	53	53	98	35	35
Tot	234	100		280	100	
RIM	**42,5**			**50,9**		

Table 3. Comparison between "genetic cost" into the farms

Parity	Genetic residual value	FARM A		FARM B	
		N° EL	Theore-tical cost	N° EL	Theore-tical cost
1	100	26	2600	56	5600
2	86	13	1118	25	2150
3	72	13	936	24	1728
4	58	9	522	11	638
5	44	5	220	16	704
6	30	20	600	22	660
7	16	24	384	28	448
>7	0	124	0	98	0
TOT			**6380**		**11928**

We tried to compare the data and the economic loses of farm B compared to farm A. Farm B bought 46 gilts more than farm A. Analysing the residual genetic value ,farm B spent 5548 € more than farm A and moreover they spent 4600 € more than farm A to replace with new gilts.
We evaluate a small productive parameter not usually analysed, because in the farm we have a lot of secondary parameters that can have an important weight on the financial statement if aggregated.
We measured a difference of more or less 10000 € between the two farm evaluating only the "genetic cost" and without considering the cost related to the different production

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Field efficacy and safety study in weaned pigs with an inactivated ORF2 subunit PCV2 vaccine, an inactivated *M. hyopneumoniae* vaccine, and a modified live PRRS vaccine administered as a trivalent mixture (3FLEX) in a 750 sow single-site farrow-to-finish operation

AC Bulay, III[1], OM Penaso[1]
[1]*Boehringer Ingelheim (Phil.) Inc.*,andy.bulay@boehringer-ingelheim.com

Introduction

The multitude of pathogens concurrently present in pig farms in this part of the region makes pig farming very challenging. Consequently pigs must be protected against main pathogens almost at the same and right time in order to elicit the right immunity prior to exposure. 3FLEX™ is the trade name associated with the mixture of Ingelvac CircoFLEX®, Ingelvac MycoFLEX®, and Ingelvac® PRRS MLV swine vaccines (Boehringer Ingelheim Vetmedica, Inc., St Joseph, Missouri) and it has been proven to be safe and efficacious in North America[1].This 7-month study was conducted to evaluate the safety and efficacy of the trivalent vaccine mixture 3FLEX under Philippine farm scenario wherein multiple forms of stress negatively impact growth rate and the physiological status of the pig.

Materials and Methods

One hundred healthy pigs aged 21 days (±3d) on Day 0 were blocked by age and body weight and randomly assigned to treatment groups (Table 1). Using a digital thermometer (MDT-101A, MD+®), rectal temperatures were individually determined from each group of 10 pigs two hours before and two hours after administering the vaccines, and then two days later. Concurrently, the injection sites from the same pig groups were visually scrutinized and manually palpated by a single observer in order to check for any post-vaccination reactions in the form of swellings, lumps, or any similar clinical manifestations that may be attributed to the vaccine injection (Table 2). Both groups tested positive serologically for PRRSV, *M. hyopneumoniae*, and PCV2 using commercial ELISA, tested prior to the start of the study, September, 2012.

Table 1. Vaccination program per group of pigs aged approximately 21 days (±3d).

GROUP	N	TREATMENT
Yellow	50	Trivalent vaccine mixture (3FLEX)
Blue	50	FLEXcombo + PRRS MLV

3FLEX was created by mixing equal volumes of the Ingelvac MycoFLEX and Ingelvac CircoFLEX products (each labeled as 1 ml/dose) and was used to rehydrate the Ingelvac PRRS MLV. The mixture was then administered intramuscularly as a 2 ml/dose vaccine administered on the right side of the neck area. For the Blue group, 2ml of the FLEXcombo mixture was injected IM on the left side while 2ml of the Ingelvac PRRS MLV was given separately on the right portion of the neck. To measure any performance differences among the treatment groups, ADG and live body weights at market (including the body temperatures) were recorded and statistically analyzed using Student's T-test.

Results

There were no significant differences in the body temperatures of the pigs from either group when measured before and after vaccination (Table 2).

Table 2. Recorded Rectal Temperatures

PARAMETERS	FLEXcombo Ingelvac PRRS MLV	3FLEX	*P*-value
2 hours PRE-Vx	39.2	39.2	*0.8663*
2 hours POST-Vx	40.8	40.6	*0.2820*
2 days POST-Vx	39.8	39.6	*0.1661*

Individual palpation and visual examination of the injection sites on each pig representing either group showed unremarkable findings, proving that with proper injection technique, either vaccine preparations were very safe to administer and systemically tolerated.

Table 3. Effect of Vaccine on Pig Performance

PARAMETERS	FLEXcombo Ingelvac PRRS MLV	3FLEX	*P*-value	*Diff.*
Avg. Birth Weight, kg	1.35	1.33	0.6762	0.02
Avg. End Nursery Weight, kg	**25.64**	**26.42**	0.4894	**+0.78**
Nursery ADG, kg	**0.366**	**0.377**	0.4894	**+0.011**
Mortality, head	1	2		-1
End of Finisher Weight, kg	**121.2**	**122.9**	**0.0064**	**+1.70**
Avg. End Finisher Age, day	207	207		0
Finisher ADG, kg	**0.585**	**0.594**	**0.0064**	**+0.009**

Conclusions and Discussion

In general, pigs vaccinated with 3FLEX did not show a statistical difference compared with the vaccinated group using separate vaccines concurrently administered (FLEXcombo + PRRS MLV) confirming under the conditions of this study, the lack of any potential negative impact due to mixing of the three components as one injection, providing the convenience of a single shot. 3FLEX group showed a statistical trend (P value of 0.0064) showing better finisher growth performance. The absence of any post-vaccination injection site lesions and body temperature differences further support earlier studies that proved the safety and efficacy of the trivalent vaccine mixture 3FLEX[2].

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Efficacy of Ingelvac CircoFLEX® compared to a local PCV2 vaccine in a farm in Southern China

T Tan[1], WY Yang[2], L Zhu[1], G Chen[1]
[1] Boehringer Ingelheim Int'l Trading (Shanghai) Co. Ltd, Beijing100004, China
[2] Huabang Farm, Yulin537000, China
tao.tan@boehringer-ingelheim.com

Introduction

PCV2 causes significant losses in the pig industry worldwide. PCV2 piglet vaccination is a useful tool in controlling PCVD [1]. The objective of this study was to evaluate the efficacy of Ingelvac CircoFLEX® and a local PCV2 vaccine by measuring wean-to-finish performance on a farm in the south of China.

Materials and methods

The study was conducted on a single-site 1200 sow farm located in the south of China. In 2010, this farm implemented Ingelvac PRRS® MLV to reduce respiratory problems in the nursery. The breeding herd in this farm was vaccinated against CFSV, PRV, FMD, PPV, JEV and atrophic rhinitis. Piglets were vaccinated against CSFV, FMD, PRV, PRRS and *Mycoplasma hyopneumoniae*.

The main clinical signs in the nursery were wasting and pale pigs, while in the fattening unit growth retardation and dermatitis were seen, suggesting PCV2 infection. Laboratory diagnostics (PCR and ELISA test) confirmed that PCV2 was the main viral pathogen. Based on these findings, a field trial was conducted from October 2011 to April 2012 to evaluate the efficacy of PCV2 vaccination with Ingelvac CircoFLEX® compared to a local PCV2 vaccine.

Five hundred-ninety one (591) piglets at 14 days of age were randomly divided into 2 groups. The piglets in group 1 were injected with Ingelvac CircoFLEX®, 1 ml intramuscularly. The piglets in group 2 were treated with a local PCV2 vaccine, 2 ml intramuscularly. The two groups were raised in different barns on the same site under the same management and housing conditions. Mortality, feed conversion rate (FCR) average daily gain (ADG) and medication costs were recorded. The mortality in the two treatment groups was compared with Chi-square test.

Results

FCR of the pigs vaccinated with CircoFLEX was reduced, while ADG increased (Table 1) by 44g compared to the control group. The mortality in group 1 was significantly lower than in group 2 ($p<0.01$).

Table1. Performance comparison between the two groups

	Group 1 (CircoFLEX)	Group 2 (local vx)	Diff.
Number of pigs	**289**	**302**	
Mortality	**5.88%**	**13.90%**	**-8.83%***
Medication costs ($)	**4.76**	**9.37**	**-4.61**
FCR	**3.21**	**3.43**	**-0.22**
ADG (g)	**657**	**613**	**+44**

* Chi-square test, $P<0.01$

Conclusions and Discussion

In this farm with PCV2 infection, Ingelvac CircoFLEX® successfully reduced the mortality from wean to finish when comparing to the local PCV2 vaccine. The nominal FCR and ADG in CircoFLEX group were improved and medication costs were reduced. The results demonstrate that CircoFLEX vaccination can control PCVD far more successfully than the local PCV2 vaccine. Based on the results of this study, the farm has been using Ingelvac CircoFLEX® since October 2011.

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Method of utilizing disease monitoring tests to improve farm productivity

F Koike, E Taniguchi, M Tomioka, S Murata, M Ooi
S.M.C., Co. Ltd., koike@swine-smc.co.jp

Introduction

In conducting biosecurity practices and vaccination program as an effective preventive strategies, disease monitoring tests have been performed for various purposes. In the state of disease in which complicated factors such as the conditions of sows are entangled, however, the relationship between the results from disease monitoring tests and the farm productivity was not clear. Therefore continuous monitoring has not been performed in many cases, and it is necessary to examine and establish a method to reflect those monitoring test results.

In this study, by considering the results from monitoring tests for multiple respiratory pathogens as uniform figures and taking them in as one of benchmarks, an effective method of utilizing such data to improve farm productivity was examined.

Materials and Methods

1. The results of monitoring tests for PRRS-ELISA, MPS-ELISA, APPII type-ELISA, PCV2 real-time PCR, PRRS-PCR were used (a total of 3357 samples obtained from the serum of healthy pigs, performed 8 times, a total of 20 commercial farms, 2008-2013). Each value was graded as 0, 10, 20, 30, 40 or 50 points based on the standards for the negative value, positive value, vaccine antibody titer and field antibody titer. The higher the probability of infection of each disease in the farm becomes, the higher the monitoring value is scored. Then the mean values for antibody titer of sows and pigs at 30, 60, 90, 120 and 150 days of age, and the PCR values pooled in each stage were applied to the respective points, and the points were summed and used as the farm monitoring score. As concerns PRRS-PCR, additionally, it was specified that the earlier the age of becoming positive would be, the higher the positive point of sows was scored. Post-weaning mortality and whole herd feed efficiency rate (FCR) during 6 month-period of time were used for this study

2. At two farms with similar production systems, the blood samples were collected at time of every month until the time of 30 days to 1 month before slaughter, and the MPS-ELISA monitoring value and the MPS lesion score of slaughter-weight pigs were investigated. At 1 month before slaughter of those pigs, additional blood sampling was performed in the whole farm to validate the monitoring score.

Results

1. The monitoring score for each farm was 110 to 600 points. It was found that the monitoring score of each farm fluctuated by 100 to 200 points depending on the order of PRRS and MPS values.

In particular, there was a high correlation with post-weaning mortality and the monitoring score. In the farm showing no correlation, such as expansion of herd size and gastrointestinal disease could be presumed.

Additionally, there was a high correlation between FCR, the monitoring score of PRRS-PCR, and PRRS-ELISA. Through the observation by each farm, the correlation between the trend in MPS-ELISA value and the trend in FCR was very high (Figure 1).

2. Comparing the monitoring score of the pigs at 30 to 150 days of age with the whole farm blood samples at slaughter of those pigs, the monitoring score of the whole farm was more linked to the lesion score in the slaughterhouse (Figure 2).

Discussion

By conducting cross sectional blood testing in continuous basis along with herd veterinarian supervision, we were able to establish the monitoring method that reflected the health status as well as productivity of the farms.

Since MPS-ELISA is influenced by sow health status or season, the evaluation by the concurrent blood sampling in the whole farm was difficult. However, it was shown that lung lesion and FCR can be predicted to some extent by checking each of this monitoring score.

By this method, the status and the trend of respiratory diseases in farm could be confirmed visually. As just described, by showing farm producers how the disease in each farm is related to the farm productivity more clearly, it is considered that the awareness of those farm producers toward the importance of continuation of monitoring tests and disease control has been increased.

In this study, by digitalizing the results of monitoring tests, a multifaceted examination has become possible as one of benchmarks. It is considered that the periodic monitoring tests are more beneficial for the future disease prevention in each farm.

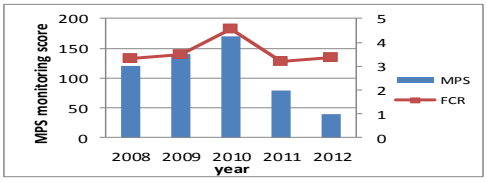

Figure1. Transition of MPS monitoring score and the farm FCR (Farm A)

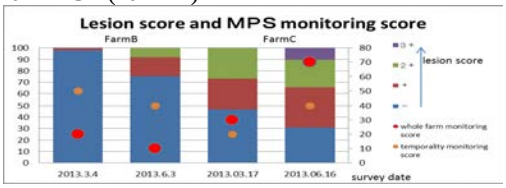

Figure2. Lesion and MPS monitoring score

Effect of tylvalosin tartrate on mortality after weaning in four pig farms with PRRS problem in Japan

S Ishizeki, H Ishikawa
Summit Veterinary Services, 382-27 Tsurugaya, Nisato, Kiryu city, Gunma, JAPAN, ishizeki@svs-jp.com

Introduction

PRRS causes serious economic losses. It has been reported that tylvalosin reduces the ability of the PRRSV to replicate (tylvalosin is the active ingredient of Aivlosin®) (1,2). We have reported the reduction of mortality in pigs after weaning when treated with Aivlosin® (3). On commercial farms, we tested the use of Aivlosin® as an intervention for cases of increased mortality caused by PRRS. From 2011 to 2013, four pig farms experienced higher mortality caused by PRRS. Here we report reduced mortality in growing pigs (25-70 days-age), when treated with Aivlosin® in these four farrow- to- finish farms.

Materials and Methods

Between 2011 and 2013, PRRS outbreaks occurred on four pig farms. After PRRS was confirmed by histo-pathology, we initiated in-feed medication with Aivlosin® Premix (ECO Animal Health Japan) at 100ppm in finished feed, starting after weaning until 70 days of age. 50 ppm Aivlosin® Premix was given in sow feed in the lactating period simultaneously.

Pathological evaluation was done for the clinical cases where respiratory symptoms were attributed to PRRS, and where mortality increased higher than usual, and with clinical observation in the nursery.

Immunohistochemical staining and histopathological examination, or PCR tests using blood or lung samples were performed to confirm the diagnosis of PRRS.

We measured the post-weaning mortality of each farm, during the period of 6 months before and 6 months after the start of the Aivlosin in-feed medication.

Results

The monthly post-weaning mortality results are shown in Figure 1-4. “m-0” means the start of medication, “m-4”: 4 months before medication, m+6: 6 months after medication.

For PRRS positive farms, mortalities ranged from 4% to 8%. After the use of Aivlosin®, mortalities were reduced to levels of less than 1% to 4%. The time period to reduce mortalities to a level lower than 2% was one month for the early responding farms and five months for the later responding farms.

Conclusions and Discussion

It has been reported that tylvalosin may inhibit the replication of the PRRSV, *in vitro*, by increasing the pH in the endosomes (1). Another report demonstrated that macrolide antibiotics such as tylosin, tilmicosin and tylvalosin diffuse into cells more rapidly than other antibiotics, *in vitro* (2). We expected activity *in vivo* and measured the effects of Aivlosin® on mortality in PRRS problem farms post-weaning.

There are various methods to reduce PRRS, for example farm closure, mass vaccination, partial depopulation, etc. However, those still need to be further evaluated. We may need to understand more about the factors that influence an improved or quicker response in the farms. It may also be related to the present pathogen load and its shedding. Farm A had only PRRSV, but other pathogens (*St.suis*, *H.parasuis*) were isolated in Farm D.

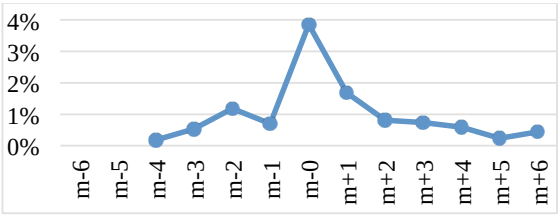

Figure 1. Mortality after weaning of Farm A (sow: 800)

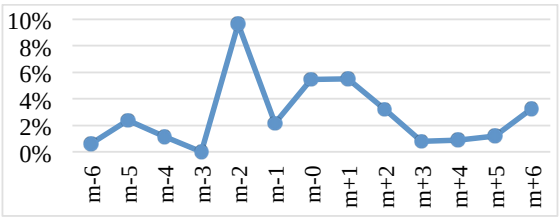

Figure 2. Mortality after weaning of Farm B (sow: 125)

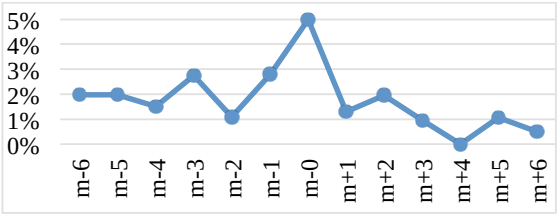

Figure 3. Mortality after weaning of Farm C (sow: 150)

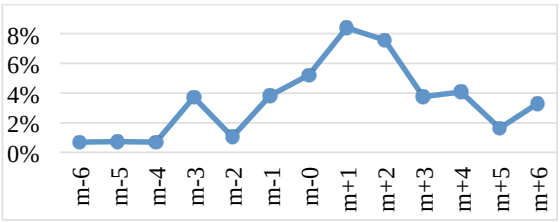

Figure 4. Mortality after weaning of Farm D (sow: 400)

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Control of Glässer's disease in nursery piglets using sow vaccination and gilt adaptation

PMS Lopes[1]
[1]*Faculty of Veterinary Medicine – ULHT, Lisboa, Portugal,* pmslopes@sapo.pt

Introduction

This paper describes the protocol used to control Glasser's disease in nursery piglets, in a farrow-to-finish 800 sow farm. The disease is caused by *Haemophilus parasuis* (HPS), piglets are infected on the first days of life (1) and express clinical signs most frequently after weaning, including polyserositis and arthritis, resulting in high mortality rates. Control strategies include early use of antimicrobials and some auto-vaccines. Because HPS has a wide variety of serotypes, vaccination success depends on matching the vaccine with the field serotype. In this case we used a commercial vaccine in sows, and gilt adaptation to prevent clinical signs in nursery piglets.

Materials and Methods

The clinical signs observed, were seen most frequently in weaned piglets from 4-7 weeks of age, and included anorexia, swollen joints, fever, dyspnea, coughing and sometimes nervous signs. Mortality rates were high, reaching over 9% in some months (last quarter 2012). Necropsy revealed polyserosites in most of the cases. Bacteriology performed in samples from lungs and heart covered with fibrin, confirmed the isolation of HPS. Serotyping technique was not available, so the serotype of HPS is not known in this case. In 2008, HPS serotype 5 and 15 were isolated from affected piglets in this farm. Because HPS is usually secondary to other pathogens or to stress factors, such as temperature changes, drafts, poor hygiene or poor nutrition, several management practices were corrected to eliminate these factors, but that alone was not enough to solve the problem.

Treatment with antibiotics, such as Tulathromicin and Tildipirosin, at weaning also proved insufficient to reduce mortality rates dues to HPS.

A vaccination strategy was implemented using Porcilis Glasser (MSD) vaccine in sows: All sows were group vaccinated with a dose of 2 ml via IM, twice with 4 weeks interval (October/ November 2012). Gils were vaccinated twice before first mating. After the second group vaccination, sows were vaccinated at 13 weeks of gestation, to allow the passing of maternal immunity to piglets via colostrum. This vaccination scheme was kept for over 12 months. No piglet vaccination was adopted.

To improve herd stabilization to HPS and PRRSV, a gilt adaptation protocol was implemented: young gilts were brought from the genetics supplier at 4 weeks of age and were directly introduced in the nursery, in a separate box, but sharing the same room. Gilts then followed the normal nursery and fattening route, and were transferred to a quarantine building at 26 weeks of age. After an 8 week vaccination program were then introduced into the gestation barn.

Results

First group vaccinations occurred in October and November 2012, and the rest of the protocol was followed to present day. The results of nursery mortality rate are shown in the graph below. (White triangles represent the beginning of the vaccination protocol). After 4 months of vaccination and gilt adaptation, mortality rates due to HPS decreased to less than 2%.

The number of runt piglets sold (i.e. not approved for fattening) between September and November 2012/ 2013 was 1722 against 447, about four times less. All year mortality rates from weaning to end of fattening were 2011=7,79%; 2012=7,52% and 2013=4,92%.

At necropsy, comparing last quarter of 2012 with first quarter of 2013, the lesions found in dead piglets changed from polyserosites and arthritis to mostly enteric lesions.

Conclusions and Discussion

In this case, vaccination of sows against HPS and gilt adaptation reduced the nursery mortality due to HPS. Vaccine used includes HPS Serotype 5 also found in the affected piglets, so this might be a reason for success. Although HPS is, in most of the cases, a secondary pathogen, in this case, all the attempts to control the disease before vaccination failed.

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Field study: Comparative efficacy of two commercial PCV2 vaccines on swine performance in A Thai commercial farm

W Navasakuljinda[1], P Ripunchaiyaupong[1], M Lumyai[2]
[1]*Zoetis (Thailand) Ltd.,* [2] *Thai-Denmark Swine Breeder Public Company limited,* Wichian.navasakuljinda@zoetis.com

Introduction

Porcine Circovirus Associated Disease caused by Porcine Circovirus type 2 (PCV2), has been one of the most economically important diseases in Thailand. Vaccination has been proven to prevent the negative impact of PCV2 on pig health and performance. This field study observed the efficacy of 2 commercial PCV2 vaccines on pig performance in a farm with a problem of PCV2 field infection in late finishing.

Materials and Methods

The observation study was conducted on a 2,800 sow farm in eastern Thailand. The operation had been using a subunit PCV2 vaccine for 4 years, but last year experienced clinical signs suggestive of PCVAD at 18-20 weeks of age (PMWS with pigs pale, jaundiced and emaciated, also respiratory signs). PCV2 presence was confirmed by PCR. The farm decided to test a different PCV2 vaccine and 600 piglets of 3 weeks of age were divided to 2 groups: Group A was vaccinated with a subunit PCV2 vaccine (2 ml) and Group B was given a vaccine containing a PCV type1–type2 chimera (2 ml). Each group was housed in a separate barn. Data were collected for calculation of ADG, FCR, death and culling losses and treatment costs. In addition, blood samples were collected from 10 pigs per group at 3, 6, 9, 12, 15 and 21 weeks of age for measurement of PCV2 antibody titer (Serelisa PCV), and at 15 and 21 weeks for detection of PCV2 by PCR (Chulalongkorn University Lab Diagnosis).

Result and Discussion

Performance parameters are shown in Table 1. Serology results are shown in Figure 1 and the % of pigs positive to PCV2 by PCR is shown in Table 2. Given that the groups were housed in separate buildings, making the barn the theoretical experimental unit, no statistical analysis was performed. The data suggest, however, that PCV2 infection occurred in Group A after 15 weeks of age, resulting in seroconversion, and that this may have been associated with a reduction in performance.

Table 1. Performance parameters

	Group B	Group A	Diff
No. pigs in	300	300	
Start weight (Kg)	27.44	28.1	-0.66
No. pigs out	295	293	+2
Finish weight (Kg)	94.1	93.4	+0.7
Feed (Kg)	48,281	47,160	+1,121
Culling (no. pigs)	3	4	-1
Mortality (no.pigs)	2	3	-1
ADG(g/day)	766.66	755.64	+11.02
FCR	2.47	2.49	-0.02
% Loss	1.6	2.3	-0.7
Treatment cost/group(US)	44.25	49.32	-5.07

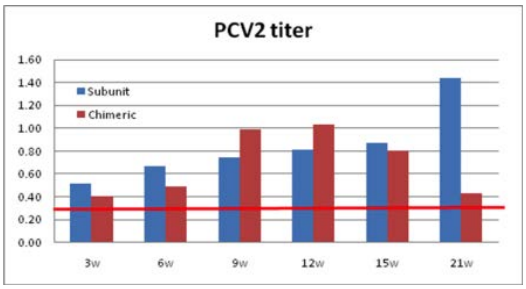

Figure 1. Serological response

Table 2. % pigs (n=10/group) positive by PCR

	Group A	Group B
15w	10	10
21w	30	10

Conclusion

Accepting the scientific limitations of a practical field trial, the chimeric PCV vaccine appeared to protect from field challenge and maintain pig performance.

Acknowledgments

This study data from Thai-Denmark Swine Breeder Public Company limited.

Field observation of efficacy of DRAXXIN on nursery pig in farms in Thailand

A Yuenyaw[1], W Nusupa[1], W Thongmak[1], W Navasakuljinda[2], S Urairong[2]
[1] Live Infomatic Co. Ltd., [2] Zoetis (Thailand) Ltd.
Wichian.navasakuljinda@zoetis.com

Introduction

Draxxin is new molecular anti-infective "Tulathromycin" that is approved to treatment pig from 3 significantly swine pathogens bacteria. However, Draxxin has used in Thailand for 2 years and worldwide used both in nursery and finishing pigs. This field observed was collected data from field used and calculated return of investment.

Materials and Methods

The trial will be conducted in 2 farms of 4,000 and 2,000 farrow-to-finishing swine operation located in western and north eastern part of Thailand respectively.

Firstly, piglets weaned from each farrowing building will be allocated into control groups (farm A 1500 heads and farm B 12500 heads) that will receive cephalpsporin (0.3 c.c.) and may receive additional antibiotic treatment if necessary**.** After each trial of control group was finishing, the trial of treatment group will be conducted. The piglets weaned from each farrowing building will be allocated into a treatment group (farm A 1500 heads and farm B 12500 heads) that will receive will receive 0.3 cc of Draxxin. Piglets in treatment pens will not receive additional antibiotic treatment until 10 days after weaning. Similar quality of feed and water will be provided ad libitum throughout the trial. Initial and final weights will be recorded individually. Feed intake will be recorded daily on a pen basis. Number of piglets died or culled will also be recorded.

Results

For a field trial on farm A (Table 1), nursery pigs treated with Draxxin seem to grew faster (296 vs 233) and were culled less (8.6 vs 17.0) than those not receiving Draxxin. The feed conversion ratio were, however, similar (1.66 vs 1.69). For a field trial on farm B (Table 2), nursery pigs treated with Draxxin grew faster (355 vs 294), had better feed conversion ratio (1.73 vs 1.93) and were culled less (8.3 vs 15.4) than those not receiving Draxxin.

Table1. Comparative nursery performance index in Farm A

Item	Farm A		
	Draxxin	Cephalosporin	Difference
No. of pigs	1,507	1,532	-25
Weight in (kg)	7.2	6.8	+ 0.4
Days in barn	38	38	-
Weight out (kg)	18.1	16.6	+ 1.4
ADG (kg)	0.296	0.233	+ 0.063
FCR	1.66	1.69	- 0.03
Mortality rate	1.7	1.7	-
Culling rate	6.9	15.3	- 8.4
Total culling rate	8.6	17.0	- 8.4
% Pig out	91.4	83.0	+ 8.4
Drug price/pig (USD)	0.95	0.76	+ 0.18

Table2. Comparative nursery performance index in Farm B

Item	Farm B		
	Draxxin	Amoxicillin	Difference
No. of pigs	11,761	13,528	- 1,767
Weight in (kg)	7.3	7.5	- 0.2
Days in barn	55	57	- 2
Weight out (kg)	22.9	21.4	+ 1.5
ADG (kg)	0.355	0.294	+ 0.061
FCR	1.73	1.93	- 0.20
Mortality rate	2.9	7.6	- 4.7
Culling rate	5.4	7.8	- 2.4
Total culling rate	8.3	15.4	- 7.1
% Pig out	91.7	84.6	+ 7.1

Conclusion

Draxxin given to nursery pigs consistently improves growth rates and reduces the number of pigs culled. Although the effects on growth rate and culling rate should be good enough to justify the more expensive cost of Draxxin.

Acknowledgments

Commercial swine farm in Northern part and North-East part of Thailand.

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Eradication of *B. hyodysenteriae* in a sow pool system

T Barmettler[1], P Scheer[1], F Zeeh[1,2]
[1] *SUISAG Pig Health Service,* [2] *Clinic for Swine, Vetsuisse Faculty Berne, University of Berne; Switzerland,*
friederike.zeeh@vetsuisse.unibe.ch

Introduction

Brachyspira (*B.*) *hyodysenteriae* is the causative agent of Swine Dysentery (SD), which has a severe impact on pig production due to illness, mortality and reduced performance (3). The Pig Health Service in Switzerland monitors SD in its member herds by clinical observation and epidemiological tracing-back (4). Until 2013, herds positive for *B. hyodysenteriae* were recommended to eradicate the pathogen by means of total depopulation or partial depopulation combined with treatment and strict cleaning and disinfection (5). With regard to "herd", all sows in a sow pool system are regarded as one unit and eradication has to be performed on every single farm of the infected sow pool.

The abstract describes an eradication of *B. hyodysenteriae* in a Swiss sow pool system.

Materials and Methods

In a sow pool system comprising 8 farms with approx. 900 sows and their offspring, *B. hyodysenteriae* was diagnosed by PCR in rectal samples of growers in April 2013 (Tab. 1). Due to diarrhea a permanent treatment with different antibiotics had been necessary.

The pool operated in a 1-week-farrowing system. Pregnant sows were housed in two sites, farrowing took place in those 2 farms and 6 other farms. Eradication was prepared immediately after diagnosis by implementation of a professional rodent control and reduction of sow number (insemination break, slaughtering of sows). Fattening pigs were slaughtered, weaned piglets and growers were sold to farms that had already received pigs from the pool. Slurry was removed from the pits. In July, medication of sows older than 270 days was performed for at least 21 days (tiamulin fumarate 10mg/kg bodyweight and day, p.o.). Minimum ten days after start of treatment, part of the one building that housed gestating sows and the tools and equipment were cleaned, disinfected (peracetic acid, hydrogen peroxide) and remaining slurry and slats were treated with cyanamide 50% ($3L/m^3$ and $0.3L/m^2$). In parallel, sows were washed thoroughly and entered the clean area through a lock. Successively the farrowing farms were emptied, cleaned and disinfected as described above and restocked within one week with sanitized sows. Finally, the second gestation barn was handled similar to the first one. In September, the eradication was finished.

A control plan (clinical monitoring, sampling of 10 pigs in 2 farms every 2 months) was established. In December, the first set of fecal samples of 10 weaners of one farm was tested.

Results

The sanitation of the complete sow pool system was finished within 9 weeks. Until now, no signs of clinical SD have been observed in the pool system. The first set of fecal samples was negative for *B. hyodysenteriae*, but still positive for *B. pilosicoli* (Tab. 1). Testing is ongoing until June 2014. The costs were calculated being approximately 450 USD per sow; however the labor costs could not be calculated precisely.

Conclusions and Discussion

Eradication of SD in a sow pool system is very complex, challenging and requires man power, drugs, disinfectants and time. Careful planning and open and precise communication to care takers are mandatory. The infection source in the sow pool could not be determined, but rodents (rats) are very likely since they were found in several farms and are known to be one of the main risk factors (1). Replacement gilts came from an unsuspicious source farm with regular testing of *B. hyodysenteriae*. Despite recommendation to test susceptibility of *B. hyodysenteriae* before starting an eradication (2), tiamulin was chosen without antibiogramme but based on own experience and resistance reports in literature (6).

Until now, sanitation of *B. hyodysenteriae* seems to be successful. Diarrhea is observed only occasionally, performance of pigs has improved and use of antibiotics has declined drastically. But outcome of the eradication has still to be proven by further testing.

B. pilosicoli and *L. intracellularis* could not be eradicated in this sow pool with the described method.

Table 1 PCR results of pooled rectal swab samples

	APR 2013		DEC 2013	
Agent	pos	neg	pos	neg
Brachyspira hyodysenteriae	3	2	0	5
Brachyspira pilosicoli	4	1	1	4
Lawsonia intracellularis	2	3	1	4

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Field observation of the efficacy of Flexcombo in finishing performance in Thailand

W Thongmak[1] , T Yong Sripanyarit [2], S Kongtes[3] , N Duangwhae[3]
[1]*Live-infomatics company* [2]*BestAgro group company* [3]*Boehringer Ingelheim (Thai) Co;Ltd*
nathaya.duangwhae@boehringer-ingelheim.com

Introduction and objective

Diseases associated with Porcine Circovirus Type 2 (PCV2) and *M. hyopneumoniae*(M hyo, enzootic pneumonia) infections are a major concern in the swine industry. M hyo has also been considered as one of the major co-factors in the development of PCVAD.[1,2] The objective of these studies was to evaluate the efficacy of both Porcine Circovirus Type 2 and M hyo vaccines when the monovalent licensed vaccines for the two agents are mixed and administered in a single combined injection in a PRRS stabilized farm.

Materials and Methods

The retrospective field observation was conducted on a 3 site production farm with 2,350 sows in Thailand. The sow herd is stabilized for PRRS by mass vaccination with Ingelvac PRRS MLV. Piglets are weaned at 26 days of age and routinely vaccinated with Ingelvac PRRS MLV at 2 weeks and Ingelvac Mhyo and Ingelvac CircoFLEX at 4 weeks of age, respectively, Pigs are shipped to contract fattening farms at the age of about 8 weeks. 14 consecutive batches with 7004 pigs were evaluated in this study. 2920 pigs from 7 batches were separately vaccinated with Ingelvac CircoFLEX and Ingelvac M hyo and 4084 pigs from 7 following batches were vaccinated with FLEXcombo (Ingelvac CircoFLEX + Ingelvac MycoFLEX, licensed to be mixed and administered in a single combined injection).. Separate as well as FLEXcombo vaccinations were applied at 4 weeks of age. All animals were kept under the same management program and feed formulation. The monitored parameters were average daily weight gain (ADWG), Feed conversion ratio (FCR) and total losses. Parameters were evaluated using standard statistical process control (SPC) performed by Statistica version 8.1.The differences between the groups were evaluated by students T-test.

Results

The results of production parameters are shown in Table 1.

The overall growth performance and FCR showed significant differences between both groups. The chart of growth parameters such as the ADG is shown in figure2.

Table 1. Evaluation of fattening pigs batches with two different vaccinations schemes.

	Separate	FLEXcombo	p-value
Prod. Batches (N)	7	7	n/a
Avg. Weight In (Kg)	20.4	19.50	n/a
Avg. Weight Gain (Kg)	97.85	95.44	0.333
ADGW (g/d)	643	683	0.045
FCR	2.66	2.54	0.022
%Total loss	4.30	6.03	0.158

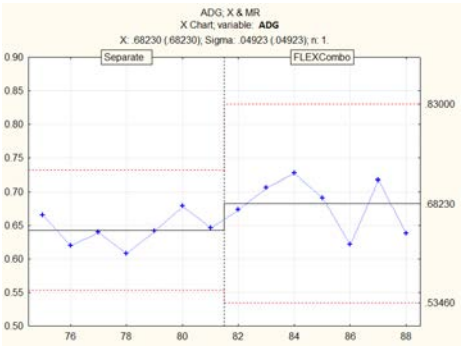

Figure1. SPC I-Charts for Average daily weight gain and FCR in finishing period

Conclusions and Discussion

The mixture of Ingelvac MycoFLEX and Ingelvac CircoFLEX delivered in a single 2 ml injection was safe and efficacious as the conventional separate vaccination scheme with Ingelvac M.hyo and Ingelvac CircoFLEX . This is in line with previous studies demonstrating the efficacy of FLEXcombo (1,2). This mixing license not only provides the protection for both pathogens, but also reduces the number of injections, pigs stress and labor requirements.

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Impact of feed change on growth and technical parameters, on a Dutch closed pig farm

MDE de Louw
Veterinary practice Ell, Ell The Netherlands, mdedelouw@gmail.com

Introduction

Several factors influence the technical results of fattening pigs during their growth period. One of these factors is the feed intake and feed quality. Other important factors are climate, water quality, genetics and health status of the animals. The objective of this paper is to give a retrospective analysis of the difference in growth, technical parameters and clinical signs after management changes on a Dutch closed pig farm.

Materials and Methods

Production- and slaughter data of a 100 sow farm with 2500 fattening places was analyzed for the period October 2010 until September 2013(Agrovision management system). Missing piglets for the total amount of fattening places are bought from another sow farm. These piglets are vaccinated with Circoflex. Twice a year blood samples were taken from sows, gilts, piglets of 5 and 10 weeks and fattening pigs of 16 and 22 weeks old for a monitoring program (Respig). No blood samples were taken from the purchased pigs. In this study PRRSV (Elisa IDEXX), Influenza (HI-SWI), APP (Elisa-OMP) Mycoplasma (Elisa IDEXX) were taken in account. A history of coughing and sneezing from 10 weeks old piglets until slaughter pigs was present at the farm; therefore a Mycoplasma vaccination was started. Vaccination with Mycoflex at 3 weeks after birth was performed form October 2010 until March 2011. From May 2011 to April 2012 a vaccination with Porcillis M. Hyo (2-shot), was executed. The first injection was administered at 1 week of age, the second 3 weeks later. Since October 2012 another change to MycoFLEX in combination with CircoFLEX was performed, at 3 weeks of age. From October 2011 the feeding of the fattening pigs changed from one to another feed supplier.

Results

Several factors changed from February 2011 to October 2012. The type of Mycoplasma vaccination changed twice, alternated each time with a period of no vaccination, and the farm changed of feed supplier. At the end of the study period no permanent coughing or sneezing in the weaned piglet or fattening unit was present. During this period of time some clinical signs of APP and Lawsonia intracellularis were seen in the fattening unit. Weaned piglets sometimes showed signs of Steptococcus suis infections at 5 weeks of age.
From the monitoring program for the different agents the following developments were observed:
APP antibody titres were high positive in 10 to 22 weeks old pigs (6,6 – 8,5) and Mycoplasma titers in the 16 and 22 weeks old pigs were low (0,0 – 0,132) for the whole observed period. PRRSV: sows of different parities were positive during the whole period (1,41 – 2,17). Since January 2013 the 10 weeks old piglets went from positive to low titres (2,93 – 0,25). Some circulation of PRRSV is seen during the fattening period but with a low response (0,015 – 1,5). Influenza titers are positive for all three Flu strains in sows and 22 weeks old pigs.

From October 2011 (change of feed supplier, vertical line fig 1) the pigs improved in growth from 780 gr/day to 830 gr/day and in technical results (fig 1);
Meat percentage increased from 57,0 % to 59,1%, the back fat thickness increased from 15,4mm to 13,9mm
Muscle percentage increased from 57,25 % to 61,92%.

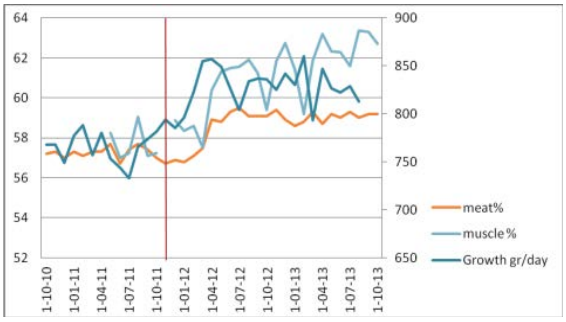

Figure 1. Average daily gain (grams/day) and muscle percentage of fattening pigs in time

Conclusions and Discussion

This retrospective analysis demonstrates that the change of feed supplier increased the growth and improved pork meat quality on a Dutch closed pig farm. The clinical signs of coughing and sneezing were not permanent at the end of the observed period. APP antibody titres were high positive, Mycoplasma titres were low, PRRSV titres were positive in sows en became low in 10 weeks old piglets. Influenza titres are positive for all strains. It is not demonstrated that the changes in vaccination strategy contributed in the improved growth and technical results. Veterinarians and pig farmers are sometimes blind to other reasons than infections on poor performance of slaughter pigs. They forget to look at the basic needs of the animals.

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Efficiency of "Doxycycline – complex" preparation for treatment of gastrointestinal diseases in swine

KI Sazykina, SA Staroverov, AA Volkov, SV Kozlov, IN Zhirkov
Saratov State Agrarian University named after N.I.Vavilov, Russia, zhircov@gmail.com

Introduction

At present, the problem of treatment of gastrointestinal diseases of different etiology is very actual in veterinary medicine. Digestive system diseases in young animals is one of the most important problems of internal pathology and it occupies the first place both for case frequency among all forms of internal non-contagious diseases and for economic damage it causes. Reduced organism resistance, caused by different unfavorable factors, can often provoke different gastrointestinal diseases, such as gastroenterocolitis, gastroenteritis, colibacillosis, especially in young swine. In this connection we elaborated and studied a liquid oral form of "Doxycycline – complex" preparation containing the following active substances: doxycycline hyclat - 100mg/ml, bromhexine hydrochloride – 5 mg/ml, lactulose – 10 mg/ml; soluphor (polyvinylpyrrolidone) was used as a solvent.

Materials and Methods

40 pigs, selected according to the principle of analogs (of "Big white" breed, 3 months of age), spontaneously fallen ill with colibacillosis, were experimental objects. Average live weight of the animals was 30 kg. "Doxycycline – complex" preparation was introduced orally, according to the instruction. Previously 100gr of sugar /1litre of water were added into prepared solution to improve its taste. It was introduced with individual and group methods 2 times a day during 5 days.

Results

100% efficiency of "Doxycycline – complex" preparation at a daily dose of 10ml/kg for treatment of colibacillosis in swine was confirmed by the experimental research results. On the second day after introduction the preparation general health condition of animals became better, diarrhea stopped, appetite was enhanced. On the third day general physiological indicators were nearly normal. Complete recovery was achieved in 4±0,02 days. Side effects of the preparation on animal's organism were not found. Bacterial inoculation confirmed normal microflora. Toxigenic colon bacillus cultures were not present. High efficiency of the elaborated liquid oral form of "Doxycycline – complex" preparation is conditioned by the presence of lactulose and the right choice of solvents, providing high bioavailability. In particular, it is stated that the most effective medical preparations are preparations in colloidal micelle systems, and concentrated preparations on the basis of organic solvents are more effective than dry antibacterial agents.

Conclusions and Discussion

In animals of 1,2 experimental and a control group recovery was achieved on the 7^{th} (7±0,03),5^{th} (5,23±0,02) and 5^{th} (5,24±0,02) day respectively. The given data confirm high therapeutic efficiency of "Doxycycline – complex" preparation for treatment of colibacillosis in pigs at a daily dose of 10mg/kg.

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Comparison of the growth performance of Improvac® male pigs with surgically castrated male pigs

K Lertphitak[1], K Sangvixienkit[1], J Thongkaew[1], M Linatoc[2]
[1]*Zoetis (Thailand) Limited,* [2]*Zoetis Inc.,* kanyarat.lertphitak@zoetis.com

Introduction

Improvac® (Zoetis, Madison, NJ, USA) is an immunological castration vaccine which is used for reducing boar taint through two injections. Improvac offers better growth performance, health and carcass quality benefits by allowing the rearing of male pigs as entire boars until shortly after the second injection. Stopping castration and using Improvac to control boar taint results in improved feed efficiency (2, 3, 6), increased cutting yield (2, 4) and reduced piglet mortality (1, 5). The objective of this trial was to compare the growth performance of pigs that were vaccinated with Improvac with surgically castrated pigs.

Materials and Methods

The study was conducted in a commercial pig farm located in Nakhonpathom province, Thailand. Ninety-two male pigs were selected at birth and divided into 2 groups: 1) immunologically castrated pigs which received a 2 ml subcutaneous dose of Improvac in the neck, close to the base of the ear at 15 and 19 weeks of age as per label instructions; and 2) surgical castrate pigs castrated within 3-7 days of age. Pigs were housed by treatment in pens of 46 and had ad libitum access to feed and water. Both groups were fed same diets and kept in the same environment. Pigs were weighed at entering to finishing unit (10 weeks of age) and a day before slaughter at 24 weeks of age. Feed intake was measured from 10 weeks of age until slaughter.

Results

The results of the study are presented in Table 1. The average weight at slaughter of the Improvac group was on average 3.5 kg more than the surgically castrated group. The average daily gain (ADG) of the Improvac group was 34 g/day better than the castrates. For feed conversion ratio (FCR) we found that the Improvac group showed an improvement in FCR of around 8% compared to the surgically castrated group.

Table 1. Comparison of the growth performance of Improvac vaccinated pigs (Imp) with surgically castrated control pigs (Cast).

	Imp	Cast	Difference
Number	46	46	0
Weight at 10 weeks (kg)	33.34	33.40	-0.06
Weight at slaughter (kg)	111.74	108.59	+3.15
Day to slaughter	96	96	0
ADG (g/day)	817	783	+34
FCR (g/feed/g gain)	2.47	2.68	-0.21

Conclusions and Discussion

In summary the Improvac vaccinated pigs had better growth performance than the surgically castrated pigs resulting in heavier pigs at slaughter and a reduced FCR. The commercial use of Improvac would enable producers to improve production efficiency while still controlling boar taint. The findings from this study confirm those reported from other countries under either controlled experimental conditions (2) or under field use conditions (6).

Acknowledgement

Thank you to the farm owner and managers of Donsala farm, who worked and shared their data with us.

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Persistence of virus, bacteria, mold, yeast and parasites in different ways of using pig manure

O Betancur[1], JA Betancourth[2], J Estrada[3], FJ Henao[3]
[1]*Novartis de Colombia SA Animal Health* [2]*Corpoica, Colombia* [3]*Departamento de Producción Agropecuaria, Universidad de Caldas, Manizales, Colombia,* fhenao@ucaldas.edu.co

Introduction

The strategic use of pig manure as raw material for feeding livestock or as biofertilizer (8) is limited by health authorities based on their possible contamination risk (4, 9). Of the different forms of use, only a few provide bioremediation potential, therefore generating the need to broaden this horizon by the means of research (9). The aim of this study was to establish the persistence of viruses, bacteria, mold, yeasts and parasites in different ways of using pig manure in Colombia.

Materials and Methods

In seven ways of using pig manure, it was assessed the persistency of: Porcine Parvovirus (PVP), Porcine Circovirus Type I (PCV–I), Porcine Circovirus Type II (PCV–II), Porcine Reproductive and Respiratory Syndrome Virus (PRRS), Porcine Herpesvirus Type I (HVP-I), *Salmonella* spp., *Clostridium* sulfite-reducing., aerobic mesophiles, *S. aureus*, Total Coliforms/*E. coli*, *Actinobacillus* spp., *Leptospira* spp, *L. monocytogenes*, *L. intracelularis*, *Aspergillus* spp., *Candida* spp., *A. suum*, *C. parvum*, *T. suis*, *B. coli*, *Metastróngylus spp.*, *G .intestinalis*, *Strongyloides* spp., *Coccidias* spp., and *Estrongilidos* spp. The forms of use were: manure heap, dryout, biodigester, silage, compost, earthwormcompost and earthworm flour. The evaluation techniques used were the recommended for each microorganism and were carried out in certified labs by the Colombian Health Authority. The evaluation was performed in duplicate in three farms.

Results

Of the assessed viruses, PCV-II was found in two farms, and it did not persist in any of the ways of use. *L. intracelularis* was found in all farms but did not persist in compost, earthworm compost, or earthworm flour. *Salmonella* spp. was found in three farms but only persisted in biodigester. *L. monocytogenes* was observed in a farm and did not persist in silage, earthworm compost or earthworm flour. Total coliforms, *Clostridium* sulfite-reducing and aerobic bacteria persisted in all the farms and routes of use. Mold and yeasts were observed in three farms, and did not persist in manure heap, dryout, compost, or earthworm flour. In all the farms *S. aureus* was observed, and did not persist in silage, compost, or earthworm flour. *Estrongilidos* spp. were found in two farms and did not persist in manure heap, biodigester or silage. *B. coli*, *Strongyloides* spp., and *Coccidias* spp. were observed in three farms, the first one did not persist in manure heap, nor in compost, the second ones did not persist in biodigester, and the third one did not persist in earthworm compost; none of these persisted in silage, dry pig manure nor earthworm flour.

Conclusions and Discussion

The best ways in the elimination of pathogens were: silage, compost, dryout and earthworm flour. In silage, most of the pathogens did not persist, similar to results obtained by other researchers (3). In dry manure and earthworm flour few pathogens persisted, possibly due to effects of dehydration (5). The results of this study agree with others for *Salmonella* spp. (7) and for mold and yeasts (11), but differ with those in the removal of coliforms. In this study PCV-II did not persist, opposed to the results of other researchers (10). The elimination of some pathogens in biodigestors was not complete, agreeing with other results (1). As in this study, persistence is reported for *B. coli* and *Coccidias* spp. in processed pig manure (2). Earthworm compost is an efficient process in the removal of pathogens (6), but the results of this study were not consistent. As stated by some authors, variability of physical, chemical and biological factors affect the persistence of pathogens in droppings (9). We do not dispose of bibliographic information about the reductiont of microorganisms by manure heaps; however, positive effects were observed. With this work it can be demostrated that the health quality of pig manure can be improved and enable its adequate use.

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Montecarlo approaches to compare the treatment efficacy of pig respiratory disease with two medicinal products containing florfenicol as active ingredient

C Vilalta[1], S Colomer[2], M Perelló[2], M Busquet[2], L Fraile[a]
[1] *Universitat de LLeida, Lleida, Spain.* [b] *Laboratorios Hipra SA, Girona, Spain*
lorenzo.fraile@prodan.udl.cat

Introduction

Antimicrobial drugs have been classified as concentration-dependent or time-dependent. The concentration-dependent are those where increasing concentrations at the locus of infection improve bacterial kill. The time-dependent are those where exceeding the minimum inhibitory concentration (*MIC)* for a percentage of the inter-dosing interval (*T>MIC)* correlates with clinical efficacy. Florfenicol is an antimicrobial widely used in swine medicine that has been described as concentration or time-dependent relying on the bacterial species involved. The goal of this trial is to foresee the clinical efficacy of a pharmaceutical product (Selectan®) whose main active ingredient is florfenicol to treat *Actinobacillus pleuropneumoniae* (APP) and *Pasteurella multocida* (PM) infection in pigs in comparison with the reference product (Nuflor®).

Materials and Methods

A model was developed to predict the likelihood of attainment of the Pharmacokinetic (PK)/Pharmacodynamic (PD) parameters that determines florfenicol efficacy on APP and PM infections in pigs. For this analysis, Montecarlo simulations were performed using the pharmacokinetic data calculated for Selectan® and Nuflor® (Laboratorios HIPRA, registration dossier data) and the MICs for APP and PM published in the scientific literature (Lizarazo et al, 2006, Gutierrez-Martin, et al, 2006). Thus, a population of 100.000 pigs and strains was created to run the simulations. Area under the curve (AUC)/MIC over 50 and T>MIC 40% of the dose interval are the PK/PD parameters to be associated with antibacterial efficacy according to the literature for florfenicol. The likelihood of attainment of the Pharmacokinetic (PK)/Pharmacodynamic (PD) parameters that determines florfenicol efficacy on both microorganisms was calculated using CrystalBall Software (V. 11.1.2.0.00; Oracle Corporation, RedwoodShores, CA, USA) as previously described (Messenger, 2012).

Results

After running the model, the probability of clinical success, using the AUC/MIC>50 and T>MIC 40% of the dose interval as threshold values for Selectan® and Nuflor® are shown in table 1.

Table 1. Probability of clinical success for Selectan® and Nuflor® using AUC/MIC>50 and T>MIC 40% as threshold values

	PK/PD parameter	
Selectan®	**AUC/MIC>50**	**T>MIC40%**
APP	90	94.7
PM	87.8	94.2
Nuflor®		
APP	86.7	91
PM	74.9	81.5

Conclusions and Discussion

The ideal situation is to know the antimicrobial susceptibility of any microorganisms before applying antimicrobial treatments. However, this information is not available in many occasions under practical conditions. On the other hand, it is necessary to apply an antibiotic treatment with a high probability of clinical success. In this trial, it is described a rational approach to compare the clinical efficacy of two pharmaceutical products based on florfenicol. In this study, it is clear that Selectan® and Nuflor® are bioequivalents from the clinical point of view and both products would be efficacious in most cases to treat pig respiratory disease due to APP and PM. As demonstrated above, he foreseen clinical efficacy of Selectan® is better than for Nuflor® for both APP and PM treatment. A possible explanation could be the higher homogeneity observed in the pharmacokinetics of Selectan® versus the reference product at population level.

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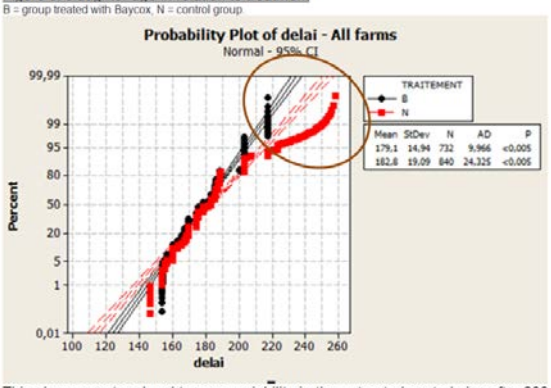
Effects of oral Toltrazuril (BAYCOX 5%®) on the growth performance of pigs up to slaughter

D Marchand, L Goureau, H Perrin, D Descamps, R Cristal, F Jean-François Perzo, M Germain,
B Santé Division Santé Animale, France, jrvm_99@yahoo.com.mx

Clinical studies have shown that the anticoccidial toltrazuril has beneficial effects on piglet weaning weight and on the intestinal health of pigs at subsequent production stages. The aim of this study was to evaluate the effects of toltrazuril (Baycox® 5% oral suspension) on the performance of pigs up to slaughter. Piglets aged 3 to 5 days at 3 pig breeding farms received either toltrazuril (group A, n = 942) or no treatment (group B, n = 801). Two or three successive feedlots were involved in the study at all three breeding farms. Slaughter weight, slaughter age, and LMP were compared between the groups by analysis of variance with treatment, gender and farm as factors. When a factor was statistically significant ($p<0.05$), additional tests were used to explain the result. For example, slaughter age distribution was examined graphically using both a box and whiskers plot and distribution histograms.
All calculations were performed by MINITAB statistical software version 16.
The results obtained showed that toltrazuril had beneficial effects on pig growth from farrowing to slaughter.

Figure 3: slaughter age in relation to treatment
B = group treated with Baycox, N = control group



This shows greater slaughter age variability in the untreated control pigs after 200 days, with a large number of animals requiring a longer fattening time.

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Malassezia spp. yeast in adult pig's ear channel

A Pulido-Villamarín[1], S Damme-Pedraza[1], A Barbosa-Buitrago[2], R Castañeda-Salazar[1], R Cubillos-Azcárate[3]
[1]*Unidad de Investigaciones Agropecuarias (UNIDIA), Departament of Microbiology, Faculty of Sciences, Pontificia Universidad Javeriana, Bogotá, COLOMBIA.* adriana.pulido@javeriana.edu.co. [2] *MV, MSc (C) Universidad El Bosque.* [3]*MV, Faculty of Animal Sciences, Universidad de Ciencias Aplicadas y Ambientales (UDCA)*

Introduction

The genus *Malassezia* ssp. belongs to the group of lipophilic yeasts that may be found as normal flora in human and animal skin, however it has been identified as an opportunistic pathogenic agent (6, 9, 10). There have been 14 different species identified: *Malassezia furfur, M. globosa, M. obtusa, M. restricta, M. slooffiae y M. sympodialis* (4), *M. nana* (5), *M. dermatis* (9), *M. japónica* (10), *M. equina, M. caprae* (1), *M. cuniculi* (2) y *M. pachydermatis* (4); The latter being the only non-lipid-dependant. In swine population, there's only few reports available, associate with this microorganism; however, Garau *et al.* in 2005 reported the presence of *Malassezia* yeast in healthy pigs, in this report, 58% belonged to *M. sympodialis* and 30% to *M. sloofiae* (3). Additionaly in 2010, Nardoni *et al.* detected the presence of *M. pachydermatis* in 13,6%, *M.furfur* in 22,7% and *M. sympodialis* in 63.6% (7). Taking these previous studies into account, the objective of the present study was to determine the species of *Malassezia* yeast present in the ear channel of adult pigs belonging to the region of Cundinamarca in Colombia.

Materials and Methods

24 otic swab samples were taken from adults pigs from three semi-technified farms. 15 out of 24 showed abundant brownish serous secretion, but 9 did not evidenced it.. Primary isolation was performed in modified Dixon Agar and the phenotypic identification was determined by biochemical testing methods such as assimilation of lipidic supplements (Tween 20, 40, 60, 80 and Cremophor-EL), amongst others (4).

Results

From the 24 samples taken, 55 isolations were obtained. From these it was determined that 18% (n=10) corresponded to *M. slooffiae*, el 7% (n=4) to *M. sympodialis*, 7% (n=4) to *M. furfur*, 6% (n=3) to *M. pachydermatis*, 4% (n=2) to *M. obtusa* and 49% (n=27) to *Malassezia* spp; the remainder 9% (n=5) corresponded to yeasts of the *Candida* spp. species.

Conclusions and Discussion

According to the results obtained and previous studies, the presence of this type of yeasts in the ear canal of adult pigs could be confirmed. The species identified were: *M. sympodialis, M. pachydermatis, M. obtusa* and *M. slooffiae.* Because of the few studies related to the presence of these types of yeasts in pigs, It has not been possible to relate it as the etiology of external otitis in pigs. This can become a new field of study in the line of pig health, being this the first study ever developed in Colombia about this topic.

Acknowledgements

Pontificia Universidad Javeriana

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Pharmacokinetics of amoxicillin in piglets after oral in-feed administration

P Poolperm[1], P Udomkusonsri[2]
[1]*Department of Farm Resources and Production Medicine,* [2]*Department of Pharmacology, Faculty of Veterinary Medicine, Kasetsart University, Thailand,* fvetpap@ku.ac.th

Introduction

Feed medication has been allowed to use in pig industry in Thailand. For veterinary standpoints, feed medication is used as a metaphylatic fashion; means to use antibiotics to minimize an anticipated disease at a specific period for a specific group of animals. Amoxycillin is one of the major antibiotics used in pig industry to control an infection of *Streptococcus* spp. during nursery and starter periods. It is well documented that antibiotics in Penicillin group are not well tolerated to acidic pH in gastric juice. This might caused differences in efficacy of antibiotics, especially amoxicillin (AMX) commercially available worldwide. The objective of this study was to determine the efficacy in Pharmacokinetics of Suramox®, an in-feed AMX commercially used worldwide.

Materials and Methods

Four cross-bred weaned piglets were randomly selected in this study. Pigs were fed non-medicated feed until study. All pigs, then, were administered amoxicillin (Suramox®, Virbac, France) orally in-feed (PO) at 20 mg/kg as a bolus administration. Blood was taken from the jugular vein at the following times: 0, 20, 30 and 45 min and 1, 2, 4, 6, 8, 10, 12, 24 and 36 hr after AMX administration. Plasma was immediately separated from blood and stored at -80°C until sample analysis was performed.

Antimicrobial assay and pharmacokinetic analysis: Plasma samples were assayed for antimicrobial activity using the agar-well diffusion microbiological assay using *Micrococcus luteus* (ATCC 9341) as an indicator organism (1). The lower limit of sensitivity of the assay was 0.05 µg/ml. The pharmacokinetic parameters of AMX on plasma were performed using PK solutions 2.0™ (Noncompartment Pharmacokinetics Data Analysis, Summit Research Services, CO, USA).

Results

The maximum drug concentrations (Cmax) were 3.62 ± 0.63 mg/kg at 3.0 ± 1.41 hr. The drug concentration was not detected after 12 hr after administration. The plasma concentration of AMX was shown in Figure 1. Elimination half-life ($t_{1/2}$) was 1.48 ± 0.13 hr. The AUC values was 18.44 ± 3.71 mg-hr/L.

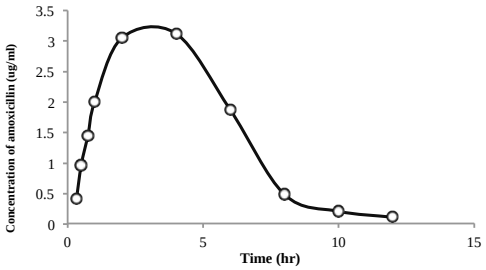

Figure 1. The mean plasma concentrations (mg/L) of amoxicillin in piglets following oral in-feed (PO) administration of amoxicillin at 20 mg/kg body weight.

Conclusions and Discussion

Pigs received a bolus of in-feed Suramox® could maintain its concentration level in plasma for at least 12 hours, however, in real life, pigs are continuing receive AMX in-feed *ad libitum*. From veterinarian stand point, pigs received Suramox® in-feed had maximum concentration in plasma within 3 hours. The AMX concentrations in plasma were higher than MIC of AMX against common pathogens in swine, especially *Streptococcus suis* (2,3). These findings could not apply to others commercial or chemical AMX used in-feed.

Acknowledgments

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Methane and biogas production from slurry of finishing pigs fed with fibrous diets

B Berenchtein[1], A Abdalla[2], H Louvandini[2], A Abdalla Filho[2], P Lima[2], D Danashekaran[2], P Santos[2], A Souza[2], L Castilho[2], M Peçanha[2], N Beltrão[1]

[1]*Institute of Social Sciences, Education and Animal Science, Federal University of Amazonas, Laboratory of Studies and Researches of Poultry and Swine Nutrition and Production (LEPPNAS), Parintins, AM,* [2]*Centre of Nuclear Energy in Agriculture, University of São Paulo, Animal Nutrition Laboratory, Piracicaba, SP,* bernardob@ufam.edu.br

Introduction

The possibility of using forages and other hays as feedstuffs in swine production had already been theorized (1). However, further studies are needed to evaluate the pollution potential of excreta and about the slurry anaerobic digestion, whereas the animals present limited capacity of the digestive tract to process this fibrous material. Methane (CH_4) is a greenhouse gas and recent inventories have shown that livestock manure makes a significant contribution to global CH_4 emissions. Although, by using controlled anaerobic digestion of animal manure, the CH_4 emissions can be used as a substitute for fossil fuels, serving as a CO_2 neutral energy source.

Therefore, the objective of the present study was to quantify the production of biogas and methane production from slurry of finishing pigs fed with fibrous diets.

Materials and Methods

Twenty male finishing pigs (70 kg ± 2.4 kg) were randomly allotted in metabolic cages and fed with basal diet (BD) or basal diet + 20% of Tifton Hay (TH), Citric Pulp (CP) and Soybean hull (SH). The BD was formulated to meet all requirements for protein, amino acids, minerals, and vitamins. The wastes were collected on the metabolic cages and bulked by feed. The substratum (slurry) was composed by waste (feces and urine) diluted with water. A laboratory scheme was used, with four treatments (BD, TH, CP and SH) and five replications, consisting of twenty digesters (total volume was 3.125 L) exposed to 39 ºC (anaerobic incubator) for 7 days and gas production measurement at regular intervals. After each reading of the gas production, an aliquot of gas was stored in ”vacuntainers” previously identified and submitted to the vacuum. For further quantification of methane, the sampled gas was analyzed in chromatograph of gaseous phase, model CG-2014 GAS (Shimadzu). The calibration of the equipment was made with standard gas. It was evaluated the total volumetric production of biogas and methane (ml), under these conditions. The analysis of variance was performed using PROC GLM of SAS (2) and the means was compared by Tukey test ($p<0.05$).

Results

The average biogas and methane production (mL) are shown in Table 1. No significant difference ($p> 0.05$) between treatments was founded.

Conclusions and Discussion

Different result was showed by (3) who explain that CH_4 production depends on the dietary content of fermentable fibre; less fermentable fibre in the diet and in slurry, resulting in less CH_4 production. In this trial with this conditions, the different sources of fiber like soybean hulls, citrus pulp and Tifton hay did not affect the production of renewable energy, such as biogas and methane.

Table 1. Biogas and methane production (mL) from slurry of finishing pigs fed with Basal Diet (BD), Basal Diet with 20% of Tifton Hay (TH), Citric Pulp (CP) and Soybean hull (SH), and Coefficient of Variation, % (CV)

Variable	BD	TH	CP	SH	CV
Biogas	2551.8[a]	2530.3[a]	2400.2[a]	2320.0[a]	31.1
Methane	166.75[a]	154.40[a]	112.46[a]	128.06[a]	54.3

(a, b) Superscripts indicate statistically significant differences within main effect ($p \leq 0.05$)

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Milk spot liver lesions in slaughtered pigs in Italy: Prevalence and preliminary results on herd risk factors

A Luppi [1], P Bonilauri[1], G Mingarelli[2], E Ferrari[3], G Maioli[1], G Biasi[1], A Rosamilia[1], C Corino[5], M Dottori [1]

[1]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER),* andrea.luppi@izsler.it

[2]*Azienda ASL Reggio Emilia,* [3]*Azienda ASL Modena,*

[5]*Department of Health, Animal Science and Food Safety,Università degli Studi di Milano*

Introduction

Ascariasis is the most important internal macro-parasitism present in farmed pigs world-wide. A number of negative effects have been attributed to it including depressing growth rates, decrease in feed conversion efficiency, interference with the pig immune modulation and condemnation of livers at the slaughterhouse (1). Aims of the present study was to evaluate the prevalence of milk spots in Italian slaughtered pigs and the risk factors influencing the occurrence of these lesions.

Materials and Methods

From January to October 2012 a total of 4764 batches (667.028 pigs) belonging to Italian herds were inspected at the slaughterhouse for the presence of milk spot liver lesions. Following the results obtained at the survey described above, 18 herds, producing heavy pigs (160 kg slaughter weight, aged 9–10 months), were selected from October and August 2012-2013. At the slaughterhouse 70 pigs per batch (140 pigs on average) belonging to each herd selected were examined for white spot (WS) liver lesions using a quantitative lobar scoring system: score 0 (no WS); score 1 (1-5 WS); score 2 (6-10 WS) and score 3 (>10 WS). Each farm was visited by a swine veterinarian and data about farm characteristics were collected: operation type, herd size, number of fattening pigs in the farm, type of floor and anthelmintic treatments, were collected using a questionnaire. Pooled floor fecal samples were randomly collected in pigs 50, 70 and 100 kg body weight (BW), to establish if adult worm infection was present. The samples were examined by McMaster method to detect the level of eggs of *Ascaris suum* per gram of feces. The average of liver lesion scores and the prevalence of WS per each batch were analyzed for their association with questionnaire results using a one way ANOVA test ($p<0.05$). The Odds ratio was calculated considering the risk factor "type of floor in the growing phase" and the risk to have an intra-batch prevalence of WS higher than 10%.

Results

Milk spot liver lesions where recorded in 92.766 pigs (13.9%) belonging to 815 batches (17.1%). The intra-batch prevalence ranged from 8% to 100%. About the questionnaire results the data collected are the following. The 50% of the herds applied a farrow-to-finish production system, whilst the other 50% were fattening herds. Six farms were classified as small (≤ 700 pigs), 5 as intermediate (700–5000 pigs) and 5 as large (> 5000 pigs). Two farms did not answer to this question. In the growing units 9 (50%) farms had a concrete floor, whereas 9 (50%) had a slatted floor. In the fattening units 15 herds (83%) had a concrete floor or concrete floor with a slatted area and only 3 (17%) herds had a slatted floor. About the anthelmintic treatments the herds were divided into three groups: 1) no treatment (28%); 2) treatment of pigs at the beginning of the growing phase or in the sows (39%); 3) treatment in both the categories previously reported (33%). The pig fecal samples examination showed negative results in 9 herds out of 18. During this preliminary phase of the study 1297 liver were evaluated with an overall WS lesions average value and frequency of 0.35 and 24% respectively. The proportion of liver with WS lesions and the WS lesions average value was significant lower ($P<0.05$) in the herds with negative parasitological results if compared with herds with a positive results. The other risk factors considered in this work did not show statistical significant association with the average value and the frequency of WS lesions. Nevertheless the farms having a concrete floor in the growing phase showed a probability four time higher to have an intra-batch frequency of WS liver lesions >10% (OR=4.00 c.i.95% 0.56-28.39).

Conclusions and Discussion

The prevalence obtained in this study (13.9%) revealed that ascariasis is a persistent problem in current farm production. The preliminary work performed on evaluation of risk factors associated to WS liver lesions at the slaughterhouse allowed to validate the method for scoring WS. A significant statistical association was observed between the proportion of WS lesions and pen floor fecal positivity for *A.suum* eggs in pigs while no statistical significant association was observed considering the other risk factors studied. These preliminary results have been influenced by the relative low number of herds included in the study that will be incresed in the progression of the study.

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Haematological indices as early indicators of iron status in piglets at weaning

S Bhattarai[1], ME Busch[2], B Friendship[3], G–P Martineau[4], T Framstad[5], JP Nielsen[1]
[1] *HERD-centre, Department of Large Animal Sciences, University of Copenhagen, Denmark* [2] *Pig Research Centre, Danish Agriculture & Food Council, Denmark* [3] *Department of Population Medicine, OVC, University of Guelph, Canada* [4] *Department of Animal Production, National veterinary School, Toulouse, France* [5] *Norwegian School of Veterinary Science, Norway,* jpni@sund.ku.dk

Introduction

Over the past decade, highly prolific sows with increasing litter sizes are kept in intensive pig production. Although large piglets tend to grow faster than the smaller ones, the iron dosing regimes used in practical pig production are often based on standard doses of 200mg iron irrespective of the birth weight, growth rate or weaning age of the piglets. The iron stores following injections in the first days of life may be depleted around weaning and this is the critical time for iron deficiency and suboptimal hematological values in piglets. The objective of our study was to investigate if large piglets at weaning are at higher risk of having low iron status compared to smaller ones based on haematological values.

Materials and Methods

Five conventional high performing farrow-finish sow herds were recruited by courtesy of two large specialized pig-practices in Denmark. The selection criteria used were: Herd size of at least 1000 sows, weekly farrowing batches of minimum 40 litters, good record keeping, and provision of a single injectable iron (either I/M or S/C) supplementation during the first few days of life. Within each herd, 20 litters belonging to a weekly farrowing batch were selected randomly before weaning. From each litter the largest piglet (large), a random piglet and the smallest piglet were investigated. Random piglets were identified as the sixth individual when counting snouts from the pen side of the observer, while the largest and the smallest piglets were judged visually. EDTA and non-stabilized blood samples were taken from the anterior Vena cava of each piglet and analysed for complete haematology including serum iron and total iron binding capacity (TIBC). The difference in the measured parameters among the three types of piglets was evaluated using either one-way ANOVA or a non-parametric Kruskal-Wallis test.

Results

A total of 296 piglets belonging to 100 litters from five herds were included in the study. The haemoglobin concentrations (Hb) in large, random and small piglets were 119.6 (SD: 15.5), 121.5 (SD: 15.1) and 121.5 (SD: 13.1) g/l respectively. The mean Hb concentrations among the three groups were not different (p=0.75).

Large piglets had lower mean corpuscular haemoglobin (MCH), reticulocyte cellular volume (MCVr), reticulocyte haemoglobin content (CHr), mean reticulocyte corpuscular haemoglobin concentration (CHCMr) and serum iron compared to random (p=0.02, 0.003, 0.0005, 0.001 and 0.001, respectively) and small (p=0.03, 0.005, <0.0001, <0.0001 and 0.001, respectively) piglets. In accordance with this, large piglets had higher red blood cell distribution width (RDW), reticulocyte red cell distribution width (RDWr) and total iron binding capacity (TIBC) compared to random (p=0.003, 0.01 and 0.007, respectively) and small (p=0.02, 0.0005 and <0.0001, respectively) piglets.

Conclusions and Discussion

The current study shows that several haematological parameters were significantly different among different sized piglets at weaning. Based on these values the large piglets showed signs of sub-optimal iron status compared to random and small piglets. However, the level of haemoglobin, which is widely used as an indicator of iron deficiency and anaemia was not statistically different among the piglet types. This suggests that other haematological parameters (MCH, RDW, MCVr, CHr, CHCMr, RDWr, serum iron and TIBC) may serve as early indicators of iron deficiency rather than the traditionally used haemoglobin values. Since erythrocytes have a slow turnover rate of 120 days, it is not surprising that the measured Hb values do not provide a reliable estimate of recent bone marrow erythropoietic activity. However, the association of the early indicators with the health and productivity of the pigs needs to be further assessed.

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A pilot study on a methodological approach for reporting treatment incidence by indication on farm level

M Sjölund[1], S Strandberg[2], G Ståhle[2], C Greko[1]
[1]National Veterinary Institute, Uppsala, Sweden [2]Sigill Kvalitetssystem AB (Seal Quality Systems Ltd.), Stockholm, Sweden,
marie.sjolund@sva.se

Introduction

The network European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) has since 2009 published reports on the sales of veterinary antimicrobials[1]. The data presented has not yet been collected by animal species and age group. However, a proposal has been developed for standardized collection of national data on usage by species and age category. However, data on use of antimicrobials is also needed on herd level as a tool for farmers and veterinarians in the herd management schemes for improving health, productivity and as a consequence, reducing the need for antimicrobials. The aim of this study was to develop and pilot a simple and easy-to-use methodology for presenting treatment incidence by indication on herd level for Swedish pig herds.

Materials and Methods

Herds affiliated to a quality assurance system (the accredited standard IP) were contacted for participation in the study. The herds agreed to provide data on the number of animals treated by age category (piglets, weaners, fatteners and adult pigs) and by product during one year. Data on treatment indications were also collected by age category. Treatment indications were grouped as follows: diarrhea, respiratory disorders, lameness, wounds/skin disorders, other symptoms and udder related diseases for sows.

To allow for comparison between different herds irrespective of herd size, production type and completeness in delivered data, treatment incidence was calculated to be shown for 100 sows in production for piglets, weaners and adults. Treatment incidence among fatteners was calculated per 1000 fatteners. In the case that data was not collected during an entire year, the number of treatments was estimated from the number of reported treatments to fit a period of one year.

Results

Nineteen herds agreed to participate and 10 delivered data. Herd data are shown in table 1. Piglets and adults were the two age categories where most treatments were performed but treatment incidence varied between herds. Treatment indications for piglets and sows are shown in tables 2 and 3, respectively. Diarrhea and lameness were the most common indications in piglets. Treatments for lameness in piglets were performed in all herds but treatment incidence varied. Udder related disorders was the most common treatment cause in sows followed by lameness.

Table 1. Herd information and reporting period for herds reporting antimicrobial treatments by indication

Herd	Production type	# Sows	# Fatteners	Reporting period
1	Farrow-to-finish	180	4000	12 months
2	Farrow-to-finish*	240	2900	8 months
3	Farrow-to-finish	140	3350	3 months
4	Multiplier	112	1750	7,5 months
5	Farrow-to-finish	195	4300	12 months
7	Farrow-to-finish*	260	5800	9 months
9	Fattening	-	15000	10 months
11	Farrow-to-finish	85	1700	12 months
14	Nucleus	108	1650	12 months
17	Sow pool central unit	2200	-	6 months

* Sells growers

Table 2. Treatment incidence by indication for piglets expressed as the number of treatments per 100 sows/year

Herd	Diarrhea	Respiratory	Lameness	Skin/Wounds	Other
1	845		207		104
2	279	6	544	43	1
3			2160		
4	234	3	151	4	7
5	237	3	142	11	3
7	1181		758		72
11			320		
14			410		

Table 3. Treatment incidence by indication for sows expressed as the number of treatments per 100 sows/year

Herd	Udder	Lameness	Skin/Wounds	Other
1	66	7		3
2	53	17	9	
3	46	3		3
4	26	11		
5	56	8	7	25
7	17	21		20
11	12	12		4
14	20	36		
17	12	12	1	1

Conclusions and Discussion

Results for herds with a reporting period less than a year must be considered with caution as calculations may result in over- or underestimates of the true incidence.

The simple methodology developed for presenting treatment incidence by age category can aid farmers and veterinarians in identifying health disturbances by age category so that appropriate measures can be taken to improve health and thereby reduce the need for antimicrobials.

Acknowledgments

Participating farmers and the Swedish Board of Agriculture for funding (Dnr: 29-13194/10).

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4. 1. Sales of veterinary antimicrobial agents in EU/EEA countries – 1st, 2nd, 3rd ESVAC Reports

Antimicrobial usage in Swedish farrow-to-finish herds

M Sjölund[1], A Backhans[2], U Emanuelson[2], A Lindberg[1], C Greko[1]
[1]*National Veterinary Institute, Uppsala, Sweden,* [2]*Swedish University of Agricultural Sciences, Uppsala, Sweden,*
marie.sjolund@sva.se

Introduction

Statistics on sales of antimicrobials for veterinary use have been monitored since 1980 in Sweden[1]. Sales of antimicrobials for animals are among the lowest in the European Union[2], but data per production type and age group is not readily available. Such data are valuable as part of herd management schemes for improving health, productivity and as a consequence, reducing the need for antimicrobials. The aim of this study was to obtain estimates on antimicrobial usage in Swedish farrow-to-finish herds.

Materials and Methods

Data were collected within the MINAPIG research project (www.minapig.eu). Treatment records and total number of animals produced during one year were collected from 59 farrow-to-finish herds. Antimicrobial use was recorded per age category (piglets, weaners, fatteners and adult pigs). The total amount of active substance used was calculated per kg body weight for the different age categories.

Fixed standard weights were assigned for each category: 2 kg (piglets), 10 kg (weaners), 50 kg (fatteners) and 250 kg (adults). To estimate treatment incidence, daily doses and treatment lengths based on information in the Summary of Products Characteristics (SPC) were used to calculate course doses for each product. Mean values were used for products where ranges for dosing and treatment length were given.

Results

Results on amounts used per age category and active substance are shown in table 1. The numbers of treated pigs expressed per 100 pigs per year by age group are shown in table 2. Treatments of sows are also shown per 100 farrowings.

Conclusions and Discussion

Treatment of individual animals, by injection or drench, was the most common approach. Group treatments were rare and only applied in weaners and fatteners. The low use of antimicrobials in weaners may be due to prophylactic use of high doses (≥2000 ppm) of zinc oxide in 26 of the herds. Piglets and adults were the two age categories where most treatments were performed. Penicillin was most frequently used. Higher doses of penicillin than recommended in the SPC are often used which might have resulted in a false high number of treated pigs. Further, when assessing the treatments of sows, the average number of farrowings (x=2.2/year) should be taken into account.

Although antimicrobial use is low in Sweden in general[2], there is still room for improvement with special attention paid to sows and piglets. Data on herd level usage would support such work, as a tool for benchmarking.

Table 1. Amount of active substance (mg/kg body weight) used in 59 farrow-to finish herds during a year by age group

Active substance	Piglets	Weaners	Fatteners	Adults
Individual				
Penicillin	9.76	0.81	0.91	19.27
Amoxicillin	3.81	0.79	0.11	0.92
Penicillin-DHS	0.94	0.03	-	0.98
Trim-Sulph.	7.57	0.39	0.01	12.09
Oxytetracycline	0.51	0.13	0.15	0.41
Tylosin	0.28	0.27	0.08	0.16
Tulathromycin	-	-	<0.01	-
Neomycin	0.43	<0.01	-	-
DHS	0.01	<0.01	-	-
Enrofloxacin	0.16	<0.01	<0.01	0.07
Colistin	0.11	0.52	-	-
Tiamulin	-	<0.01	<0.01	-
Group				
Doxycycline	-	0.12	0.10	-
Tylosin	-	2.80	0.07	-
Tiamulin	-	0.29	0.04	-
All treatments	30.1	6.7	2.1	46.7

Table 2. Estimated number of treated pigs per 100 pigs or 100 farrowings per year in 59 farrow-to-finish herds shown by age group

Active substance	Piglets	Weaners	Fatteners	Adults	Farrowings
Individual					
Penicillin	27.11	2.28	2.57	53.52	24.33
Amoxicillin	6.36	1.34	0.19	1.53	0.70
Penicillin-DHS	0.74	0.02	-	0.77	0.35
Trim-Sulph.	16.83	0.87	0.02	26.86	12.21
Oxytetracycline	1.47	0.38	0.43	1.17	0.53
Tylosin	0.47	0.45	0.13	0.26	0.12
Tulathromycin	-	-	<0.01	-	-
Neomycin	0.17	<0.01	-	-	-
DHS	0.01	<0.01	-	-	-
Enrofloxacin	2.11	0.03	0.02	0.90	0.41
Colistin	0.38	1.74	-	-	-
Tiamulin	-	<0.01	0.01	-	-
Group					
Doxycycline	-	0.14	0.11	-	-
Tylosin	-	7.56	0.19	-	-
Tiamulin	-	0.61	0.09	-	-
All treatments	55.6	15.4	3.8	85.0	38.64

Acknowledgments

The MINPIG research team, participating farmers, the Swedish Animal Health Service and the Swedish Research Council Formas for financial support.

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2. Sales of veterinary antimicrobial agents in 25 EU/EEA countries in 2011 – 3rd ESVAC Report

Scandinavian pig veterinarians' views on the use of antimicrobials in pig production

A Backhans[1], EO Nielsen[2], M Sjölund[3], A Lindberg[3], U Emanuelson[1], V Visschers[4]
[1]Swedish University of Agriculture, Uppsala, Sweden, [2] Danish Pig Research Centre, Danish Agriculture & Food Council, Copenhagen, Denmark, [3]National Veterinary Institute, Uppsala, Sweden, [4]ETH Zurich, Institute for Environmental Decisions, Zurich, Switzerland, annette.backhans@slu.se

Introduction

The antimicrobial (AM) usage in animals is low in Denmark and Sweden compared to many other European countries (1). In both countries veterinarians have no income from AM sale, there is a central registration of all prescriptions and growth promoters have been prohibited since 1986 (SE) and 2000 (DK). However, the pig production in Sweden and Denmark differs in size and structure. The aim of this study was to investigate Scandinavian pig veterinarians' opinions on AM use in pigs, as part of the multi-country MINAPIG project (www.minapig.eu).

Materials and Methods

A questionnaire was prepared for veterinarians consisting of 50 questions related to: AM usage in pig production, policy measures to reduce AM usage, ways of reducing AM usage and alternatives to AM, relationship with farmers and veterinarians' own interest in further education in pig health. Most questions were statements where the respondent could mark agreement on a six-point scale from "do not agree at all" to "fully agree". Respondents were also asked about their average time allocated to pig practice, gender, age and year of graduation as a veterinarian. Danish (n=140) and Swedish (n=257) veterinarians working in pig medicine were asked to participate in an on-line questionnaire. In addition, in Sweden, a paper version was sent to veterinarians with unknown e-mail addresses (n=75).

Results

The response rates were 42% in Denmark and 20% in Sweden. Respondents using less than 25% of their time in pig medicine were excluded from the Danish results. The majority (85%) of the Danish vets spent **more than** 75% of their time in pig practice. In Sweden, the corresponding figure was 20% while 63% of Swedish respondents spent **less than** 25% of their time in pig practice. Among questions regarding AM usage in pig production, the statements with highest agreement in both countries were "I can think of other ways to prevent bacterial diseases in pigs, without using antibiotics" and "The use of antibiotics in pig farming is a danger to human health". Swedish vets were more concerned about the health risks for humans carrying resistant bacteria than Danish vets, and female vets from both countries were more concerned than male vets. The most popular policy measure was that pig farmers would be obliged to develop a plan together with their veterinarian on how to reduce antibiotic usage to a certain level, whereas fining farmers if the antibiotic consumption is above the allowed national threshold was considered less acceptable. On the issue of reducing AM usage and alternatives to antimicrobials, vets agreed that they regularly advised farmers on how to reduce their AM usage and that with the help of veterinarians it is possible to reduce AM usage at pig farms. A possible reduction of AM in the next 5 years, economically manageable for farmers, was estimated to 15.4% (DK) and 29.5% (SE). The vets agreed that they support their pig farmers in implementing or using alternative measures instead of antibiotics to keep their pigs healthy and they had the perception that their farmers to a high degree adopt the preventive measures and treatment options that they suggest to them. Finally, the respondents stated that they were very interested in keeping up-to-date with pig health and alternative measures in pig husbandry and a vast majority had taken continuing courses after graduation.

Conclusions and Discussion

In Sweden, the questionnaire was distributed to all vets that registered **any** AM usage in pigs, including a number of companion animal vets, which might explain the low response rate. The Danish vets in this study were much more specialized in pig medicine. This difference reflects that pig farming is a much bigger industry in Denmark than in Sweden, where in many areas pig farms are few, and therefore herd veterinarians are often general practitioners. Nevertheless, opinions on issues regarding AM usage in pig production were in general very similar between the two countries. Swedish vets were more concerned about risks related to AM usage, but were also more optimistic regarding the possibility for future reduction of AM usage.

Acknowledgments

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Pig farmers' self-reported antimicrobial usage and perception of antimicrobials in five European countries

A Backhans[1], L Collineau[2], E grosse Beilage[3], S Loesken[3], M Postma[4], M Sjölund[5], V Visschers[6]
[1]*Swedish University of Agriculture, Uppsala, Sweden;* [2]*SAFOSO, Bern, Switzerland and UMR BIOEPAR, Oniris, INRA, LUNAM, Nantes, France;* [3]*University of Veterinary Medicine Hannover, Field Station for Epidemiology, Germany;* [4]*Veterinary Epidemiology Unit, Faculty of Veterinary Medicine, Ghent University, Belgium;* [5]*National Veterinary Institute, Uppsala, Sweden;* [6]*ETH Zurich, Institute for Environmental Decisions, Zurich, Switzerland,* annette.backhans@slu.se

Introduction

Antimicrobial (AM) resistance is an increasing problem in human and veterinary medicine and has been related, among other factors, to the extensive use of AM in pig production. To be able to reduce AM usage in this sector, it is important to know which characteristics and psychosocial factors of pig farmers are related to their AM treatment practices and their perception of policy measures relevant for AM usage in this sector. As part of the European MINAPIG project (www.minapig.eu), we conducted a survey among pig farmers in five European countries that differ in AM usage and pig production systems to examine these issues.

Materials and Methods

A paper and pencil survey was conducted in Belgium, France, Germany, Sweden and Switzerland between November 2012 and December 2013. The 7-page questionnaire included items about farmers' concerns related to their pigs' health, AM resistance and financial and legal issues related to pig farming, their perceived benefits, perceived risks and perceived need of AM, self-reported AM usage, and demographics. Most items were measured on 6-point Likert scales.

Results

In total, 291 pig farmers completed the survey. Overall, all farmers seemed to believe that their own AM usage was less than that of the others. There were significant differences in the estimations of their own AM usage compared to other farmers in their own country and to farmers from different countries. Swiss and French farmers estimated their own AM usage compared to their countrymen to be lower than Belgian and Swedish farmers. Also, Swedish and Swiss farmers estimated their own AM usage to be lower compared to that of farmers in all countries (see Fig. 1). The French, Belgian and German farmers of our sample believed their AM usage was the same or higher than that in Sweden and Switzerland but less than that in Belgium and Germany.

All farmers were significantly more concerned about financial/legal issues than about AM resistance and their pigs' health. Moreover, farmers from different countries had different levels of concern. Swiss and Swedish farmers were less concerned about their pigs' health than about AM resistance and about financial/legal issues, and overall less concerned than French and Belgian farmers.

Farmers' perceived benefits of AM were relatively high, whereas the perceived need for AM in pig farming was relatively low. Moreover, farmers from different countries only differed on the perceived need for AM in pig farming and not on perceived benefits and risks of AM. That is, Swedish, Swiss and Belgian farmers perceived a higher need for AM than French farmers.

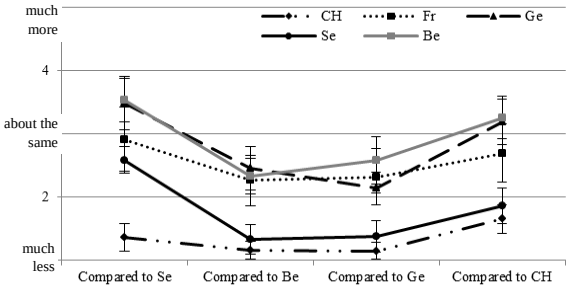

Figure 1. Estimated AM usage compared to farmers in own and other countries, among Swiss (CH), French (Fr), German (Ge), Swedish (Se) and Belgian (Be) farmers

Conclusions and Discussion

In sum, all farmers appeared to estimate their AM usage as rather low compared to that of others farmers in their own country and that of most other countries. Our farmers may have had an optimistic bias regarding their own AM usage (i.e., they are unrealistically optimistic about their usage) (1). Moreover, Swedish and Swiss farmers showed an even more positive view on their AM usage and appeared less worried about their pigs' health than farmers from the other three countries. Whether the latter are correctly more optimistic compared to other countries needs to be verified by relating farmers' actual AM usage data and disease incidences to their perceptions and estimations, and by comparing them between countries.

Acknowledgments

We would like to thank all farmers participating in the study and the MINAPIG consortium for helping to develop the questionnaire.

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Global full value pigs survey: Health

M Rostagno[1], G Pelger[1]
[1]*Elanco Animal Health, Greenfield, IN, USA,* rostagno_marcos@elanco.com

Introduction

Full Value Pigs (FVP) is a profitability-focused business model that takes a population of pigs and constraints of facility and market to determine the optimum performance for a swine operation. A Full Value Pig is a pig that achieves at least 90% of the Margin Over Feed Cost (MOFC) of the single optimum pig as determined by feed intake, feed costs and payment grids. While some producers are currently benchmarking their FVPs compared to historical data, many are still using single point production parameters that are solely cost-focused or misleading. The four key areas that constitute the pillars for the production of Full Value Pigs area: Health, Feed, Output and Access. These areas influence one another, and are essential for efficient pork production. Therefore, a global survey was conducted to obtain detailed information about these four areas of pork production, and develop a global Full Value Pigs framework. This report is focused on the survey results for the area of health.

Materials and Methods

A survey containing 23 questions, based on the four key areas of FVP (Health, Feed, Output, and Access) was designed and applied. The questions consisted of multiple select or single answers. The survey was applied to swine producers, production managers, veterinarians, nutritionists and influencers in 35 countries in multiple regions of the world. Surveys were either administered via an internet site or completed by hand. Entries were validated and transferred by a third-party for summarization. All surveys were completed within 7 months of each other during the year of 2013.

Results

A total of 565 surveys were returned (346 from USA/Canada, 80 from Europe, 78 from Asia/Pacific, and 61 from Latin America). Due to differences in production systems, not all questions applied to all in the surveyed population. The most commonly identified health challenges are presented in table 1. Globally, the most common diagnostic methods of health challenges identified were: Veterinary consultation (74.8%), clinical observation (60.8%), laboratory submission (58.2%), onsite necropsy (55.4%), and performance records (33.7%). On the level of clinical incidence of a health challenge that would trigger the need for a group treatment, responses were: 0-5% (26.9%), 6-10% (37.3%), 11-15% (21.3%), 16-20% (8.9%), and >21% (5.6%). Overall, improved animal health was the most frequently identified factor (43.3%) leading to feed optimization, whereas disease was identified as the most important factor preventing the production of more Full Value Pigs (43.5%). In table 2, results are presented on the severity of respiratory diseases and ileitis in pork production systems.

Conclusions and Discussion

Health challenges are a major concern to pork producers worldwide, causing significant financial losses (1,2). Results of the global FVP survey on the impact of health challenges on feed optimization, and as the main limiting factor for production of FVPs serve to demonstrate the existing concerns. The top 4 health challenges identified in this survey were in agreement with the previous FVP survey (3), confirming the persistent relevance of PRRS, *M. hyopneumoniae*, *E. coli*, and *S. suis* to the global pork industry.

Table 1. Most commonly identified health challenges

Health Challenge:	Proportion of Responses:
PRRS	50.1%
Mycoplasma hyopneumoniae	41.7%
Escherichia coli	35.6%
Streptococcus suis	34.8%
Swine Influenza	34.8%
Ileitis	34.4%
Haemophilus parasuis	28.6%
PCV2AD	25.4%
Actinobacillus pleuropneumoniae	21.3%
Swine Dysentery	17.5%
Worms/Parasites	17.1%
Gastric Ulcers	16.1%
Pasteurella multocida	11.5%

Table 2. Severity of respiratory diseases and ileitis over time in pork production systems

Resp. disease	Health challenge has:	Ileitis
22.6%	become more serious	8.4%
27.4%	become less serious	28.6%
50%	remained the same	63%

Acknowledgement

This study was sponsored by Elanco Animal Health.

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Global full value pigs survey: Feed

M Rostagno[1], G Pelger[1]
[1]*Elanco Animal Health, Greenfield, IN, USA,* rostagno_marcos@elanco.com

Introduction

Full Value Pigs (FVP) is a profitability-focused business model that takes a population of pigs and constraints of facility and market to determine the optimum performance for a swine operation. A Full Value Pig is a pig that achieves at least 90% of the Margin Over Feed Cost (MOFC) of the single optimum pig as determined by feed intake, feed costs and payment grids. While some producers are currently benchmarking their FVPs compared to historical data, many are still using single point production parameters that are solely cost-focused or misleading. The four key areas that constitute the pillars for the production of Full Value Pigs area: Health, Feed, Output and Access. These areas influence one another, and are essential for efficient pork production. Therefore, a global survey was conducted to obtain detailed information about these four areas of pork production, and develop a global Full Value Pigs framework. This report is focused on the survey results for the area of feed.

Materials and Methods

A survey containing 23 questions, based on the four key areas of FVP (Health, Feed, Output, and Access) was designed and applied. The questions consisted of multiple select or single answers. The survey was applied to swine producers, production managers, veterinarians, nutritionists and influencers in 35 countries in multiple regions of the world. Surveys were either administered via an internet site or completed by hand. Entries were validated and transferred by a third-party for summarization. All surveys were completed within 7 months of each other during the year of 2013.

Results

A total of 565 surveys were returned (346 from USA/Canada, 80 from Europe, 78 from Asia/Pacific, and 61 from Latin America). Due to differences in production systems, not all questions applied to all in the surveyed population. Responses about how much feed represents of the cost of raising pigs to market weight are presented in table 1. Feed represents >60% and >70% of the cost of raising pigs to market weight in 83.8% and 59.8% of the cases, respectively. Moreover, the vast majority (78.2%) predicted that the cost of feed as a proportion of total expenses will stay the same (29.3%) or be greater (48.9%) in the next few years. Considering as percent of total revenue, responses indicate that the average margin over feed cost is usually ≤40% for most (78%), with only 5% responding that their margin was >60%.

In table 2, results are presented for the question on what has had the most positive impact on the ability to optimize feed.

Conclusions and Discussion

It is well known that feed constitutes the main input and cost in pork production (1,2). Results of the FVP survey confirm that for the majority of respondents feed is the main production cost (more than 60%). Moreover, most of the respondents also agree that this percentage will stabilize or grow in the next three years, and that reducing disease was identified as a critical factor to improve feed optimization. While most respondents are working under low margin conditions (78% have <40% MOFC), feed optimization can be the difference between profit and loss, particularly when considering that the definition of Full Value Pigs is 90% of optimum MOFC.

Table 1. Feed as a percentage of the cost of raising pigs to market weight

Feed as percentage cost	Responses
50% or less	4.4%
51 to 60%	9.6%
61 to 70%	24%
71 to 80%	52.4%
More than 81%	7.4%

Table 2. Factors leading to feed optimization

Factors:	Responses:
Improved Animal Health	43.3%
Changed Genetics	25.2%
Adjusted Feeders	11.1%
Changed Nutrition	7.9%
Limited Feed Intake	7.9%
Used a Feed Additive	4.6%

Acknowledgement

This study was sponsored by Elanco Animal Health.

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Global full value pigs survey: Output and access

M Rostagno[1], G Pelger[1]
[1]*Elanco Animal Health, Greenfield, IN, USA,* rostagno_marcos@elanco.com

Introduction

Full Value Pigs (FVP) is a profitability-focused business model that takes a population of pigs and constraints of facility and market to determine the optimum performance for a swine operation. A Full Value Pig is a pig that achieves at least 90% of the Margin Over Feed Cost (MOFC) of the single optimum pig as determined by feed intake, feed costs and payment grids. While some producers are currently benchmarking their FVPs compared to historical data, many are still using single point production parameters that are solely cost-focused or misleading. The four key areas that constitute the pillars for the production of Full Value Pigs area: Health, Feed, Output and Access. These areas influence one another, and are essential for efficient pork production. Therefore, a global survey was conducted to obtain detailed information about these four areas of pork production, and develop a global Full Value Pigs framework. This report is focused on the survey results for the areas of output and access.

Materials and Methods

A survey containing 23 questions, based on the four key areas of FVP (Health, Feed, Output, and Access) was designed and applied. The questions consisted of multiple select or single answers. The survey was applied to swine producers, production managers, veterinarians, nutritionists and influencers in 35 countries in multiple regions of the world. Surveys were either administered via an internet site or completed by hand. Entries were validated and transferred by a third-party for summarization. All surveys were completed within 7 months of each other during the year of 2013.

Results

A total of 565 surveys were returned (346 from USA/Canada, 80 from Europe, 78 from Asia/Pacific, and 61 from Latin America). Due to differences in production systems, not all questions applied to all in the surveyed population. Most respondents (86.3%) indicated >100Kg as target pig weight at harvest, with 79% taking >140 days to reach the target weight. However, 83.5% of the respondents estimated that <15% of the pigs are below the target weight at harvest. There were some regional differences. The survey also revealed that 68.1% of the respondents are able to evaluate growth rate and feed utilization of each batch of pigs at close out. In table 1, the proportion of pigs raised for meat export market is presented. There were differences between regions, with Latin America and Asia/Pacific having the highest proportion of pigs raised for internal meat market (>80% for both regions), in comparison to USA/Canada and Europe. In table 2, the proportion of respondents that keep track of the Full Value Pigs produced is presented per region. Globally, 69.5% of respondents report >70% of FVPs per group. However, the vast majority of the respondents (79.7%) estimate that >U$10 are lost per pig that do not reach full value, with 19.7% of respondents estimating a loss of >U$25 per pig.

Conclusion

Monitoring of Full Value Pigs produced is critical to determine efficiency and profitability. Diseases constitute the most important factor preventing production of more Full Value Pigs.

Table 1. Proportion of pigs produced for meat export market

Proportion of pigs	Responses
0%	61.7%
1-20%	13%
21-40%	7.8%
41-60%	5.2%
61-80%	2.6%
81-100%	9.6%

Table 2. Proportion of respondents that tracks Full Value Pigs produced

Region	Responses:
US/Canada	59.6%
Latin America	41%
Europe	50%
Asia/Pacific	48.7%
Global	52.2%

Table 3. Most important factors preventing the production of more Full Value Pigs

Factor	Proportion of respondents:
Disease	43.5%
Variation	18.5%
Missed target weight/age	11.6%
Facilities/Handling	9.9%
Attrition	9.1%
Feed Optimization	7.4%

Acknowledgement

This study was sponsored by Elanco Animal Health.

Comparison of diagnostic potential of serological, molecular and cell culture methods for detection of chlamydiosis in pigs

K Niemczuk, M Szymańska-Czerwińska
Department of Cattle and Sheep Diseases, National Veterinary Research Institute, Pulawy, Poland kniem@piwet.pulawy.pl

Introduction

Diagnosis of chlamydiosis in pigs based on clinical symptoms is unattainable; thus, different laboratory techniques are used to detect the infection. Routine diagnosis is based on serological studies. Serological techniques are of limited validity because they often fail to detect *Chlamydia sp.* shedding animals and show different sensitivities. However, little is known about the correlation and the significance of the results of different laboratory methods. The aim of the study was to compare the diagnostic potential of complement fixation test (CFT), conventional PCR, (r) PCR and cell culture.

Materials and Methods

Serological tests (CFT) were carried out on 840 serum samples from pigs. The molecular and cell culture tests were carried out on biological material collected from the same animals. In total, 295 placentas, 420 vaginal swabs and 125 specimens of the internal organs of aborted foetuses were examined. DNA extraction was performed with using DNA Qiagen mini kit. Nested-PCR for *Chlamydia* sp. was performed according to previously published procedure[1,3]. Species-specific real-time procedures were performed on *Chlamydiaceae*-positive samples according to Pantchev et al[2]. Isolation was performed on Vero cell line (ATCC® CCL-81™) grown in MEM. Pearson's chi-square test was used to compare the results obtained using the 4 methods. Correlation coefficients were calculated for all the methods used. All analyses were conducted using the program STATISTICA version 10 (Software StatSoft, Inc.) For the purpose of this study, the following guidelines for interpreting the degree of correlations were used: r = 0 to 0,09: no or negligible r = 0.1 to 0.29: weak relationship; r = 0.3 to 0.49: moderate; r = 0.5 to 0.69: strong; r = 0.7 to 0.99 = very strong; r = 1: full.

Results

The χ^2 test confirmed that in most cases the results obtained by means of different methods correlated with each other ($P<0.05$), but the correlations had different values. The highest correlation coefficient was observed in the case of real time PCR and nested PCR, but it was higher for placenta and swab samples (r=0.82-0.84) in comparison with the samples from aborted foetuses (r=0.73) (table1). However, based on the established criteria, all results were classified into the group of "very strong relationship". The comparison between the methods used to detect the infectious agent (cell culture isolation, real time PCR and nested PCR) showed a moderate degree of correlation (r=0.32-0.44). The relationship between (r)PCRs and CFT was moderate (r=0.35-0.47), but it was the lowest when the comparison was made between the test results of samples isolated from placentas. No correlation or a weak to moderate relationship was observed when the comparison was made between cell culture isolation and all the other methods.

Table 1. Results of statistical analysis of correlations between methods used for detection *Chlamydia* sp.

method	placenta		aborted fetus		swab	
	PCR	rPCR	PCR	rPCR	PCR	rPCR
r PCR	0.82	-	0.73	-	0.84	-
cell	0.41	0.44	0.33	0.32	0.37	0.34

Conclusions and Discussion

In the veterinary diagnosis of chlamydiosis, different kinds of methods are used. The types of assays largely depend on the type and number of samples for investigation, the availability of diagnostic tests in a laboratory, and the size of the herd tested. In particular, there is very little data showing comparative studies of serological assays with molecular biology and cell culture techniques. Laboratory diagnosis of chlamydiosis should be based on the interpretation of results obtained by different kind of methods both detecting the serological response as well as the presence of pathogen. To sum up the values and results obtained, it can be concluded that the statistical analysis of data from a comparison of the four diagnostic methods has shown that serological, molecular and culture methods can be used in practice for the diagnosis of chlamydiosis. However, their diagnostic potential and the level of correlation between them was variable and it is necessary to use several methods simultaneously, preferably CFT for serological studies and nested PCR or real-time PCR for pathogen detection.

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Efficacy of Tiamulin (Denagard® 45%) treatment on piglets vaccinated against PCV2 under field conditions in Brazil

G Machado[1], R Pinheiro[1]
[1]*Integrall!- Soluções em Produção Animal Ltda.*, glauber@integrall.org, ronie@integrall.org

Introduction

Recently porcine circovirus type 2 (PCV2) associated diseases (PCVAD) have emerged as a major problem causing significant morbidity and mortality in pigs worldwide. The most common manifestations of PCVAD observed are severe systemic and respiratory disease. After PCV2 vaccines were made available and had their efficacy proven, many farms weakened their management and medication protocols mainly as an immediate attempt to reduce costs. There were then many cases of new outbreaks arising even in vaccinated herds. These findings were observed at field level and brought up the question on what would be the potential benefit of consistent reliable medication protocols in addition to PCV2 vaccination. The objective of this trial is to evaluate the effect of Tiamulin (Denagard®) on the performance and health of piglets previously vaccinated with CIRCOFLEX® under field conditions and naturally exposed to Porcine Circovirus type 2 (PCV2), Mycoplasma hyopneumoniae, Haemophilus parasuis and Pasteurella multocida type A.

Materials and Methods

A total number of 900 feeder pigs weighing around 24 kg live-weight were divided in 2 treatments: T1 - vaccinated with CIRCOFLEX®, 1.0 ml at weaning, NOT medicated; T2 - vaccinated with CIRCOFLEX®,

8.8 mg/kg with 2 pulses of 5 days each (65-70 days and 105-110 days). The following parameters were evaluated and compared between treatments: Weight at 65 (entrance weight), 101 and 145 days of age, Average Daily Gain, Feed Conversion, Mortality rates, Incidence of runts, culled animals within groups, Faeces scoring (diarrhea scores), Coughing scoring, Cost benefit.

Results

Treatments did not differ statistically when compared for weights at 101 days of age. At 145 days of age, a numerical difference was observed between treatments as far as the average live-weight was concerned, but such a difference was not statistically significant. Control group had 99.25 kg, while medicated animals had 102.46 kg of live-weight (p=0.09). However, there was statistical difference between treatments regarding weight gain from 101 until 145 days of age (p=0.003) and also regarding total weight gain throughout the trial period (p=0.02), as seen in Table 1.

Table 1. Initial weights, weights at 101 and 145 days of age and daily weight gains

Treatment	Ini Wt	Wt 101	Wt 145	DWG 101	DWG 145	Total DWG
1 (contr.)	23.31	51.89	99.25	0.816	1.07B	0.961A
2 (medic.)	23.51	51.99	102.46	0.813	1.15A	0.999B
CV %	8.157	6.463	4.875	5.693	5.042	4.289
P value	NS	NS	0.09	NS	0.003	0.02

No group difference in feed intake was found (Table 2). Nevertheless, there was statistical difference on feed conversion between medicated animals for the period between 101 and 145 days of age (p=0.004) and also for the whole trial period (p=0.003) (Table 2). Medicated animals were consistently more efficient than non-medicated animals on feed conversion.

Table 2. Feed intake and feed conversion individual & whole trial period

Treatment	Feed Int101	Feed Int 145	Total Int	FC 101	FC 145	Total FC
1 (contr.)	52.74	130.2	182.95	1.88	2.85B	2.47B
2 (medic.)	52.47	130.2	182.67	1.84	2.52A	2.27A
CV %	6.808	6.772	6.101	5.562	10.11	6.18
P value	NS	NS	NS	0.27	0.004	0.003

Significantly lower coughing index, percentage of runt pigs and a lower pleuropneumonia index was found in the group 2 (Table 3).

Table 3. Clinical parameters and cost benefit evaluation

Treatment	Coughing index	Removed pigs	Pleuropneumonia	Cost Benefit
1 (control)	6.50A	4.44A	0.70	1
2 (medicated)	2.02B	2.1B	0.55	3.57

Conclusions

Under the conditions of this trial, we can conclude that: Medicated animals had higher total weight gain and average daily gain than non-medicated animals, during the trial period. Animals medicated through drinking water were more feed efficient than animals which were vaccinated but not medicated. There was a higher incidence of runt pigs removal within non-medicated animals than within medicated animals. Medicated animals had a lower incidence of coughing, although the incidence of diarrhea did not differ between treatments. Animals which were not medicated presented a higher prevalence of lung lesions and pleuropneumonia index at slaughter. A higher return on investment was calculated for group 2 vs. group 1 (3.57 : 1).

Acknowledgement

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Rapid detection of swine JEV with loop-mediated isothermal amplification

H Li, X Yin, Z Ha, B Rao, L Zhang, H Liu*
Institute of Animal Science and Veterinary Medicine, Shanghai Academy of Agricultural Sciences, liuhl71@yahoo.com

Introduction

Japanese encephalitis virus (JEV) can cause central nervous system disorders to animals and human beings. It has caused heavy losses in pig farm in recent decades [l]. RT-PCR is the commonly used for pathogen detection [2,3]. Loop-mediated isothermal amplification (LAMP) is a new technique for nucleic acid amplification [4]. The amplification of nucleic acids in LAMP proceeds under isothermal conditions at a certain temperature for 1 h or less and the accumulation of large amounts of various lengths products make detection much easier [5]. Furthermore the result can be observed in naked eyes which make detection simplified. The object of the study is to establish a RT-LAMP approach for JEV genotype I & III detection and used for clinical sample detection.

Materials and Methods

Six primers were designed by software Primer Explorer V4, including two outer primers, two inner primers and two loop primers. It can identify eight different conserved regions of JEV E gene of genotype I & III. F3 and B3 primers were also used for PCR amplification. SA14 (genotype III, GenBank: U14163.1), SA14-14-2 (genotype III, GenBank: AF315119.1) and! VN88/Viet Nam/2001/Swine blood (Genotype I, GenBank: AY376464.1) RNA were used as template for establishing the method. Swine influenza virus (SIV), transmissible gastroenteritis virus (TGEV), Classical swine fever virus (CSFV) or Porcine reproductive and respiratory syndrome (PRRSV) were used as for specificity detection. The result of RT-LAMP was observed either by agarose gel electrophoresis or by naked eyes observation. 1500 serum samples were collected at slaughter houses and used for JEV detection using RT-LAMP and RT-PCR.

Results

RT-LAMP and RT-PCR for JEV detection were established. RT-LAMP was tenfold more sensitive than conventional RT-PCR in agarose gel electrophoresis (Tab.1). Six of 1500 serum samples were positive both in RT-LAMP and RT-PCR assay.

Table 1. Comparisons of RT-PCR and RT-LAMP methods for detecting sensitivity of JEV RNA

Methods	Concentrations of JEV RNA (copies/μL)							
	1.5×10^{7}	1.5×10^{6}	1.5×10^{5}	1.5×10^{4}	1.5×10^{3}	1.5×10^{2}	1.5×10^{1}	1.5×10^{0}
RT-PCR	+	+	+	+	+	+	-	-
RT-LAMP								
Naked-eye inspection	+	+	+	+	+	+	-	-
Agarose gel analysis	+	+	+	+	+	+	+	-

Conclusions and Discussion

RT-LAMP method in this study was highly sensitive and specific. Sensitivity of RT-LAMP (15copies/μL) is superior to conventional RT-PCR (150copies/μL) in electrophoresis, and with no cross reaction with other swine viral pathogen. Furthermore RT-LAMP method is far more convenient by observing results with naked eye inspection. More than 1500 clinical serum samples were tested using RT-LAMP and RT-PCR. Positive samples were reasonable concordance between the two methods, which means RT-LAMP in the study can be an alternative approach for detection of JEV in clinical application. Another advantage of RT-LAMP is that it does not rely on expensive and sophisticated facilities such as thermal cyclers. The test can be carry out under certain temperature, which simplified the protocol, and the result can be observed in less than an hour. It may be widely used in clinical pathogen detection.

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Efficacy of a formulation of avian immunoglobulins against the PRRSV in the reproductive herd and in viremic piglets, in a farm of central Mexico

W González[1], E Lucio[1], J Munguía[1]
[1] *Investigación Aplicada S.A. de C.V.*, wgonzalez@grupoidisa.com

Introduction

Numerous control and prevention measures have been developed to face the porcine reproductive and respiratory syndrome (PRRS) due to its extension and economic impact. Among the strategies we have the use of inactivated and live virus vaccines; nevertheless both strategies have not been 100% efficacious in controlling the disease [(1)].

Immunoglobulins obtained from egg yolk (IgY) have been used in several health and profilaxis applications, even in humans [(2)].

Research works by Akita and Nakai[(3)] were able to demonstrate that the protective function of IgY's against pathogens is attributable to their capacity to prevent colonization or neutralize toxins.

The production of IgY specific against PRRS is an innovative technology, highly attractive, which would directly influence a decrease in the expenses associated with vaccination processes and diminishing productive losses linked with the virus.

Materials and Methods

The farm subject of this study is a full cycle facility with 500 sows in production located in western central Mexico. The farm produces piglets negative to PRRSV which are contaminated along their productive life.

The reproductive herd is under a program of avian immunoglobulins which consists in the application of 1 mL to the entire herd every four months, with a repetition 15 days later; afterwards weekly applications are performed at the 70th and 85th days of gestation.

Breeding stock sows are monitored by means of ELISA (IDEXX, PRRS X3) every other month in order to evaluate viral circulation, trying to collect blood samples from the same animals. Piglets are monitored in site 1 (three weeks), by means of real time PCR to discard the presence of viremia.

Piglets show clinical evidence of viremia between weeks 6 and 8 of age, and in the next flow they were given 3 mL of a highly concentrated formulation of avian immunoglobulins against PRRS. Ten days after the application, the same animals were monitored by means of the same diagnostic technique.

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Results

Antibody levels in sows of the reproductive herd show a slow decrease in time (see figure 1).

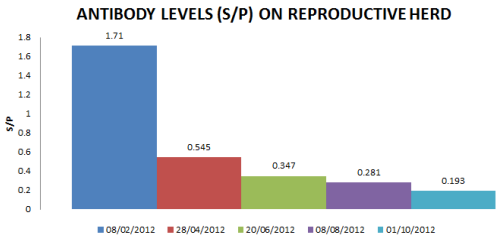

Figure 1. Antibody levels in reproductive herd

The 30 monitored piglets were grouped in 5 sample pools with the following results (see table 1).

Table 1. Viral load in piglets

IDENTIFICATION	RESULTS	VIRAL LOAD
WEANING 6	POSITIVE	6.16 X 10^5
WEANING 6	NEGATIVE	
WEANING 6	POSITIVE	4.98 X 10^6
WEANING 7-8	POSITIVE	5.54 X 10^6
WEANING 7-8	POSITIVE	2.24 X 10^7
WEANING 7-8	POSITIVE	1.21 X 10^7

Results of the monitoring ten days after the application of the highly concentrated immunoglobulins demonstrated 100% of the sampled units as negative to the presence of the genetic material of PRRSV in sera.

Conclusions and Discussion

In this study, the application of avian immunoglobulins was capable of maintaining the reproductive herd stable to PRRSV for the 12 months of the evaluation. Stability was evaluated with S/P levels by means of ELISA, nevertheless a background of circulation during weaning was neutralized with the use of a highly concentrated avian immunoglobulins formula.

Keeping a program of immunoglobulins in the breeding stock avoids viral circulation and can neutralize viremia in weaned piglets with 10^7 viral particles per mL.

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In vitro comparison of several matrices for the individual or collective sampling of oral fluids in pigs for PRRSV detection by quantitative RT-PCR

E Gibert[1], E Pileri[1,2], E Cano[1], GE Martín-Valls[1], Mateu E[1,4]
[1]*Centre de Recerca en Sanitat Animal (CReSA),* [2]*Dept. Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain,* enric.mateu@uab.cat

Introduction

Analysis of oral fluids (OF) for the detection of porcine reproductive and respiratory syndrome virus (PRRSV) is becoming a widely used technique (1). Most often, oral fluids are collected in the pen and therefore are used to perform a collective diagnosis. However, in other instances (e.g. boar monitoring, determination of viral shedding, experimental studies), individual information is of importance. While collective sampling is made by means of cotton ropes, this method is not as useful for individual sampling since the amount of oral fluids produced by a single pig is too scarce. In those cases, the use of specific devices for OF collection may be useful but it is necessary to know how the different matrices may affect the performance of the test. The purpose of the present study was to compare the use of three different matrices and the effects of the storage on the performance of the qRT-PCR for PRRSV using individual OF.

Materials and Methods

The three matrices used were: untreated cotton ropes, Salivette® devices (Sarstedt AG & Co.) and non-treated cotton gauzes. Eighty-seven ml of OF collected from experimental PRRSV-negative piglets were pooled and used for the study. A sample of saliva was spiked with the equivalent of 10^5 copies of the genotype 1 isolate 3267 and subsequent decimal dilutions up to 10^0 were made with the pooled OF. The different matrices (cotton ropes, Salivette® and gauzes) were plunged into the different concentrations of virus for 1 minute. Afterwards, samples were centrifuged at 800 x g for 5 min and the OF were recovered and divided in three aliquots. One aliquot was analyzed immediately, a second one was kept at 4°C for 24 h and the third was frozen at -80°C for 24 h, thawed and analyzed. At the same time, virus was diluted in RNAse-free water or OF in the same range of concentrations as above, stored at 4°C for 2 h and tested in duplicate in qRT-PCR in order to see if OF produced a degradation of viral RNA. Viral RNA was extracted using the Nucleospin® RNA Virus extraction kit (Macherey-Nagel GmbH and Co.). The real-time qRT-PCR reaction was performed with a commercial mix One-Step RT-PCR Master Mix (Applied Biosystems) and primers that were designed to bind specifically the ORF7. A series of decimal dilutions (10^0-10^7 genomic copies) of a standard (ORF7 amplicon) were included. Statistical analyses were done in order to compare the three matrices and the three storage methods using StastDirect 2.8.0.

Results

Non-significant differences were found for the spiked samples stored under different conditions (immediately processed, 4°C for 24 h or frozen at -80°C) using different matrices provided that they contained at least 10^1 copies of the viral genome. Table 1 shows the results for the storage at 4°C for 24 h. The use of OF instead of water had no effect on the detection of PRRSV.

Table 1. Mean PRRSV Ct detected in samples stored at 4ºC by qRT-PCR using rope, Salivette® and gauze.

Log_{10} viral copies	Rope	Salivette®	Gauze
0	39.72	Neg	38.47
1	35.23	36.30	35.45
2	31.80	32.34	31.96
3	27.57	28.62	27.94
4	24.31	24.47	23.90
5	19.83	20.46	20.15

*NA=not applicable

Conclusions and Discussion

The data obtained in the present study showed that the three matrices and methods of storage assayed have no different effect on the performance of the PRRSV qRT-PCR. Under these conditions, sampling of individual pigs may be carried out using ropes, Salivette® or gauzes indistinctly. In our experience, individual sampling of OF in small pigs can be done most conveniently by leting the pigs chewing Salivette® devices. This is convenient for further processing in the lab since the device is contained in an ad hoc tube for the retrieval of OF after centrifugation.

Acknowledgments

This work was supported in part by project RTA2011-00119-00-00 of INIA. E. Gibert has a fellowship from INIA. E Pileri is supported by a fellowship of Universitat Autònoma de Barcelona.

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Impact of co-infection with PRRSV on duration of PCV2 viremia in field conditions

K Podgórska[1], K Kus[1], K Szymanek[1], K Stępniewska[1], A Jabłoński[1], A Szczotka-Bochniarz[1], T Stadejek[2]
[1]National Veterinary Research Institute, Department of Swine Disease, 57 Partyzantów Str., Puławy, Poland.
Department of Pathology and Veterinary Diagnostics, Faculty of Veterinary Medicine, Warsaw University of life Sciences, Warsaw, Poland, kp@piwet.pulawy.pl

Introduction

Porcine reproductive and respiratory syndrome virus (PRRS) and porcine circovirus type 2 (PCV2) infections cause serious economic losses to the global swine industry (1, 2). It has been reported that PRRSV enhance and prolong PCV2 replication and shedding in co-infected pigs what may result in enhanced respiratory disease and severity of associated lesions (2, 3).
The objective of this study was to determine if co-infection with PRRSV and management practices influence the duration of PCV2 viremia in field conditions.

Materials and Methods

The study was carried out in 22 polish farms with different size of sow herds (80-1100) and production systems. Serum samples were collected cross-sectionally in 2 week intervals from several age groups of swine. Samples were tested with ELISA for the presence of antibodies specific to PRRSV (IDEXX) and PCV2 (Ingenasa). Real-time (RT-)PCR was used for detection of PRRSV (Tetracore) and PCV2 (Opressnig et al. 2003) viral genetic material.

Results

Seventeen examined farms were infected with PRRSV (table 1). Infection with PCV2 was confirmed in all farms by PCR/serology or serology only (No. 9, 12, 14, 16). In 11 PRRSV-positive farms co-infections with both pathogens were detected in the same age groups (No 1-8 and 19-21).
In herds 1-8 where health status was described as poor and co-infection with PRRSV was identified, PCV2 viremia lasted from 6 to 16 weeks. On the other hand, in farms 9-13 with similar poor health/biosecurity status but no PRRSV/PCV2 co-infection PCV2 viremia was not detected or lasted up to 6 weeks.
In 3 herds with satisfactory health/biosecurity status viremia lasted 6 weeks or longer. Two of those herds (No 20 and 21) were co-infected with PRRSV. In the other herds with similar conditions PCV2 viremia was either not detected (No 14) or short.

Conclusions and Discussion

In herds with poor biosecurity and the presence of PRRSV co-infections, vaccination against PCV2 did not reduce the duration of PCV2 viremia. PCV2 viremia was previously identified as a factor affecting growth pigs even in herds with subclinical PCV2 infections (1). Therefore prolonged viremia may affect farm productivity. The results of this study clearly show that proper management practices are very important in reducing the impact of PCV2 on the health status of the herd, even in herds where PCV2 immunoprophylaxis is already implemented.

Table 1. Summary results.

Farm no.	Duration of PRRSV viremia (weeks)	Duration of PRRSV/PCV2 co-infection (weeks)	Duration of PCV2 viremia (weeks)	PCV2 vaccination	Health/biosecurity status
1	2	2	14	+	very poor
2	8	6	6	+	very poor
3	9	9	13	+	poor
4	10	10	10	+	poor
5	7	3	9	-	poor
6	8	8	14	+	poor
7	8	8	16	+	poor
8	2	2	13	-	poor
9	8	nd	nd	+	very poor
10	nd*	nd	6	+	poor
11	nd	nd	6	+	very poor
12	11	nd	nd	+	poor
13	nd	nd	from 20**	+	poor
14	12	nd	nd	+	satisfactory
15	6	nd	1***	+	satisfactory
16	15	nd	nd	+	satisfactory
17	nd	nd	7	+	satisfactory
18	nd	nd	nd	+	satisfactory
19	8	2	2	-	satisfactory
20	12	6	6	-	satisfactory
21	12	12	12	-	satisfactory
22	9	nd	from 23**	-	satisfactory

*not detected, ** last sampling, ***one age group positive in PCR

Acknowledgements

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P.307

Monitoring of epidemiological situation concerning African swine fever in Poland in 2013

I Markowska-Daniel, E Kozak, K Urbaniak, Z Pejsak
Department of Swine Diseases, National Veterinary Research Institute, Pulawy, Poland, iwonamd@piwet.pulawy.pl

Introduction

New era of African swine fever (ASF) started in 2007, after introducing of ASFV to Georgia. From this time it's circulating in the Caucasian region and the Russian Federation. In 2012, 2013 and 2014 the outbreaks of ASF were also registered in Ukraine, Belarus and Lithuania, representing high risk of its further spread to neighboring countries.

An increased risk of transmission of ASFV force high level of diagnostic preparedness. Therefore in 2011 Chef Veterinary Officer decided to implement the monitoring of the epidemiological status of North and East regions of Poland for ASF, by performing suitable laboratory tests. All examinations were done in the National Reference Laboratory (NRL) for ASF located in National Veterinary Research Institute in Pulawy. NRL has an accreditation for both, ELISA and PCR and is regularly updating the laboratory diagnostic skills and prove the ability to diagnose ASF by regular participation in the inter-laboratory comparison tests, organized yearly by EURL.

In 2013 the program of wild boars and domestic pigs monitoring for ASF was introduced throughout the territory of Poland, with special emphasis to regions located 40 kilometers from East border of the country.

Materials and methods

Biological samples (sera, whole blood and organs) from wild boars were collected. They were taken from both, the dead animals (due to different reasons, including car accidents), as well as from shot animals in normal hunting practices. Additionally, the samples from pigs that died with symptoms of infectious disease were analyzed. In total 14772 samples taken from 13365 animals (1672 pigs and 11693 wild boars) were examined.

Serological survey. In total 1015 sera (191 from pigs and 824 from wild boars) were checked using commercial ELISA (PPA Ingezim Compact 11.PPA.K3, Ingenaza). Positive or questionable samples were retested using OIE ELISA as well as immunobloting (IB) and Immunoperoxidase test (IPT) - as confirmatory techniques. Reference positive, limit and negative sera were included in the study.

Molecular survey. For the isolation of DNA various biological materials were used. In total 14772 samples: 2507 from pigs (691 blood samples, 276 sera, 61 kidneys, 481 spleens, 401 tonsils, 452 lymph nodes, 144 lungs, 1 long bone) and 12265 from wild boars (9203 blood samples, 329 sera, 857 kidneys, 942 spleens, 142 tonsils, 200 lymph nodes, 582 lungs, 3 breastbones, 7 long bones) were examined. The Real-Time PCR based on the VP72 gene sequence was used.

Results

Serological survey. Using commercial ELISA all samples except 4 blood samples of wild boars which gave the questionable and the 12 others which gave weak positive results were negative. One of mentioned sample gave questionable result in OIE ELISA. We didn't confirm the presence of the antibodies specific to ASFV in the mentioned sera using confirmatory tests - IB and IPT, more over these samples were also testing by PCR and they were negative, therefore taking into account the results of all testes performed all samples were finally evaluated as negative.

Molecular survey. All the results of 14772 blood, sera and organ samples tested by Real-Time PCR were negative - no presence of genetic material of ASFV was evident.

Conclusions and Discussion

ASF is considered as one of the global animal health priorities. It is producing great socio-economic impact in affected countries. The risk of disease spreading to new areas is increased by high resistance of ASFV to environmental factors, uncontrolled movements of wild boars and lack of the commercial vaccine. We conclude that monitoring studies conducted by NVRI were very useful to prove epidemiological status of Poland concerning ASF. The results of serological and molecular surveys clearly illustrate that up to now Poland is free from ASF. This is very important not only for Polish government and agriculture but also for the EU since Poland is an Eastern border of the EU. Serological studies allow to early detection of antibodies and detection of carriers animals. Molecular biology techniques are useful for early identification of viral DNA, before clinical symptoms appearance in infected animals. Due to the high risk preventive action with special emphasis to strict follow of biosafety rules, disinfection of vehicles and confiscating of food at the East Polish border are necessary to protect against the appearance of ASFV. The risk of transmission of ASFV to the EU is much higher nowadays therefore the monitoring program is continuing in Poland.

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Currently isolated PEDV in Korea

H Jang[1], JG Kang[1], HJ Shin[2]
[1]*WOOGENEBNG,* [2]*College of Veterinary medicine ChungNam Nat. Univ.* hjang@woogenebng.com

Introduction

Porcine epidemic diarrhea caused by porcine epidemic diarrhea virus (PEDV) infection severe damage to swine industry in Asia and USA. Several attenuated strains such as CV777 and SM98 were used to vaccine production but recently field isolated strain were different from vaccine strain genetically as well as serologically. Currently isolated PEDV in Asia and USA show more contagious and more severe mortality compare with previous PEDV. Continuous vaccine strain development is essential for effective PEDV prevention and control therefore new field strain isolation and cultivation in the laboratory is first step for effective vaccine development against new variant. In this study show pathogenecity and immunogenicity of currently isolated CNU-1 strain in Korea.

Materials and Methods

Clinical samples for PEDV isolation are prepared from feces and intestinal homogenates of piglets that was show typical PEDV infected symptoms. Spike protein specific real-time RT-PCR was doing for identification of PEDV positive sample. RT-PCR positive samples were mixed with sterilized PBS and centrifuged at 4,200*g* for 10 minutes at 4°C. Centrifuge supernatant s are filtered through a 0.22-micron filter and used as inoculums for virus isolation.

Virus isolation and culture was doing in Vero cell line. PEDV virus culture method follow standard Vero cell culture method but PEDV culture in Vero cell was need media containing specific concentration typsin.

Virulenecy and immunogenicity test using $10^{5.0}$ $TCID_{50}$/ml of PEDV virus

Phylogenetic analysis was performed using the nucleotide Spike protein total sequences of the six PEDV viruses from this study as well as other PEDV strains.

Results

Virus isolation was attempted PEDV-PCR positive feces and PEDV-PCR positive intestine homogenates on Vero cells. RT-PCR positive sample inoculated Vero cell show typical PEDV CPE such as cell fusion, syncytia formation (Fig.1). CPE observed PEDV was cultured in order to isolated viruses can be efficiently propagated and maintained in Vero cell cultures. After doing more than 30 continuous passage cultures, isolated PEDV titer was reach to $10^{6.5}TCID_{50}$/ml(Fig. 3). Isolated PEDV (CNU-1 strain) Spike protein nucleotide sequence was different from previous isolated PEDV (Fig. 2)

Figure 1. Cytopatic effect of PEDV CNU-1 strain in Vero cell culture.

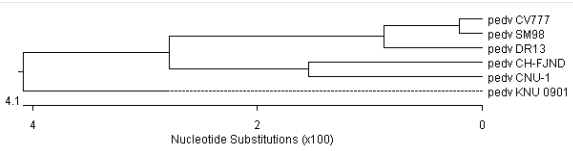

Figure 2. Pylogrntic analysis of PEDV CNU-1 strain.

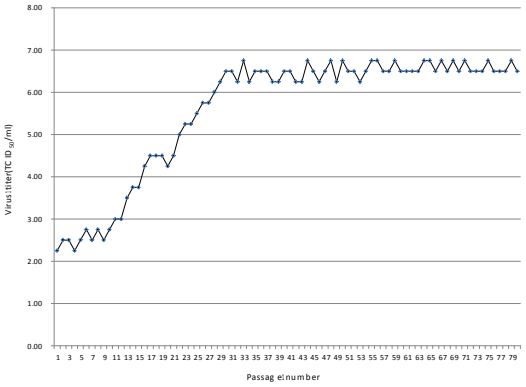

Figure 3. PEDV CNU-1 strain titer increase according to the passage culture number.

Conclusions and Discussion

In this study we isolate new variant PEDV in Korea and also successfully cultured on the Vero cell. Phylogenetic analyses show new PEDV (CNU-1) completely different from previous PEDV. CNU-1 is important to effective vaccine development.

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Complete genome sequence of a novel PEDV in Eastern China

D-Q Yang, F-F Ge, H-B Ju, J Wang, J Liu, K Ning, P-H Liu, J-P Zhou
Shanghai Animal Disease Control Center, Shanghai, People's Republic of China, dequan_yang@yahoo.com

Introduction

Since December 2010, outbreaks of porcine epidemic diarrhea (PED) have been observed on most swine breeding farms in most of the provinces of China (4). In order to investigate whether there is a novel porcine epidemic diarrhea virus (PEDV) variant circulating in China, we sequenced the complete genome of SHQP/YM/2013.

Materials and Methods

Intestinal tracts were collected from dead piglets during an outbreak of diarrhea in an immunized-swine breeding farm in Shanghai in 2013.

Primers were designed to anneal to sites that are highly conserved among PEDV sequences available in the GenBank database, and complete genome sequencing was determined by primer walking strategy. The 5' and 3' end regions were amplified using 5' and 3' full RACE kits (TaKaRa, Dalian, China) according to the manufacturer's instructions. These plasmids were sequenced by Invitrogen Company (Life Technologies, Shanghai, China). Each nucleotide was determined from three identical results. Sequences were assembled and analyzed by using the DNAstar software package (DNAstar Inc., Madison, WI, USA).

Results

The complete genomic sequence of SHQP/YM/2013, excluding the poly(A) tail, comprises 28,038 nucleotides(nt), with 5′ (292 nt) and 3′ (334 nt) ends containing untranslated regions (UTRs). Seven coding regions, respectively, encode 4 structural proteins (S [nt 20634 to 24794], E [nt 25449 to 25679], M [nt 25687 to 26367], and N [nt 26,379 to 27,704]), 2 nonstructural proteins (replicase 1a [nt 293 to 12601] and replicase 1b [nt 12601 to 20637]), and the only accessory protein, ORF3 (nt 24794 to 25468).

The complete genome of SHQP/YM/2013 has 96.6% to 99.7% nucleotide sequence identities with those of the reference strains reported in GenBank. The S gene contained 4,161 nt and was 9 nt longer than that of the PEDV reference strain CV777. Compared to CV777, two insertions (in positions 56-59 and 140) and one deletion (in position 156-157) were observed. These regions are also found in the S genes of Korean PEDV isolates (3). The ORF3 and M gene did not have nucleotide deletions or insertions but did have point mutations.

Phylogenetic analyses of the S gene nucleotide sequences revealed that all PEDV strains in this study could be separated into two groups (Figure 1): SHQP/YM/2013 belonged to Group 2, which also contained nine Korean field strains (Chinju99, Spk1, KNU-0801, KNU-0802, and KNU-0901–KNU-0905) and ten Chinese strains, which occurred in severe PED outbreaks of China during 2011-2013.

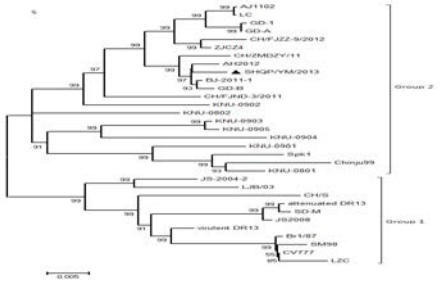

Figure 1. Phylogenetic analysis by neighbor-joining method (bootstrapping for 1000 replicates with a value >50%) based on nucleotide sequences of S gene

Conclusions and Discussion

Similar to other coronavirus, the S gene has been hypothesized to be important in the virulence and pathogenesis of PEDV infections (1,2,5). The same amino acids insertions and deletion of the S gene were observed among prevailing PEDV strains (except SD-M) in China and two Korean (KNU-0802 and KNU-0902) strains in South Korea during 2008-2009. These insertions and deletion could be responsible for the unique character of the novel PEDV variants that have circulated in China.

Phylogenetic analysis demonstrate that prevalent PEDV isolates (except SD-M) in China are more closely related to each other and to Korean field strains (Chinju99, Spk1, KNU-0801, KNU-0802, and KNU-0901-KNU-0905) rather than reference strains (CV777 and Br1/87), Korean vaccine strain (attenuated DR13), Korean field strains (virulent DR13 and SM98) and Chinese strains (JS-2004-2, LJB/03, CH/S, LZC, JS2008 and SD-M). These results suggest that PEDV isolates prevalent in China may have originated from Korean ones.

Acknowledgments

Shanghai Animal Disease Control Center, Shanghai 201103, People's Republic of China

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Biological properties of current PED Viruses in South Korea

S-H Kim, J-M Lee, J-K Oem, Y-H Kim, M-H Lee, K-K Lee
Animal Disease Diagnostic Division, Animal and Plant Quarantine Agency, AnyangDong, Gyeonggido, South Korea, soho923@korea.kr

Introduction

Porcine epidemic diarrhea virus (PEDV) is the etiological agent of enteropathogenic diarrhea in piglets in Korea. PED has been consistently detected since 1993 (2,3). To determine the extent of genetic variation among PEDVs in Korea, 27 PEDV isolates from diarrhea and intestines collected from pig farms located in several Korean provinces between 1998 and 2013 were sequenced and their spike (S) and ORF3 sequences were compared with those of reference strains from other countries. The antigenic properties of the virus were determined using serum neutralization assays with pig antisera raised against PEDVs, a vaccine virus, and field viruses. Furthermore, the biological properties of the field viruses were investigated in commercial piglets by oral inoculation.

Materials and Methods

Viral RNA was extracted from 300 µl of diluted diarrhea using an RNeasy® Mini Kit (Qiagen). Reverse transcription (RT) was performed using the extracted RNA, oligo (dT), and a PrimeScript First Strand cDNA Synthesis Kit (Takara Bio Inc.) according to the manufacturer's instructions. A cDNA fragment was produced by PCR from the RT product using an Advantage® 2 PCR Kit (Clontech) according to the manufacturer's protocol. Each sequence was aligned using ClustalW and phylogenetic trees were constructed using the neighbor-joining method. Pig antisera raised against a selection of vaccine and field viruses by intramuscular injection and oral infection, respectively, at 2-week intervals were used to confirm antigenicity. To confirm host biopathogenesis, three 2-day-old pigs, which were serologically free from PEDV, were inoculated orally with 2 ml of 30 $TCID_{50}$/pig of two field viruses and monitored for clinical signs and changes in weight for 14 days.

Results

A phylogenetic analysis of the S genes from the viruses revealed that most Korean isolates were divided into two groups (G1 and G2) with two subgroups (G2-1 and G-2-2). Homology analyses of the S genes showed that the Korean isolates were 91.4-99.9% and 89.7-99.8% homologous at the nucleotide (nt) and amino acid (aa) levels, respectively. Furthermore, three field strains were found to be similar to American strains detected in 2013 (1), with 99.2-99.8% nt and 98.8-99.7% aa homology. The Korean isolates showed 91.8-99.3% homology with vaccine strains used in Korea, which corresponded to 90.5-98.9% homology at the aa level. In the ORF3 region, cell adaptation strains and some field strains showed large deletions.

Variable serologic cross-reactivity was observed between the SM98 vaccine and field viruses. Limited cross-reactivity, however, was detected between the P5V vaccine and field viruses, with a reduction in the serum neutralization titer. The clinical signs observed in piglets infected with each virus (KDGG13HWN and KDGG13DJ) ranged from diarrhea to vomiting and dehydration. Approximately 10^4 copies of PEDV were shed in diarrhea on day 1, with the copy number decreasing until 8-10 days after inoculation. The villous height and crypt depth ratio in the small intestines of the infected pigs were also measured and found to be significantly reduced compared to those of the controls. Although the two viruses were similar in terms of pathogenicity, KDGG13HWN was more virulent than KDGG13DJ in terms of weight loss and the number of viral copies in diarrhea.

Conclusions and Discussion

Our sequencing and phylogenetic analysis results for the S and ORF3 genes of PEDV indicate genetic diversity among field isolates. Furthermore, current PEDVs in Korea were found to be virulent, causing severe villous atrophy, and somewhat different in their antigenicity. This information will further our understanding of the prevalence of PEDVs in Korea and aid in the evaluation of vaccines against PED.

Acknowledgments

This study was supported by a grant from the Animal, and Plant Quarantine Agency, Korean Ministry of Agriculture, Food and Rural Affairs.

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PEDV outbreaks in Taiwan, 2013-2014

CN Lin, WB Chung, SW Chang, CC Wen, H Liu, CH Chien, MT Chiou
National Pingtung University of Science and Technology, Pingtung, Taiwan, mtchiou@mail.npust.edu.tw

Introduction

Porcine epidemic diarrhea viruses (PEDVs) are enveloped viruses with a large, capped and polyadenylated RNA genome of approximately 28,000 nucleotides (1). PEDVs belong to the genus *Alphacoronavirus*, family *Coronaviridae*, and order *Nidovirales*. Other members of this subgroup include human coronavirus (HCoV) 229E, HCoV NL63, and Bats coronavirus 512/05 (2). Although PEDV was first identified in Europe, it has become increasingly problematic in many Asian countries (1) and North America (3). However, there is no retrospective study on the emergence of PEDV in Taiwan.

Materials and Methods

Samples from 32 pig farms in central and southern Taiwan from December 2013 through January 2014 were submitted to the animal hospital of National Pingtung University of Science and Technology. All of the pig farms had similar disease histories, clinical signs, and lesions, including the presence of a sudden outbreak and rapid spread in farms, high mortality in young pigs, and diseases affecting animals of all age, notably, vomiting and diarrhea. The clinical specimens were collected and tested for rotavirus, transmissible gastroenteritis virus, and porcine epidemic diarrhea virus.

Results

All of the specimens were negative for rotavirus and transmissible gastroenteritis virus. A total of 68 samples from 25 pig farms were confirmed as positive for PEDV by reverse transcription PCR.

All 8 PEDV isolates collected from this outbreak shared 99.5-100% DNA sequence identity of the partial S gene. Two major clusters, based on the phylogenetic relation of the partial nucleotide sequences of the COE domain in the S gene, were detected (Figure). The first cluster was comprised of prototype isolates (TW1/A/2013 and TW2/B/2013) and Chinese strains LJB/03 and DX/2007. The second cluster consisted of all 8 Taiwanese isolates from this outbreak and 7 US isolates (Figure 1). One nonsynonymous mutation was observed in recent Taiwanese PEDV strains and US strains at nucleotide positions 1540-1542 (GTA) of the S gene compared with PEDV CV777 (GTC), PEDV AH2012 (GTC), or the Taiwanese prototype.

Conclusions and Discussion

Our data suggest that all recent Taiwanese PEDV isolates are genetically similar to US isolates identified in 2013. These strains were responsible for the recent PEDV outbreak in Taiwan and produced a similar mortality rate and pathologic effects of US isolates. Taken together, our findings indicate that these PEDV outbreaks in Taiwan share a common evolutionary origin with the S gene of US strains of PEDV. Additional PEDV cases should be investigated using continuous surveillance and sequence analysis.

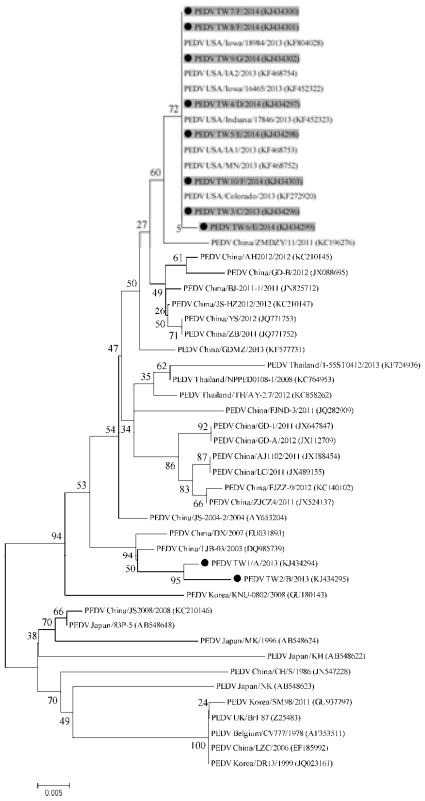

Figure 1. Phylogenetic relationships based on the partial COE domains of the S genes of Taiwanese PEDV isolates and reference strains. *The analysis was performed employing the maximum likelihood method based on 1,000 replicates using MEGA 5 software. Light grey underlay: Taiwanese PEDVs isolated in this severe outbreak. Solid circles: Taiwanese PEDV isolates in the present study.*

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Preliminary virology and pathology of PEDV in the United States, spring 2013

SL Swenson[1], JJ Schiltz[1]; R Lomkin[2]; M Jenkins-Moore[1]; L Koster[1]; AS Predgen[3], AD Lehmkuhl[3]
[1]*United States Department of Agriculture, Animal and Plant Health Inspection Services, Veterinary Services, National Veterinary Services Laboratories, Diagnostic Virology Laboratory, Ames, Iowa;* [2]*USDA, APHIS, VS, Colorado Office;* [3]*USDA, APHIS, VS, NVSL, Pathobiology Laboratory, Ames, IA,* sabrina.l.swenson@aphis.usda.gov

Introduction

Porcine epidemic diarrhea (PED) virus is a coronavirus similar to transmissible gastroenteritis (TGE) virus, causing severe vomiting, diarrhea, dehydration, and death in pigs. It was first identified in the United Kingdom in 1971, causing epidemics in Europe during the 1970s and 1980s before spreading to Asia; PED was not previously known to be in the western hemisphere. The virus spreads by the fecal-oral route and can cause 80-100% mortality in piglets while adult pigs typically recover. Cases were identified in April 2013 in three states and were discovered in 11 additional states in May and June. As of February 20, 2014, the AASV website reports infection in 25 states. Virus isolation, real time reverse transcription polymerase chain reaction (RT-PCR), sequencing, and histopathology were performed at the NVSL on field submissions.

Both TGE virus and PED virus are members of the of the *Coronaviridae* family, are highly contagious, and can cause vomiting, diarrhea, dehydration and death in pigs of multiple ages; disease is more severe in young pigs. Typical gross and microscopic lesions of TGE and PED may include fecal staining of the perineum, flaccid, thin and transparent small intestinal walls, and small intestinal villous atrophy with epithelial cell degeneration or necrosis. The diseases caused by these viruses are very similar and impossible to differentiate based on clinical signs and pathological changes.

Materials and Methods

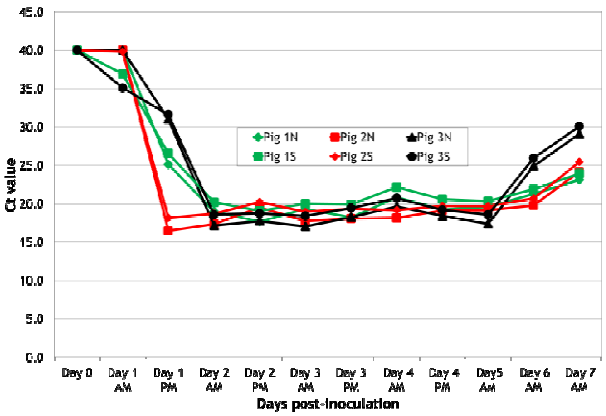
Three, approximately 4 week-old piglets were orally inoculated with feces and intestinal homogenate from a field submission case and were monitored for three weeks for clinical signs, seroconversion, and fecal shedding of viral nucleic acid. Pigs were inoculated with the feces/tissue homogenate (20%) both AM and PM of Day 0. Fecal swabs were collected on Day 0 AM, Days 1-4 AM and PM, and Days 5-7 AM. Blood samples were collected on Day 0, 7, and 15. Real-time RT-PCR was conducted on all fecal swabs and IFA testing, with end point determination, was conducted on serum samples collected.

Results

All three pigs developed vomiting and diarrhea, seroconverted, and viral RNA was detected in fecal swabs by real-time RT-PCR. Vomitus was noted in the pen 24 – 48 hours after initial inoculation. Diarrheic stools were noted 48-72 hours after initial inoculation, with gradual return to a more normal consistency. All serum samples were negative at Day 0 and Day 7; Day 15 samples were positive at 1:100.

Conclusions and Discussion

Figure 1. PEDV rRT-PCR Ct values in experimentally inoculated pigs



Real time RT-PCR results show all animals were positive for PED viral RNA by 36 hours post initial inoculation, maintained Ct values in the high teens for Days 2 through 5, had increasing Ct values on Days 6 and 7.

Collectively, the PCR results show a high degree of uniformity between animals and between the two reactions.

Antibodies to PEDV were not detected at Day 7 but were present at 15 dpi.

The study demonstrated the infectious nature of the inoculum, the reproduction of typically reported clinical signs, and molecular and serological confirmation of PED virus presence in the original inoculum.

Molecular epidemiology of PEDV in South China during 2011-2014

J Mo[1], J Niu[2], G Zhang[2], Y Chen[2], X Zeng[1], B Sun[1], Y Bi[1], J Ma[1]*

[1]*College of Animal Science, South China Agricultural University, Tianhe District, Wushan Road, Guangzhou 510642, Guangdong, P.R. China.*[2]*Guangdong Wen's Foodstuff Group Co., Ltd., Yanjiang Street, Xinxing 527400, Guangdong, People's Republic of China.* majy2400@scau.edu.cn

Introduction

Porcine epidemic diarrhea virus（PEDV）was well known as a widely spread virus with high morbidity rate and high fatality rate for newborn piglets in China since the end of 2010, causing huge economic losses to the swine industry (1). To identify strains of outbreak in South China resent years, the full-length of S gene of 116 PEDV strains from 2011 to 2014 were sequenced and compared with PEDV reference strains obtained from NCBI. Phylogenetic analysis based on S gene of PEDV was conducted.

Materials and Methods

From February 2011 to January 2014, 116 PEDV positive samples were collected from swine herds in South China, including 12 samples in 2011, 4 samples in 2012, 86 samples in 2013 and 11 samples in January 2014. The full-length nucleotide sequences of S gene were amplified by reverse transcription polymerase chain reaction (RT-PCR). Phylogenetic tree was constructed using the MEGA 5 with the maximum likelihood method (2).

Results

Phylogenetic analysis of the nucleotide sequences of S gene revealed that all PEDV strains used in this study could be divided into three groups, Group 1, 2 and 3 which were represented by CV777, KNU-0801 and HnN, respectively. Most strains used in the present study belong to the group 3. Several earlier strains, especially the strains obtained in 2011, had a close relationship with the earlier domestic strains, such as CHGD-01.While the strains collected from 2013 to 2014, form an independent branches.

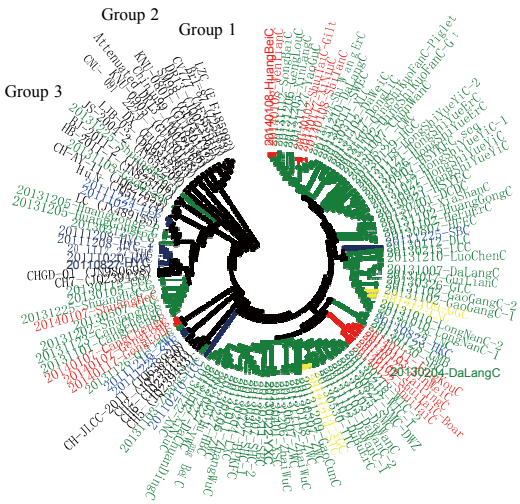


Figure 1. Phylogenetic relationship of the 2011(Blue), 2012(Yellow), 2013(Green) and 2014(Red) strains identified from South China with other PEDV reference strains (Black) based on the nucleotide sequences of full-length S gene.

Conclusions and Discussion

Epidemiological evidence found in the present study demonstrated that most of recent PEDV occurred in South China during 2013-2014 belong to Group 3 of phylogenetic tree, and was genetically different from those earlier PEDV strains. Moreover, long distance between field strains and tradition vaccine strains, such as CV777, which belonged to G1, may provide less protection. Novel vaccines should be developed to use in PEDV controlling and the variants of PEDV circulated in swine herds should be pay more attention.

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Serological survey of transmissible gastroenteritis virus and porcine respiratory coronavirus in Korean conventional pig farms

KK Lee, SH Kim, JK Oem, YH Kim, JY Chung, MH Lee
Animal and Plant Quarantine Agency, Anyang, Gyeonggi-do, Republic of Korea, naturelkk@korea.kr

Introduction

Transmissible gastroenteritis virus (TGEV) causes a highly contagious enteric disease of swine, with acute diarrhea, severe dehydration and a high mortality rate in sucking piglets. Porcine respiratory coronavirus (PRCV) is a mutant of TGEV with a spontaneous deletion at N-terminus of the spike (S) gene. In contrast to TGEV, PRCV replicates in epithelial cells of respiratory organs and causes mild respiratory clinical signs. Since PRCV induces the production of antibodies able to neutralize TGEV, the wide distribution of PRCV led to a marked decrease in the number of TGEV outbreaks. In Korea, although the prevalence of TGEV infection was not high, the sporadic TGEV cases have been occurred in several regions, recently. To prevent and control TGE effectively, it is important to understand accurately about situation of outbreak and immunity of TGEV determined by surveillance. In the present study, we investigate the serological condition against TGEV and PRCV in Korean conventional pig herds.

Materials and Methods

Twenty two pig farms were randomly chosen from Gyeongnam province of Korea in 2013. All farms were conventional farrow-to-finish farms including 2 TGE outbreak farms. To analyze the antibody levels of every growing stage, blood samples were collected from 6 sows, 2 gilts, 8 sucking piglets of bled sows, and 5 pigs at 40, 70, 100, 130 days of age, respectively. A total of 888 sera were tested by commercial blocking ELISA (SVANOVA, Sweden) differentiating TGEV and PRCV antibodies. Antibody titers were calculated as percent inhibition (PI) against TGEV and PRCV specific monoclonal antibodies (MAbs). PIs greater than 60% were considered the presence of competing antibody.

Results

Seven farms were confirmed sero-positive herds against TGEV (31.8%). Among these positive farms, the sows of 3 farms have been vaccinated for TGEV. One of 2 TGE outbreak farms showed strong antibody response in pigs of all ages while the other outbreak farm has sero-positive groups at sows, suckling piglets and weaned piglet. The remaining 2 TGEV positive farms showed weak antibody response in parts of growing stage. PRCV antibody-positive pigs were detected in 20 farms (90.9%). Four patterns of serological response in PRCV positive farms were observed at growing stages; (I)! negative response of all ages, (II) strong or moderate positive response of all ages, (III) ever-increasing response from sucking piglets, (IV) increasing response until 40 days of age followed by rapid decrease. Of 684 sera excluding positive sera of pigs vaccinated or infected with TGEV, 14 sera (2.1%) were TGEV antibody positive and 317 sera (46.4%) were PRCV antibody positive (Table 1).

Table 1. Results of antibody response against TGEV and PRCV in Korean pig herds.

	farms	pigs	TGEV positive		PRCV positive	
			farms (%)	pigs (%)	farms (%)	pigs (%)
including farms vaccinated or infected with TGEV	22	888	7 (31.8)	112 (12.6)	20 (90.9)	471 (53.0)
excluding farms vaccinated or infected with TGEV	17	684	2 (11.8)	14 (2.1)	15 (88.2)	317 (46.4)

Discussion

Although the prevalence of TGEV infection was low, PRCV was distributed widely in conventional pig herds of this study. The antibody-positive pigs in 1 unvaccinated farm were converted negative in re-examination after 6 months, presuming a few vaccinated sows were introduced into the farm. The re-examination of the other positive farms showed similar results to a previous test providing the possibility of TGE outbreak. However, clinical signs and antigenic detection of TGEV did not occur at the farm since then. The antibody-positive rate and titer of PRCV were diverse in farms and growing stages. The average positive rate of farms vaccinated or infected for TGEV was higher than that of TGE negative farms. In conclusion, to prevent and control TGEV in Korea, further sero-surveillance of TGEV and PRCV is needed at many other regions, followed by investigating the risk of TGEV outbreak.

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Molecular identification of the PEDV

F Diosdado[1], A Martínez[1], G Socci[1], E Carrera[1], D Córdova[1], A García[1], V Quintero[2], R Fajardo[3], R Flores[1]
[1]CENID-.Microbiología, INIFAP. Km. 15.5 carretera México-Toluca, 05110, México DF. [2] FES-Cuautitlán, UNAM. [3] CIESA, FMVZ, UAEMX. diosdado.fernando@inifap.gob.mx

Introduction

Porcine epidemic diarrhea (PED) caused by PED virus (PEDV) is an infectious disease, highly contagious and clinically similar to porcine transmissible gastroenteritis (TGE) and porcine group A rotavirus (GAR). PEDV was identified in the United States for the first time on 29 April 2013, having 99.5% nucleotide homology with AH2012 strain, which caused outbreaks in China (2). In México, by early July 2013, pig breeders and veterinarians reported a number of suspect cases, which met the signs of PED. So the objective of this study was to develop the molecular diagnostics for a rapid, differential and specific detection of PEDV.

Materials and Methods

Small and large intestine, and stool samples from piglets with suspect PED episodes from complete cycle farms were studied. To detect PEDV, TGEV and GAR, RT-PCR was used. Targeted sequences were respectively: 503 bp for the S gen of PEDV (3), 160 bp for TGEV, and 416 bp for GAR (1), at same conditions to amplify sequences. Amplimers were purified and sequenced using a brand kit (Wizard® SV Gel and PCR Clean-Up System). Determined sequences were analyzed using the Blast program.

Results

Eleven out of 13 samples were positives for the PEDV (Fig. 1).

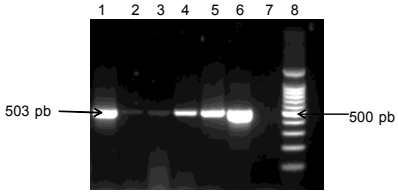

Figure 1. PEDV identification by RT-PCR from piglets with suspect diarrhea episodes. *Lines: 1, large intestine; 2, small intestine; 3, large intestine; 4, stool; 5, large intestine; 6, small intestine; 7, negative control; 8, weight molecular markers.*

TGEV and GAR were not detected in any sample. The sequence alignment of amplimers gave an homology of 99% with the USA/Colorado/2013 strain (2).

Conclusions and Discussion

RT-PCR was rapid and effective to detect PEDV from porcine intestinal tissues and stools. After differential diagnosis, all samples were negative for TGEV and GAR detection. Sequencing results confirmed the PEDV presence in the clinical samples.

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Development of indirect and blocking ELISAs for detection of antibodies against PEDV

S Lawson, F Okda, X Liu, A Singrey, T Clement, J Christopher-Hennings, E Nelson *Veterinary & Biomedical Sciences Department, South Dakota State University, Brookings, SD,* USA
eric.nelson@sdstate.edu

Introduction

Porcine epidemic diarrhea virus (PEDV) was first described in Europe in the 1970s with more recent and severe outbreaks in Asia (1,2,3). PEDV is an enveloped, single-stranded, positive-sense RNA virus infecting swine and is a member of the *Coronaviridae* family. PEDV was first identified in the U.S. in May 2013 (4) and has since been confirmed in multiple states and Canada. The initial PEDV strain identified in the U.S. has high sequence identity with recent variant strains identified in China in 2011 and 2012 (4). In the U.S., PCR assays were quickly developed to detect the presence of PEDV RNA in intestinal contents or fecal material. These assays provide an important tool in control of the virus; however, well-validated, high-throughput assays to detect antibodies following infection would provide a valuable additional diagnostic tool for the swine industry. The first generation of indirect fluorescent antibody (IFA) serological assays are now being offered by several U.S. diagnostic laboratories but they have drawbacks including challenges propagating the virus, somewhat subjective interpretation, difficulty adapting to high throughput testing and limited ability for adaptation to oral fluid testing.

Materials and Methods

In response to these needs, we developed a serological enzyme-linked immunosorbent assay (ELISA) based on recombinant expression of a full length PEDV nucleoprotein (NP). The NP gene was cloned and expressed as a 51 kDa, 6x His tag protein which reacted to PEDV positive sera and a 6x His-specific monoclonal antibody via immunoblotting. The test was evaluated for sensitivity and specificity for the serodiagnosis of PEDV antibodies in serum samples of known status. Known PEDV negative sample sets included samples from selected high biosecurity herds with no history of PEDV and archived serum samples collected prior to the emergence of PEDV in the U.S., including samples testing positive for the related swine coronaviruses, TGEV and PRCV. Known positive samples were collected from pigs that were naturally infected at least 3 weeks prior to collection and were previously positive by PCR. Additionally, monoclonal antibodies developed against the PEDV NP were biotinylated and used to develop a blocking ELISA using the same NP antigen.

Results

Receiver operating characteristic analysis (ROC) was performed using swine serum samples of known serostatus (n=1521). ROC analysis of the indirect NP-ELISA results showed estimated sensitivity and specificity of 97.6% and 97.9%, respectively, with a diagnostic cutoff of 0.390. Inter-rater agreement (kappa association) is a statistical measure of test agreement and was calculated to be 0.932 between IFA and NP-ELISA demonstrating a significantly high level of agreement between tests. The assay was further employed to investigate the kinetics of an antibody response in infected pigs over time. The sensitivity of the ELISA showed PEDV-NP seroconversion as early as 7 to 10 DPI. Additionally, none of the known positive TGEV or PRCV samples tested (n=>50) were shown to cross-react on the PEDV NP-ELISA. Full validation of the blocking ELISA is in progress and preliminary data suggests a higher level of assay specificity.

Conclusions and Discussion

These results indicate that the purified nucleoprotein may be a useful antigen for the serodiagnosis of PEDV. ELISA testing demonstrated a high degree of sensitivity and specificity using swine serum. Kappa values indicate that both the IFA and ELISA tests agree significantly with each other. Also, most animals seroconverted between 7-14 DPI and there was no antibody cross reactivity between PEDV and either PRCV or TGE. These assays should be of value in controlling the spread of the disease in North America and in seroprevalence studies. Ongoing studies include adapting the assays to oral fluid and milk testing and to a fluorescent microsphere immunoassay (FMIA) format.

Acknowledgments

This work was funded by the South Dakota Animal Disease Research & Diagnostic Laboratory and the South Dakota Agricultural Experiment Station (AES Hatch Project H-392).

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Development and diagnostic application of monoclonal antibodies to PEDV

A Singrey, S Lawson, F Okda, X Liu, T Clement, J Nelson, J Christopher-Hennings, E Nelson
Veterinary & Biomedical Sciences Department, South Dakota State University, Brookings, SD, USA
eric.nelson@sdstate.edu

Introduction

Porcine epidemic diarrhea virus (PEDV) was first described in Europe in the 1970s with more recent and severe outbreaks in Asia (1,2,3). PEDV was first identified in the U.S. in May 2013 (4) and has since been confirmed in multiple states and Canada. PEDV is an enveloped, single-stranded, positive-sense RNA virus infecting swine and is a member of the *Coronaviridae* family. Due to the recent emergence of PEDV in North America, availability of specific monoclonal antibodies (mAbs) is limited. Therefore, the purpose of this study was to develop high quality, readily available reagents for detection of PEDV antigen in diagnostic tests, such as virus isolation, immunohistochemistry and a variety of immunofluorescence-based techniques.

Materials and Methods

Mice were immunized with selected recombinant PEDV proteins including a 51 kDa full length PEDV nucleoprotein (NP) or portions of the spike (S) protein containing putative epitopes associated with virus neutralization. Splenocytes from immunized mice were fused with NS-1 myeloma cells and cultured on 24-well plates with selective HAT medium. Cell culture supernatants were screened by ELISA and IFA then positive wells were subcloned, expanded and retested. Immunoglobulin isotyping of the resulting mAbs was performed using a commercial lateral flow assay.

A fluorescent focus neutralization (FFN) assay was also developed for rapid detection of neutralizing antibodies developed in response to PEDV infection or vaccination. Heat-inactivated swine serum samples were diluted in 2-fold dilution series in MEM plus 2.5µg/ml TPCK-treated trypsin in 96-well plates. An equal amount of PEDV stock at 100 to 200 foci forming units/100µl was added to each well and plates incubated for 1 h at 37C. The virus/serum mixture was then added to washed confluent monolayers of Vero-76 cells and incubated for 1h at 37C. Plates were then washed again and incubated 24h to allow for replication of non-neutralized virus. Plates were then fixed with 80% acetone and stained with FITC conjugated mAb SD6-29 to allow visualization of infected cells. Endpoints were determined as the greatest serum dilution resulting in a 90% or greater reduction in fluorescent foci relative to controls.

Results

Ten hybridoma clones producing mAbs against the PEDV nucleoprotein and two producing spike-specific mAbs were isolated. Most mAbs were of the IgG_1 isotype though several IgM mAbs were also produced. Further characterization of epitope specificity was performed using immunoprecipitation and immuno-blotting methods. The SD6-29, SD17-103 and other mAbs recognized native PEDV nucleoprotein in Western blots. Fluorescein conjugated mAbs were prepared for direct fluorescence applications including verification of virus isolation attempts, virus titrations and the FFN assay. Virus neutralization titers of PEDV naïve pigs as determined by the FFN assay were <1:20 while most infected animals demonstrated titers of 1:80 to 1:1280 by 3 weeks post-exposure.

Conclusions and Discussion

The mAbs and related reagents produced in this project should be of substantial value in the detection of PEDV antigen in a variety of applications including: early verification of virus isolation attempts and in virus titrations; immunohistochemistry staining of fixed tissues and fluorescent antibody staining of fresh tissues; development of field-based antigen capture assays such as lateral flow devices; and ELISA applications (competitive ELISA and antigen capture). They are also a key component of the fluorescent focus neutralization (FFN) assays for assessment of neutralizing antibodies produced following PEDV exposure. Evaluation of neutralizing antibody responses may provide insight into protective immunity. This assay is currently being used as a tool in efforts to understand duration of immunity and identify the most effective feedback and management strategies.

Acknowledgments

This work was funded by the South Dakota Animal Disease Research & Diagnostic Laboratory and the South Dakota Agricultural Experiment Station (AES Hatch Project H-392). The PEDV isolate was provided by NVSL. We thank the staff of the SD-ARW for assistance with animal care and Amanda Brock and Dr. David Knudsen for immunohistochemistry assistance.

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Environmental stability of a cell culture adapted U.S. isolate of PEDV

J Nelson, F Okda, R Parmar, A Singrey, S Lawson, X Liu, J Christopher-Hennings, E Nelson
Veterinary & Biomedical Sciences Department, South Dakota State University, Brookings, SD, USA
eric.nelson@sdstate.edu

Introduction

Porcine epidemic diarrhea virus (PEDV) was first described in Europe in the 1970s with more recent and severe outbreaks in Asia (1,2). PEDV is an enveloped, single-stranded, positive-sense RNA virus infecting swine and is a member of the *Coronaviridae* family. PEDV was first identified in the U.S. in May 2013 (3) and has since been confirmed in multiple states and Canada. The initial PEDV strain identified in the U.S. has high sequence identity with recent variant strains identified in China in 2011 and 2012 (3). Real-time PCR can be used to detect the presence of PEDV nucleic acid following environmental stability or disinfection studies but detectable nucleic acid may not correlate with the presence of viable, infectious virus. Several research groups have used a swine bioassay system to evaluate environmental stability of PEDV and assess the efficacy of disinfection protocols. However, the swine bioassay uses live animals and is expensive and time-consuming, thus limiting the number of samples that can be tested. Therefore, the purpose of this study was to evaluate an *in-vitro* system involving re-isolation of cell culture adapted PEDV to assess virus stability under various treatment conditions.

Materials and Methods

The PEDV-CO isolate at passage 5 was provided by Dr. Sabrina Swenson at the National Veterinary Services Laboratories (NVSL). The virus was further adapted to Vero-76 cells in the presence of 2.5 µg/ml TPCK-treated trypsin. By passage 15, titers of 10^7 fluorescent focus units (FFU)/ml were regularly obtained. Environmental stability of PEDV was evaluated by drying cell culture adapted virus in petri dishes and subjecting it to various temperatures or freeze/thaw cycles over time. Dried virus was re-suspended, serially diluted and endpoint titers determined using re-isolation and our monoclonal antibody-based fluorescent focus titration methods, thus negating the need for a swine bioassay. Similar trials were conducted with PEDV in liquid media.

Results

Air dried cell culture preparations of PEDV were demonstrated to be very stable at room temperature, showing an approximate 1 log drop in titer of recoverable infectious virus after 14 days. No infectious PEDV was recoverable after 10 days at 37°C. Freeze/thaw cycles substantially reduced the amount of recoverable PEDV but infectious virus was still detected after 7 freeze/thaw cycles.

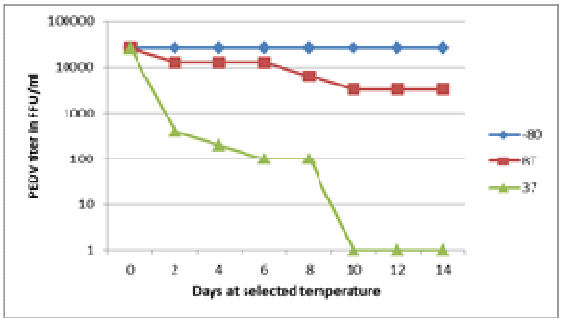

Figure 1. PEDV was dried in petri dishes and held for times designated at -80°C, room temperature or 37°C for 0-14 days. *Dried virus was re-suspended, diluted and endpoint titers determined using re-isolation and monoclonal antibody-based fluorescent focus titration.*

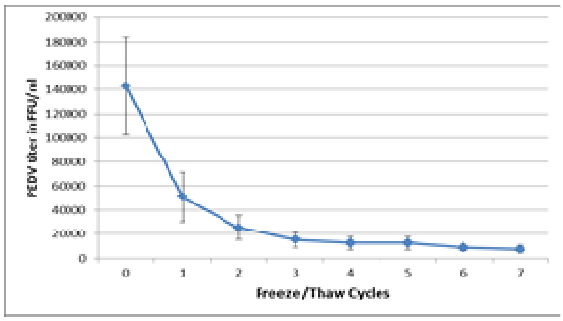

Figure 2. PEDV in liquid MEM was subjected to 1 to 7 freeze-thaw cycles prior to virus titration as above.

Conclusions and Discussion

Re-isolation of cell culture adapted PEDV provides a model system for preliminary assessment of virus stability and viability under various environmental conditions or following disinfection protocols. PEDV can be very stable when dried at moderate temperatures, presenting risk of spread by contaminated materials.

Acknowledgments

This work was funded by the South Dakota Animal Disease Research & Diagnostic Laboratory and the South Dakota Agricultural Experiment Station (AES Hatch Project H-392). The PEDV-CO isolate was provided by NVSL.

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Diagnosis and genotyping of PEDV in Taiwan

C-C Chang, T-Y Cheng, Y-T Yang, B-Y Lin
Department of Veterinary Medicine, National Chia-Yi University, Taiwan, ccchang@mail.ncyu.edu.tw

Introduction

Storm-like outbreaks of porcine epidemic diarrhea (PED) were reported worldwide from 2011-2012 in China, 2013 in the United States, and recently early 2014 in Canada, Japan and Taiwan. Economic loss and the impacts on the pig industries due this disease are incalculable. Ongoing outbreaks of PED in Taiwan actually started from late 2013 till now. The newborn piglets showed severe clinical signs of fatal diarrhea and dehydration. Piglets with between 7 days to weaned ages show a downward-ladder-like mortality of 90 to 10%. Growing and fattening pigs showed severe diarrhea, but with limited mortality. In this study, we identified and sequenced the PEDV from a commercial Taiwanese pig farm. Also the protective efficacy for the newborn piglets provided by sows fed feedback of intestinal content of diseased piglets was also reported.

Materials and Methods

This clinical outbreak of PED-suspected case started from late January in a 12,000-head farrow-to-finish pig farm in southern Taiwan. Clinical signs typical of TGE-like diarrhea originally occurred in the farrowing facilities. Ten diarrhea piglets were sent to our lab for etiological diagnosis. Systemic organs were collected for pathological and molecular diagnosis. A mixture of lung and lymph nodes was tested for PRRSV, PCV2 and PRV by RT-PCR and PCR. Intestinal contents were also collected and tested for differential diagnosis TGEV and PEDV. The S gene was used for the target and amplified by RT-PCR. (Ref. 2) Positive samples were further sequenced and a phylogenetic tree was constructed by genetic software with some known standard reference strains. Furthermore, we noted the survival rates of piglets born afterward after the process of feedback of intestinal homogenates orally fed back to the sow population.

Results

Only the amplifid product of 651 base pairs of PEDV S gene was found in intestinal samples of those ten piglets, but negative for PRRSV, PCV2, PRV, and TGEV. Further sequencing data of the S gene (Taiwan PEDV-CC-01) were phylogenetically analyzed. The genetic identities were 99% and 98% to US genogroup 2a and China genogroup 2b isolates, respectively (Figue 1). The survial rates of newborn piglets born from the feedback process were also noted in Table 1.

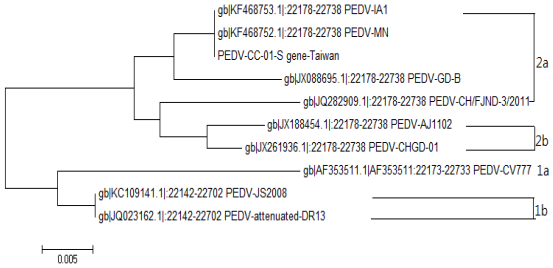

Figure1. Genotyping of Taiwnese PEDV-CC-01 (PEDV 2a) based on the S gene of PEDV was constructed with some standard eference isolates: USA PEDV-IA1 and MN, China GD-B and JND (PEDV 2a), China AJ1102 and CHGD-01(PEDV 2b), prototype CV 777 (PEDV 1a), and China JS 2008 and Korea attenuated-DR 13 (PEDV 1b). *Please refer to reference 1 for detail informations.*

Table 1. Piglets born and survived after feedback (FB) to the sow population in a Taiwanese pig farm

Days after FB	litters	pigs	Survive after 2 wks	Survival rate (%)
0-10	142	1533	0	0
11	12	137	13	9.5
12	5	63	9	14.3
13	16	161	61	37.9
14	21	200	70	35.0
15	8	58	38	65.5
16	3	31	20	64.5
17-22	23	247	240	97.2

Conclusions and Discussion

Phylogenetical analysis rvealed that Taiwan PEDV-CC-01 is closely related to 2013 US and 2011/12China PEDV isolates. However furhter investigation for the origin of Taiwan PEDV is needed. Also, we found that a succesful feedback protocol might take about 16 days for sows to develop an effective immunity for the newborn piglets.

Acknowledgments

Eatlive Industrial Co.LTD Taiwan.

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Circulation of PED in South China from 2013 to 2014

X Zeng[1], Y Chen[2], Z Li[3], G Zhang[2], J Niu[2], J Mo[1], B Sun[1], Y Bi[1], J Ma[1]

[1]South China Agricultural University, Tianhe District,Wushan Road, Guangzhou 510642, Guangdong,People's Republic of China, majy2400@scau.edu.cn, [2]Guangdong Wen's Foodstuff Group Co., Ltd., Yanjiang Street, Xinxing 527400, Guangdong, People's Republic of China, [3]Henan University of Science and Technology, 263, Kaiyuan Road, Luoyang 471023 , People's Republic of China

Introduction

Porcine epidemic diarrhea virus (PEDV) played an important role in the disease of porcine diarrhea since the end of 20101, and PED had become one of the most important swine diseases in piglets. In the present study, we monitored the PED prevalence in some farms located in South China.

Materials and Methods

Data of forty-seven PEDV infected farms from January 2013 to February 2014 in South China were collected, which included the mortality of suckling piglets, the morbidity of pregnant sows and nursing sows.

Results

The percentage of mortality of suckling piglets and morbidity of pregnant sows and nursing sows was showed in Figure 1. From the result we can see that PED existed all year round, the percentage showed a downtrend with the arrival of summer. The high mortality of suckling piglets was in September 2013 and January 2014 especially the later. The curve trend of morbidity of pregnant sows and nursing sows were consistent with the mortality of suckling piglets

Discussion

The measures to control PED mainly rely on vaccines in China at present, but the disease still existed. Whether the field PEDV had been mutated, enhanced virulence lead to the current vaccines does not provide adequate protection was not very clear at present. Strengthen the managements and develop new vaccines are extremely urgent. Our last monitor day was on February 13, 2014 while is still showed a high level mortality, which indicated that the situation of PED control is still severe.

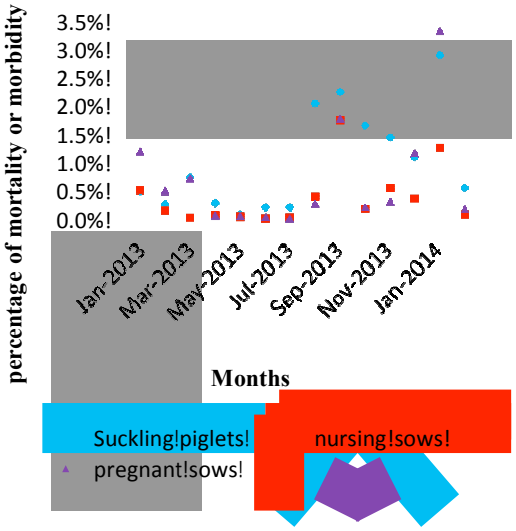

Figure 1. Percentage of mortality of suckling piglets and morbidity of pregnant sows and nursing sows

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Study of the antiviral activity of glycirrhicic acid on the Aujeszky disease virus, *in vitro* model

A Jiménez[2], MZ Urbán[1], H Ramírez[3], E. Hernández[2], D Quintanar[1], S Mendoza[2]
[1] *Laboratorio de Investigación y Posgrado en Tecnología Farmacéutica FESC-UNAM, México.* [2] *Laboratorio de Virología y Microbiología de Enfermedades Respiratorias del Cerdo, FESC-UNAM, México.* [3] *Laboratorio de Virología, FMVZ-UNAM, México.* elspacio7@yahoo.com.mx

Introduction

The importance of eradicating Aujeszky's disease virus (ADV) lies on three main aspects: 1st.- Sanitary. The severity of the clinical disease, as well as the persistence of the virus in the farm for prolonged periods after an outbreak of the disease. 2nd. Economic, since the loss of fertility causes important losses in the pig farm; there were is an increased period between parturitions, infertility of boars, as well as stillborn and dead piglets at birth. Secondary bacterial infections and increased expenses caused by treatments and vaccination of the whole farm. and 3rd Commercial, since the presence of the disease limits the possibility of exporting pigs or pig carcasses', as many countries require certified animals from Aujeszky's disease free countries. Also mobility within the Country is limited to within disease free areas [(1)]. The objective was to evaluate *in vitro* glicyrrhicic acid (GA) in Aujeszky's Disease Virus (ADV) infected cells.

Materials and Methods

ADV was titrated in the MDBK (Madin-Darby Bovine Kidney) cell line, which was used through this study. Several concentrations of glicyrrhicic acid (GA) from 0.1mg/ml to0.9 mg/ml were prepared using RPMI medium and were tested for cytopathic effect on MDBK cell monolayers. The effect was evaluated in microplates in the following manner: the complete monolayers were infected with 1 LD_{50} of ADV, 24 hours after infection, the cells were treated with various amounts of GA [(2)], and were evaluated by microscopy at 24, 48 and 72 hours post-treatment with the drug. The cytopathic effect on the cells was evaluated by means of the Trypan Blue exclusion method, as well as the MTT colorimetric method.

Results

This study consisted of an *in vitro* evaluation of the GA and its antiviral activity against the ADV (3). The maximum non-toxic dose of GA was 0.8 mg/ml. The viral titer was $10^{5.46/ml}$ while in the infected cultures subjected to the treatment with the GA the viral titer vas lower, ($10^{3.24}$).

With constant virus, the monolayer's treated with the drug survived the 72 hours without change. Both the reduction of titer and the protection of the monolayers by the GA indicate a possible inhibitory effect. However the mechanism of virus inhibition was not determined at this time.

Several hypotheses have been advanced, but at this early stage of the study, none can be selected based on the data available. (4) In this trial, the inhibitory effect was observed from 0.1 mg/ml. With the trypan blue and MTT tests, the cell viability was higher with the GA.

Conclusions and Discussion

At low concentrations, the GA was not citotoxic. The highest dose that could be used without toxic effects on the monolayer was 0.8 mg/ml. The GA had an inhibitory effect on the ADV both in a titration and against constant virus.

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D22G mutation in the hemagglutinin protein found in mild case of 2009 pandemic influenza A (H1N1) in Poland

A Kowalczyk, K Urbaniak, I Markowska-Daniel, Z Pejsak
Department of Swine Diseases, The National Veterinary Research Institute,
57 Partyzantów Avenue 24-100 Pulawy, Poland, andrzej.kowalczyk@piwet.pulawy.pl

Introduction

A novel influenza A virus subtype H1N1, discovered in April 2009, spread globally in the human and swine population and since then the genetic script has revealed successive mutation. Although influenza A virus infection has already been detected in swine in Poland, these circulating viruses have not been genetically characterized and subtyped. Therefore, in this study, we genetically characterized the whole genome of all 3 pandemic SIV isolated from pigs in 2012.

Materials and Methods

In the present study we analysed 7 virus isolates from 3 cases with mild clinical outcomes collected between March and October 2012. The isolates were grown on 9-day-old embryonated chicken eggs as described previously [21]. No more than 1 passage was made. Three cases were assigned with numeric characters as virus isolates names were termed: A, B, C. Total 29 nasal swabs were collected from two presented cases (A and B) with noticeable respiratory signs such as sneezing, coughing, conjunctivitis and fever. Lung tissues were collected from one 5-6-week-old necropsied pig in the farm named as C. The amplified PCR products were sequenced in Genomed-DNA analysis service (Poland). The nucleotide sequences were compared initially by using the clustal W alignment algorithm method. Phylogenetic trees of each gene were determined with Neighbour-Joining method using MEGA 4 software (MEGA4, USA). Sequences distance were analysed with Megalign unit software (Lasergene, USA).

Results

The general sequence analysis revealed these swine pH1N1 viruses had all genes, including HA and NA genes closely related to the gene constellation of SIV circulated worldwide after pandemic human pH1N1 virus. All eight genes of these viruses were classified as pandemic and in none of them were indicated as reassortats due to first human and swine isolates in 2009. The NS, NP, PB1, PB2 and PA gene segments are in the SIV triple reassortant lineage. Viruses that seeded this lineage, originally of avian origin or entered swine in North America around 1998. In the phylogenetic tree of matrix genes, Polish isolates were found in the avian-like Eurasian lineage, but concurrently they were report in the outside the lineage with SIV prevalence from last two decades in European pigs. However, viruses from A and C cases showed genetic drift from the other related viruses indicate matrix sequences as a new mutation pattern of the evolution. The isolate from farm C remain distinguish also in HA antigenic analysis. Substitutions noted in the position 222 of HA gene was observed in Swine/Poland/134312/12 virus sequences, virus from the farm C.

Conclusions and Discussion

This genetic drift from the other related viruses indicate that HA, PB1 and M sequences of 15620-farm viruses and 03195-farm viruses constituted a new mutation pattern of the evolution. This may show independent evolution of pandemic SIV in Polish population of pigs, where two lineages can appear just for three genes.

The evidence of D222G mismatch was confirmed only in one reported case, where mild course was observed. In the genetic study over pdm isolates in Tunis, 3 different viruses with D222G substitution were isolated from three different clinical observations: mild, severe and fatal (Moussi, 2013). Also in humans several specimens possessing D222G substitution were obtained from mild H1N1pdm cases during two waves of the epidemic in Japan (Yasugi, 2012). In addition to the D222G substitution, two other changes D187E and Q223R were recognised. Several reports have shown that the D222G substitution was associated only with severe, and sometimes fatal, cases of H1N1pdm (Melidou, 2010). Kilander at al reported the occurrence of an amino acid substitution, aspartic acid to glycine in position 222 (D222G) in the HA1 subunit of the viral samples derived from clinical specimens from 11 out of 61 cases analysed in Norway with severe outcome (Kilander, 2010). Such mutants were not observed in any of mild cases investigated, thus the frequency of this mutation was significantly higher in severe (including fatal) cases.

Acknowledgments

European Surveillance Network for Influenza in Pigs 3 (ESNIP 3)

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Four-year surveillance of influenza virus infection in a closed swine herd in Argentina

M Dibarbora[1,3], J Cappuccio[1,3,4], MI Lozada[2], MA Quiroga[2], E Perez[2], H Barrales[4], M Machuca[2], A Pereda[1,3], CJ Perfumo[2]

[1]*Institute of Virology, CICVyA, INTA Castelar,* [2]*Department of Special Pathology, Faculty of Veterinary Sciences, National University of La Plata,* [3]*CONICET,* [4]*Large Animals Clinics, Faculty of Veterinary Sciences, National University of La Plata,* mdibarbora@cnia.inta.gov.ar

Introduction

Influenza A virus (IAV) infection is one of the main causes of respiratory disease in pigs. Subtypes H1N1, H3N2 and H1N2 have been commonly detected in the swine population both throughout the world (4) and in Argentina (2). However, there are multiple antigenic and genetic variants within subtypes. In 2008, an outbreak of swine influenza was reported in a swine herd. Phylogenetic analysis revealed a human-like H3N2 IAV (1) and in 2009, the same farm reported a clinical respiratory outbreak associated with a novel reassortant δ2 H1N1 (3). In this study, we summarize previously published information (1,2,3) plus new one obtained in the last two years. The surveillance of IAV infection comprised a total of four years (November 2008 to December 2012).

Materials and Methods

The study was carried out in a closed, multisite farm of 6,000 sows and comprised visits (30), necropsies of dead or severely clinically affected pigs and sampling for complementary studies. Presumptive diagnosis was achieved by observation of main gross changes. Patterns and extension of lung lesions were recorded and tissue samples were taken for histopathology.

Virological studies: Lung samples from suspicious cases were individually collected. In addition, nasal swab samples (n=270) were obtained from a cross-sectional study in December 2012. All samples were processed as described previously (2). Phylogenetic analysis of the HA, NA and M genes of the strains isolated was performed (4).

Results

Anatomopathological studies: a total of 413 necropsies were performed. Gross lung lesions were observed in 103 cases (25%): 8 (7.7%) in 2008, 21 (20.4%) in 2009, 16 (15.5%) in 2010, 30 (29.1%) in 2011, and 28 (27.2%) in 2012. Bronchopneumonia (57%), pleuritis (23%) and lobular consolidation and chessboard-like lesion (19%) were the most frequent gross lesions described. Necrotizing bronchiolitis was found in 32% of the cases, followed by circulatory changes (16%), and suppurative bronchopneumonia (13%).

Anatomopathological studies: a total of 413 necropsies were performed. Gross lung lesions were observed in 103 cases (25%): 8 (7.7%) in 2008, 21 (20.4%) in 2009, 16 (15.5%) in 2010, 30 (29.1%) in 2011, and 28 (27.2%) in 2012. Bronchopneumonia (57%), pleuritis (23%) and lobular consolidation and chessboard-like lesion (19%) were the most frequent gross lesions described. Necrotizing bronchiolitis was found in 32% of the cases, followed by circulatory changes (16%), and suppurative bronchopneumonia (13%).

Conclusions and Discussion

The pathological study carried out revealed the subclinical endemic form of IAV infection, associated with the persistence of necrotizing bronchiolitis. During the study period, three different events of reassortment were observed: δ2 H1N1 in 2010, H3N2 in 2011 and δ2 H1N2 in 2012, and the phylogenic analysis of the HA gene showed 98% of nucleotide identity between the isolates of 2010 and 2012 (1, 2, 3). All reassortant events compromised the HA and NA genes from non-contemporary human origin and internal genes of pdm H1N1. However, this genotype was not isolated in that farm. Pandemic H1N1 has become the most prevalent subtype in Argentina (2). These results support the evidence of co-circulation of more than one subtype of IAV in a closed farm, as previously described (2). Long-term pathological and virological surveillances on farms or slaughterhouses are useful tools not fully used in the studies of emerging diseases such as IAV infection. Besides, partial or complete genomic sequences of the IAV strains isolated improves our understanding of the ecology and evolution of IAV and its implications on human health.

Acknowledgments

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Genetic characterization of Italian swine influenza viruses: 2011-2013

Ch Chiapponi[1], A Moreno[2], L Baioni[1], A Luppi[3], S Faccini[4], E Foni[1]

[1] *Istituto Zooprofilattico Sperimentale Lombardia ed Emilia Romagna, Parma, Italy,* [2] *Istituto Zooprofilattico Sperimentale Lombardia ed Emilia Romagna Virology, Brescia, Italy ,* [3] *Istituto Zooprofilattico Sperimentale Lombardia ed Emilia Romagna, Reggio Emilia, Italy,*[4] *Istituto Zooprofilattico Sperimentale Lombardia ed Emilia Romagna, Mantova, Italy.* chiara.chiapponi@izsler.it

Introduction

The genome of Influenza A virus consists of eight single stranded, negative-sense RNA segments. The hemagglutinin (HA) and neuroaminidase (NA) segments define the subtype. The segmented structure of the viral genome, in case of mixed infection by two viral strains, facilitates the exchange of genes, the process may lead to a "genetic reassortment". Swine influenza viruses (SIVs) have become enzootic in the pig population and subtypes H1N1, H1N2 and H3N2 are currently circulating among pigs worldwide, but genetic lineages can occur within each subtype depending on the geographical location. Viruses from the avian like swine H1N1 ($H1_{av}N1$), the human-like reassortant swine H1N2 ($H1_{hu}N2$) and the human like reassortant swine H3N2 lineages are the main strains detected in European pig population. Moreover after the spread of pdmH1N1 virus in human population, infection in European pigs was reported (1).

Materials and Methods

In 2011, 2012 and 2013 a total of 3206 clinical samples from outbreaks of respiratory disease in Italian pig farms were examined for SIV presence by molecular and virological methods. Viral isolates were subtyped by PCR (2) and a selected group was submitted to genetic studies regarding HA and NA genes. Viral RNA was extracted, sequences of genome segments and genetic analysis were obtained as described previously (3, 4).

Results

219 SIVs were isolated on cell cultures. Among them 129 were sub-typed as H1N1, 46 as H1N2 and 44 as H3N2. A total of 55 (25%) viral strains were selected for genetic studies: 30 H1N1 (23,2%), 16 H1N2 (34,7%) and 9 H3N2 (20,4%). **H1N1**: we observed the circulation of the endemic SIVs $H1_{av}N1$ with HA and NA genes clustering with European $H1_{av}N1$ SIVs and the circulation (n=10) of the pdmH1N1 strain related to the reference strain A/California/04/2009. A reassortant H1N1 ($H1_{av}N1$ and pdmH1N1 derived genes) was detected in 2013. **H1N2**: A variegate picture was observed due to the circulation of three different lineages. The Italian $H1_{hu}N2$ with the deletion of two amino-acids in the HA1 region derived from the reference Italian strain A/sw/Italy/1521/1998 and the N2 gene of '97-'98 H3N2 human influenza origin was detected. An increasing isolation (12/46) of $H1_{av}N2$ reassortant strains was observed in the period. Two groups of reassortant strains were pointed out: the first (n=6) with NA gene similar to the N2 of H3N2 human influenza virus circulating in 1997-1998 year and the second (n=6) with NA gene derived from the European H3N2 swine lineage. One $H1_{hu}N2$ strain with the HA and the N2 genes similar to the co-circulating European cluster of H1N2 SIVs was isolate from an imported pig. **H3N2**: HA and NA genes of this subtype clustered with other European H3N2 SIVs of human origin.

Conclusions and Discussion

The genetic studies performed on 55 strains show the lasting of the European $H1_{av}N1$ SIV in Italian pig farms. It was confirmed the established circulation of the Italian $H1_{hu}N2$ with the deletion of two amino-acids in the HA1 region and the human derived N2 gene (3). On the other hand the study highlights the variability of Italian H1N2 SIVs with the detection of 12 $H1_{av}N2$ reassortant strains. Among these, 6 strains showed N2 of human origin circulating in Italy since 2003 and 6 strains showed NA gene derived from the European H3N2 swine lineage. Moreover $H1_{hu}N2$ strain clustering with the European group isolated from pigs on arrival stresses the role of trading movements in SIV epidemiology. The ascertained circulation, even if at low morbidity, of the pdmH1N1 and of a pdmH1N1 reassortant strain in Italian pigs generate a potentially emergence of new reassortants to investigate. Further work is needed to better investigate the genetic content of circulating SIVs isolates. A deeper evaluation of the evolution of the Italian SIVs genome is indeed required in order to provide insights into the emergence of influenza viruses with epidemic potential in swine and humans.

Acnowledgement

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Immune response and reproduction parameters in pregnant gilts infected with swine influenza virus

I Markowska-Daniel, K Kwit, M Pomorska-Mól
Department of Swine Diseases, National Veterinary Research Institute, Pulawy, Poland, iwonamd@piwet.pulawy.pl

Introduction

Influenza virus is widely spread pneumotropic pathogen of human and animals. In the course of influenza in pregnant women, as well as in females of various animals' species, respiratory or systemic infection is sometimes accompanied with abortions.

The aim of the project was to study the immune response, pathogenesis of infection and selected reproduction parameters in pregnant gilts infected with swine influenza virus (SIV).

Materials and Methods

In total, 45 sexually mature gilts, seronegative and free from SIV RNA, were inseminated. The pregnancy was confirmed with the use of ultrasonography at 24 and 58 days post insemination. Pregnant gilts (in the first, second or third month of pregnancy) were infected intranasally (IN) or intratracheally (IT) with H1N2 SIV. Non infected gilts were used as a control of the study Clinical signs; presence of viral RNA in nasal swabs, placenta and lungs of piglets; hematological parameters; specific and unspecific humoral and cellular immune response (HI titer, T-cell specific immunity, concentration of cytokins (IL-4, 6, 10, IFN-γ, TNF-α) and acute phase proteins (CRP, Hp), as well as selected production and reproduction parameters were evaluated. The presence of RNA in the tested samples was determined with the use of real-time reverse transcription PCR based on Matrix gene. The titer of antibodies specific to SIV was determined using haemagglutination inhibition assay. An antigen-specific proliferation of lymphocytes was determined with the use of lymphocyte proliferation test. Concentrations of CRP, Hp and cytokines were analyzed with the use of commercial ELISAs.

All necessary controls were included in all tests.

Results

No fever or any other significant clinical symptoms typical for SI were found in all inoculated and control gilts. Nevertheless, the experimental infection was successful because specific antibodies against SIV were detected in all infected gilts from 14 dpi and an antigen-specific proliferation from 6 dpi. The virus shedding in nasal swabs was recorded from 1 to 4 dpi.

The gestation length was from 113 to 116 days. Any of the infected gilts do *not* have any signs of *pregnancy pathology*. The average number of piglets per litter was from 11.66 to 12.33, an average birth weight was around 1.42 kg and an average litter weight was around 17 kg. No significant differences were found between control gilts and those infected in the first month of pregnancy with regard to hematological parameters, and concentrations of cytokines and APP. In contrast gilts infected in the second and third month of pregnancy, especially those from IT infected group, had significantly higher concentration of IL-6, IL-10 and TNF-α from 4 to 7 dpi, as compared to the control groups. Similar trend was observed with regard to the concentration of HP. The concentration of CRP remained stable and had the similar level as in controls. No SIV RNA were found in samples taken from newborn piglets as well as from placenta.

Conclusions and Discussion

Reproductive failure can be caused by a variety of non-infectious and infectious factors, including the influenza virus. According to the results obtained previously the influenza virus can cause abortions and other reproduction disorders in both, humans and pigs. Abortions observed during influenza infection may be a result of high fever and proinflammatory cytokines, as well as transplacental transmission of the virus, however the knowledge about the possibility of intrauterine infection of fetus with influenza virus is limited. The results of our study indicate a lack of intrauterine transmission of H1N2 SIV. Our results tend to support the hypothesis that in the course of SI high fever and inflammation play a main role in the pathogenesis of abortion.

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Nationwide surveillance and population dynamics of swine influenza virus in South Korea

M-H Lee, J-Y Park, J-K Oem, Y-H Kim, K-K Lee, S-H Kim
Animal Disease Diagnostic Division, Animal and Plant Quarantine Agency, AnyangDong, Gyeonggido, South Korea, vetlee@korea.kr

Introduction

Since the first report of an outbreak of pandemic (H1N1) 2009 (pH1N1) in humans in April of 2009, commercial pigs in several swine-raising countries have been confirmed to be infected with the pH1N1 virus. The present state of transmission of pH1N1 suggests that the virus is efficiently transmitted between humans as well as within pig populations (3). For this reason, the Animal and Plant Quarantine Agency (QIA) launched a nationwide swine influenza virus (SIV) surveillance program in 2009 to determine whether pig populations in South Korea were infected with pH1N1 virus. Here, we describe that surveillance system and the genetic characterization of SIVs detected during the surveillance period.

Materials and Methods

There are ~6,500 commercial pig farms and 204 breeding pig farms and artificial insemination (AI) centers in South Korea. The nationwide SIV surveillance program assumed a prevalence of 1% and confidence level of 99%; thus, 1,000 pig farms were examined (1). Swab samples were collected from the pig farms at random in each province, and all breeding pig farms and AI centers were inspected during the fourth quarter of the year. Nasal swabs were collected from 17 heads per farm by staff of the Livestock Health Control Association and transported with an ice pack to the QIA within 1 day after collection. This sample size was selected based on the classical swine fever surveillance program in South Korea, assuming a prevalence of 0.5% and a confidence level of 95% (2). Samples were taken from 12 heads of growing and finishing pigs, and 5 heads of relatively poorly growing pigs. An initial analysis of allantoic fluid and the culture supernatant of the nasal swab samples by reverse transcription-polymerase chain reaction (RT-PCR) confirmed the presence of influenza virus (4). Final confirmation of the SIVs was achieved by sequencing.

Results

Overall, 34 pH1N1 and 7 influenza A H3N2 variant [(H3N2)v] viruses were detected from 2009-2013. Furthermore, novel reassortant viruses derived from reassortment between endemic SIV and pH1N1 were identified beginning in 2010. Movement restrictions were imposed on pH1N1- or (H3N2)v-confirmed pig farms for a period of time, during which pH1N1 or (H3N2)v RT-PCR assays were conducted at 1-week intervals; restrictions on farms that tested negative during this period were lifted. The shipment of individual pigs to market was permitted during the restriction period, so long as that animal had tested negative for pH1N1 or H3N2v by RT-PCR. The number of pigs from pH1N1- or (H3N2)v-confirmed pig farms from which samples were taken was determined according to the modified FAO guideline (2009).

Conclusions and Discussion

Using this nationwide surveillance program, pH1N1, (H3N2)v and novel reassortant viruses were confirmed to be widespread among Korean pigs in each province. Given the coexistence of SIVs in pig populations, there is a strong possibility of interspecies transmission of the reassortants from pigs to humans. Therefore, it is necessary to conduct continuous surveillance of pig farms to detect the emergence of variants.

Acknowledgments

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Serological responses in 11 Italian herds after vaccination with a combined vaccine against H1N1, H3N2 and H1N2 swine influenza virus subtypes

G Leotti[1], E Foni[2], E Arioli[3], E Bongiovanni[3], M Bresaola[4], A Codato[3], R Donna[3], M Faccenda[5], F Fruttero[5], F Gamba[3], M Giorgiutti[6], F Salvini[3], B Zizioli[3], F Ostanello[7], O Merdy[8], T Vila[8], F Joisel[8]

[1]*MERIAL SpA, Milano, Italy;* [2]*IZSLER, sezione di Parma, Italy;* [3]*DVM, Lombardia, Italy;* [4]*DVM, Veneto, Italy;* [5]*DVM, Piemonte, Italy;* [6]*DVM, Friuli V.G., Italy;* [7]*UNIBO, Bologna, Italy;* [8]*MERIAL SAS, Lyon, France,*

francois.joisel@merial.com

Introduction

Protection against viral infections is achieved through a large array of immune mechanisms. Priming pigs toward antigens is able to produce specific memory cells later responsible for a faster onset and a higher intensity of the immune response when animals are infected with the field viruses. The aim of the present study was to evaluate on a large scale and under Italian current field conditions the serological responses of pigs vaccinated or not vaccinated with a commercial inactivated Swine Influenza (SI) vaccine containing H1N1, H1N2 and H3N2 strains. A secondary objective was to assess if a common field off label use of the two vaccines, i.e. dilution of the dry pellet of a Pseudorabies (PR) MLV vaccine in the SI vaccine was not impairing the safety nor the immune response of the SI vaccine.

Materials and Methods

Eleven farrow-to-finish farms in the North of Italy were studied during the year 2013: in each farm two groups of 12-14 pigs were vaccinated with GRIPOVAC®3 against SI concomitantly to PR vaccination (AKIPOR ®6.3) scheme i.e. twice before turn-to-fattening (70-90 days of age (DoA) and 3 weeks later) and at 180 DoA during fattening: one group received separated injections of the two vaccines and one group was vaccinated following dilution of the AKIPOR 6.3 dry pellet in GRIPOVAC 3. A control group of 12-14 pigs was also included in the study and were vaccinated only with AKIPOR 6.3 at the same dates. Blood samples were roughly collected at around 70, 125, 180 and 250 DoA (just before slaughter) for antibody titration against recent Italian SIV subtypes H1N1, H3N2, and H1N2 by inhibition of hemagglutination test (HI) (1). A sample was considered positive for one specific subtype if the titre was ≥20; one farm was classified as positive against one given subtype if at least 2 sera showed an HI titre ≥20 against this subtype. Mitigation of the results was performed to account for cross-reactivity between strains. Statistical analyses were performed using non-parametric tests.

Results

No adverse event related to safety was seen in any of the farm for any of the vaccination schedules.

No clinical signs evoking a flu passage was observed except in one farm in which a H3N2 flu passage was confirmed both virologically following nasal swabbing and serologically. However, a raise of the SIV antibodies titres in control pigs, induced a suspicion of a field infection in all herds: 2/11 herds for H1N1, 4/11 for H3N2, 4/11 for H1N2, and 1/11 for both H1N1 and H3N2, thus stressing a high incidence of swine influenza cases in fatteners.

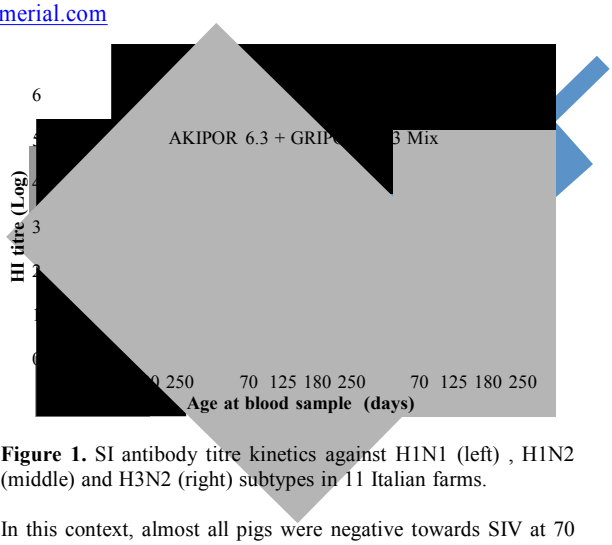

Figure 1. SI antibody titre kinetics against H1N1 (left) , H1N2 (middle) and H3N2 (right) subtypes in 11 Italian farms.

In this context, almost all pigs were negative towards SIV at 70 days of age before primo-immunization. One month after the second vaccination, a clear seroconversion was seen in both groups vaccinated against SI: the pigs vaccinated with GRIPOVAC 3 displayed indeed significantly higher HI titres as compared to the controls ($p<0.001$). Seroconversion was then definitely observed in the control groups from mid-fattening but in the groups vaccinated with GRIPOVAC 3 HI titres were significantly higher than in the controls ($p<0.04$) except for H3N2 titres at the third sampling but with a trend of significance ($p=0.06$). No difference between the vaccinated groups was evidenced in HI titres over the whole fattening period except in H1N2 titres at 125 days of age which tended to be higher when the vaccine were injected separately ($p=0.06$).

Conclusions and Discussion

Despite no severe SI clinical signs were detected during the study, seroconversion of control pigs indicated frequent SIV infections. Whatever the vaccination protocol, GRIPOVAC 3 appeared capable of inducing a significant antibody response against the SIV strains circulating in Italy.

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Genomic analysis of influenza a virus from captive wild boars in Brazil reveals a human-like H1N2 influenza virus

N Biondo[1], R Schaefer[2], D Gava[2], ME Cantão[2], S Silveira[2], MAZ Mores[2], JR Ciacci-Zanella[2], DESN Barcellos[1].
[1]*Federal University of Rio Grande do Sul, Porto Alegre, Brazil;* [2] *Embrapa Swine and Poultry, Concórdia, Brazil*
rejane.schaefer@embrapa.br

Introduction

Influenza is an acute respiratory disease that affects human and several animal species worldwide (3). In Brazil, H1N1, H3N2 and 2009 pandemic H1N1 (A(H1N1)pdm09) influenza A viruses (IAVs) circulate in domestic swine herds(4, 5). Whereas wild boars are susceptible to IAV infection, the close contact with humans andother animal species brings concern about the infection of captive wild boars with IAVs. Here we describe the histopathological, virological and genomic analyses of lungs from captive wild boars presenting lung consolidation, suggestive of IAV infection.

Materials and Methods

Lung samples from 60 captive wild boars presenting gross lesions of consolidation were collected at slaughter.The sampleswere screened by RT-PCR (1) andquantitative real-time PCR (qRRT-PCR) (2)for IAV detection.Additionally, virus isolation (VI)was performed inembryonated chicken eggs and the chorioallantoic fluids were tested by the hemagglutination test.One virus sample (GenBankaccession nº: KF572613–KF572620)wasfully sequenced using Illumina'sgenome analyzer platform (MiSeq). The obtained sequences were assembled using the Newbler Assembler V.2.9 andphylogenetic analysis was performed using the Neighbor-Joining method in the MEGA 5.2 software (6). For pathological analysis, lung samplespositive to IAV by qRRT-PCR were fixed in formalin and processed by HE and immunohistochemistry (IHC) (7).

Results

Eleven out of 60 lungs (18.3%) were positive to IAV by RT-PCR and seven out of eleven were also positive for A(H1N1)pdm09 by qRRT-PCR, with viral load ranging from 4.65 to 3863copies/uL. No IAV was isolated on ECE, eitherembryo death was observed.Chronic diffuse bronchopneumonia was observed in all samples and IHC analysis was negative for influenza A antigen. The genomic analyses revealed that the HA and NA genes clustered with IAVs of the human lineage and the six internal genes were derived from the H1N1pdm09 IAV. The analysis of the H1 gene showed that it grouped with H1-δ cluster and is closely related to seasonal human influenza viruses from 2002-2003. The N2 gene grouped to seasonally human H3N2 viruses from the late 1990s. The six remaining internal genes (PB2, PB1, PA, NP, M and NS) were almost identical (99-100%) to A(H1N1)pdm09 viruses.This is a novel reassortant H1N2 IAV carrying genes derived from human H1N2, H3N2 and A(H1N1)pdm09 influenza viruses.

Conclusions and Discussion

This is the first report of a H1N2 influenza virus infection in captive wild boars in Brazil.Although IAV infection is endemic in commercial pig herds,outbreaks of clinical swine influenza were only reported after the introduction of A(H1N1)pdm09 influenza virus in pigs (4, 5). Moreover, there is little information about IAV infection in captive wild boars and about the genetic composition of IAVs. The human-like H1 influenza virus was first detected in pigs in Canada in 2004 and it has been recognized as the dominant genotype in the U.S (3).Added to this, theviruses belonging to the δ-cluster were shown to be paired either with an N1 or an N2 gene of human lineage (3). The histological lesions observed suggest that the IAV infection occurred earlier and it could explain the negative results in IHC and VI. In summary, a reassortant human-like H1N2IAV circulates in captive wild boar populations in Southern Brazil. Prevention and control of the transmission of IAVs, among captive wild boars, should be considered to minimize their impact in both swine production system and public health.

Acknowledgements

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Vaccination against swine influenza virus results in a high variability of individual antibody responses

GE Martín Valls[1], YL Li[1,3], L Planasdemunt[2], Martín M[1,3], Casal J[1,3], Mateu E[1,3].
[1]*Centre de Recerca en Sanitat Animal (CReSA),* [2]*AVP Planasdemunt Associats S.L., 17400, Girona,* [3] *Dept. Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain*
gerard.martin@cresa.uab.cat

Introduction

In Spain, only two commercial vaccines against swine influenza virus (SIV) are available in the market but little is known about the cross-reactivity of the antigens present in the vaccines with the SIV strains currently circulating in Spanish pigs. Considering the big diversity of SIV in Europe (1) and the fact that SIV strains of which vaccines are made were isolated more than 10 years ago it was expected beforehand that some current field may not react with the antibodies induced by vaccination. It has been shown before that repeated vaccination can generate heterologous recognition of influenza viruses (2). The objective of the present study was to determine if different protocols using one vaccine alone or combining products enhanced the recognition of heterologous SIV isolates.

Materials and Methods

Four groups of 20 pigs naïve to SIV were immunized with the commercial vaccines available in Spain Gripork® (GP; HIPRA) and Gripovac 3® (G3; Merial). Group A received two consecutive doses of GP, group B received one dose of GP and a booster with G3; group C was administered one dose of G3 and a second immunization with GP and group D received two consecutive doses of G3. Unvaccinated pigs were kept as controls (group E). Two weeks after the last vaccination, animals were sampled to obtain sera. Ten pigs from each vaccination protocol were randomly selected in order to test their sera by the haemagglutination inhibition assay (HI) against 6 SIV isolates, 2 H1N1 (S1 and S2), 2 H1N2 (S3 and S4) and 2 H3N2 (S5 and S6). HI titer ≥40 was considered as positive.

Results

None of the control pigs was seropositive against the SIV isolates used in the present study. Vaccination protocol A produced positive sera against strains S2, S4, S5 and S6 (9, 1, 9 and 10 pigs, respectively) and vaccination protocols B, C and D induced antibodies against all strains at least in two pigs/group, although all protocols resulted in a high variability of antibody titers. For example, in protocol B titers against strain S1 ranged from negative to 1280. In contrast, pigs from group A did not recognize strain S1 at all. One H1N2 isolate was only recognized by two pigs in group D in spite that the vaccination protocol included a similar strain. Figure 1 summarizes the distribution of results.

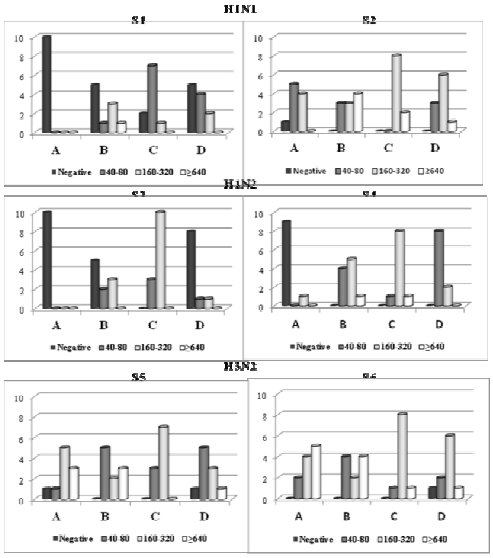

Figure 1. Distribution of HI titers of vaccinated pigs (groups A to D) against each of the SIV isolates tested. *A= 2 doses of Gripork®; B= Gripork + Gripovac 3®; C= Gripovac 3® + Gripork®; D = 2 doses Gripovac 3®*

Conclusions and Discussion

Response to vaccination was very diverse within a group and suggests that individual factors may have an important role in developing immunity against SIV after vaccination. The fact that two SIV isolates reacted very weakly with the sera of animals vaccinated against those subtypes emphasizes the need for a continuous surveillance of SIV variants circulating in pigs to better determine the actual spectrum of European commercial vaccines.

Acknowledgments

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Evaluation of a rapid test for detection of influenza A combined with virus isolation for routine use in veterinary diagnostic laboratory in Brazil

FA Vannucci, MR Henriques, KCP Reis, LEM Bouillet, WV Guimaraes, DL Santos, LF Santos, JL Santos.
Microvet – Microbiologia Veterinaria Especial, Vicosa, MG, Brazil, fvannucci@microvet.com.br

Introduction

Influenza A virus (IAV) is an important respiratory disease in the swine industry. Although the virus has become endemic to the majority of pig populations in Brazil, the diagnosis routine based on virus isolation is still limited to few research laboratories (1). Recently, rapid test for antigen detection has been validated and used at farm level (2). The objective of this study was to evaluate the use of a rapid influenza A diagnostic test to optimize the routine isolation of swine influenza.

Materials and Methods

Analytical sensitivity: Six archived IAV isolates (H3N2/n=2; H1N1/n=2; H1N2/n=2) were chosen based on the most common strains that are found in Brazilian swine herds. These strains were isolated and subtyped as described previously (3). The hemagglutination (HA) titers were determined for each strain. Starting from the 128 HA unit, serial dilutions (10^{-1}, 10^{-2}, 10^{-3} e 10^{-4}) were performed in order to define the minimal concentration of virus that the rapid test (FluA Dot, Secure Detect Co. Ltd, Beijing, China) is able to detect compared with the HA test.

Clinical specimens: The isolation of influenza A virus from 16 clinical samples was performed based on cytopathic effect (CPE) in MDCK cells followed by HA test. The same viral culture supernatants used in the HA test were submitted to the rapid test. The agreement between HA test and FluA Dot rapid test was calculated using Kappa coefficient (4).

Results

Based on the serial dilutions from archived samples, the FluA Dot rapid test was able to detect viral culture supernatants with $\geq$ 0.128 HA unit (Figure 1) of all the three subtypes (H3N2, H1N1 and H1N2).

HA Dilution 10^{-1} 10^{-2} 10^{-3} 10^{-4}

Rapid Test + + + –

Figure 1. FluA Dot rapid test. Serial dilution of influenza A virus (H1N1) starting from 128 HA. *Small dot in the lower zone represents the quality control for all reactions. Grayish-blue ellipse in the upper zone represents positive reactions for 12.8 (10^{-1}), 1.28 (10^{-2}) and 0.128 (10^{-3}) HA units.*

In the clinical specimens, the Kappa coefficient showed moderate agreement between HA and FluA Dot rapid test (κ = 0.48; 95% Confidence Interval 0.10-0.85) according to the guidelines previously established (5). The Table 1 shows the results for both tests (HA and FluA Dot).

Table 1. Comparison between HA and FluA Dot rapid test from clinical samples.

		HA test		Totals
		+	–	
FluA Dot rapid test	+	11	3	14
	–	0	2	2
Totals		11	5	16

Kappa = 0.48; Standard error = 0.19; 95%CI 0.10-0.85

Conclusions and Discussion

The results demonstrated that the rapid test for detection of influenza A was more sensitive than HA test when applied to viral culture supernatants after the first passage during the routine viral isolation. The Kappa coefficient revealed a moderate agreement between both tests. However, three samples did not showed detectable HA titers but they were positive by FluA Dot rapid test. This finding demonstrated a higher chance of having false negative results when the isolation is only based on HA test. Additional studies with a larger sample size are necessary to confirm the sensitivity of this rapid test using clinical samples. In conclusion, the present study showed the usefulness of this rapid test during the routine viral isolation especially when there is low viral load in the culture supernatants after the first passage.

Acknowledgments

All the colleagues from the Microvet Laboratory.

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Phylogenetic study of a swine influenza H1N1 virus

Tufiño-Loza C[1], Rojas-Anaya E[1], Loza-Rubio E[1], Diosdado VF[1]
[1]*CENID-Microbiología, INIFAP, Mexico;* edith_ra23@hotmail.com

Introduction

Swine flu is a common disease in pigs and is caused by a virus belongs to the family *Orthomyxoviridae* Because of to the zoonotic potential of influenza virus it is important to carry out surveillance in populations that are at risk and can act as transmitters of the virus such as pigs (1). In recent years it has been observed that the major subtypes circulating in Mexico is the H1N1 and H3N2 (2). This study aimed to analyze the genetic variability of subtype H1N1 virus that was obtained from a healthy pig in Guanajuato, Mexico.

Materials and Methods

Sample of nasal swabs pigs of Guanajuato, Mexico were collected and subsequently analyzed by real time RT-PCR to detect the matrix gene of influenza virus type A (3). For molecular characterization, genes HA, NA, M and NS were amplified by RT-PCR using universal primers described previously (4). Sequencing was performed at the IBT-UNAM. Editing, assembling and sequence analysis was made using the Clustal X software MEGA 5.0, and BLAST. Complete gene sequences were aligned with American, Mexican, Asian and European sequences of avian, human and swine origin. Phylogenetic trees were inferred by maximum likelihood with 1000 bootstrap replicates. Additionally, amino acid sequences were deduced to discuss possible changes related to virulence.

Results

Analysis of the nucleotide sequence, showed that this isolate had 97-100% identity with sequences of swine origin H1N1 Veracruz, Querétaro and Mexico State recently reported (GeneBank, 2010), as well as with American sequences and some of Asian origin. The trees generated (Figure 1) for the analyzed genes showed a similar distribution, greater genetic relationship to porcine sequences Mexican states of Veracruz, Mexico and Queretaro was observed, grouped in the same branch, and less relationship with European porcine sequences. Five patterns of distribution were identified in phylogenetic trees, which are grouped into Mexican, American and Canadian pig clusters. In other clades sequences of human, avian, and pandemic Eurasian origin were pooled.

Conclusions and Discussion

Analysis of the sequences of the genes suggests that the isolate obtained is of Mexican and porcine origin, apparently non-recombined. The observed changes in the nucleotide sequence did not change the amino acid regions that are related to pathogenicity factors reported in the literature.

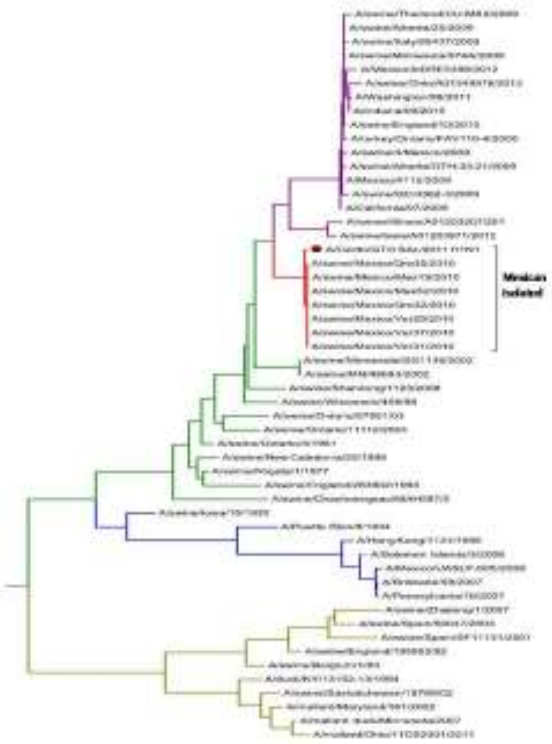

Figure 1. Phylogenetic analysis of the nucleotide sequences of the HA1 gene. *The tree was generated by the maximum likelihood method with 1000 bootstrap replicates in Mega 5.0 program.*

This agrees with the fact that the sample was taken from an apparently healthy animal. This shows the importance of active epidemiological surveillance in these populations, since it is known that the pig can be a mixer viral strains that may eventually cause epidemics.

Acknowledgments

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Seroprevalence of SIV on PRRS suspected farms in Belgium and the Netherlands

HG Prüst[1], FX Tribó[2], D Llopart[2]
[1] *HIPRA BENELUX, Brusselsesteenweg, Belgium,* [2]*HIPRA HQ, Amer, Spain,* herman.prust@hipra.com

Introduction

Influenza A virus (SIV) is endemic on swine population worldwide and they are one of the major causes of acute respiratory disease outbreak in pigs. Acute outbreaks of swine influenza are characterized by high fever, depression, loss of appetite, tachypnea, abdominal breathing and, less frequently, coughing (1). Besides, SIV circulation may be came endemic in many farms. The aim of this study is to summarize the results of SIV serology performed in 24 farms from Belgium and Netherlands in 2013.

Materials and Methods

A total of 1207 blood samples belonging to 24 different farms which were suffering reproductive problems were analyzed for SIV serology. Breeders were grouped into 5 groups according to sow parity (group 1: Gilts; group 2: Sows of 1-2 parities; group 3: sows of 3-4 parities; group 4: sows of 5-6 parities; and group 5: sows of >6 parities). Pig samples were grouped in 3 groups according to the age (4, 7 and 10 weeks of age). If fatteners were present (above 10 weeks of age) also these were sampled with approximately 4 week age-interval. From these blood samples serum was collected, frozen and sent by cool transport to DIAGNOS, HIPRA in Spain. Samples were analyzed for antibodies with ELISA (CIVTEST® SUIS INFLUENZA; HIPRA).

Results

Serology results show 100% of the farms were seropositive. A total of 61.1% of the samples were found seropositive against SIV. Moreover, 82.9%, 42.8% and 44.7% of sows, nursery pigs and fatteners respectively, were seropositive for SIV.

Table 1. Number of positive, negative and doubtful SIV samples on 4, 7 and 10 week-old (W) pigs.

	4W	7W	10W
N samples	93	104	102
Seronegative	34	41	34
Doubtful	14	25	17
Seropositive	45	38	51
% SEROPOSITIVE	48.4%	36.5%	50%

Table 2. Number of positive, negative SIV samples on fatteners.

	early	mid	end
N samples	96	88	98
Seronegative	45	44	30
Seropositive	38	44	54
% SEROPOSITIVE	39.6%	50.0%	55.1%

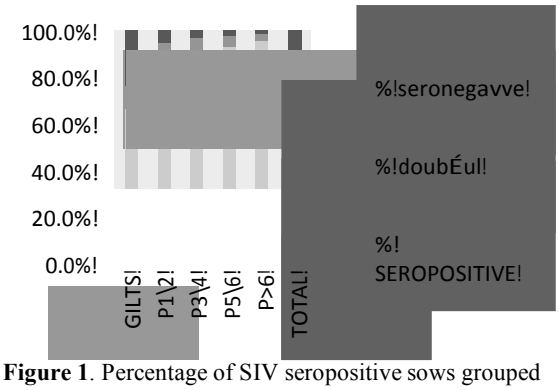

Figure 1. Percentage of SIV seropositive sows grouped by parities. N=557

Conclusions and Discussion

Serumprofile results have shown all tested farms were SIV seropositive indicating a high SIV circulation in both countries. More important the virus circulation in most of farms showed to be endemic, because virus circulation was detected between 4 to 10 weeks old pigs (most nursery buildings presented high SIV circulation). Thus makes necessary to take into consideration SIV on farms experiencing reproductive problems and consequent respiratory problems in nurseries.
The blood sampling will continue the next year.

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Exploratory study on TTSuV loads evolution in chemically immunosuppressed and immunestimulated pigs

M Aramouni[1], J Segalés[1, 2], D Nieto[1], T Kekarainen[1]

[1] *Centre de Recerca en Sanitat Animal (CReSA), UAB-IRTA, Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain.* [2] *Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain,* Tuija.kekarainen@cresa.uab.es

Introduction

Torque teno sus virus (TTSuV) infections are worldwide distributed and adapted to pigs and wild boars (Kekarainen and Segalés, 2012). Some studies have demonstrated higher prevalence and viral loads of TTSuVs in diseased than in healthy animals. It was therefore hypothesized that TTSuVs may act as triggering factors in disease occurrence or, more likely, they can be up-regulated by the immune system under illness situations. To get further insights into the investigation on the role of TTSuV in disease and elucidate the up-regulation of these viruses in an immunocompromised scenario, naturally infected animals were immunosuppressed or immunestimulated chemically. TTSuV loads were evaluated and compared to age-matched control animals.

Materials and Methods

Two month-old farm pigs, naturally co-infected with TTSuV1 and TTSuV2 were used. They were randomly allotted to three groups: Immunosuppressed group (SP): five animals treated with Dexamethasone (DM); Immunestimulated group (ST): nine treated with keyhole limpet hemocyanin in incomplete Freund's adjuvant (KLH/ICFA); Controls: eight non-treated controls. All groups were reared in the same pens. DM was administered IM at a dose of 5 mg/kg, for a total of 8 doses, one dose each 48 hours. KLH/ICFA was administered IM at 2.0 ml/1.0 mg once a week for two weeks. The immune status of the animals was followed up during the experiment by means of white blood cells (WBC) counts, INF-γ ELISPOTS, and flow cytometry. TTSuV loads measured by quantitative PCR were evaluated and compared on days 0, 19 and 28.

Results

WBC counts and ELISPOT values of control group animals were taken as the base value. In comparison, SP animals showed a decreasing WBC count (with leucopoenia and neutropoenia), while in ST pigs WBC count increased (figure 1). Flow cytometry showed declination of CD4+ cells in SP pigs, while ST and control ones showed a similar, but raising CD4+ values. INF-γ ELISPOTs showed no stimulation on both days 19 and 28 in IS animals.

No significant differences were found when comparing TTSuVs viral loads on days 0, 19 and 28, nor when comparing between groups.

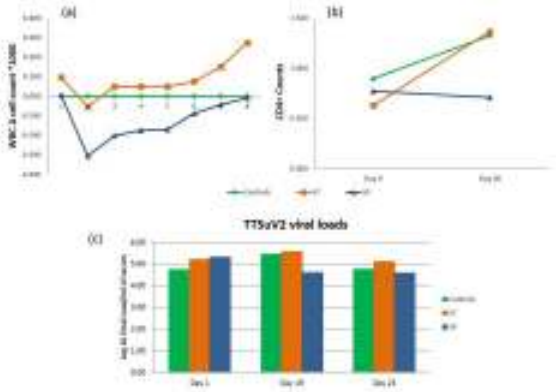

Figure 1. (a) Total white blood cells (WBC) count at different time points with control group as reference on each point. (b) CD4+ counts previously to the dosing and at the day the animals were necropsied. (c) TTSuV2 loads at different times during the experiment.

Conclusions and Discussion

DM and cortisol affect cell-mediated immunity of pigs by producing depletion of CD4+ T-cells and neutrophilia in peripheral blood (Kawashima et al., 2003). In this study, we aimed to elucidate a possible role of the cell-mediated immunity on TTSuVs load. As previously suggested, TTSuV2 loads were found to be increased in immunocompromised pigs compared to healthy ones (Aramouni et al., 2011: Aramouni et al., 2013). In this particular exploratory study, with limited number of animals, although immunosuppression was achieved, TTSuV loads were not affected as hypothesized. This may be explained by the fact that this particular experiment did not allow the imitation of the natural and multiple factors that could be affecting the immune system under disease conditions. Furthermore, the interaction of the immune system with the virus could be mediated by other defence mechanisms such as humoral immunity that in this case was not tackled.

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Rotavirus B in American and Japanese Pigs: VP6 classification, genetic diversity, intragenic recombination, and reassortment

D Marthaler[1], T Suzuki[2], K Rossow[1], M Culhane[1], J Collins[1], S Goyal[1], H Tsunemitsu[2], M Ciarlet[3], J Matthijnssens[4]

[1]*University of Minnesota Veterinary Diagnostic Laboratory, St. Paul, MN* [2]*Viral Disease and Epidemiology Research Division, National Institute of Animal Health, National Agriculture and Food Research Organization, Ibaraki, Japan* [3]*Clinical Research and Development, Novartis Vaccines & Diagnostics, Inc., Cambridge, Massachusetts* [4]*Laboratory of Clinical and Epidemiological Virology, Department of Microbiology and Immunology, Rega Institute for Medical Research, University of Leuven, Belgium,* marth027@umn.edu

Introduction

Rotaviruses (RVs) are classified into eight species (RVA-RVH) based on sequencing on the viral protein 6 (VP6) (1). Rotavirus B (RVB) causes diarrhea in rats, humans, cattle, lambs, and swine. RVB has been recognized as an important cause of diarrhea in pigs. A RVB VP7 genotype nucleotide cut-off value of 80% has recently been proposed, generating 20 RVB G genotypes. We investigated the RVB VP6 genetic diversity and reassortment dynamics from 80 newly sequenced RVB VP6 genes of American (n=64) and Japanese (n=16) porcine strains and those of previously reported of rat (n=1), human (n=25), and bovine (n=4) RVB strains.

Materials and Methods

The 64 USA RVB VP6 sequences were generated using previously described protocols (2,3) while the 16 Japanese RVB VP6 sequences were generated using previously described protocols (4). The 80 novel porcine and 30 GenBank RVB VP6 sequences were aligned using clustal W and pairwise identity frequency graphs and phylogenetic trees were constructed. The RVB VP6 sequences were tested for recombination using the RDP software.

Results

Using RVB VP6 nucleotide pairwise frequency chart and phylogenetic tree from all available RVB VP6 sequences, an 81% nucleotide cut-off value was applied, resulting in 13 RVB VP6 (I) genotypes (Fig 1). Swine RVB VP6 genetic diversity contained 10 I genotypes. Although the RVB I genotype diversity appeared to be region- and host-specific, the RVB I13 genotype contained both Japanese and American strains. Our analyses also revealed a high frequency of reassortment events involving the RVB VP7 and VP6 gene segments, confirming that RVB co-infections must occur frequently, with RVB VP7 genotypes G4 and G16 possibly being only associated with RVB VP6 I10 and I13 genotypes, respectively. Also, an intragenic recombinant RVB VP6 Japanese strain PB-85-I3 from 2008 was related to RVB strains closely PB-70-H5 and PB-Taiheiyo strains, detected in 2007 and 2000, respectively.

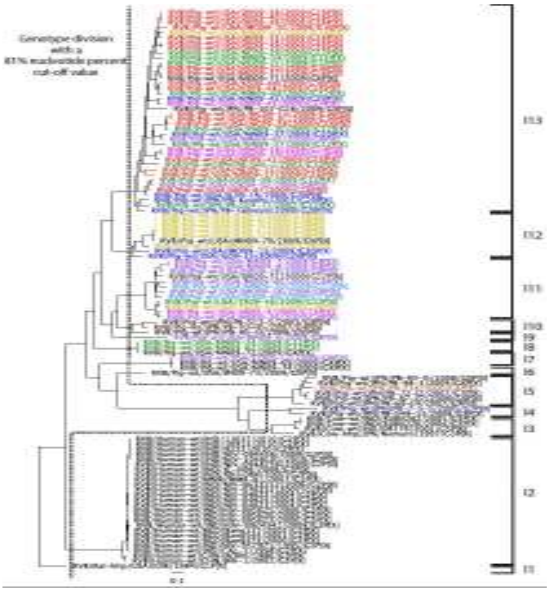

Figure 1

Conclusions and Discussion

In conclusion, an 81% nucleotide percent cut-off value is proposed based on pairwise identity chart and phylogenetic tree. Reassortment events with porcine RVB strains are common.

Acknowledgments

National Institute of Animal Health, National Agriculture and Food Research Organization, Japan and University of Minnesota Veterinary Diagnostic Laboratory

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Isolation of BEDV from pigs with respiratory diseases, decreased growth rates, and without characteristic signs of BED

A Martínez[1], MA Coba[1], L Gómez[1], F Diosdado[1], D Córdova[1], G Socci[1], S Cuevas[1], J Santiago[2], E Carrera[1], LE Zapata[1]

[1]CENID-Microbiología, INIFAP. Km. 15.5 carretera México-Toluca, 05110; [2]Instituto Nacional de Enfermedades Respiratorias, SS; México DF. atalomartinez@yahoo.com.mx; martinez.atalo@inifap.gob.mx

Introduction

Blue eye disease (BED) is one of the most important viral diseases of pigs in Mexico. The BED virus (BEDV) causes reproductive, neurological, and respiratory disorders (1). At field BEDV is involved in the porcine respiratory disease complex (PRDC) associated with other agents, such as PRRS and PCV2 (2), complicating the pathological stage. Frequently opacity of the cornea (OC) is not evident during BEDV infection of the pig. Therefore the aim of this study was to isolate the BEDV from pigs with respiratory signs and slow growth or instead OC, from Mexican pig farms.

Materiasl and Methods

Farms in: a) endemic states, Michoacán and Guanajuato; b) near-endemic satates, Querétaro; and c) BEDV-free states, Puebla, and Veracruz, were visited. At each farm two pigs 1.5 months old, with respiratory signs and slow growth, were humanely sacrificed. Samples from lung, nervous, lymphatic, and renal tissues were taken, and immediately frozen in liquid nitrogen (-196°C) until processed. For virus isolation, tissues were macerated 1:5 w/v in MEM supplemented with 500 UI/µg/ml penicillin-streptomycin. Homogenates were centrifuged at 2,147 g, and recovered supernatants were filtrated through 0.45 µm sterile membranes. Filtrates were inoculated in duplicate into MARC-145 and PK-15 cells in 24-well plates at a confluence of 50-60%, adding 200 µl of inoculum per well. Two wells were inoculated with a lab strain of BEDV (positive control), or MEM alone (negative control). Cells were incubated at 37°C, for 5 days. All specimens were inoculated for at least three consecutive passages searching any cytopathic effect. After this, cells and media were harvested and analyzed by RT-PCR with primers specific for BEDV.

Results

BEDV was isolated from 4/10 farms in Querétaro; 3/8 farms in Guanajuato; and 2/2 farms in Michoacán. One pig with OC from a negative farm gave negative results for virus detection. We obtained virus isolates from tonsils, lungs, lymphatic nodes, kidney, and nervous tissue. As expected BEDV was not isolated from farms in Puebla, and Veracruz states (Table 1).

Table 1. BEDV isolates obtained from farms in different Mexican States.

State	Farm num.	Total samples	Farms (+)	Farms (-)
Querétaro	10	63	4 (15/24 s)*	6
Guanajuato	8	46	3 (7/12 s)	5
Michoacán	2	7	2 (4/7 s)	0
Puebla	3	10	0 (0/10 s)	3
Veracruz	1	3	0 (0/3 s)	1

*s, samples positive/negative.

Conclusion and Discussion

Our results show that i) BEDV was isolated from pigs with respiratory signs and slow growth, which had neither nervous symptoms nor OC; ii) BEDV was isolated from farms in Querétaro, an state near to endemic Mexican region, in which recently had had no evidences from BED; iii) accordingly to literature, this is the first report of BEDV isolation on MARC-145 cells (3).

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Co-infection of classic swine H1N1 influenza virus in PorPV persistently infected pigs

JF Rivera-Benitez[1], J De la Luz[1], M Saavedra-Montañez [1], I Sánchez-Betancourt[2], J Reyes-Leyva[3], J Hernández[4], A Pérez-Torres[5], H Ramírez-Mendoza[1]

[1]*Departamento de Microbiología e Inmunología, FMVZ, UNAM.* [2]*Departamento de Medicina y Zootecnia de Cerdos, FMVZ, UNAM.* [3]*Centro de Investigación Biomédica de Oriente, IMSS.* [4]*Centro de Investigación en Alimentación y Desarrollo, A.C.* [5]*Facultad de Medicina, UNAM. México.*

betosram@yahoo.es; expide@yahoo.com

Introduction

Respiratory diseases in pigs are considered a primary health problem and are responsible for great economic losses in the swine industry worldwide (1). Viruses that cause respiratory disease and pneumonia in growing pigs include PRRSV, SIV, PRV, PorPV, PCV-2 and PRCV. All these viral agents can act individually or through interaction, but associations with other infectious agents of bacterial origin can also occur. PorPV persistent infection could facilitate the establishment of secondary infections. There are no previous studies of experimental or natural infection of PorPV with SIV. However, in the swine farms of central and western-central regions of Mexico, seropositivity for both viruses in growing pigs is common. These regions are the most important national swine production regions in Mexico (2, 3). A primary infection with PorPV is common in pigs under field conditions, and it may become persistent (4). As a consequence, persistently infected pigs have greater susceptibility to secondary infections and these infections may become exacerbated. The aim of this study was to analyse the pathogenicity of classic swine H1N1 influenza virus (swH1N1) in growing pigs persistently infected with porcine rubulavirus (PorPV).

Materials and Methods

Conventional 6-week-old pigs were intranasal inoculated with PorPV, or swH1N1, and PorPV/swH1N1. A mock-infected group was included. All groups were of four pigs in each. The co-infection with swH1N1 was at 44 days post-infection (DPI). Clinical signs, seroconversion (IH test), viral excretion and viral load in tissues were evaluated (qRT-PCR). Histological analysis was included in all experimental groups. The pigs of all groups were euthanised at 2 different points during the experiment: 46 and 52 DPI (three pigs in each group). A student's *t*-test assuming unequal variance and a significance level of $P \leq 0.05$ was used to compare rectal temperatures and the viral load of PorPV and swH1N1 in different samples (nasal and oral swabs, respiratory tissues and BALT) between the single-infected groups to the co-infected group.

Results

The pigs of the co-infection group presented an increase of clinical signs compared to the simple infection groups. In all infected groups, the most recurrent lung lesion was hyperplasia of the bronchiolar-associated lymphoid tissue and interstitial pneumonia. Very marked interstitial pneumonia was observed only in one pig at 52 DPI in the co-infected group. The viral excretion of PorPV in nasal and oral fluid was recorded at 28 and 52 DPI, respectively. In oral swabs, the excretion of PorPV was more prolonged, and positive samples were detected from day 1 up to 43 DPI in PorPV/Mock group. In the PorPV/swH1N1 group after co-infection with swH1N1, negative samples were recorded at 46 DPI and viral load was detected again at 50 and 52 DPI. PorPV persist in several samples from respiratory tissues (RT), bronchus-associated lymphoid tissues (BALT) and bronchoalveolar lavage fluid (BALF). In BALT only in 46 DPI statistical differences ($P = 0.015$) were observed between PorPV/Mock and PorPV/swH1N1 groups. For swH1N1 the viral excretion in nasal fluids was significantly higher in single-infected swH1N1 pigs than co-infected group (Fig 2). However, in the co-infection group seems to be an increase in the presentation of swH1N1 in RT, BALT and BALF at two days of co-infection.

Conclusions and Discussion

In conclusion, the results obtained confirm infection, seroconversion, excretion, and distribution of PorPV and swH1N1 in growing pigs. The observations included an increase in the clinical signs in the co-infected group, compared to simple infections. Other evaluations such as rectal temperature, macro- and microscopic lesions, and viral loads of both viruses do not have statistical differences between analysed groups. However, primary infection with PorPV seems to have a positive impact on the spread and viral load of swH1N1 in respiratory and lymphoid tissues in early stage of co-infection, whereas the shedding in nasal and oral secretions was not enhanced. In the present study the interaction of swH1N1 infection is demonstrated in pigs persistently infected with PorPV. Under field conditions, preventing PorPV infection could also reduce the clinical effect of co-infections with other viral or bacterial agents or, as in this case, SIV.

Acknowledgments

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Detection of antibodies anti-BVDV in finishing pigs slaughtered in the State of São Paulo -Brazil

IRH Gatto[1], HMS Almeida[1], ASR Medeiros[1], SI Samara[1], MEF Oliveira[1], LG Oliveira[2]

[1] *Department of Preventive Veterinary Medicine and Animal Reproduction, College of Agricultural and Veterinary Sciences, São Paulo State University (UNESP), Jaboticabal, São Paulo, Brazil* [2] *Department of Veterinary Clinic and Surgery, College of Agricultural and Veterinary Sciences, São Paulo State University (UNESP), Jaboticabal, São Paulo, Brazil* luis.guilherme@fcav.unesp.br

Introduction

The *Pestivirus* genus, of the *Flaviviridae* family comprises three virus of great epidemiological importance, they are: the Classical Swine Fever Virus (CSFV), Bovine Viral Diarrhea Virus (BVDV) and the Border Disease Virus (BDV). The BVDV has the capacity of infecting swines, especially in properties where pigs are bred together with cattle and/or other ruminants. Usually the disease occurs in a subclinical form, however the birth of weak piglets, abortion and stillbirths may happen in herds with infected animals. Because of the occurrence of cross-reactivity in serological tests with other virus of the same family, mainly the CSFV, knowing the epidemiology of the disease caused by BVDV in pigs is of utter importance, to promote CSFV eradication programs and also to prevent production losses due to infection in swine herds.

Materials and Methods

817 swine blood samples were collected during the bloodletting moment in a slaughterhouse located at the State of São Paulo, which also slaughter animals from other states. These samples were taken to the Laboratory of Viral Reproduction Diseases – Faculty of Agricultural and Animal Science of the São Paulo State University (Unesp) and centrifuged. Aliquots of the serum were stored at -80ºC. All samples were tested through the virus neutralization test, for the detection of anti-BVDV antibodies, according to the method described in the Manual of Diagnostic Test and Vaccines for Terrestrial Animals (OIE). The virus used in the test was the BVDV type 1- strain Singer. Although the pigs are coming from free's CSFV area, the positive samples will be tested by ELISA, to avoid false positive results due to the cross reactivity.

Results

The seroprevalence of neutralizing anti-BVDV 1-Singer antibodies, using the virus neutralization test was 2.32%. The results found are shown in table 1.

Table 1. Percentage of positive samples and antibodies titles obtained using the virus-neutralization test.

Total number of samples	Positive	Titles			
		1/10	1/20	1/40	1/80
817	**19**	10	7	1	1
Percentages	**2.32%**	**52.63%**	**36.84%**	**5.26%**	**5.26%**

All positive samples will be tested by CSFV; however these results are not finalized.

Conclusion and Discussion

The occurence of ruminant *Pestivirus* infection in finishing pigs was low in this study. Other researches show seroprevalence of BVDV infections in domestic pigs of 2.2% (BVDV strain NADL) in Norway (Loken et al., 1991) and 6.4% (BVDV strain Ug59) in Denmark (Holm Jensen, 1985). Breeding ruminants and pigs in the same farm is considered the main risk factor since the interspecies transmission route of the BVDV was proved (Liess and Moennig , 1990). In intensive farms the disease can show up due to the disrespect of the biossecurity norms. According to Wieringa – Jelsma et al., (2006) the transmission among pigs is limited, although some data show that sows can act as a resevoir spreading the disease among the piglets. It is highly probable that ruminant *Pestivirus* infections in pigs difficults classical swine fever (CSF) epidemiological surveillance programs especially in countries that has the CSF free status. Pigs which were infected with BVDV when challenged by the CSFV, they respond with higher anitbodies titles resulting a false-negative diagnostic for CSF (Wieringa – Jelsma et al., 2006). Thus, it is of extreme importance that cattle and swines are not bred together in farms. Separating those two species is the main way of preventing the occurence of ruminant *Pestivirus* infections in swines.

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Occurrence of ruminant pestivirus infection in pigs from small farms of the Mossoró city – Rio Grande do Norte, Brazil

IRH Gatto[1], HMS Almeida[1], AI Leite[1], GCP Silva[1], ASR Medeiros[1], MEF Oliveira[1], IS Dutra[2], SI Samara[1], LG Oliveira[3]

[1] *Department of Preventive Veterinary Medicine and Animal Reproduction, College of Agricultural and Veterinary Sciences, São Paulo State University (UNESP), Jaboticabal, São Paulo, Brazil* [2]*Department of Support Production and Animal Health, College of Veterinary Medicine, São Paulo State University (UNESP), Araçatuba, São Paulo, Brazil.* [3] *Department of Veterinary Clinic and Surgery, College of Agricultural and Veterinary Sciences, São Paulo State University (UNESP), Jaboticabal, São Paulo, Brazil,* luis.guilherme@fcav.unesp.br

Introduction

The *Pestivirus* genus is mainly composed of three types of virus: the classical swine fever virus (CSFV), the Border Disease Virus (BDV) and the Bovine Viral Diarrhea Virus which has 2 types, (type 1 and type 2). Recently reports of BVDV infection in pigs are becoming more common and consequently more importance is being attributed to these cases. One of the main risk factor for BVDV infection in swine is breeding pigs and cattle together in the same farm or even in the same stockyard. This is a common practice among small farmers in Brazil. Usually the disease in swines occurs in a subclinical form however the birth of weak piglets, abortion and stillbirths may happen in swine herds with infected animals. There are no reports of the occurrence of this disease in pigs at Brazil.

Materials and Methods

412 samples of swine blood were collected in the city's slaughterhouse in the bloodletting moment, from pigs of 20 different small farms in the city of Mossoró – Rio Grande do Norte State, Brazil. The serum obtained from those blood samples was used in the virus neutralization test for anti-BVD antibodies detection, according to the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE). The virus used was BVDV type 1 Singer strain. The positive samples will be tested for Classical Swine Fever by ELISA, due to the cross reactivity between these two *Pestivirus*. The results of tests were tabled and analyzed.

Results

Nine out of 20 (45%) farms had at least one positive animal. 4.13% of the animals (17 pigs) were positive in the virus neutralization test. Among the positives the highest title observed was 640 and the lowest 10. The titles distribution is represented in table 1. Seven out of 12 sows that were positive had presented abortion during pregnancies, which represents 58.33%. All positive samples will be tested by CSFV, however these results are not finalized.

Table 1. Antibody title distribution of the positive samples obtained using the virus-neutralization test

Titles	10	20	40	80	160	320	640	Total
Nº of animals	3	4	2	2	3	1	1	17

Conclusions and Discussion

Loeffen et al, (2009) found a 2.5% prevalence of BVDV infection in sows and 0.42% in finishing pigs of Netherlands. Loken et al (1991) found 2.2 % of infected animals in Norway. In this study the prevalence was of infected animals 4.13%, higher than the ones found in both studies. This might happen because of the animals used in this reasearch were not from specilaized production farms. In the Northeast region of Brazil, where the samples were collected, the swine supply chain is not tecnified, which means that those animals are more exposed to risk factors and consequentely have greater risk of being infected by BVDV. That could be the possible explanation for 45% of the farms, in this study, had at least one animal positive. Breeding ruminants and pigs in the same farm is considered the main risk factor since the interspecies transmission route of the BVDV was proved (Liess and Moennig , 1990). That might be the main risk factor to which the animals are exposed, since breeding ruminats and pigs together is quite common among small farmers, resulting in higher prevalence compared to other places. 58.33% of infected sows presented abortion during pregnancies, what could be associated to BVDV infection once it can cause birth of weak piglets, growth retardation, abortion and stillbirths. It is of great importance further epidemiological studies about ruminant *Pestivirus* infection in swines since it is present in at least one part of the brazilian swine herd and can cause economic losses.

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First report of porcine teschovirus, porcine sapelovirus, and enterovirus G in pig herds of Brazil

DG Donin[1], R de Arruda Leme[1], C Feronato[1], DR Silva[1], BLD Molinari[1], AF Alfieri[1], GC Alberton[2], AA Alfieri[1]
[1] *Laboratory of Animal Virology, Department of Veterinary Preventive Medicine, Universidade Estadual de Londrina, Londrina, Paraná, Brazil,* alfieri@uel.br
[2] *Department of Veterinary Medicine, Universidade Federal do Paraná, Palotina, Paraná, Brazil*

Introduction

Porcine enteric picornaviruses are widely distributed in different age pig populations of many countries (2,4,5,6). *Porcine teschovirus* (PTV), *porcine sapelovirus* (PSV), and *enterovirus G* (EV-G) infections are usually non-pathogenic and circulate in asymptomatic domestic pigs. Occasionally, depending on the serotype involved in the infection, these picornaviruses may be considered important etiological agents of enteric, respiratory, reproductive, or neurological disorders. Clinical manifestations may include diarrhea, pneumonia, febrile illness, vesicular diseases, reproductive failure (SMEDI syndrome), myocarditis, and polioencephalomyelitis (3). There are no reports of PTV, PSV, or EV-G infections from pigs of South American countries. The present study describes the first molecular survey for the detection of PTV, PSV, and EV-G in Brazilian pig herds.

Materials and Methods

Forty pig fecal samples from herds located in the Southern, Southeast, and Midwest regions of Brazil were evaluated. Seventeen feces of normal consistency from suckling piglets (*n*=10) and nursery pigs (*n*=7), and 23 diarrheic feces from each pig age group (suckling piglets, *n*=12 and nursery pigs, *n*=11) were selected. The samples were deriving from a collection of pig feces from 2010 to 2013 and were stored at 4°C. The nucleic acid extraction was performed with combination of phenol/chloroform/isoamyl alcohol and silica/guanidinium isothiocyanate methods (1). The RNA was eluted in 50 µl of ultra-pure DEPC-treated sterile water. An aliquot of ultrapure autoclaved water was used as a negative control during the nucleic acid extraction procedures. RNA samples were submitted to RT-nested-PCR assays with specific primers for each virus species, targeting the 5'-non-coding region of porcine enteric picornavirus genomes (3). One amplified product for each of the virus species was selected for sequencing analysis to confirm the results.

Results

Porcine enteric picornaviruses were detected in both suckling piglet and nursery pig age groups in single and mixed infections. Considering all of the samples, PTV was detected most frequently (45%, 18/40), followed by EV-G (40%, 16/40). PSV was the less (17.5%, 7/40) common virus present in the fecal samples evaluated, and was not detected in the suckling piglets. The Midwest region presented positive results for PTV and EV-G in co-infection. The South and the Southeast regions presented the three tested viruses. The similarities between porcine enteric picornaviruses nt sequences in this study and others available in GenBank varied from 95.3% to 99.2% for PTV, 94.2% to 98.5% for PSV, and 86% to 100% for EV-G. The phylogenetic tree formed three well-defined clusters according to the viruses.

Conclusions and Discussion

The results presented herein confirm the occurrence of infection by PTV, PSV, and EV-G in the regions that represent the three major Brazilian pig-producing areas. This is the first molecular detection and characterization of porcine enteric picornaviruses in pig herds of a South American country, and the results suggest the possibility of endemic circulation of these viruses in Brazilian pig herds. Further investigations are needed to provide additional information about the infections and molecular epidemiology of PTV, PSV, and EV-G in Brazilian commercial pig herds.

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Seroprevalence of foot-and-mouth disease in slaughtered pigs in Ibadan, Southwestern Nigeria

CO Aiki-Raji[1], IA Adeyemo[1], AI Adebiyi[1], DO Oluwayelu[1]
[1]*Department of Veterinary Microbiology and Parasitology, University of Ibadan, Nigeria* coaikiraji@hotmail.com

Introduction

FMD is classified as an OIE List A disease (1). It is caused by the foot-and mouth disease virus (FMDV) of the genus Aphthovirus and family Picornaviridae (2). FMD is of major constraint to international trade in livestock and animal products and of great economic importance resulting in serious loss in trade and market (3). FMD has been shown to be endemic in cattle in Nigeria but there is paucity of information on the seroprevalence in the pig population. However, in most parts of Africa including Nigeria, FMD outbreaks are often underreported resulting in lack of information of FMD in pigs (4). Hence, occurrence and distribution pattern of the known serotypes in pigs still remain poorly understood. Therefore, the objective of this study was to find out the seroprevalence of FMD among slaughtered pigs at the Bodija municipal abattoir, Ibadan, Southwestern Nigeria.

Materials and Methods

364 Blood samples were collected between June and August, 2013 from pigs slaughtered at Bodija Municipal abattoir, Ibadan, Southwestern Nigeria. Pigs slaughtered were brought in from different locations within the region. The sera from 241 female and 123 male pigs were screened to detect the O type antibodies of porcine FMD using the GreenSpring FMD-Ab ELISA kit, Version 2012-2, Shenzhen, China. Data was subjected to Chi square analysis using Medcalc statistical software version 13.0.4 (Medcalc software bvba, Ostend, Belgium).

Results

Results showed an overall seroprevalence of 45.6% with the difference between the females and males being statistically significant ($P<0.05$) (Figure 1).

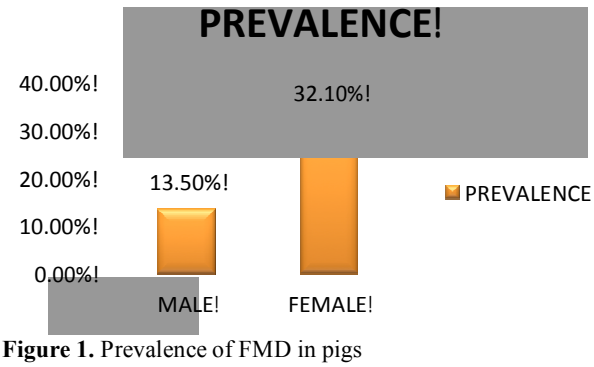

Figure 1. Prevalence of FMD in pigs

Conclusions and Discussion

These findings suggest high prevalence of porcine foot and mouth disease in slaughtered pigs at Bodija abattoir, as the animals were not vaccinated. This, therefore calls for continuous monitoring of the disease among pigs in Nigeria. This will help to ascertain the burden of the disease so that appropriate control measures can be put in place to curtail its further spread among pigs and possible transmission between pigs and cattle.

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Development and use of a dual real-time quantitative polymerase chain reaction assay for detection and differentiation of PCV2 genotypes 2a and 2b in PCV2 survey in Shanghai area

J Liu, F-F Ge, H-B Ju, D-Q Yang, J Wang, J-P Zhou, P-H Liu
Shanghai Animal Disease Control Center, jianjian115@sohu.com

Introduction

In General, PCV-2 strains from different geographical areas were also shown to be differentiated into genetic groups 1 and 2. PCV-2 groups 1 and 2 were found to contain 1,768 and 1,767 nucleotides, respectively (1,2). In phylogenetic trees, group 1 viruses were classified in the same cluster of PCV-2b and were mostly reported from European countries and China, whereas group 2 viruses were from countries throughout the world. Therefore, the dual real-time quantitative polymerase chain reaction (drtqPCR) assay was developed to detect with high sensitivity PCV-2 strains present in tissue samples of pigs. Furthermore, this polymerase chain reaction (PCR) assay was able to identify PCV-2 genotypes by targeting the ORF2 gene.

Materials and Methods

For general detection of PCV-2, a conserved region of PCV-2 genomes was targeted by the drtqPCR using the following primers and probe: C-F (5'-CCA, GAA, TTC, AAC, YTT, AAC, CTT, YCT, TAT-3'), C-R (5'-GRC, RGT, GGA, CAT, GMT, GAG, A-3'), C-2a-Probe (FAM-AGG, GTA, TAG, AGA, TTT, TGT, TGG, TCC, CCC, CTC-BHQ), and C-2b-Probe (HEX-CAA, ACC, CCC, kCw, CTG, TGC, CCT, TTG, A-BHQ) (Y=C or T; M=A or C; R=A or G; K=G or T; W=A or T).

In the dual reaction, 2 µl of sample containing the DNA template was added to a 28-µl reaction mixture (Final concentration: dATP, dTTP, dGTP and dCTP 0.24mM respectively; Mg^{2+}2.4mM; C-F and C-R 0.32µM; C-2a-Prob and C-2b-Probe 5µM; Taq1U/µL; Taq protection 0.2U/µL). The PCR reaction started with an initial denaturation and polymerase-activating step of 94℃ for 3 min, followed by 40 amplification cycles of a 3-step PCR (94℃ for 15 sec; 58℃ for 40 sec and detecting fluorescent light). Fluorescence channels are FAM and HEX.!

A total of 367 tissue samples from pigs were randomly selected from Shanghai swine farms. 173 samples were dead pigs tissue for infectious diseases, and 194 samples were healthy pigs tissue.

Results

164 samples were PCV-2 positive in 367 tissue samples. From 2009 to 2011, the positive ratio in PCVAD samples was 54.17%, 54.05%, 65.79% respectively, and the positive ratio in healthy samples was 20.00%, 22.64%, 39.69%.

From 2009 to 2011, PCV-2a single positive ratio was 13.33%, 4.17% and 16.88% respectively, PCV-2b single positive ratio was 86.67%, 91.67% and 75.32%, and PCV-2a/2b co-infection ratio was 0%, 4.17% and 7.79%.

In PCVAD samples, the PCV-2a single positive ratio was 7.69%, 3.57% and 0 in 2009, 2010 and 2011 respectively, the PCV-2b single positive ratio was 92.31%, 91.07% and 88.00%, and the PCV-2a/2b co-infected ratio was 0, 5.36% and 12.00%.

In healthy swine farms samples, the PCV-2a single positive ratio was 50.00%, 12.50% and 23.08% in 2009, 2010 and 2011 respectively, the PCV-2b single positive ratio was 50.00%, 87.50% and 71.15%, and the PCV-2a/2b co-infected ratio was 0, 0 and 5.77%.

Conclusions and Discussion

The ratio in dead pigs was higher than in healthy, and both of them raised in recent years of Shanghai area. This suggests that dead pigs for infectious diseases was related of PCV-2, but not all pigs infected with PCV-2 virus may lead to death, and pigs infected PCV-2a/2b could be no any clinics. 9 PCV-2a/2b co-infections were also observed but at a lower prevalence in Shanghai area (2.45%). It was remarkable that the co-infection ratio was rising in recent years. The ratio of PCV-2b was much higher than PCV-2a. The ratio of PCV-2a/2b co-infected rose from 2009 to 2011, whereas PCV-2a was low. This suggests PCV-2b was the most viruses in Shanghai area, and PCV-2a/2b co-infected would impact the farms of Shanghai in the future.

In conclusion, the new drtqPCR diagnostic assay is suitable for the sensitive identification and differentiation of PCV-2 and is a more convenient approach than RFLP(3), partial or entire viral genome sequencing, and conventional PCR techniques(4). Furthermore, it could efficiently quantify PCV-2 in submitted samples and provide a risk assessment in regards to the odds of developing PCVAD.

Acknowledgments

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Efficacy of Ingelvac CircoFLEX® in a PCVAD affected Australian piggery

S Megson, G Stuart, RT Lising, M Howard
Boehringer Ingelheim Animal Health Pty Ltd Australia, roel.lising@boehringer-ingelheim.com

Introduction

PCV2 is considered an endemic disease in all major pig producing countries around the world (1), with affected herds showing a wide variety of symptoms including wasting, diarrhoea and respiratory disease - collectively known as Porcine Circovirus Associated Diseases (PCVAD) (2). PCV2 vaccination is a significant aid in the control of PCVAD (3). The objective of this study was to evaluate the efficacy of Ingelvac CircoFLEX® on a high health farm, by measuring growth performance and mortality.

Materials and Methods

This study was conducted in a high health multi-site herd in Australia. Piglets received a pasteurella vaccine at 10 weeks of age. Weaners and growers received low dose pulsing of lincomycin in water. The most apparent clinical signs of disease in the herd were porcine dermatitis and nephropathy syndrome (PDNS), diarrhoea, coughing and poor body condition, in growers 12 to 14 weeks of age.

This trial was conducted over 1 week's worth of production, using approximately 2600 piglets from four farrowing units. Piglets were randomly allocated to treatment groups at an average of 14 days of age. 50% of piglets within the batch received 1mL of Ingelvac CircoFLEX®, the other 50% remained unvaccinated controls. All pigs were individually identified with electronic tags and individually weighed at 14 days (vaccination) and 18 weeks (end weight) of age. Mortalities and culled pigs were also recorded during the study. Statistical analysis was performed using Statistica© version 9, comparing mortalities with chi-square tests and growth differences using t-test analysis.

Results

The pooled results in Table 1 indicate that when compared with controls (c), vaccinated (v) pigs were heavier at end weight (c=78.35, v=81.51, p<0.001) and had a higher start to end Average Daily Gain (ADG) (c=612, v=640, p<0.001). Mortalities and culls were significantly lower in the vaccinated group (c=102, v=61, p=0.0066).

The vaccinated group had a higher frequency of heavier pigs and a lower frequency of lighter pigs compared to the control group (Figure 1).

Table 1: Pooled Growth Performance Data

	Vaccinates	Controls	Difference	p-value
Number of pigs	1141	1090	51	N/A
Start wt - 14 days of age (kg)	5.2	5.18	0.02	0.826
End wt - 18 wks of age (kg)	81.51	78.35	3.16	<0.0001
Start to end gain (kg)	76.17	72.9	3.27	<0.0001
ADG start to end (g)	640	612	28	<0.0001
Mortalities and culls	61	102	41	*0.0066

*Mortality p-value was analysed using Pearson Chi Square

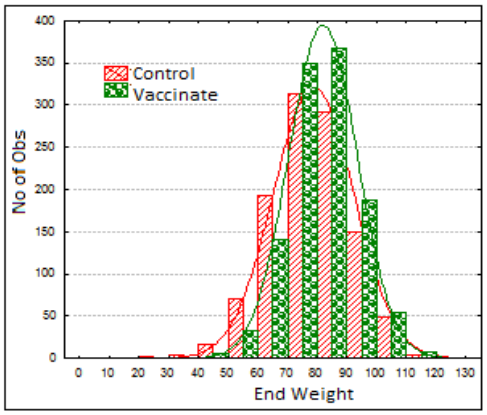

Figure 1. End weight (kg) distribution vaccinates vs controls

Conclusions and Discussion

Pigs vaccinated with Ingelvac CircoFLEX® were 3.16kg (p<0.0001) heavier at end weight, and grew 28g (p<0.0001) more per day from 2 to18 weeks of age, than the unvaccinated control group. There were also 41 fewer mortalities and culls in the vaccinated group. It must also be considered that this trial was performed in co-mingled batches with unvaccinated pigs. From our experience and understanding of the disease and the vaccine, greater results may be achieved with whole group vaccination, due to reduced viral challenge pressure.

Acknowledgements

John Glassbrook, Boehringer Ingelheim Australia.
Gabbrielle Brooke, Boehringer Ingelheim Australia.

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Influence of Ingelvac CircoFLEX® on growth performance in a mild PCVAD herd in Australia

B Lloyd[1], G Brooke[2], R Lising[2], M Howard[2]
[1]Dr. Barry Lloyd Pty Ltd, [2]Boehringer Ingelheim Animal Health Pty Ltd Australia
roel.lising@boehringer-ingelheim.com

Introduction

Porcine Circovirus type-2 (PCV2) is the primary pathogen of a group of clinical syndromes named Porcine Circovirus Associated Diseases (PCVAD). PCVAD has a serious impact on the economical parameters of pig herds (1). PCVAD can manifest in various forms, including respiratory and enteric illness. Vaccination with Ingelvac CircoFLEX® has been shown to reduce PCV2 viraemia in growing pigs (2), increase finishing weight and reduce the incidence of mortalities and culls (3). The objective of this study was to assess the influence of PCV2 vaccination with Ingelvac CircoFLEX® on growth performance and mortalities in a mildly affected PCV2 positive Australian pig farm.

Materials and Methods

This study was conducted on a two site farrow to finish piggery in Australia. The most apparent clinical sign of disease in the herd was poor body condition in finisher pigs between 14 and 20 weeks of age. At the time of the study, the herd was serologically positive for *M. hyopneumoniae* and *A. pleuropneumoniae.* Piglets were receiving mycoplasma vaccination at 5 days of age, growers 50 ppm Lincomycin in feed, and finishers 50ppm Kitasamysin or 50ppm Tylosin, on alternate months.

One batch of piglets (n=1,200) were randomly allocated to treatment groups at an average age of 12 days. 50% of the piglets received 1mL of Ingelvac CircoFLEX® and the remaining 50% were unvaccinated controls. All pigs were identified with radio frequency identification (RFID) tags and individually weighed at 12 days (vaccination) and 18 weeks of age. Mortalities were recorded during the study. Piglets were randomly split into 5 straw based shelters at weaning (3 weeks of age) with pigs of both treatments co-mingled for the duration of the study. Statistical analysis was performed using Statistica version 9, comparing mortalities with chi-square tests and growth differences using t-test analysis.

Results

The data shows that vaccinated pigs grew faster than controls, from 12 days to 18 weeks of age by 21 g/d ($p<0.005$), resulting in consistently heavier pigs at 18 weeks of age (+2.24kg, $p<0.005$) (Table 1).

The distribution of live end weights were skewed heavier for vaccinated pigs compared to controls. This distribution of end weights should translate into heavier carcass weights and lower discounts with fewer undersized carcasses (Figure 1).

Mortalities were numerically lower in the vaccinated group (Table 1).

Table 1. Weight, Average Daily Gain and Mortalities in vaccinated and control groups

	Ctrl*	Vx*	Vx-Ctrl	p-value
12 days of age (kg)	4.35	4.3	-0.05	0.341
18 weeks of age (kg)	78.04	80.28	2.24	< 0.005
12d-18wk Gain (kg)	73.69	75.97	2.28	< 0.005
12d-18wk ADG (kg)	0.657	0.678	21	< 0.005
Mortality (no. pigs)	21	14	-7	0.201

*V = Vaccinated group; Ctrl = Control group

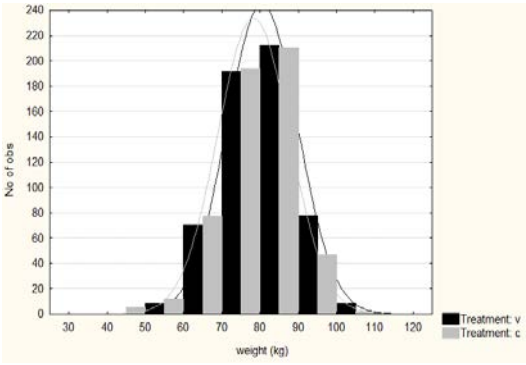

Figure 1. Batch data showing end weight (kg) distribution for vaccinated and control groups.

Conclusions and Discussion

Results from this study show a positive influence of Ingelvac CircoFLEX® on growth performance in a mildly affected PCV2 positive herd. Pigs vaccinated with Ingelvac CircoFLEX®, grew 21g per day faster resulting in a 2.28kg greater end weight ($p<0.005$) than their unvaccinated cohorts.

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Acknowledgements

Lisa Knobben, Boehringer Ingelheim Australia
Shaun Megson, Boehringer Ingelheim Australia

PCV2 viral profile in non-vaccinated Australian pig herds

G Brooke, RT Lising, M Howard
Boehringer Ingelheim Animal Health Pty Ltd Australia, roel.lising@boehringer-ingelheim.com

Introduction

Porcine circovirus type 2 (PCV2) is ubiquitous in the swine population and is associated with multiple disease complexes in most pork producing countries (1). The diagnosis of porcine circovirus-associated disease (PCVAD) in pigs requires the presence of characteristic clinical signs and corresponding gross and histopathological changes (2), along with the detection of viral DNA or antigen in tissues from affected pigs (3). This paper investigates via quantitative polymerase chain reaction (qPCR), the prevalence of PCV2 antigen in 40 unvaccinated pig farms across 5 states of Australia.

Materials and Methods

Sera collected from 1,969 animals across 40 unvaccinated herds were tested for PCV viral antigen using real-time qPCR. In all herds, at least 15 serum samples were collected at time-points from 6 weeks through to finishing. Multi-site systems and farrow-to-finish herds were represented. Herd production parameters and disease status was collected for each herd (data not shown).

Results

qPCR detected PCV2 viral antigen in all 40 unvaccinated pigs herds which covered 5 Australian states. All unvaccinated herds included samples > 2.3 *log10 (> log 2.3) PCV2* copies/mL of serum, with 59.4% of all samples > log 2.3 (Table 1).

Figure 1 shows the amount of PCV2 Ag detected in serum with qPCR increases with age, with 8-10 weeks being the transitional point for Australian herds at the time of this data collection. From 12-14 weeks of age, 80-85% of samples had detectable PCV2 viral Ag.

50% of the samples were positive (>log 4) at 10-12 weeks of age (Figure 2), reaching a peak at 12-14 weeks of age with 66% of samples positive.

Table 1. Distribution of serum qPCR PCV2 viral Ag in unvaccinated Australian herds

	Log<2.3 (Undetectable)	Log 2.3-4 (Suspect)	Log 4-10 (Positive)	Total
Unvaccinated	800	434	735	1969
%	40.6	22	37.4	

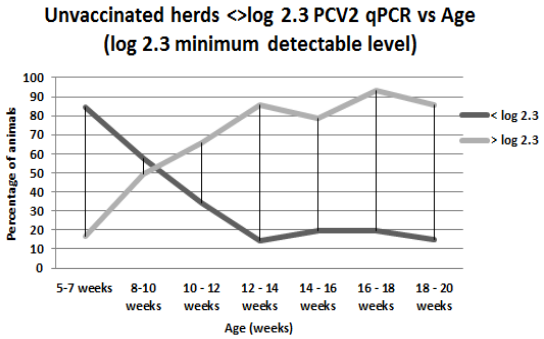

Figure 1. log 2.3 qPCR PCV2 serum viral antigen vs age in unvaccinated pig herds in Australia

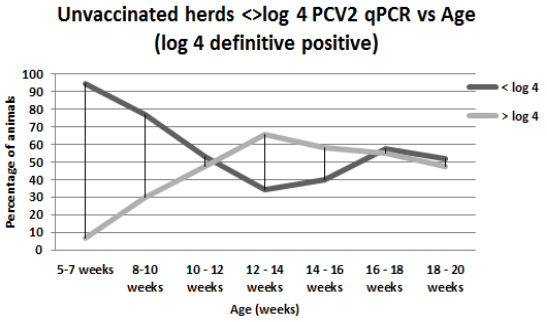

Figure 2. log 4 PCV2 qPCR serum viral antigen vs age in unvaccinated pig herds in Australia

Conclusions and Discussion

This study shows the endemic status of PCV2 across Australia. PCV2 qPCR is a useful tool to characterise the viral load of a population before vaccination. The level of PCV2 serum viral antigen varies with age, with the majority of PCV2 infections in Australia at the time of the data collection appearing to occur 4-5 weeks after weaning (8 weeks onwards). This is perhaps a reflection of waning maternal immunity.

Acknowledgements

The author wishes to thank all managers, owners, and veterinary consultants of farms involved, and the Boehringer Ingelheim Australia Swine team for blood collection.

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Efficacy of Ingelvac CircoFLEX® in an Australian piggery with mild PCVAD

R Dunlop[1], S Megson[2], G Stuart[2], R Lising[2], M Howard[2]
Chris Richards and Associates[1]
Boehringer Ingelheim Animal Health Pty Ltd Australia[2], roel.lising@boehringer-ingelheim

Introduction

Porcine Circovirus type 2 (PCV2) is a ubiquitous infection which has devastated the pig industry leading to major production losses (1). PCV2 is considered a key agent in the expression of a number of diseases termed Porcine Circovirus Associated Diseases (PCVAD) (1). These diseases can manifest in various forms including respiratory and enteric illness (2), and affect production through reduced growth rates and increased mortalities and culls (3). The objective of this study was to determine the effect of Ingelvac CircoFLEX® on growth performance of piglets compared to unvaccinated controls.

Materials and Methods

This study was conducted in a high health multi-site production pig herd in Australia. Piglets received Enterisol® Ileitis vaccination at 5-6 weeks of age, and weaners received amoxicillin-medicated water daily for 1 week post-weaning. Growers and finishers received tylosin in water for 2 days every second week. There were no apparent clinical signs of PCVAD disease in the herd.

This co-mingled trial was conducted over 4 x weekly batches of production. 520 piglets/week were used and all piglets were sourced from a single farrowing unit. Piglets were randomly allocated to treatment groups at weaning (average 25 days). 50% of piglets within the batch received 1mL of Ingelvac CircoFLEX® at weaning (Treatment v), the other 50% remained unvaccinated controls (Treatment c). All pigs were individually identified with electronic tags and individually weighed at weaning (vaccination) and end weights at 19 weeks (females) and 20 weeks (males) of age. Females and males were housed separately from weaning on straw bedding. Statistical analysis was performed using Statistica© version 9 using t-test analysis to analyse differences between weights of treatment groups.

Results

Table 1 shows female vaccinated pigs were 1.41 kg heavier (p=0.01) at 19 weeks of age, and grew 12g/day faster than unvaccinated females. Male vaccinates were 2.37kg heavier (p=0.00002) at 20 weeks of age, and grew 19g/day faster than the unvaccinated males. Figure 1 shows that the vaccinated males and females had a higher frequency of heavier pigs and a lower frequency of lighter pigs compared to the control treatment group.

Table 1. Growth performance of female pigs

	Control	Vaccinated	Difference (corrected)	p-value
Pig Numbers	545	542	-3	
Start Weight	7.19	7.14	-0.05	0.603
End Weight (19)	80.19	81.6	1.41	0.01
Start - End Gain	73	74.46	1.46	0.004
ADG (gm/d)	664	676	12	0.004

Table 2. Growth performance of male pigs

	Control	Vaccinated	Difference (corrected)	p-value
Pig Numbers	532	547	15	
Start Weight	7.11	7.26	-0.02	0.1
End Weight (20)	82.46	84.83	2.37	0.0002
Start - End Gain	75.35	77.56	2.21	0.0002
ADG (gm/d)	633	652	19	0.0002

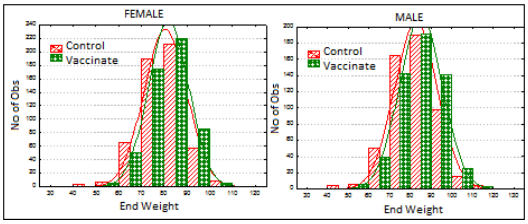

Figure 1: Histograms of Male and Female End weight.

Conclusions and Discussion

This trial demonstrated that Ingelvac CircoFLEX® significantly improved growth rates in both female and male pigs from weaning to 19 and 20 weeks of age respectively, on a farm with no clinical evidence of PCVAD. The increase in weight gain was largely attributed to a reduction of light pigs in the vaccinated group.

Acknowledgements

Gabbrielle Brooke, Boehringer Ingelheim Australia.
Lisa Knobben, Boehringer Ingelheim Australia.
John Glassbrook, Boehringer Ingelheim Australia.

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Highly efficacious piglet PCV2 vaccinati

G Stuart, S Megson, RT Lising
Boehringer Ingelheim Animal Health Pty Ltd Australia. merideth.howard@boehringer-ingelheim.com

Introduction

Porcine circovirus type 2 (PCV2) has been linked with many disease syndromes collectively termed porcine circovirus associated diseases (PCVAD) (1). Vaccination with Ingelvac CircoFLEX®, is effective in controlling symptoms of PCVAD and reducing PCV2 viraemia in growing pigs (2). The objective of this study was to quantify differences in PCV2 viraemia and growth rate between pigs vaccinated and not vaccinated with Ingelvac CircoFLEX®.

Materials and Methods

This study was done on a farrow to finish piggery in Australia. Production concerns encountered on farm included uneven growth in pigs, and respiratory illness. Pigs routinely receive *M. hyopneumoniae* and *A. pleuropneumonia* vaccinations.

The trial was conducted over one batch of female only piglets (n=881). Piglets were ear tagged at birth for individual identification, and randomly allocated to treatment groups at an average of 18 days of age. 50% were randomly selected and vaccinated with 1mL of Ingelvac CircoFLEX®, while the remainder were unvaccinated controls. Pigs were comingled for the duration of the trial. All pigs were individually weighed at 18 days and 20 weeks of age.

At 6 weeks of age, 15 vaccinated pigs and 15 control pigs were randomly selected for serial bleeding for PCV2 quantitative polymerase chain reaction (qPCR). Individual blood samples were collected from these 30 pigs fortnightly, from 6 to 20 weeks of age. qPCR PCV2 assays were performed by the Elizabeth McCarthur Agricultural Institute (EMAI) laboratory. A two-sample t-test was used to compare viraemia and growth rates (Statistica® 9.0).

Results

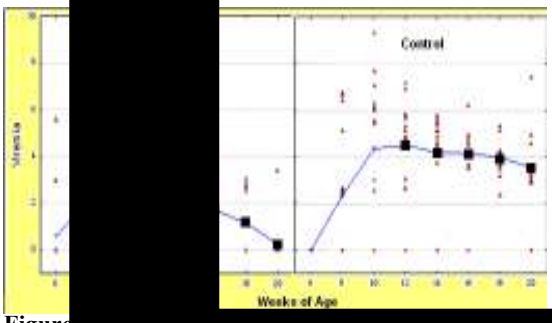
A significant difference in favour of vaccinates was observed in End Weights at 20 weeks (+8.68 kg, p = 0.009) and Average Daily Gain (ADG) (67 g/day, p=0.01) (Table 1).

qPCR results show average viraemia was significantly reduced in vaccinates compared with controls, from 12 to 20 weeks of age (Figure 1).

A decline association with end weight was seen as average viraemia increased in the control group. This is supported with a low multiple regression p-value (p = 0.02) and high coefficient of determination (R^2 = 0.42). No relationship can be observed in the vaccinated treatment (Figure 2).

Table growth performance

	Vaccinated	Difference	p-value
18 days	6.28	0.18	0.689
20 wk W	85.95	8.68	0.009
ADG (g)	635	67	0.01



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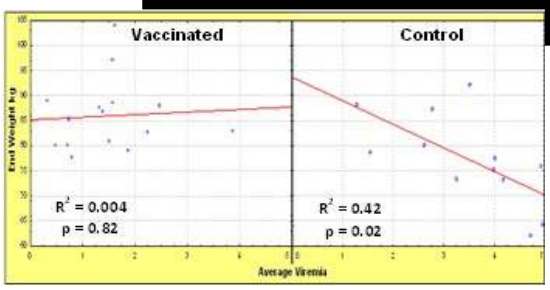

Figure 2. End weight (kg) plotted against average viraemia (Log10) of each serial bled pig.
Viraemia values as Log10 copies DNA/mL.

Conclusions and Discussion

This study shows a trend of decreasing viraemia levels in growing pigs from 12 to 20 weeks of age after vaccination with Ingelvac CircoFLEX®. Vaccinated pigs also had significantly higher end weights and ADG than unvaccinated control pigs.

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Increased growth performance following Ingelvac CircoFLEX® vaccination

S Megson, G Stuart, RT Lising, M Howard
Boehringer Ingelheim Animal Health Pty Ltd Australia, merideth.howard@boehringer-ingelheim.com

Introduction

PCVAD (Porcine Circovirus Associated Disease) is the name given to a number of diseases associated with porcine circovirus type 2 (PCV2) infection (1). Infection with PCV2 weakens the immune system, causing disease that negatively impacts production (2). Vaccination with Ingelvac CircoFLEX® has been shown to aid in the prevention and control of diseases associated with PCV2 infection, increasing finisher average daily gain (ADG) (3), and reducing the incidence of mortalities and culls (4). The objective of this study was to assess growth performance improvements associated with Ingelvac CircoFLEX® vaccination in growing pigs in Australia.

Materials and Methods

This study was conducted in a high health farrow to finish piggery in Australia. Piglets received Mycoplasma vaccination during the first week of life, Enterisol® Ileitis vaccination at 2 weeks of age and pasteurella vaccination at 8 weeks of age. Weaners and growers received low dose pulsing of tylosin and lincomycin in water. The most apparent clinical signs of disease in the herd were porcine dermatitis and nephropathy syndrome (PDNS), diarrhoea, coughing and poor body condition, in late stage weaners through to finishers.

This trial was conducted over 6 batches, using approximately 45% of female only pigs out of each batch (Batch 1 n=294, Batch 2 n=318, Batch 3 n=296, Batch 4 n=276, Batch 5 n=336, Batch 6 n= 303. Total n=1823). Piglets were randomly allocated to treatment groups at an average of 14 days of age. 50% of piglets within a batch received 1mL of Ingelvac CircoFLEX®, the other 50% remained unvaccinated controls. Vaccinated and unvaccinated pigs were co-mingled. All pigs were individually identified with bar coded tags and individually weighed at 14 days (vaccination), 14 weeks (mid weight) and 18 weeks (end weight) of age. Mortalities and culled pigs were recorded during the study. Statistical analysis was performed using Statistica® version 9, comparing mortalities with chi-square tests and growth differences using t-test analysis.

Results

The pooled results in Table 1 indicate that compared with controls (c), vaccinates (v) were heavier at mid weight (c=46.95, v=48.74 P=<0.001) and end weight (c=71.48, v=74.14 P=<0.001) and had a higher Average Daily Gain (ADG) for start-mid (c=495, v=515, p<0.001), mid-end (c=867, v=898, p=0.005) and start-end (c=589, v=612, p<0.001) periods. Mortalities and culls were significantly lower in the vaccinated group (c=140, v=98, p<0.0023). The vaccinated group had a higher frequency of heavier pigs and a lower frequency of lighter pigs compared to the control group (Figure 1).

Table 1. Pooled growth performance data

!!	Vaccinates!	Controls!	Difference!	p-value!
Number!of!pigs!	919!	904!	15!	N/A!
Start!wt!-!14!days!of!age!(kg)!	5.41!	5.33!	0.08!	0.19!
Mid!wt!-!14!wks!of!age!(kg)!	48.74!	46.95!	1.79!	<0.0001!
End!wt!-!18!wks!of!age!(kg)!	74.14!	71.48!	2.66!	<0.0001!
ADG!start!to!mid!(g)!	515!	495!	20!	<0.0001!
ADG!mid!to!end!(g)!	898!	867!	31!	0.005!
ADG!start!to!end!(g)!	612!	589!	23!	<0.0001!
Mortalities!and!culls!	98!	140!	42!	*0.0023!

*Mortality!p-value!was!analysed!using!Pearson!Chi!Square!

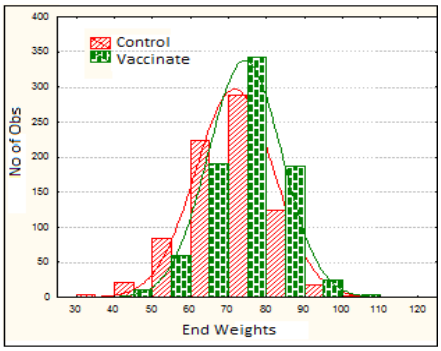

Figure 1. End weight (kg) distribution of vaccinates compared to controls

Conclusions and Discussion

Pigs vaccinated with Ingelvac CircoFLEX® grew faster, resulting in a higher proportion of heavier pigs in the vaccinated group.

Acknowledgements

John Glassbrook, Boehringer Ingelheim Australia.

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2. Genzow M. et al. 2009. Can J Vet Res 73(2): 87-90.
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4. Lising R. et al. 2012. Proc IPVS conf p897.

Efficacy of Ingelvac CircoFLEX® in a high health Australian piggery

G Stuart, SMegson, RT Lising, M Howard
Boehringer Ingelheim Animal Health Pty Ltd, Australia. merideth.howard@boehringer-ingelheim.com

Introduction

Porcine circovirus type 2 (PCV2) is ubiquitous in pig herds worldwide, including Australia (1). PCV2 is considered a key agent in the expression of a number of diseases termed Porcine Circovirus Associated Diseases (PCVAD) (1). These diseases can manifest in various forms including respiratory and enteric illness (2). Vaccination with Ingelvac CircoFLEX® has been shown to reduce PCV2 viraemia in growing pigs (3), increase finishing weights and reduce the incidence of mortalities and culls (4). The objective of this study is to determine the efficacy of Ingelvac CircoFLEX® in growing pigs in a high health piggery.

Materials and Methods

This study was conducted in a high health farrow to finish piggery in Australia. This herd does not express any signs of *M. hyopneumoniae* or *A. pleuropneumoniae.* Piglets and growing pigs do not receive any vaccines. Weaners and growers received 50ppm of olaquindox in feed, and finishers receive low dose pulsing of tylosin (4 days per fortnight) and lincomycin (2 days per fortnight) in water. The most apparent clinical signs of disease in the herd were diarrhoea and poor body condition in finisher pigs between 16 and 24 weeks of age.

This trial was conducted using two batches of piglets (Batch 1 n=626, Batch 2 n=1014, Total n=1640). Piglets were randomly allocated to treatment groups at an average of 12 days of age. 50% of piglets received 1mL of Ingelvac CircoFLEX®, the other 50% remained unvaccinated controls. All pigs were individually identified with radio frequency identification (RFID) tags and individually weighed at 12 days (vaccination) and 19 weeks (end weight) of age. Mortalities and culled pigs were also recorded during the study. Piglets were split into sexed groups at weaning with pigs of both treatments co-mingled for the duration of the study. Statistical analysis was performed using Statistica® version 9, comparing mortalities with chi-square tests and growth differences using t-test analysis.

Results

The results, as seen in Table 1, show that in both Batch 1 and 2, the vaccinated pigs were significantly heavier at 19 weeks of age (+3.65 and + 2.99kg respectively, $p<0.0001$) with a higher Average Daily Gain (ADG) (+32kg and +26g/day respectively, $p<0.0001$) than their unvaccinated cohorts. The vaccinates in Batch 1 and 2 also had a higher frequency of heavier pigs and a lower frequency of lighter pigs compared to their respective control groups (Figure 1). Mortalities and culls were numerically lower in vaccinates of both Batch 1 and 2 (-6 and -7 respectively).

Table 1: Weight, Average Daily Gain and Mortalities in vaccinated and control groups

	Batch 1			Batch 2		
	Vx*	Ctrl*	p-value	Vx*	Ctrl*	p-value
Number of pigs	313	313	N/A	509	505	N/A
12 day wt (kg)	4.53	4.48	0.45	4.52	4.48	0.53
19 wk wt (kg)	82.92	79.27	<0.0001	82.24	79.23	<0.0001
ADG (g)	700	668	<0.0001	677	651	<0.0001
Mortalities/Culls	36	42	0.47	51	58	0.45

*Vx = Vaccinated group; Ctrl = Control group

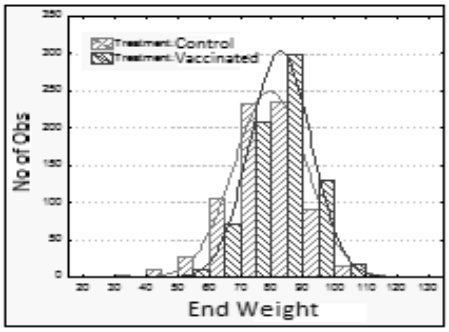

Figure 1. Pooled data showing 19week weight (kg) distribution for each treatment group.

Discussion

Pigs vaccinated with Ingelvac CircoFLEX® grew 28 grams more per day ($p<0.0001$) and were 3.25 kg ($p<0.0001$) heavier than the control group at 19 weeks of age. There were also numerically fewer mortalities and culls in the vaccinated groups.

Acknowledgement

John Glassbrook, Boehringer Ingelheim Australia

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Co-infection of PCV2 and pporcine hokovirus in wild boars

S Vilcek, I Sliz, M Vlasakova, A Jackova
University of Veterinary Medicine and Pharmacy, Kosice, Slovakia, vilcek@uvm.sk

Introduction

Porcine hokovirus (PHoV) or porcine parvovirus 3 belongs to family *Parvoviridae*. Its genome is represented by approximately 5 kb long single-stranded DNA. PHoV was detected in Europe, Asia, Africa and North America not only in domestic pigs (2,5,7) but also in wild boars (1,4).

PCV2 is etiological agent of PMWS – porcine multisystemic wasting syndrome (3). Viral genome is composed of 1.7 kb DNA. PCV2 was often found in swine with other co-infecting viruses as PRRSV, TTSuV1, TTSuV2 (8,9). Recently co-infection of PCV2 and PHoV was detected in domestic pigs in Europe and Asia (5,7).

In present work we study distribution of PCV2, PHoV and co-infection with both viruses in wild boars originating from Slovakia.

Materials and Methods

Tissue homogenates of lymph nodes, spleen and kidney of 194 wild boars hunted in 2012 in 64 out of 79 districts representing all 8 regions of Slovakia were used for the detection of PCV2 and PHoV. A group of animals analysed was composed of young (age under 1 year) and old (age over 1 year) wild boars. Total DNA was isolated with Chelex resin (Bio-Rad). PCV2 and PHoV were detected by a single PCR with specific primers (4,6). The selected collection of 20 PHoV PCR amplicons was sequenced. Nucleotide sequences were analysed by ClustalW method (MegAlign program from DNASTAR, Lasergene) and by phylogenetic analysis using Neighbour-joining method in MEGA 4.0.

Results

PCV2 was more prevalent in young (48.3%) than in old (36,8%) wild boars. Virus was detected in all of 8 regions of Slovakia in range from 31.3% to 56.3%.

On the other hand, PHoV was detected similarly in young (18.6%) and old (19.7%) wild boars. In comparison to PCV2, distribution of PHoV indicated geographic variations between regions – 0.0% to 50.0%, with highest prevalence in the region of Western Slovakia. When testing animals for PCV2 and PHoV co-infection, similar percentage of co-infected animals was observed in the group of young and old animals, 11.0% (13/118 animals analyzed) and 11.8% (9/76 animals), respectively.

The VP1 gene sequences of PHoV from Slovakia were 98% similar to each other and share more than 96% similarity to isolates originating from other parts of the world.

Phylogenetic analysis of VP1 (356 bp DNA fragment) also confirmed high similarity of viral isolates originating from different countries including Slovakia.

Conclusions and Discussion

Results of this work demonstrate that wild boars in Slovakia were more often infected with PCV2 than PHoV. Co-infection of animals with both viruses was observed only in 22 of 194 wild boars tested representing 11.3% of samples. When compare our results with published data the PCV2 and PHoV co-infection in domestic pigs (data on wild boars were not published until now) was lower in Hungary – 3.3% (5) but higher in China – 20.2% (7).

The co-infection with both viruses did not result in any clinical sign in wild boars. At present it is unclear what is particitation of the PCV2 and PHoV infections on health status of those animals.

Acknowledgments

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The efficacy of Ingelvac CircoFLEX compared with a chimeric circovirus commercial vaccine on a 1200 sow farm in Taiwan

CH Yu[1], CM Maala[2]
[1]*Boehringer Ingelheim Taiwan Limited, Taipei, Taiwan,*
[2]*Boehringer Ingelheim Animal Health Regional Marketing Asia,* ken.yu@boehringer-ingelheim.com

Introduction

Porcine circovirus type 2 (PCV2) is a small, non-enveloped, circular, single-stranded DNA virus, which causes serious economic impact in swine production worldwide. The prevalence of PCV2 infection at herd level in Taiwan was 92% [1]. Two commercial vaccines are available at the moment and showed varying efficacy in field. The objective of this study is to compare the efficacy of different PCV2 vaccines in a farrow to finish farm in Taiwan.

Materials and Methods

This study was conducted in a 1200 sow level, farrow-to-finish pig herd with a weekly production system. Pigs are weaned at 24-25 days of age and stay in the nursery for 6 weeks, before being transferred to the grower unit for another 6 weeks. Afterwards they enter/are transferred (to) the finishing unit. A chimeric circovirus vaccine was used to control PCVD since May of 2012. In January of 2013, increased mortality and cull rate were observed in 18 weeks old pigs; 5 sick pigs were sent to a diagnostic center and PCV2 was detected by PCR in all of them. In February the farm switched to use Ingelvac CircoFLEX® (Boehringer Ingelheim Animal Health). In order to compare the efficacy of these two commercial vaccines, performance data including cull rate in nursery and grow-finish stage, ADG in nursery, Days to market (DTM) and market weight (MW) were collected from 6 batches of pigs before (group A: n=2383) and 6 batches after switch to Ingelvac CircoFLEX (group B: n=2607). These data were analyzed by Statistical Process Control (SPC).

Results

The average cull rate in group B was reduced to 1.79% from 3.68% in nursery (Fig.!1) and to 3.61% from 9.73% in grow-finish period (Fig.!2). Less variation was observed in both stages of production. Other performance parameters were (group A/B): ADG 408 g/418.33!g, DTM 179 / 180.92!days, and MW 111.76!kg/ 110.32!kg.

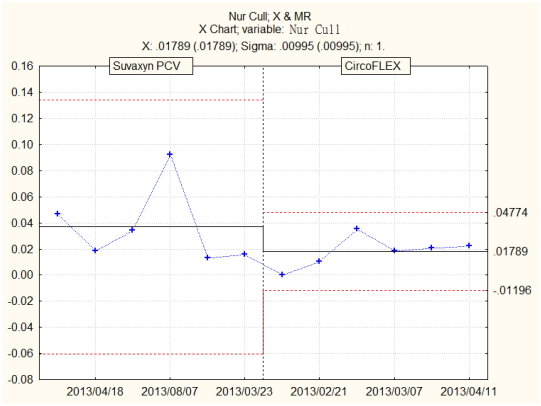

Figure 1. Statistical Process Control chart of cull rate in nursery stage

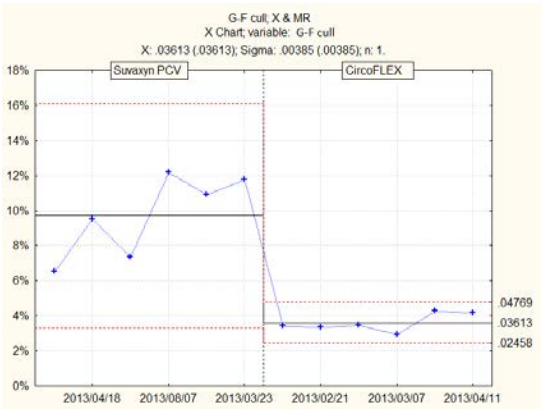

Figure 2. Statistical Process Control chart of cull rate in grow-finish stage

Conclusions and Discussion

In this study, pigs vaccinated with Ingelvac CircoFLEX showed an extremely better performance in cull rate of grow-finish stage compared to the chimeric vaccine. It suggests that not every commercial PCV2 vaccine is equally efficacious under field conditions.

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Molecular characterization of PCV2 in vaccinated and non-vaccinated farms

T Kekarainen[1], A Llorens[1], J Segalés[1,2]
[1]*Centre de Recerca en Sanitat Animal, UAB-IRTA, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain*
[2]*Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain* ,
kovac@uvm.sk

Introduction

PCV2 is one of the most important pathogens for domestic swine worldwide, causing significant economic losses. In the last 3 years, the vaccines against this virus have become the most used ones in pigs, displaying high levels of efficacy and excellent return on investment (1). Vaccinated animals can still get infected with PCV2, although with lower viremia loads than non-vaccinated ones. Therefore, current vaccines do not provide sterilizing immunity (2).

Molecular variation of PCV2 is of great importance, especially in scenarios where non-sterilizing vaccination is applied and viral evolution under vaccination pressure may occur. Therefore, the goal of this study was to characterize the variability of PCV2 in farms where vaccination has never been applied and in farms where pigs were vaccinated against PCV2 at least for two years.

Materials and Methods

Conventional pig farms from Northeast Spain were selected based on their history of PCV2 vaccination; farms that had never been vaccinated against PCV2 and farms that had been vaccinating animals against PCV2 for at least two years. Vaccination in the farms was done according to the manufacturer's recommendations with currently registered PCV2 vaccines. DNA was extracted from serum samples and full-length genome of PCV2 was amplified with proof-reading polymerase. Amplicons from total of 16 samples from five different non-vaccinated (NV) farms and total of 8 samples from two vaccinated (V) farms were pooled resulting in NV and V pools. Deep sequencing of pools was performed using the Ion Torrent platform. Subsequent bioinformatics analysis were performed using FastQ, SegminatorII, DNAsp and Mega5 programs using a Spanish PCV2 sequence (GU049341) as reference.

Results

Polymorphism analysis was performed in the whole genome, ORF1 (replicase) and ORF2 (capsid) genes for NV and V pools reporting sites with frequencies above 5%. Analyses showed that viral populations were heterogeneous in both pools (table 1).

Altogether there were 32 variable sites that were unique in one or the other pool, from which most (23) were detected in the NV animals. According to the phylogenetic analysis of the *capsid* gene, the main genotype variants circulating in the NV farms were of PCV2b genotype, while the dominating ones in V farms were of genotype PCV2a. However, both genotypes were found in both types of farms. Novel amino acid changes were identified both in Replicase and Capsid.

Table 1. Summary of results in both V and NV pools.

Parameter	Vaccination status	Genome (1-1795)	*Rep* (51-992)	*Cap* (1734-1036)
Number of polymorphic sites	NO	109	27	79
	YES	96	26	65
Number of mutations	NO	114	27	83
	YES	98	28	66
Synonymous changes	NO	72	25	47
	YES	55	20	35
Non synonymous changes	NO	38	2	36
	YES	39	8	31

Conclusions and Discussion

By applying next generation sequencing on PCV2, we were able to confirm that, at a given moment, several different viral variants are circulating in farms. With this technique, low frequency variants were also identified. This approach is useful to analyze circulating viral populations *en masse* but does not provide information per animal or farm level. The obtained results show that highly variable PCV2 populations are circulating in the analyzed farms and that this variability can differ depending on vaccination. However, it is still unknown how vaccination may affect the PCV2 evolution and therefore similar analysis on more controlled and elaborated settings, like experimental infections of NV and V animals, are underway.

Acknowledgments

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CIRCOVAC® vaccination in piglets reduces the antimicrobial use in Danish slaughter pigs throughout the fattening period

R Soegaard[1], P Mortensen[1], O Merdy[2]; T Vila[2], F Joisel[2]
[1]*MERIAL Norden, Copenhagen, Denmark;* [2]*MERIAL SAS, Lyon, France;* rikke.soegaard@merial.com

Introduction

CIRCOVAC is a PCV2 vaccine for vaccination of gilt or sow using a dosage of 2 ml, and for vaccination of piglets from 3 weeks of age using a single dose of 0.5 ml. In the piglets, CIRCOVAC vaccination has shown to improve productive parameters such as average daily weight gain, feed conversion ratio, mortality and reduction in the cost of medication (1,2).

In the current study, the objective was to evaluate the reduction in use of antimicrobials throughout the fattening period from 30 – 100 kg of body weight, in piglets vaccinated with CIRCOVAC at the age of 3 weeks. The study included herds initiating vaccination with CIRCOVAC in the study period as well as herds initially using another PCV2 vaccine but changing to CIRCOVAC during the study period.

Materials and Methods

In Denmark all antimicrobials used in production animals are registered in a national database, "Vetstat". The registration is performed at herd level and within each species there are different age groups. The antimicrobial usage is measured in ADD (Average Daily Doses). The national limit value for slaughter pigs (30-100 kg) is 7 ADD.

Nine Danish farms were included in the current study, 3 farms that initiated the PCV2 vaccination program during this study using CIRCOVAC, 6 farms previously using a sub-unit PCV2 vaccine: 3/6 with Vaccine A (1ml, IM) and 3/6 with Vaccine B (2ml, IM).

To evaluate the effect of the new PCV2 vaccination, a "before" period was included covering a 6 months period spanning from 4 months before initiation or change of PCV2 vaccination until one month after this event. The following 3 months were defined as the vaccination period and not included in the data evaluation since during this period a mixture of animals with different vaccination status was expected to be present in the fattening unit. The following 4-6 months were named the "after" period and represent the period where there are only CIRCOVAC vaccinated pigs present.

The monthly use of antimicrobials was recorded for each farm in ADD during the "before" and the "after" periods. Based hereon, monthly average in ADD were calculated for each of the periods per farm, then compared using a Wilcoxon test(s).

Data were obtained and allowed to be used per courtesy of both veterinarians and farmers.

Results

The number of slaughter pigs in each herd varied from 825 to 3300 with an average of 1719 pigs.

As shown in Table 1, a clear reduction in antimicrobial use was detected following CIRCOVAC implementation ($p<0.001$), even when restricting the comparison to farms in which competitor vaccines were already in use ($p<0.01$).

Table 1. Average monthly antimicrobial use measured in ADD during the "Before" and "After" periods. [a, b]: $p<0.01$

Type of PCV2 piglet vaccination against PCV2 before the implementation of CIRCOVAC	Before	After
Unvaccinated	3.71	0.70
Unvaccinated	2.83	0.17
Unvaccinated	2.91	1.82
Vaccine A	2.15	1.18
Vaccine A	2.13	0.74
Vaccine A	2.91	1.33
Vaccine B	1.78	1.48
Vaccine B	2.46	2.10
Vaccine B	3.24	1.18
Total (Vaccinated)	*2.45[a]*	*1.34[b]*
Total	*2.68[a]*	*1.19[b]*

Conclusions and Discussion

This study highlights the benefit of CIRCOVAC piglet vaccination in order to decrease antimicrobial usage in pig farms which is a special concern in the pig industry nowadays.

Even if relatively low and below the national limit, the antimicrobial usage was clearly reduced following piglet vaccination with CIRCOVAC. The effect was observed even in farms already vaccinating with other PCV2 vaccine.

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Anti-PCV2 vaccination significantly reduces viremia and shedding after experimental infection of conventional gilts

F Ostanello[1], S Panarese[1], C Bianco[1], G Galeati[1], ML Bacci[1], M Dottori[2], P Bonilauri[2], G Leotti[3], T Vila[4], F Joisel[4], G Sarli[1]
[1]*Department of Veterinary Medical Science, Bologna University, Bologna, Italy;* [2]*IZSLER, Italy*
[3]*Merial Italia SpA, Milano, Italy;* [4]*Merial S.A.S., Lyon, France;* giuseppe.sarli@unibo.it

Introduction

Recurrent viremia in pigs naturally exposed to PCV2 infection and the shedding mainly through the fecal route are the two most important epidemiologic phenomena that allows both horizontal and vertical PCV2 transmission (1). One way to test the ability of a strategy to reduce PCV2 circulation is to measure its influence on viremia and on shedding through natural routes (nasal, fecal, vaginal). In this paper we present quantitative data on the effect of PCV2 vaccination in 2 trials run in the same conditions and aimed to assess PCV2 viremia and shedding in conventional gilts from a farm with high serological and viral prevalence of PCV2.

Materials and Methods

Animals were purchased from a PCV2 infected farm in which no PCV2 vaccination was performed. Thirty pure Large White gilts were randomly divided in 3 groups and stabled in three different rooms as follows: vaccinated-infected (VI) group (n=6 trial 1 and n=6 trial 2), first vaccination at 16 weeks of age, the second one month later (CIRCOVAC®, MERIAL, France- 2 mL, IM), non-vaccinated infected (NVI) group (n=6 trial 1 and n=6 trial 2), and control (C) group (not-vaccinated not-infected) (n=3 trial 1 and n=3 trial 2). Hormonal estrus synchronization was followed by artificial insemination with a single (trial 1) or double (trial 2) semen dose (PCV2 negative by PCR) spiked with 0.2 ml of a suspension containing $10^{3.9}$ $TCID_{50}$/25 µl of PCV2 (VI and NVI groups), and one or two doses (trial 1 or 2, respectively) with no viral particles (C group). After ultrasonography at 29 days post-insemination (DPI), empty animals were euthanized at 30 DPI whilst pregnant ones between 52 and 56 DPI. Vaginal, nasal and rectal swabs, and blood samples were weekly collected from -2 DPI till the end of the experimental period and tested by real time-PCR for PCV2. During necropsy tissues from each fetus as well as placentas were collected and submitted to PCV2 real time-PCR (qPCR).

Results

Results of trials 1 and 2 were pooled since both the experimental conditions and the readouts were equivalent. In each of the VI and NVI groups respectively 7 and 6 out of the 12 gilts were pregnant at 29 DPI, while 4 out of 6 in the C group. VI group showed a significantly lower proportion of qPCR positive *in vivo* collected samples: 88/224 (VI) vs 174/306 (NVI) and 80/165 (C) (Chi Square =45.2.; P<0.0001), Table 1.

Table 1. Percentage of PCV2 positive samples in the 3 groups

Sample type	VI (%)	NVI (%)	C (%)
Serum	8.7 [a]	38.9 [b]	25.6 [b]
Vaginal swab	32.0 [a]	53.8 [b]	45.2 [b]
Nasal swab	34.7 [a]	61.5 [b]	52.4 [b]
Fecal swab	42.7 [a]	71.8 [b]	69.0 [b]
Fetal tissues	9.1 [a]	26.3 [b]	5.4 [a]
Placenta	56.0 [a]	75.3 [b]	46.9 [a]

[a, b]: Different superscripts mean significant difference (p<0.05, Chi-square test or Fisher's exact test)

Results of these two trials show a significantly lower proportion of viremia and shedding in the VI group compared to the NVI and C groups, but not between these latter two groups.
The percentage of fetal tissues positive to PCV2 by PCR was significantly higher in NVI group compared to the other two but not between VI and C and the same observation was apparent also for placenta.

Conclusions and Discussion

The vaccine has clearly demonstrated an active role in reducing viremia and shedding even after an experimental infection. A probable consequence of the reduced viremia is the lowest percentage of placentas and fetal tissues PCV2 positive by qPCR. The synthesis of data strongly highlights a conclusive role of vaccination to reduce as well as the horizontal also the vertical transmission of PCV2. The choice to use in these trials conventional gilts from a PCV2 infected farm was aimed to produce data on the vaccination effect as close as possible to field condition and by using a category of animals, the gilt, in which, due to the highly variable level of antibodies after natural exposure, the possibility of PCV2 infection is higher (2).

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®CIRCOVAC is a registered trade mark of MERIAL in Italy and elsewhere.

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Application of the diagnostic protocol for PCV2 in experimental reproductive pathology of gilts

C Bianco[1], F Ostanello[1], S Panarese[1], M Dottori[2], P Bonilauri[2], G Leotti[3], T Vila[4], F Joisel[4] , G Sarli[1]
[1]*Department of Veterinary Medical Science, Bologna University, Bologna, Italy;* [2]*IZSLER, Italy;*
[3]*Merial Italia SpA, Milano, Italy;* [4]*Merial S.A.S., Lyon, France;* giuseppe.sarli@unibo.it

Introduction

PCV2 is involved in clinical and subclinical reproductive failure (1). In addition to clinical evidence, the clinical form is identified by microscopic lesions within foetal tissues (heart, liver or lymphoid tissues) and PCV2 antigen or PCV2 DNA within foetal tissues. The subclinical PCV2 *in utero* infection is only identified by the detection of PCV2 DNA in foetal tissues without the presence of microscopic lesions. The most used diagnostic algorithm in PCV2 diseases encompasses, after a screening by PCR, the *in situ* demonstration of PCV2 by *in situ* hybridization or immunohistochemistry (IHC) (2). In this paper we present the results of IHC to PCV2 in cases of experimentally induced infections of fetuses.

Materials and Methods

We conducted 3 trials that overall included 45 gilts of which: 9 (3 in each trial) were non-vaccinated and non-infected animals (controls; C); 18 (6 in one trial (vaccine A) and 12 in another trial (6 vaccine A and 6 vaccine B)) were gilts vaccinated against PCV2 and infected; 18 (6 for each trial) were non-vaccinated but infected gilts (NVI). Vaccine A (2 mL/dose, IM, administered twice) (group VAI) is a commercial inactivated PCV2 vaccine licensed for sows and piglets; Vaccine B (1 mL/dose, IM, administered twice) (group VBI) is a commercial vaccine based on an ORF2 capsid protein expressed in a baculovirus system, and licensed for use in piglets.

Samples from fetuses and placentas were obtained at mid-gestation (55 days post-insemination) from conventional gilts experimentally infected with PCV2 spiked ($10^{3.9}$ TCID50/ 25 µl) semen. Tissues (heart, liver, spleen) from foetuses and the corresponding placenta were fixed in formalin, then underwent routine histology (haematoxylin-eosin stain H&E) and IHC according to a previous protocol (3). The same samples were also frozen for PCV2 genome assessment by real time PCR (RT-PCR).

Results

From the 3 trials were collected 233 fetuses (7 resorptions) and 230 placentas. From the 9 gilts of the group C were collected 51 fetuses and 51 placentas. From INV 107 placentas, 107 fetuses and 5 resorptions. From VAI group 53 placentas and 51 fetuses and 2 resorptions, while from VBI gilts 19 placentas and 19 fetuses.

No gross lesions suggestive of PCV2 in fetuses and placentas were recorded. The number of real time PCR positive tissue samples, separately as for placentas and fetuses are reported in table 1. Histology allowed to disclose focal placental necrosis (1 case, group NVI) or necrotic placentitis (1 case, group VBI) both PCV2 positive by IHC. In fetal tissues IHC was positive in liver and heart of one fetus (group VBI) of which only the liver showed necrotic foci, and the liver in another fetus (group NVI) that not disclose changes by H&E stain.

Table 1. Results on placentas and fetuses samples.

Samples	Groups			
Placentas	**C(n/%)**	**VAI(n/%)**	**VBI(n/%)**	**NVI(n/%)**
RT-PCR +	18/51(35)	29/53(55)	19/19(100)	64/107(60)
RT-PCR>10^8	0/51(0)	0/53(0)	4/19(21)	0/107(0)
Lesion (H&E)	0/51(0)	0/53(0)	1/19(5)	1/107(1)
Positive IHC	0/51(0)	0/53(0)	1/19(5)	1/107(1)
Fetuses				
RT-PCR +	2/51(4)	4/51(8)	8/19(42)	33/112(29)
RT-PCR>10^8	0/51(0)	0/51(0)	1/19(5)	4/112(4)
Lesion (H&E)	0/51(0)	0/51(0)	1/19(5)	0/112(0)
Positive IHC	0/51(0)	0/51(0)	1/19(5)	1/112(1)

Conclusions and Discussion

Presence of the viral genome is frequent, although in the absence of macro or microscopic lesions. The demonstration of PCV2 by IHC, even if sporadic, overlaps the histopathological identification of fetoplacental lesions. In the presented cases placental necrosis, placentitis and foetal hepatic necrosis were the lesions observed. It seems that 10^8 PCV2 genomic copies/ml is a good cut-off to predict fetoplacental tissues damage. The IHC is highly specific but not sensitive. The RT-PCR positivity of conceptuses can be interpreted as a risk factor for the PCV2 circulation in breeding sector, then the real time PCR assessment can represent a “risk assessment” and not only a diagnostic tool. Finally, the description of PCV2 induced placental lesion enrich the spectrum of PCVDs.

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Identification of variants of swine influenza virus by RT-PCR restriction analysis

GP Ávalos[1], OME Trujillo[1], BJI Sánchez[1]
[1]*Facultad de Medicina Veterinaria y Zootecnia, UNAM*, paulina_morina@hotmail.com

Introduction

Porcine influenza is a viral disease that affects a great variety of species. The high mutation rate of the virus, its capacity to modify its genetic material by recombination with other influenza-virus species and the broad number of host that can infect; are the main factors that allow the virus to have a very effective transmission rate; those factors are also involved in the generation of new highly pathogenic and contagious serotypes that can infect several species including humans. (1) (2) (3)

Materials and Methods

Lung samples from different slaughterhouses were used. All positive samples were inoculated in chick embryos. Viral RNA from allantoic fluid was extracted with fenol-chloroform technique. Viral genes was amplified using ONE STEP RT-PCR KIT (QIAGEN®. Cat. Number 210212). The designated primers pair used for NA1 gene as follows: N1F5´-GGTTCCAAAGGAGACATTTTTG-3´, N1R5´-CTATCCAAACACCATTGCCATA-3´. Amplifications conditions consisted of 1 cycle of 50°C for 30 min, 95°C for 15 min followed by 40 cycles of 94°C for 15 min, 55.5°C for 60 seg, 72°C for 60 seg, and a final extension of 72°C for 7 min. Restriction sites in the PCR products were simulated with the Clone Manager software using the reference strain [A/swine/New Jersey/11/1976(H1N1)]. The choice of restriction enzymes was based on differences in sequence. The PCR products were digested with four restriction enzymes, *BsgI, HaeIII, HpaII* y *MboII* (Biolabs, New England). The amplicon DNA (754 bp) were individually digested with all four enzimes for 1 h at 37°C. The resulting products were electrophoresed through 3.5% agarose gel in Tris–borate buffer containing ethidium bromide and visualized under UV ligth.

Results

After an enzymatic digestion of 7 amplicons of N1 using *Bsg*I, *Hae*III, *Hpa*II y *Mbo*II enzymes, it was observed that the cutting pattern was similar to the simulation produced with the Clone Manager software, with exception of the pattern produced by the *Mboll* enzyme.
We detected with the *MboII* enzyme, it is possible to identify strains with variations that are located in the target sequence of this enzyme; getting up to 4 strains with differences in the digestion pattern from a total of 7.

Conclusions and Discussion

Pandemic or epidemic presentations of human and avian influenza often cause great damage to human society and the economy. Therefore, it is necessary to use specific, rapid and accurate diagnostic tools for detecting variants of swine influenza virus. The objetive of this work was to identified and differentiated the variant strains NA1 gene using the RT PCR RFLP´s in Mexico during the period September 2009 to February 2011. It was possible to identify four strains with differences in the cutting pattern with enzyme *MboII*. Therefore it can be concluded that the swine influenza virus shows variability in the NA1 gene.

Table 1. Restriction sites of NA1 [A/swine/New Jersey/11/1976(H1N1)]. Amplicon of 754 bp.

Enzyme	Cleavage	Size
BsgI	1	570, 184
HaeIII	2	410, 273, 71
HpaII	1	263, 491
MboII	1	471, 283

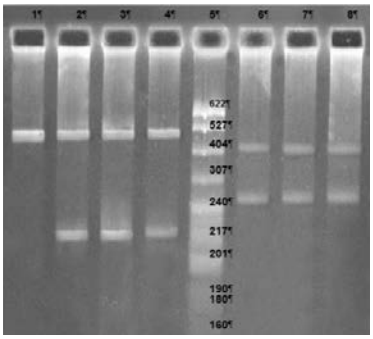

Figure 1. PCR restriction patterns for the NA1 gen of H1N1 influenza viruses with the *MboII* digestión enzyme. Enzyme *Mbo*II. Amplicon of 754 pb N1. Lanes 5 mpm (pBR 322). Lane 1 sample (0), lane 2 sample (1), lane 3 sample (2), lane 4 sample (20), lane 6 sample (31), lane 7 sample (49), lane 8 sample positive control. The enzyme cutting, yielding two fragments. Lane 1-4 were obtained two fragments of 527 bp and a 217 bp approx. Lane 6-8 two fragments were obtained of 404 bp and a 240 bp approx.

Acknowledgments

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Prevalence of different respiratory pathogens during post-weaning and fattening period in Belgian and Dutch pig herds using a tracheo-bronchial swab technique

F Vangroenweghe[1]
[1]*Elanco Animal Health Benelux, BU Swine & Poultry, Plantijn en Moretuslei 1A, 2018 Antwerp, BELGIUM*
vangroenweghefr@elanco.com

Introduction

Besides *M. hyopneumoniae(M.hyo)*, many other viruses and bacteria can be concurrently present during respiratory problems in pigs, provoking the disease complex known as Porcine Respiratory Disease Complex (PRDC). Diagnosis of infections with these pathogens can be performed using different approaches, including the detection of the pathogen through Polymerase Chain Reaction (PCR) assays. Recently, a new sampling technique (1) has been developed and validated for the detection of *M.hyo* in pigs using PCR, namely the tracheo-bronchial swab (TBS) technique. With this technique, pathogens present at the level of the trachea-bronchial junction can be recovered and analysed through PCR-analysis.

The aim of the present study was to obtain data on the distribution of different pathogens involved in PRDC in closed pig herds in Belgium and the Netherlands using the TBS technique.

Materials and Methods

Three hundred and four pig farms were sampled using the TBS technique. In every herd, at least 30 coughing piglets were sampled in at least two age groups (3-5, 6-11 and 12-20 weeks of age). TBS were collected as described previously. Following collection, samples were transported under cooled conditions to the laboratory and analyzed using mPCR and/or dPCR (IVD GmbH, Hannover, Germany). A multiplex PCR assay (mPCR) [porcine respiratory coronavirus (PRCV), porcine reproductive and respiratory syndrome virus (PRRSV), *M. hyopneumoniae*(*M.hyo*) swine influenza virus (SIV), porcine cytomegalovirus (PCMV), porcine circovirus 2 (PCV2)] and duplex PCR assay (dPCR) [*Actinobacillus pleuropneumoniae* (App), *Haemophilus parasuis* (Hps)] were used to detect the different pathogens in the TBS samples. PCR results were reported as negative or positive for the presence of PRCV, SIV, PCMV, PCV2, App and Hps. For PRRSV, strain type EU/US or both was also reported.

Results

The prevalence of *M. hyorhinis* was overall high with 86.7% in piglets of 3-5 weeks of age, 96.7% in piglets of 6-11 weeks of age and 98.7% in fattening pigs (12-25 weeks of age). Overall, Hps with virulence factor was present in 83.8 , 91.4 and 83.1% of the pigs samples at 3-5, 6-11 and 12-25 weeks of age. In piglets of 3-5 weeks of age, the most prevalent pathogens were SIV (26.5%), PCMV (18.6%), PRRSV-EU (10.5%) and *Mhyo* (8.2%), whereas in piglets of 6-11 weeks of age, PCMV (21.9%), PRRSV-EU (23.1%), SIV (21.9%) and *Mhyo* (10.6%) were the most prevalent pathogens. In older pigs (12-25 weeks of age), coughing was mostly provoked by *Mhyo* (54.6%), PCV2 (34.5%) and PRRSV-EU (29.8%), whereas SIV prevalence was much lower (13.1%). Combined infections between PRRSV-Mhyo, PRRSV-SIV and PRRSV-PCV2 did also occur at an overall prevalence level of 6.3%, 4.3% and 5.7% in pigs of 3-5, 6-11 and 12-25 weeks of age, respectively. Besides the combined infections involving PRRSV, other combinations such as *Mhyo*-SIV, *Mhyo*-PCV2 and SIV-PCV2 were also present. The overall prevalence of triple infections were as following: PRRSV-*Mhyo*-SIV 0.7%, PRRSV-PCV2-SIV 1.0%, PRRSV-*Mhyo*-PCV2 1.2% and *Mhyo*-SIV-PCV2 0.6%.

Conclusions and Discussion

The present study clearly shows that different viral pathogens responsible for PRDC may already be present during the post-weaning period. Concerning PRRSV, the most prevalent type was PRRSV-EU, whereas PRRSV-US was far less frequent. It is clear that in several herds, *M.hyo* is already present in piglets at weaning, further increasing in the second part of the nursery period. These observations are in accordance with Villarreal et al. and Meyns et al. (2,3,4). Hps was more prevalent than previously assumed, however, only in a smaller percentage of farms clinical relevance (polyserositis, polyarthritis, …) was also apparent. The prevalence of co-infection and triple infections of PRRSV with SIV, *M.hyo* or PCV-2 also may occur, but their prevalence is rather low as compared to dual infections. In conclusion, the present study showed that under Belgian and Dutch conditions, PRDC in post-weaning piglets is provoked by SIV, PCMV, PRRSV-EU and *M.hyo*. PRRSV is involved in many of the combined infections.

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Use of oral fluids to monitor PRRS disease in a commercial pig production system using commercial kits

HE Aguilar[1], NR Carreón[1], GR Martínez[1], ESE Mendoza[3], RA Reynoso[2], GR Mendoza[2], RA Pensado[2]
[1]*Departamento de Medicina y Zootecnia de Cerdos FMVZ-UNAM, México City* [2]*Departamento de Servicios Técnicos Veterinarios GCM, Veracruz México* [3]*FES-Cuautitlan UNAM, México,* mvzenrique.ah@gmail.com

Introduction

For decades, the sample most used to diagnose and monitor pig reproductive and respiratory syndrome (PRRS) has been blood serum. However, there is at present an alternative to carry out such monitoring using oral fluids [(1,2)]. These have a marked diagnostic value as they contain a high concentration of non-blood immunoglobulins, plasmatic proteins, desquamated epithelial cells and defense cells, among others [(3)]. The purpose of this study was to compare the number of positive samples obtained using blood serum and oral fluids, applying a commercial ELISA test specific for each of the matrices to be evaluated.

Materials and Methods

Oral fluid and serum samples were collected at a multi-site pig production unit that produces pigs for marketing and has a history of abortions and respiratory problems. Both samples were obtained from sows of different parities, as well as boars and 3, 10, 16 and 22 week-old animals in the production line.

The oral fluid samples were obtained following the technique described by Zimmerman & Prickett in 2008 using non-treated cotton rope. They were collected individually from the breeding stock (n=35) and from the production line per pen (n=6). The blood samples were obtained by conventional bleeding of the pigs using the Vacoutainer® system. Samples were individual in the case of the breeding stock (n=35), and were obtained from 5 animals of each age (n=30) from the production line per pen (155 blood samples and 59 oral fluid samples per sampling). In order to insure repetitivity, sampling was carried out twice and provided a total of 310 blood samples and 118 oral fluid samples.

The samples were analysed with commercial ELISA kits to detect antibodies against PRRS disease: IDEXX PRRS Oral Fluids Ab Test for the oral fluids analysis and IDEXX PRRS X3 Check Ab for the serum samples, following the indications established by the manufacturer (IDEXX Laboratories, Inc).

The s/p values of each test were compared individually for the breeding stock and for the production line per pen. In this last case, in order to compare the per pen results among tests, the s/p of the serum per pen were averaged to classify a pen as positive according to the cutoff point of the kit and then compared with the s/p obtained with the oral fluids test.

The information obtained will be analysed considering the number and percentage of positives for each test.

Results

The number of animals in the breeding stock detected as positives with the oral fluids test was greater than that recorded for the serum (Table 1). Also, the production line provided a different number of positives for the 3 week-old animals in the two samplings. From 10 weeks of age onwards, the number of positives obtained with both tests was the same.

Table 1. Number and percentage of positive samples per area of production

AGE OF ANIMAL	SAMPLE NUMBER PER AREA	SAMPLE (+)SERUM KIT	SAMPLE (+)OF KIT
ADULT	70	54 (77.14%)	64 (91.42%)
3 WK	12	9 (75%)	12 (100%)
10 WK	12	12 (100%)	12 (100%)
16 WK	12	12 (100%)	12 (100%)
22 WK	12	12 (100%)	12 (100%)
TOTAL	**118**	**99 (83.89%)**	**112 (94.91%)**

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Conclusions and Discussion

This study proves that it is possible to detect the same or more positive samples using the Kit and oral fluids samples, compared with the blood serum. The results of the study suggest that the samples and the ELISA test for oral fluids are a more sensitive alternative for programmes that monitor PRRS in pig herds.

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Effect of CIRCOVAC® vaccination in sows' reproductive parameters

A Ruiz[1], C Contreras[2], G Lennon[3], P Quilodrán[1]
[1]*Departamento de Patología y Med. Preventiva, Facultad de Cs. Veterinarias, Universidad de Concepción, Chile,*
[2]*Sucesión Salvador Yanine Abadi*, Chile, [3]*Merial-Chile*, aruiz@udec.cl

Introduction

The impact of Porcine Circovirus type 2 on reproductive disorders has been demonstrated in experimental conditions (1) and associated to abortion and an increase in rates of mummified, macerated, stillborn and weak-born piglets as well as early embryonic death (2).
The aim of this study is to report the impact of the sow vaccination with CIRCOVAC® in their reproductive parameters.

Materials and Methods

The present study was conducted in the site 1 of an 1100-sow farm, located in the central region of Chile. This farm was serologically positive to PCV2, and it has been demonstrated the presence of the virus by immunohistochemistry among other technique. Sows were vaccinated at 80 and 100 days of pregnancy. Meanwhile gilts were vaccinated at 140 and 160 days in puberty. Both group received a booster at 15 days of lactation. The data (total born piglets, live born piglets, mummified rate and fertility rate) were collected and compared whit the historical record of the farm, corresponding to the two months previous to the beginning of the vaccination, using the T student test. In order to discriminate the possible genetic effect on the assay, because the farm was doing at the same time a change in genetic, sows were classified into Group A (entire reproductive herd) and group B (excludes genetics that was incorporated)

Results

A marked difference was observed in total born piglets between vaccinated and non-vaccinated sows in group A, showing an minimum increase of 0,8 piglets when May and September (Autumn-Spring) were compared (Table 1), which was statistically significant. However, in group B this difference was reduced to a minimum of 0,2 piglets, which was not statistically significant, a clear tendency was observed in favor of vaccinated group (Table 1).
In live born piglets there is a similar pattern, showing a minimum difference of 0,7 piglets between May and September in group A (Table 1), which was statistically significant. Similar tendency was observed in group B, showing an increase in this parameter in vaccinated group in comparison with non-vaccinated (Table 1). Although there is not a significant statistical difference in mummified rate between vaccinate and unvaccinated sows, in both groups, there is a tendency to decrease the number of mummified piglets in vaccinated sows.
Additionally, the fertility rate showed a clear tendency to improve in vaccinated sows, in both groups (Table 2). However this deference was only statistically significant between the average of non-vaccinated and vaccinated sows (90,59% and 93,79% respectively) in group A.

Table 1. Reproductive parameters of non-vaccinated (NV) and vaccinated (V) sows with CIRCOVAC®, classify by the genetic effect (Group A and B).

	Group A						Group B					
	May	Jun	Jul	Aug	Sep	Oct	May	Jun	Jul	Aug	Sep	Oct
	NV	NV	V	V	V	V	NV	NV	V	V	V	V
Sows	199	195	215	214	193	122	83	87	67	48	51	40
Total born piglets	12,8	12	13,7	13,7	13,6	14,1	12,6	12,3	12,8	13,7	12,8	12,7
Live born piglets	11,8	11	12,7	12,7	12,5	12,9	11,5	11,4	11,7	12,4	11,3	11,8
Mummified rate (%)	2,66	1,39	1,79	1,69	1,37	1,97	2,49	1,40	3,26	1,37	1,54	1,57

Table 2. Fertility rate among non-vaccinated (NV) and vaccinated (V) sows with CIRCOVAC®, classify by the genetic effect (Group A and B).

	Group A					Group B				
	Jun	Jul	Aug	Sep	Oct	Jun	Jul	Aug	Sep	Oct
	NV	NV	V	V	V	NV	NV	V	V	V
Sows	249	240	287	244	210	45	54	23	11	27
Fertility Rate (%)	89,9	91,2	93,9	94,6	93,8	84,4	92,6	95,7	72,7	100

Conclusions and Discussion

During this study, it can be observe an improvement of the sows' reproductive parameters in vaccinated animals. However, the sample size of group B was to low. The increases in total piglets born and piglets born alive, in group A, were considerable, being statically significant. The progressive increase in the reproductive parameters measured through the months, suggests a positive impact of sows vaccination with CIRCOVAC®, in concordance with others studies (3, 4, 5).

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Investigating the causes of poor ADG: A study of the correlation between the results of blood tests and fecal tests for *L. intracellularis* and PCV 2

VNAM Geurts[1], GJR Groenland[2], ALM Cruijsen[1]
[1] *MSD-AH Intervet Nederland BV, Boxmeer, The Netherlands. victor.geurts@merck.com*
[2]*De Heus Voeders b.v. Ede, The Netherlands,* victor.geurts@merck.com

Introduction

Lawsonia intracellularis (Li) is one of the most important intestinal infections in growing/finishing pigs. Porcine circo virus type 2 (PCV2) can also cause intestinal infection in the same age pigs. Both infections can cause growth retardation and thus economic losses without clinical signs. Blood samples are tested routinely by serology and PCR all over the world. For Li, sero prevalence can be much greater than that evidenced by bacteriology/PCR [1]. A critical threshold level of Li which will cause a reduction in ADG has been demonstrated in challenged pigs [2]. The aim of this study was to investigate the correlation between blood test results (Li and PCV2 serology, and PCV2 PCR) and the results of fecal tests (Q-PCR for Li and PCV2) to determine the feasibility of using blood tests to diagnose Li or PCV2 infections causing poor average daily weight gain (ADG) in the absence of further clinical signs.

Materials and Methods

On the "de Heus" trial farm, 656 fattening pigs from 3 different farms were housed separately in nine units each containing eight pens. The piglets were vaccinated for *Mycoplasma hyopneumoniae* and PRRS, but not against Li or PCV2. The animals in each pen were weighed and their feed intake measured. Feces (pooled)[3] and blood samples were taken from 18 pens (two per unit) at 10, 15, 20 and 25 weeks of age. The feces samples were analyzed for Li and PCV2 at the GD-lab in Deventer by Q-PCR. The Lawsonia Elisa (Bioscreen), Alphalisa PCV2 (MSD-AH in-house antibody test of the ORF2 protein) and qPCR PCV2 were done on serum samples.
Li and PCV2 intestinal infection and the phase of infection (acute = increase of pathogens; recovery = decrease of pathogens) were recorded and a possible correlation with the blood test results was calculated.

Results

Table 1: Li test results

farm:	test:	10 w	15 w	20 w	25 w
1	Feces:Li PCR(log10)	2.58	4.87	1.6	0.53
1	Li elisa (serum)	19.2	67.21	67.1	61.61
2	Feces:Li PCR(log10)	0.41	3.39	0	1.7
2	Li elisa (serum)	13.92	43.38	49.5	49.17
3	Feces:Li PCR(log10)	4.48	3.75	0	0
3	Li elisa (serum)	28.08	70.68	65.3	54.14

– Lawsonia correlation in feces (PCR) and serum (antbodies)

Farm		week 10 - 15 Elisa Li	week 20-25 Elisa Li
1	PCR Li feces	0.26	0.2
2	PCR Li feces	0.81*	-0.043
3	PCR Li feces	-0.32	____

* statistically significant p-value <0.05

Table 2: PCV2 test results

farm:	test:	10 w	15 w	20 w	25 w
1	Feces:PCV2 PCR(log10)	4.86	6.45	5.2	4.7
1	blood:PCV2 PCR(log10)	0.16	2.32	1.4	0.4
1	PCV2 (serum) log2	3.45	6.76	10.3	9.1
2	Feces:PCV2 PCR(log10)	1.57	5.78	6.3	2.4
2	blood:PCV2 PCR(log10)	0	2.34	3.1	2
2	PCV2 (serum) log2	2.15	3.28	8.9	10.7
3	Feces:PCV2 PCR(log10)	5.73	6.36	5.2	3.5
3	blood:PCV2 PCR(log10)	0.84	3.33	1.4	0.4
3	PCV2 (serum) log2	3.94	8.41	10.2	10.8

– PCV2 correlation in feces (PCR) and serum (PCR and antibodies)

Farm		week 10 - 15 alphalisa	week 10 - 15 PCR serum	week 20-25 alphalisa	week 20-25 PCR serum
1	PCR feces	0.61**	0.70**	0.27	0.68**
	PCR serum	0.81**	____	0.34	____
2	PCR feces	0.62**	0.63**	-0.14	0.54
	PCR serum	0.54	____	-0.27	____
3	PCR feces	0.67	0.66	-0.02	0.43
	PCR serum	0.97**	____	0.03	____

** statistically significant p-value <0.05

Conclusions and Discussion

-Li levels in feces and serum antibodies were strongly correlated in piglets during the acute phase of the infection. However, during the recovery phase (farm 1 and 2), there was only poor correlation between the antibody titers and Li shedding in feces. This means that Li serology is useful for diagnosing Li infections, but is only related with poor ADG if the samples are taken in the acute phase of the infection, which is impractical when Li infections are subclinical.
Lawsonia qPCR testing on feces is more suitable for diagnosing Li infections as cause of suboptimal ADG [2, 3]
-PCV2 virus levels in feces and serum were positively correlated in all phases of the infection. Furthermore, similar to Li, serum antibody levels were positively correlated during the acute phase of infection but poorly correlated in the recovery phase.
Therefore, PCV2 serology is useful to demonstrate infection. For diagnosing PCV2 infections with poor ADG, PCV2 PCR has to be used.

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Scanner photography: effective technique to investigate needle free device injection dispersion pattern

C Bianco[1], F Ostanello[1], D Lorini[2]; G Leotti[3], T Vila[4], O Merdy[4], F Joisel[4], G Sarli[1]
[1]*Department of Veterinary Medical Science, Bologna University, Bologna, Italy;* [2]*Giordano Poultry Plast, Cuneo, Italy;* [3]*Merial Italia SpA, Milano, Italy;* [4]*Merial S.A.S., Lyon, France;* giuseppe.sarli@unibo.it

Introduction

Vaccination, allowing to contain infections and to prevent health problems is a crucial strategic management tool in today's pig industry. However, it also represents a cost item and therefore condition of vaccine application must be optimized. In the recent years needle free injection devices (NFID) have utilized various techniques for transcutaneous vaccine injections. Among the benefits to be highlighted there are: no broken needles in the meat, reduced inter animal transmission of diseases, respect of animal welfare (1). In this paper a scanographic (scanner photography) technique will be illustrated, for analyzing and quantifying transport / pattern of penetration and dispersion of a vaccine administered by NFID in piglets focussing on macroscopical visual injection outcome.

Materiasl and Methods

Conventional piglets of 5-20 kg lbw were vaccinated on the left side of the neck with NFID Valery® (Giordano Poultry Plast, Caraglio Cuneo, Italy) with black china ink spiked CIRCOVAC® (MERIAL, Lyon, France), 1 cc ink/100 cc of vaccine), according to datasheet (0.5 ml/piglet) and immediately underwent euthanasia. Freezing of hanged piglets was carried out (48 h, -20°C) and subsequently frozen cross-sectional slices were obtained (thickness about 1 cm). Images were acquired by means of an ordinary flatbed scanner protected by a glass platen (hp, Palo Alto-CA, USA).

Results

Scanographies are shown in Fig 1 & 2.

Conclusions and Discussion

Mechanism of liquid jet injectors relies on the principle of forcing fluids through a small orifice, generating a high pressured stream that penetrates into the skin with high velocity (2, 3). There are numerous predictive "pre-skin" biophysical trials and predictive models (4), but there are rare models of evaluation of intramuscular penetration, and dispersion characteristics in vivo. Therefore the proposed method provides valuable and reliable information, is objective, repeatable and reproducible, not expensive, and allows the comparison of *in vivo* efficacy of various NFID.

Acknowledgments

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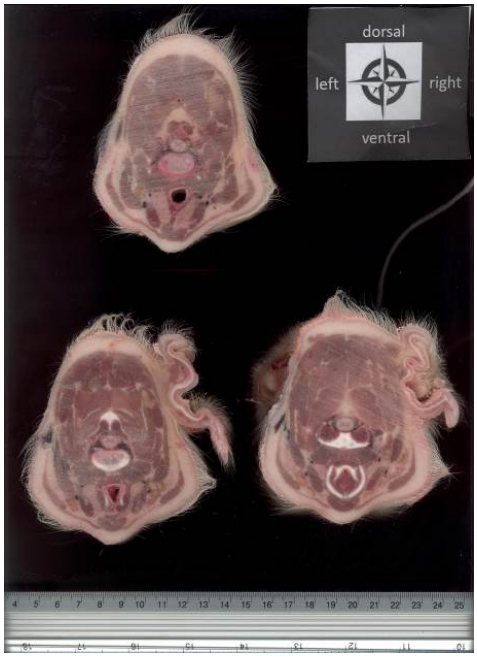

Figure 2. Example of a scanography of 3 serial cross section. (Jpeg, 1200 dpi; 10400X14040 pixels)

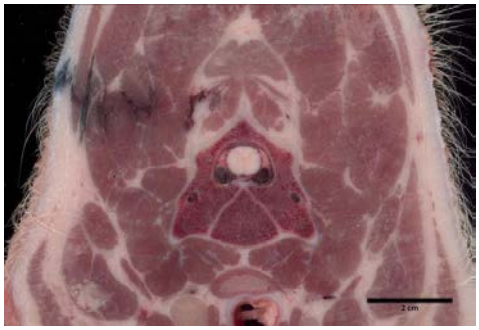

Figure 3. Intradermal, fascial and intramuscular dipersion pattern traced by black ink

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Comparison of sensitivity and specificity of PRRSV antibody ELISA kits and application for clinical samples collected from Korean swine farms

W-I Kim, H-S Cho, B-S Kim
College of Veterinary Medicine, Chonbuk National University, Jeonju, Korea, kwi0621@jbnu.ac.kr

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) causes major economic losses in the pig industry worldwide. Two types of PRRSV have been reported: the European type (type 1) and the North American type (type 2). To control this virus, reliable tools for monitoring, import controls, outbreak investigations, and follow-up studies are necessary. Several serological tests for routine diagnostics of PRRS have been developed and ELISA tests based on recombinant nucleocapsid protein of PRRSV have been used most commonly for PRRS diagnostics (1). Because both PRRSV types can be found in most of the countries positive for PRRSV infection, it is important to detect antibodies against both types of PRRSV. In the current study, two commercial PRRSV antibody ELISA kits have been compared for their sensitivity and specificity using experimental samples collected from animal challenge studies with Korean PRRSV strains and clinical samples from Korean swine farms.

Materials and Methods

Two commercial PRRSV ELISA kits (Bionote PRRS Ab ELISA and IDEXX PRRS 3XR Ab ELISA) were evaluated in the study. Five hundred serum samples sequentially collected from 5 different animal studies in which 4-week old, PRRS-negative pigs were challenged with various type1 and type 2 PRRSV strains were evaluated with the two PRRSV ELISA kits as known positive samples to determine the sensitivity of those ELISA kits. In addition, one thousand serum samples collected from 20 different PRRS-negative farms were tested to determine the specificity of the Bionote PRRS Ab ELISA kit. All of the test results were analyzed using SPSS statistical software. Kappa statistic was used to measure agreement between the two different kits.

Results

Based on preliminary results, there was good agreement between test results from the two ELISA kits (Kappa coefficiency=0.917) and sample-to-positive (S/P) ratios produced by the ELISA kits also correlated very well (R^2=0.78). In addition, both of the ELISA kits effectively detected antibodies against type 1 and type 2 PRRSV strains.

Conclusions and Discussion

Because both of the ELISA kits utilized recombinant PRRSV nucleocapsid proteins as detection antigens, there was good agreement on test results from the ELISA kits, including S/P ratios and diagnostic interpretations. Therefore, these preliminary results indicate that the two commercial PRRS ELISA kits are effective tools in conducting accurate PRRS diagnostics though accurate sensitivity and specificity of the kits are being currently evaluated using over 1,500 known positive or negative serum samples.

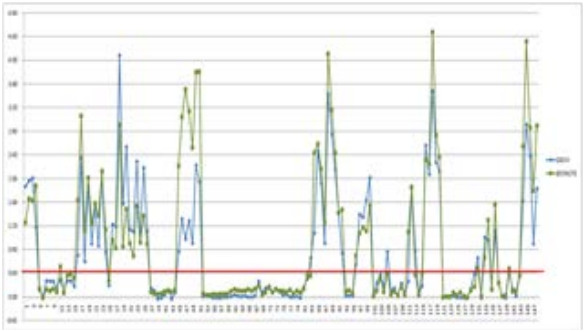

Figure 1. Comparison of S/P results on experimental and clinical samples between two commercial PRRSV antibody ELSIA kits

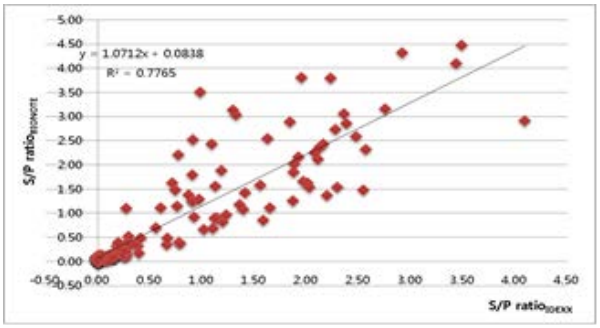

Figure 2. Correlation of S/P ratios on experimental and clinical samples between two commercial PRRSV antibody ELSIA kits

Acknowledgments

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Development of a multiplex PCR assay for simultaneous detection of five single-stranded DNA viruses in pig lungs

D Gava[1]; R Schaefer[1]; AMG Ibelli[1]; MC da Silva[2]; JR Ciacci-Zanella[1]
[1] *Embrapa Swine and Poultry Research Center, Animal Health Laboratory, Concórdia, Brazil.* [2] *Diagnostic Center for Animal Health (CEDISA), Concórdia, Brazil.* danielle.gava@embrapa.br

Introduction

Porcine parvovirus (PPV1) and porcine circovirus type 2 (PCV2) cause clinical disease in swine (1, 3). Porcine parvovirus 4 (PPV4), torque-teno sus virus (TTSuV1 and TTSUV2) have been implicated as co-factors for PCV2 disease development, but their epidemiology and pathology remains unclear (1, 2). In order to simultaneously detect these five DNA viruses (PCV2, PPV1, PPV4, TTSuV1 and TTSuV2), a multiplex polymerase chain reaction (mPCR) was designed, optimized and tested in pig lungs.

Materials and Methods

Primer design: Multiple sequences of each virus retrieved from GenBank database were aligned using MEGA 5.2. The primer set was designed using Primer3Plus software targeting a conserved region of each virus.

Positive controls: Amplicons of TTSuV1 (101bp), PCV2 (284bp), TTSuV2 (341bp), PPV4 (440bp) and PPV1 (561bp) were cloned into pCR4 plasmid (TOPO TA Cloning Kit, Invitrogen) and purified using the PureLink Quick Plasmid Miniprep Kit (Invitrogen). All cloned amplicons were verified by sequencing and quantified using a ND-1000 spectrophotometer.

Sensitivity and specificity: A 10-fold serial dilution of 1.2×10^{10} up to 1.2×10^{0} DNA copies/µL was carried out to evaluate the sensitivity of the mPCR. The specificity was tested using influenza A virus and PCV1.

Multiplex PCR: The mPCR was performed in a 30µL reaction and contained 4mM $MgCl_2$, 1.5× PCR Buffer, 0.4mM dNTP, 0.05µM TTSuV1, TTSuV2 and PCV2 primers, 0.16µM PPV1 primer, 0.4µM PPV4 primer and 1.8U Platinum *Taq* DNA Polymerase (Invitrogen). The PCR cycling conditions consisted of an initial denaturing at 95°C for 30s followed by 35 cycles at 95°C for 1 min, 60°C for 1 min and 72°C for 1 min followed by a final extension at 72°C for 10 min. The amplicons were analyzed by electrophoresis in a 2% agarose-TBE gel and stained with ethidium bromide.

Clinical samples: Seventy-five lung samples were collected from 15-180 day-old pigs, from 2009 to 2013 in Southern Brazil. Viral DNA was extracted using DNeasy Blood & Tissue Kit (Qiagen) and the mPCR was performed as described above.

Results

The sensitivity of the mPCR was 1.2×10^{3} DNA copies/µL and no amplification was observed with other pathogens. All clinical samples were positive for at least one virus. Sixty-eight (90.7%) samples were positive for PPV4, 38.7% for TTSuV1, 37.3% for TTSuV2, 17.3% for PCV2 and 16% for PPV1. Among the 75 samples, PPV4 was present as the only virus in 29.3% of the tested samples. Co-infection by PPV4 and TTSuV1 was observed in 14.7% samples, co-infection by PPV4 and TTSuV2 occurred in 12% and triple co-infection by PPV4, TTSuV1 and TTSuV2 was detected in 10.7%. A single infection by PCV2 or PPV1 was not observed.

Conclusions and Discussion

A rapid, sensitive, specific and cost-effective mPCR assay is described and applied for simultaneously and differential detection of TTSuV1, PCV2, TTSuV2, PPV4 and PPV1 in pig lungs. In current intensive swine production, pigs can be infected at the same time with two or more viral pathogens (1, 2, 3). The five viruses included here are involved in multifactorial diseases that cause significant economic losses in swine production worldwide (1, 2). A rapid and reliable detection of these viruses is important for herd management and the prevention of disease spread. Moreover, the detection of viral co-infections that have been shown to enhance the severity of PCV2 infection would allow the implementation of control measures directed against these possible 'trigger' factors.

Acknowledgements

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Internal genes amplification of Mexican swine influenza virus by RT-PCR

VM Carrera-Aguirre[1], MC Mercado-García[1], SE Mendoza-Elvira[2], JI Sánchez-Betancourt[1]
[1]*Departamento de Medicina y Zootecnia de Cerdos. FMVZ-UNAM.*
[2] *Facultad de Estudios Superiores Cuautitlán. UNAM.* vickcarrera@gmail.com

Introduction

Because of the continuous change of SIv and the difficulty of its diagnosis, the internal genes PB2, PA, NP and M have been founded rearanged continuously in virus from different species. The quick genomic identification of these rearrangements should be done to identify its origin and for subsequent studies.

Materials and Methods

In this study were used seven SIv isolates during differents outbreaks in central zone of Mexico. These virus were compared by RT-PCR assay again A/swine/NewJersey/11/76(H1N1) and A/swine/Minnesota/9088-2/98(H3N2). The RNA extraction was carried out with the GIBCO-BRL protocol (Life Technologies, 1996) and the next protocol for RT-PCR using the One Step RT-PCR kit (QIAGEN). The RT-PCR using oligonucleotides designed individually for the identification of the genes PB2 (384pb), PA (596pb), NP (475pb) and M (330pb) (Table 1). Oligonucleotide design was done using Clone Manager® software V.7.0 and its specificity was proved by a GenBank BLASTn analysis.

Table 1. Primers used for amplification of internal genes by One-Step RT-PCR

Gen	Tamaño pb	Secuencia	Amplicón pb
PB2	Fw 25	5´GATCTGATGTCGCAGTCCGCGCACTC´3	384
	Rw 22	3´TCTGAAGTGGACAGGGCCGAAG´5	
PA	Fw 22	5´GAGACCGAATCATGGCCTGGAC´3	596
	Rw 20	3´TGACCGCTGATGGCAAAGAG´5	
NP	Fw 26	5´GCGCCAAGCAAACAATGGTGAAGATG´3	475
	Rw 23	3´AGCAGGCAGGCAAGACTTATGTG´5	
M	Fw 25	5´CTCAAAGCCGAGATCGCGCAGAGAC´3	330
	Rw 20	3´GCCCATGCAACTGGCAAGTG´5	

Results and Discussion

With the RT-PCR protocol, the PB2, NP and MP genes were efficiently amplified. The PA gene amplicons obtained from the isolates, were different (about 300bp) than the expected at 596pb, observed in the reference virus amplicon. Due to intrinsic RNA polymerase characteristics prone to error, sequence variations been generated, including own subunits of polymerase. Sequencing could show variations regarding reference virus. See figure 2.

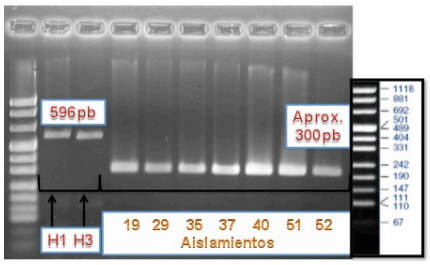

Figure 2. Amplification of PA gen in viral isolates and reference virus.

Some authors have amplified successfully the entire influenza virus genome, with multiple modifications of the RT-PCR technique,[1,2,3,4] however, we suggest the use of our primers if pretend looking for Mexican sequences reported in the GenBank to obtain favorable results (Table 2).

Table 2. Viral isolates used to amplify internal genes

Aislamiento	Nomenclatura
1	(A/swine/México/Mex19/2010(H1N1))
2	(A/swine/México/Ver29/2010(H1N1))
3	(A/swine/México/Qro35/2010(H1N1))
4	(A/swine/México/Ver37/2010(H1N1))
5	(A/swine/México/Mich40/2010(H3N2))
6	(A/swine/México/Mex51/2010(H3N2))
7	(A/swine/México/Mex52/2010(H1N1))

Conclusions

The oligonucleotide design is the most important factor to determine the sensitivity and specificity of protocols based on RT-PCR technique for detect specific strains previously reported.
It is important to consider the evolution of these genes because they play a significant role in virulence.

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Assessment of specific IgG avidity to discriminate between recent and chronic *Toxoplasma gondii* infection in pigs

W Basso[1, 2], F Grimm[1], P Deplazes[1], X Sidler[2]

[1]*Institute of Parasitology, Vetsuisse-Faculty, University of Zurich, Winterthurerstrasse 266a, CH-8057, Zurich, Switzerland,* [2]*Department of Farm Animals, Division of Swine Medicine, Vetsuisse-Faculty, University of Zurich, Winterthurerstrasse 260, CH-8057 Zurich, Switzerland,* Walter.Basso@access.uzh.ch

Introduction

Toxoplasma gondii (Protozoa, Apicomplexa) infections in pigs are frequently asymptomatic. However, several cases of clinical disease (dyspnoea, general weakness, anorexia, fever, cyanosis, diarrhoea, and even death) and reproductive failure in sows have been described in the last years (Dubey, 2009). In symptomatic cases, clinical signs are supposed to occur during the acute phase of the infection. Current serological assays used to detect anti-*T. gondii* antibodies in pigs do not distinguish between recent and chronic (asymptomatic) infections.

The measurement of specific IgG avidity (functional affinity) is used in the diagnosis of recent or past *T. gondii* infections in humans. Avidity assays are based on the fact that antibodies produced early after an infection have lower binding affinities than those produced later on. Low affinity antibodies can be eluted in serological tests by including an additional incubation step with urea (Björkmann et al, 1999).

Materials and Methods

Six 4.5 week-old piglets were orally inoculated with 5,000 *T. gondii* oocysts. Serial serum samples were obtained and tested with a commercial ELISA (PrioCHECK Toxoplasma Ab porcine ELISA, Prionics, Switzerland) (Basso et al., 2013). All absorbance values were normalized by calculating a percentage of positivity (PP) value relative to the O.D. of the positive control as suggested by the manufacturer (PP Sample = O.D. 450 nm Sample/O.D. 450 nm Positive Control x 100).

For the assessment of IgG avidity, positive sera were titrated in duplicate in the commercial ELISA (fourfold dilutions, starting at 1:12.5) and treated by an additional incubation step, either with 6M urea in sample buffer (10 min, at room temperature) or with sample buffer alone. For each serum, two end point titers – with and without urea treatment – were determined, using PP 15 as cut-off. Avidity index (AI) was calculated as endpoint titer with urea/ endpoint titer without urea x 100.

Results

All animals showed antibody levels above the threshold suggested by the manufacturer after 2 weeks post inoculation (wpi.) (Fig.1). The specific IgG avidity increased during the course of infection in all 6 inoculated animals. Until 4 wpi, AIs remained below 12%. At 6 wpi, AIs were between 14% and 30%. Avidities >40% were observed at 8 wpi or later. Interestingly, while 5 pigs showed high AI values (48-76%) at 11 wpi, the AI remained low (≤28%) in one animal during the whole study (Fig 2).

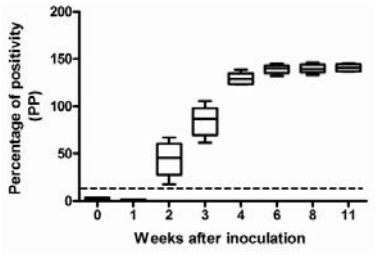

Figure 1. Anti-*T. gondii*-IgG antibody levels in experimentally inoculated pigs.

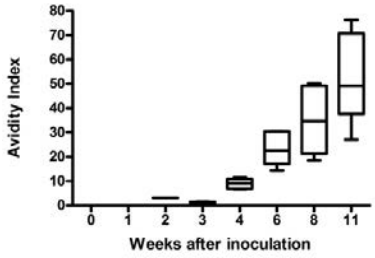

Figure 2. Avidity of anti-*T. gondii*-IgG antibodies in experimentally inoculated pigs.

Conclusions and Discussion

Although low AI values might be suggestive of recent infections, low values can persist in some animals for at least 11 weeks. On the other side, a high AI (>40%) could be used to rule out a recent (<8 weeks) infection. The avidity-ELISA might represent a valuable diagnostic tool for the diagnosis of clinical *T. gondii* infections in pigs.

Acknowledgments

P Buholzer and Firma Prionics AG, Switzerland for providing the ELISA Kits.

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Case report: Innovative diagnostic approach and subsequent adapated vaccination strategies to reduce the antibiotic use in post-weaning piglets under Belgian field conditions

F Vangroenweghe[1], M Verduyn[2]
[1]*Elanco Animal Health, Antwerp, Belgium,* [2]*DAP Verduyn, Meulebeke, Belgium,* vangroenweghefr@elanco.com

Introduction

For several months, a closed pig farm (n = 500 sows) with 2-site production (sow-piglet/fattening pigs) and a 4-week batch management system had continuous coughing in its post-weaning facilities, characterized by increased mortality (> 6%), a major increase in antibiotic consumption (colistin, doxycyclin, amoxicyclin, tylosin) and decreased growth and runt pigs at the end of the production cycle. Moreover, acute coughing occurred following introduction of their SPF gilts into the main sow group. Piglets were not vaccinated at all, whereas gilts were vaccinated for PRRSV (EU strain), parvovirus and *Erysipelothrix rhusiopathiae*. Overall, antibiotic use during the post-weaning period (4 – 13 wk of age) was extremely high with a treatment incidence (TI) of 1800, meaning that 1800 treatments were administered every day per 1000 piglets present.

The present case report documents how an innovative diagnostic approach and subsequent technical advice on vaccination schedules and management strategies induced a better overall herd performance and a significant reduction in antibiotic use.

Materials and Methods

Diagnostic approach consisted of trachea-bronchial swab (TBS) sampling in piglets at 6-10-14 wks of age and coughing gilts following introduction into the main sow herd. TBS swabs were analyzed using multiplex PCR (PRRSV, *M. hyo*, SIV, PCV-2) analysis (IVD GmbH, Hannover, Germany).

Diagnostic Results

Piglets were positive for *M. hyo* at 6-10 wks of age and during the entire post-weaning period for PCV-2. Additionally, PRRSV-EU was detected and slight circulation of SIV was present during the post-weaning period. Coughing gilts were positive for *M. hyo*, PCV-2 and SIV.

Technical Advice on Vaccination Schedules

Subsequent veterinary advice on vaccination schedules resulted in the application of a one-shot *M. hyo* vaccine (Stellamune One, Elanco) at 1 week of age and a PCV-2 vaccine at 17 days of age. Gilts were additionally vaccinated for *M. hyo*, PCV-2 and SIV during the quarantine-adaptation period.

Follow-up Data

To check for improvement following the introduction of the corrective measures, 2 additional samplings were performed 1 and 2 years after the initial problems occurred. From the 1st year onwards, gilts did not suffer from coughing anymore following the extended vaccination schedule. In piglets, no early *M. hyo* infection could be observed and PCV-2 circulation decreased significantly. The clinical picture and piglet performance were significantly improved. Nevertheless, focus on good application of PCV-2 vaccination was sharpened. Follow-up after 2 years resulted in overall good performance during gilt introduction and post-weaning piglet performance. Moreover, due to the intensive vaccination schedule, the treatment incidence (TI) during the post-weaning period decreased with 84% from 1800 to 300. Whereas before the diagnosis and adaptation of vaccination schedule, piglets were treated with one to two antibiotics throughout the post-weaning period, antibiotics were only used during the first 10 days after weaning for treatment of post-weaning diarrhea after the introduction of all vaccination and management measures.

Conclusions and Discussion

From the present case report it is clear that before effective management and vaccination strategies are implement on a problem farm, a thorough problem analysis and diagnosis is performed in order to obtain detailed information on the primary pathogens involved in the problem. The case has clearly demonstrated that effective preventive measures can help in reducing the use of preventive and curative antibiotic treatments, with a concurrent improvement of overall farm performance at post-weaning piglet level.

In conclusion, detection of the etiologic agents through an extended diagnostic approach, followed by correct veterinary advice results in a significant reduction in antibiotic use. It is key to continue the evaluation of all implemented measures and to impose additional corrective actions if expected results remain insufficient.

Comparative study between ID and IM vaccination and the course of seroconversion in PRRSV-negative gilts following vaccination with Porcilis® PRRS

T Worku[1], R Tabeling[2], S von Berg[2]
[1]*Veterinary practice Dr. Tesfaye Worku, Mühlhausen/Thüringen,* [2]*Intervet Germany / MSD Animal Health, Businessunit Livestock, Unterschleissheim,* stephan.von.berg@msd.de

Introduction

The porcine reproductive and respiratory syndrome virus (PRRSV) is still a major issue in the German swine industry. The virus can lead to severe reproductive disorder in sows and also, mainly by a strong immunosuppressive effect, to massive respiratory infections within the porcine respiratory disease complex (PRDC). Economic losses can reach up to € 125.00 per sow per year (1). The most effective way to control PRRSV is vaccination of sows and/or piglets with a modified live vaccine (MLV). Two alternatives to administer the vaccination are available: intramuscular injection, or, for one vaccine, intradermal injection with a special air pressure injector (IDAL) (2) The aim of the present study was to compare humoral immune response following vaccination via ID or IM route under field conditions.

Materiasl and Methods

A PRRSV positive farm, housing 650 productive sows (Hypor), introduces new gilts negative for PRRSV and *Actinobacillus pleuropneumoniae* (APP) on a regular basis. In quarantine, the following vaccination program is performed: PRRSV upon arrival (Porcilis PRRS®, Intervet/MSD), one week later Influenza A (Respiporc Flu3®, IDT-Biologika), earliest from the 180th day of life Parvoruvac® (Merial). These vaccinations are boostered subsequently 3 weeks after the first vaccination.

For the study, blood samples were taken from 20 gilts upon entering the quarantine. These sows were already marked individually by ear tags and were clinically healthy. Two groups of 10 animals each were formed randomly. All gilts were vaccinated with a genotype I MLV (Porcilis PRRS®); 10 sows intramusculary, 10 intradermal with the IDAL – applicator. Three weeks later, prior to the second PRRS vaccination, blood samples were taken again.

The pre-vaccination samples were analyzed in a commercial Laboratory as follows: APP (ApXII inhouse ELISA), Influenza A (HAH), PRRSV Antibodies (IDEXX PRRS X3 ELISA) and PRRSV genome (LDL Virotype PRRS). The three weeks post-vaccination samples were just analyzed for PRRSV specific antibodies, using the aforementioned test.

Results

On arrival, all gilts were negative in all performed tests, including the PRRSV Ab ELISA (Tab. 1&2). The three weeks post-vaccination samples were consistently positive for PRRSV specific antibodies, regardless of the vaccination technique used (Tab. 1 & 2).

Table 1. PRRSV Ab before (b.v.) and post (p.v.) im vaccination

Gilt Nr.	24474	24475	24472	24473	24477	24448	24452	24450	24454	24449
Titer b.v.	0,018	0,006	0,036	0,025	0,011	0,000	0,015	0,000	0,010	0,015
Titer p.v.	1,093	0,806	1,312	1,490	1,349	1,831	0,670	1,253	0,560	1,221

Table 2. PRRSV Ab before (b.v.) and post (p.v.) id vaccination

Gilt Nr.	24464	24468	24469	24467	24466	24461	24458	24463	24457	24456
Titer b.v.	0,010	0,011	0,013	0,004	0,004	0,004	0,122	0,017	0,010	0,015
Titer p.v.	0,851	0,516	2,006	1,206	2,077	1,618	2,192	1,612	1,907	1,444

Conclusions and Discussion

Our results demonstrate that the vaccination with a PRRS genotype I MLV (Porcilis PRRS) induces a humoral immune response in PRRS naïve pigs. There was no difference in the antibody response between intramuscularly and intradermally vaccinated animals. These results are in good accordance with other authors (2), (3) and confirm their experimental results on the occurrence of PRRSV specific antibodies after vaccination of PRRSV-naïve animals via intradermal vaccination with the IDAL injector in the field.

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The impact of PCV2 vaccination compliance on exposure timing and PCVAD onset: A case study

M Potter[1], S Henry[1], L Tokach[1], S Dritz[2]
[1]*Abilene Animal Hospital, P.A., Abilene, KS,* [2]*Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS,* mpotter@aahpa.com

Introduction

Different porcine circovirus type 2 (PCV2) vaccination strategies have been tried to improve compliance, build immunity at younger ages or reduce cost. Some strategies have resulted in protection but others have not, strengthening the argument that successful immunization with PCV2 vaccines is an important part of the health program.

Materials and Methods

Three farms were involved in this case study. In 2012 these farms switched to a common source of 3 week old gilts for development into replacement gilts. This sow farm also supplied Farm 1 with 380 barrows along with 20 gilts for an off-site nursery.

Vaccination history for Farms 1, 2, and 3:

Farm 1: Administration of a PCV2 vaccine dose at weaning was discontinued at the nursery after the source switch as reports from the sow farm indicated 2 mL Circumvent® PCV (Merck Animal Health, Summit, NJ) was given prior to weaning. All pigs received 2 mL Circumvent® PCV M (Merck Animal Health, Summit, NJ) at 5 to 7 weeks of age (WOA).

Farms 2 and 3: All gilts receive 2 mL Circumvent® PCV (2nd dose) and 1 mL Myco Silencer® ONCE (Merck Animal Health, Summit, NJ) at 6 WOA.

Case description and diagnostic response:

In May 2013, attending veterinarians received a call regarding scouring pigs from the producer finishing the barrows from Farm 1 nursery. Health and vaccination history for finisher groups on feed was requested from the attending veterinarian for the nursery. According to the finishing farm's naming scheme, these were groups 14 and 15 (25 and 17 WOA, respectively). The veterinarian shared that the current nursery group (group 16) was showing signs of PCV2 infection including wasting, porcine dermatitis and nephropathy syndrome (PDNS), and increased mortality (9.5%). For comparison, groups 14 and 15 had 1.3% and 0.25% nursery closeout mortality, respectively. Subsequently, group 16 nursery closeout mortality reached 33%.

Clinical signs in group 15 included wasting, diarrhea, pallor, and PDNS. A diagnosis of porcine circovirus associated disease (PCVAD) with ileitis was suspected. Serum was collected from both finishing groups for PCV2 indirect fluorescent antibody assay (IFA). The veterinarian for the source sow farm was contacted for health history and testing. The veterinarian for the nursery also had serum samples from previous groups stored which were then tested by PCV2 IFA and polymerase chain reaction (PCR).

Results

The IFA titers were variable even after pigs had received their final PCV2 vaccine dose. This was not group dependent and led to an unexpected discovery. The order database for the source sow farm had a no-PCV2 vaccination request flag (placed for Farm 1 prior to the source switch) on the standing order. This was not known to the Farm 1 attending veterinarian or the source farm veterinarian. Thus, pigs in groups 13, 14, 15, and 16 only received 1 dose of PCV2 vaccine at approximately 6 WOA.

The protocol was changed so the 1st PCV2 vaccine dose was administered at the sow farm prior to weaning. The next delivery (group 17) was delayed to the nursery to allow thorough cleaning and disinfection of facilities.

Group 17 had 1% nursery mortality with confirmed PCV2 infection on 2 dead pigs. Clinically, about 10% of the pigs were still affected.

Meanwhile in Farms 2 and 3, increased mortality was noted in July 2013 in PCV2-vaccinated weanling replacement gilts about the time of 2nd vaccination. Interstitial pneumonia with lymphoid depletion was present. Immunohistochemistry was PCV2 positive.

Conclusions and Discussion

Vaccination at weaning was not appropriate in these cases as pigs were becoming PCV2-viremic prior to administration of the 2nd dose of PCV2 vaccine. In addition, the missed 1st vaccination in Farm 1 pigs appears to have contributed to earlier onset of PCVAD. The PCV2 vaccination at the sow farm was changed to approximately 10 to 14 days of age with the intent to allow pigs to begin to develop immunity prior to entry into the nurseries. The 2nd dose of PCV2 vaccine was administered at approximately 3 weeks after arrival into the nursery as in the past. To date, these adjustments have allowed control of clinical PCVAD.

Acknowledgements

Appreciation is expressed to Merck Animal Health for their financial support of diagnostic testing and technical expertise as well as to PIC and Dr. Larry Coleman for their collaboration on these cases.

Coinfection of *S. enterica* serovar choleraesuis and PCV2 in pigs with PMWS in Brazil

MR Henriques, FA Vannucci, KCP Reis, LEM Bouillet, WV Guimaraes, DL Santos, LF Santos, JL Santos Microvet
Microbiologia Veterinaria Especial Ltda, Vicosa/MG, Brazil, fvannucci@microvet.com.br

Introduction

Porcine circovirus type 2 (PCV2) and *Salmonella enterica* serovar Choleraesuis are two leading causes of serious economic loss in the swine industry (1). Recently, reports have demonstrated co-infection of *S.* Choleraesuis and PCV2 in the USA (2), Japan (3) and Korea (4). However, so far, concurrent infections of these two pathogens have not been reported in Brazil. The objective of this study was to describe the clinical, microbiological and pathological features involved in the co-infection of *S.* Choleraesuis and PCV2 in animals with clinical signs of PMWS in swine herds in Brazil.

Materials and Methods

Thirty animals at the nursery age (21 to 60 days) from eight different herds in Brazil were submitted to diagnosis with history of typical symptoms of PMWS. At necropsy, total of 141 samples from lung, liver, kidney, lymph nodes and spleen were collection for bacterial isolation, followed by biochemical tests. Serotyping method for characterization of *Salmonella* sp. Isolates was performed using a commercial kit (Probac®). In addition, the samples were evaluated by histopathology and immunohistochemistry (IHC) using streptavidin method with a rabbit polyclonal antiserum to PCV2, as previously described (5).

Results

Non-collapsed lungs associated with varying degrees of interlobular edema and petechial hemorrhage, hepatomegaly, splenomegaly and lymphadenopathy were the macroscopic changes most frequently observed. Histopathological examination of lymphoid tissues showed different levels of lymphoid depletion, histiocytic replacement and, occasionally, follicular necrosis (Figure 1A). Multiple foci of virus-positive histiocytes and follicular dendritic cells were observed in all cases (Figure 1A). Acute interstitial pneumonia with presence of fibrin thrombi in interalveolar capillaries were the histological changes observed in the affected lungs. In the liver, microscopic changes were characterized by multifocal necrotic hepatitis characterized by histiocytic and neutrophilic infiltration sometimes associated with the presence of "paratyphoid nodules" (Figure 1B) and in some cases associated with lymphohistiocytic portal hepatitis.

Colorless to amber bacterial colonies compatible with *Salmonella* sp. grew on Levine EMB agar. These colonies were submitted to biochemical tests which revealed negative results for fermentation of arabinose, dulcitol and trehalose. These results strongly suggested the isolation of *S.* Choleraesuis. However, as a gold standard diagnostic, the serotyping method was performed and confirmed the isolation of *S.* Choleraesuis belonging to the group C1 with somatic antigen 6,7 and flagellar group c. The bacterium was most commonly isolated from lung, liver, spleen and lymph nodes.

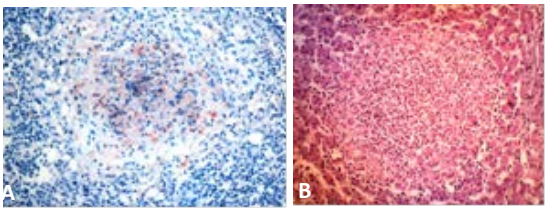

Figure 1. (A) IHC staining. Follicular necrosis in the lymph node of a *S.* Choleraesuis-PCV2 infected pig containing virus-positive cells. (B) HE staining. Paratyphoid nodule in the liver of a *S.* Choleraesuis-PCV2 infected pig

Conclusions and Discussion

Animals from different swine herds in South and Southeast of Brazil with typical signs of PMWS symptoms showed co-infection of PCV2 and *S.* Choleraesuis. Subclinically infected pigs are considered the most common source of new *S.* Choleraesuis infections (1). These findings suggest that the immunosuppression induced by PCV2 in infected animals may increase susceptibility and facilitate the clinical manifestation of *S.* Choleraesuis infection (6).

Acknowledgments

All the colleagues and colaborators from the Microvet Laboratory.

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Study of the molecular profile in strains of *Pasteurella multocida* serotype A from lung lesions in swine

CS Klein[1], R Rebelatto[1], JX de Oliveira Filho[2], MAZ Morés[1], JD Kich[1], N Morés[1]
[1]Embrapa Swine and Poultry, Brazil; [2]Department of Animal Medicine at UFRGS, Brazil. catia.klein@embrapa.br

Introduction

Pasteurella multocida A (*P. multocida*) is a common bacteria isolated from swine respiratory tract. Despite of been considered an opportunistic [5], this pathogen has been associated with lung lesions, pleurisy and pericarditis observed in field outbreaks and experimental challenges [3]. The objective of this study was to evaluate the molecular profile of different strains of *P. multocida* from eight Brazilian States. Studies included information on species-specific genes, capsular typing and virulence genes.

Materials and Methods

A total of 157 *P. multocida* strains, previously characterized, by standard biochemical procedures, like *P. multocida* serotype A were analyzed. The strains were recovered from pneumonic lungs of pigs from farms and slaughterhouses in the eight major pork producer states in Brazil. DNA samples were analysed by multiplex PCR to detect capsular genes type A, D, F and the species-specific *kmt*1 gene [6]. Virulence genes were detected by PCR specific for *toxA* (dermonecrotic toxin), *tbp*A (binding protein hemoglobin), *hgbB* (mechanisms of iron acquisition) and *pfhA* (filamentous hemagglutinin-bacterial adhesion factor) [1]. One amplified fragment from each gene was sequenced, and the specificity was confirmed by the GenBank database using the BLAST tool.

Results

All studied 157 strains were positive for the *P. multocida* species-specific gene *kmt1*. In addition, 94.9% (149/157) were positive for capsule type A and 5.1% (8/157) for the capsule type D. None strains were identified as type F. Investigation of virulence factors, showed no positive results for *toxA* and *tbpA* genes. However, 31.8% (50/157) and 82.8% (130/157) of the strains harbored the *pfhA* and *hgbB* genes, respectively. Only 14% (10/157) of the samples harbored both *pfhA* and *hgbB* genes, therefore, 85.4% (134/157) of strains were positive to a single one of these genes. *P. multocida* positive for *pfhA* and *hgbB* genes, important virulence factors, were identified in all investigated Brazilian states.

Conclusions and Discussion

All strains of this study were verified as *P. multocida* and the capsular serotype A was widely predominant. The high prevalence of hemoglobin binding protein coding by *hgbB* gene suggests that an additional advantage to increase the pathogenicity [7].The *hgbB* gene was detected in the majority of studied strains, even though *P. multocida* does not show visible and classical hemolysis [7]. Possibly, this gene's presence is related to occurrence of lesions. The genetic arrangement of *tbpA* support a possible transpositional recombination event of this genetic locus from non or low pathogenic to highly virulent *P. multocida* strains [2]. Both the *toxA* gene coding the dermonecrotic toxin and the *tbpA* gene, which was previously described factor involved in hemorrhagic septicemia in cattle [2] were not detected. The *toxA*, *tbpA* and *pfhA* as well as the capsule biosynthesis genes are important marker genes to define the pathogenic potential of *P. multocida* strains [2]. The *pfhA* gene codes a protein associated to a bacterial adhesion factor in respiratory tract. This gene was present in 31.8% of strains, in agreement with Ewers [2]. Experimental studies in pigs using different virulence genes profiles are needed to understand their effect in the pathogenicity. In conclusion, the *P. multocida* A was the most prevalent serotype and it is widely distributed in pneumonic lesions in pigs from farms and slaughterhouses in Brazil. In addition, *P. multocida* A strains harbor different virulence related genes.

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Characterization of antigen presenting cells from the porcine respiratory system

MG López-Robles[1], E Silva-Campa[2], J Hernández[1]
[1]Laboratorio de Inmunología, *Centro de Investigación en Alimentación y Desarrollo A.C, Hermosillo, Sonora, México.*
[2]*Departamento de Investigación en Física, Universidad de Sonora, Hermosillo, Sonora, México,* jhdez@ciad.mx

Introduction

In swine, data about respiratory antigen presenting cells (APCs) is scarce (1). The identification and characterization of swine respiratory APCs subsets will contribute to a better understanding of their participation in the immunopathology of porcine respiratory diseases. The aim of this study was to evaluate the phenotypic and functional properties of lung tissue-derived cells (L-Cs), lymph node-derived cells (LN-Cs) and comparing them with bronchoalveolar lavage cells (BAL-Cs).

Materials and Methods

Conventionally healthy pigs of 4 to 6 weeks-old from a PRRSV-free herd were euthanized according to the ethics of the Mexican protocols (3). To collect L-Cs and LN-Cs, lungs and LN were minced into small pieces and digested enzymatically. After, cells were fractionated by gradient separation and cultured overnight to obtain non-adherent cells. BAL-Cs were obtained from airways flushes.

A multiple staining was performed in tree steps: 1) anti-CD11R3 (2F4/11), anti-CD16 (G7), CD1a (76-7-4), anti-CD14 (CAM36A), or anti-CD206 (122D2.08). 2) Goat anti-mouse IgG (H+L) labeled with Alexa Fluor® 488 (A11001). 3) anti-MHC-II (H42A)/R-PE Cy7, anti-CD163 (2A100/11)/Alexa Fluor 647 and anti-CD172a (74-22-15)/R-PE; and in some tubes anti-CD207 (929F3.01)/R-PE Cy5 or anti-DEC-205 (1.F6F6)/R-PE Cy5. For the MLR assay, allogeneic PBLs labeled with CFSE were co-cultured for 72 h with MHC-II$^+$ MACS enriched APCs at different ratios. The uptake of dextran and latex beads by MHC-II$^+$ enriched APCs were evaluated for endocytosis and phagocytosis assays, respectively. Cells were analyzed by flow cytometry.

Statistical analyses were performed by one-way ANOVA. Differences were determined by Bonferroni test or Tukey's test. Statistical analysis were done in PRISM 5.02 software (a = 0.05).

Results

Cells were first analyzed based on FSC/SSC profile and the MHC-II^{high} expression; the subsets were then defined based on CD163 and CD172a expression. L-Cs presented four subsets: CD163$^+$CD172a$^-$, CD163$^+$CD172a$^+$, CD163$^-$CD172a$^-$ and CD163$^-$CD172a$^+$; LN-Cs three subsets: CD163$^+$CD172a$^+$, CD163$^-$CD172a$^-$ and CD163$^-$CD172a$^+$; and BAL-Cs two subsets: CD163$^+$CD172a$^-$ and CD163$^+$CD172a$^+$. L-Cs expressed CD1a^{low}CD206^{low}, LN-Cs CD1a$^{+/high}$CD206^{low} and BAL-Cs CD1a^{low}CD206$^+$. Moreover, functionality by endocytic, phagocytic and MLR were also assessed in MHC-II$^+$ enriched cells. L-Cs and LN-Cs demonstrated the most efficient phagocytosis, endocytosis and MLR abilities.

Conclusions and Discussion

In this study, we identified tree respiratory cell populations which were classified in subsets according to a previously proposed method (2). The phenotypic characterization of APCs subpopulations provides clues about their possible functions. Porcine L-C subpopulations contained low percentages of phagocytic cells, which were consistent with previous reports from human, ovine and mouse lung DCs (5, 6, 7); however, the most efficient were the LN-C CD163$^+$CD172a$^+$ and CD163$^-$CD172a$^+$ subsets. For endocytosis, the three cell types showed high percentages of endocytic cells. These results could be influenced by the elevated expression of uptake receptors in some subpopulations, such as DEC-205 and CD206. In the MRL analysis, L-Cs showed higher lymphocyte activation capability than BAL-Cs, but this was lower than LN-Cs. These data are in agreement with a previous report (1).

This work provides a full description of porcine APC from the porcine respiratory system. We performed the classification of porcine L-Cs and LN-Cs and compared with BAL-Cs. In each population, subsets were differentially distributed and presented different functional abilities. The phenotypic and functional characteristics of the cells suggest that L-Cs and LN-C could be DCs. Further studies are necessary to evaluate the role of L-C and LN-Cs subpopulations in the immune response and the immunopathology of respiratory diseases affecting pigs.

Acknowledgements

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Comparative DTH reaction of piglets vaccinated at weaning with either CIRCOVAC® or a subunit PCV2 vaccine under field conditions

A Callén[1], S Cárceles[1]; H Smits[2], T Vila[2]; F Joisel[2]
[1]*MERIAL laboratorios, S.A., Barcelona, Spain;* [2]*MERIAL S.A.S., Lyon, France;* antonio.callen@merial.com

Introduction

The specific immunological response to pathogens or vaccines involves two pathways: the humoral response and the cell-mediated immunity (CMI). In the field, the serological response to PCV2 vaccines is routinely assessed by ELISA techniques. CMI is not easy to be evaluated on a field routine basis. However, type IV delayed type hypersensitivity (DTH) reactions are known to be mediated by T_H1 cells and CD8 cytotoxic T cells (1) and have been used in other contexts to evaluate CMI after vaccination. Recent reports (2, 3, 4) have shown a potential interest of a test developed on type IV delayed type hypersensitivity (DTH) reaction to evaluate the immune response elicited by a PCV2 vaccination. The aim of the present study was to evaluate whether two PCV2 piglet vaccines were eliciting or not different CMI responses.

Materials and Methods

In a farrow-to-finish farm, 30 piglets weaned at 4 weeks of age were included in the study and randomly allocated to vaccination with 0.5 ml of CIRCOVAC® (Vaccine N, n=15) or with 2.0 ml of a PCV2 subunit vaccine (Vaccine R, n=15). Pairs of piglets vaccinated with each vaccine were constituted and placed in 15 different pens, commingled with piglets from the regular flow either vaccinated with vaccine N, or with vaccine R.

Serum samples were collected in each experimental animal at 4 weeks of age and assayed for anti-PCV2 antibodies by ELISA technique. The DTH reaction was assayed 4 weeks following vaccination (8 weeks of age). The test was conducted by intradermal injection of 0.1 ml of PCV2 antigen as previously described (2). The reaction was measured with a ruler and expressed in cm of diameter. The operator was not informed of the group allocation.

Differences in proportion between groups were tested with a Fisher's Exact test. The statistical analysis of the DTH reaction was performed with a t-test after confirming the normality of the data.

Results

The serological results confirmed the homogeneity in serological status between groups the day of vaccination.

A positive response was observed in 100% of the piglets in group group N whereas only 60% of the piglets in group R displayed a positive DTH reaction (Fisher's Exact test, p=0.008) The mean diameter of the erythema reaction area was significantly higher: 3.43 cm *vs* 1.03 cm for groups N and R respectively (t-test, p<0.0001). See Figure 1.

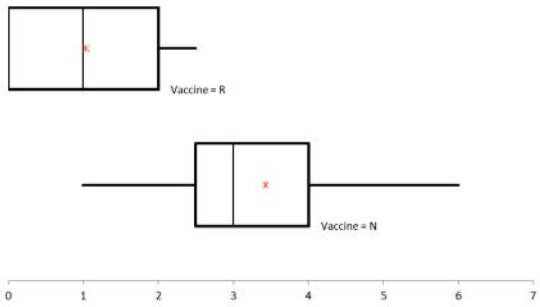

Figure 1. Box-whisker distribution of DTH reaction for the experimental groups.

Conclusions and Discussion

Under the conditions of the study CIRCOVAC-vaccinated pigs displayed a stronger DTH reaction as compared to those vaccinated with the PCV2 subunit vaccine. The results are in favour of higher levels of CMI recalled by the DTH reaction assay in CIRCOVAC-vaccinated piglets.

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Dynamics of the PCV2 delayed type hypersensitivity test response in CIRCOVAC® vaccinated piglets

G Cano[1], A Callén[2], S Carceles[2], A Morillo[1], H Smits[3], O Merdy[3], T Vila[3], F Joisel[3]
[1]*Tests&Trials, S.L., Monzón, Spain;* [2]*MERIAL laboratorios, S.A, Barcelone, Spain;*
[3]*MERIAL S.A.S., Lyon, France;* antonio.callen@merial.com

Introduction

Preliminary trials with a delayed type hypersensitivity (DTH) test based on the use of CIRCOVAC antigen suspension in CIRCOVAC vaccinated piglets in comparison with contemporary placebo injected pigs has yielded promising results (1). In order to better understand and interpret the results and assess the reliability of this test as a compliance tool in practice, it is necessary to study the dynamics or chronological evolution of the response in the animals being tested. Therefore, in this experiment we selected a group of CIRCOVAC vaccinated animals and assessed the reaction at four subsequent timepoints after antigen inoculation.

Materials and Methods

Twenty seven piglets from a commercial PRRS-positive farm were vaccinated IM in the left side of the neck with 0.5 mL of reconstituted CIRCOVAC (MERIAL, Lyon, France) at 3 weeks of age, and 14 of them were simultaneously vaccinated with a *M. hyopneumoniae* vaccine in the right side of the neck. Four weeks later, the pigs were intradermally inoculated with 0.1 mL of the antigen solution of CIRCOVAC in the lower abdomen area. Subsequently, the response to the DTH test was qualitatively and quantitatively evaluated 18 h, 24 h, 32 h and 40 h after the antigen inoculation according to the presence/absence of erythema and/or induration and/or oedema, and the diameter of the reaction was measured by means of a digital caliper. Six animals were biopsied after the 24 h evaluation, therefore reaction was consequently assessed in only 21 animals at 32 h and 40 h post-inoculation.

Results and Discussion

A remarkable reaction (red erythema) was observed at the antigen inoculation point in 27/27 (100%), 24/27 (88.9%), 12/21 (57%) and 0/21 (0%) at 18 h, 24 h, 32 h and 40 h a.i., respectively (Table. 1, Figure 1) whereas only one pig reacted with induration and no single animal showed oedema. The average and standard deviation of the erythema area diameter for those animals showing a positive reaction is shown in Table 1. There was no statistical difference between CIRCOVAC and CIRCOVAC+ *M. hyopneumoniae* groups.

Histological analyses performed in biopsy samples showed a moderate to intense generalized perivascular and periadnexal lymphoplasmatic infiltrate, thus confirming the involvement of a lymphocytic reaction. In addition, some multifocal haemorrhagic areas in the dermis were observed in conjunction with a disruption of the collagen structure and the presence of a reduced number of neutrophils.

Table 1. Characterization of the DTH reaction to the PCV2 antigen inoculation in piglets vaccinated with CIRCOVAC.

	Post-inoculation interval (h)			
	18	24	32	40
Number of . responders/ Total tested (%)	27/27 (100%)	24/27 (88.9%)	12/21 (57%)	0/21 (0%)
Erythema area diameter (mm) Mean±SD	23.0[a] ± 9.2	21.0[a] ±15.2	13,8[b] ±15.7	NA

[a,b]: $p<0.05$, NA: not applicable.

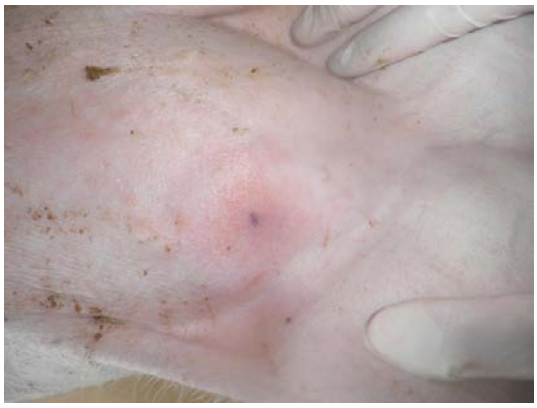

Figure 1. DTH reaction (red erythema) read 24h after ID injection of 0.1mL of CIRCOVAC antigen suspension

Conclusion

According to these preliminary results it looks like the best timepoint to evaluate the DTH response in piglets vaccinated with CIRCOVAC should be chosen from 18 to 24 h after the antigen inoculation.

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Influence of spray-dried plasma (SDP) in starter diets on production parameters associated with a conventional vaccination program for pigs

J Crenshaw[1], J Pujols[2,3], J Segalés[2], J Campbell[1], C Rodríguez[4], J Polo[1,4]
[1] *APC Inc., Ankeny, IA,* [2] *Centre de Recerca en Sanitat Animal (CReSA), UAB-IRTA, Cerdanyola del Vallès, Spain,* [3] *Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Barcelona, Spain,* [4]*APC Europe S.A., Granollers, Spain,*
Joe.Crenshaw@functionalproteins.com

Introduction

Vaccination of pigs against porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* (Mhyo) around weaning is a common practice to prevent or reduce the negative effect of these infections. Vaccination is associated with stimulation of the immune system which may cause fever, reduced feed intake, lethargy, and reduced growth for a few days after vaccination (2).Therefore, the stress caused by weaning, along with the added stress of vaccination, can result in reduced performance during the post-weaning period.

Dietary spray dried plasma (SDP) is well known as a high quality protein source in nursery pig diets. The nutrition provided by diets containing SDP supports and maintains the immune system (1) and may mitigate post-weaning growth lag associated with vaccination and weaning stress.

The objective of this study was to compare, under commercial field conditions, if the inclusion of SDP in PCV2/Mhyo vaccinated pigs at weaning affects performance parameters during the initial two weeks after weaning.

Materials and Methods

An experimental farm located in the Catalonia region (Northeastern Spain) was used in this study. Three hundred sixty-four pigs ((Large white X Landrace) X Pietrain) weaned at 21 days of age (initial BW 5.83±0.90 kg) were randomly distributed into four different treatment groups with 13 pens per treatment (Trt) and 7 pigs per pen. The groups were as follows:

Trt A- Pigs were vaccinated and fed a diet without SDP

Trt B- Pigs were vaccinated and fed a diet with 6% SDP

Trt C-Pigs were injected with a placebo and fed a diet without SDP

Trt D-Pigs were injected with a placebo and fed a diet with 6% SDP.

Basal diets were similar to standard commercial diets for weaned pigs and 6% SDP (AP820P, APC Europe, S. A.) was included in Trt B & D replacing soy protein concentrate on an equal energy and lysine basis. Diets were fed in meal form for 14 days after weaning and were formulated to contain 1.45% total lysine with 3,425 Kcal ME/kg of diet. Pigs in Trt A & B were vaccinated with a Mhyo-PCV2 combined vaccine (Ingelvac CircoFlex and Ingelvac MycoFlex, Boheringer Ingelheim) as a single injection given at day 3 post-weaning. Animals in Trt C & D received a placebo (saline) injection at day 3 post-weaning. Individual pig weight was recorded at 0, 7, and 14 days post-weaning and average daily gain (ADG), average daily feed intake (ADFI) and feed conversion rate (F:G) were calculated for these periods. The effect of vaccination and feeding of diets with or without SDP were analyzed as a completely randomized design using the multivariate analysis procedures (Statgraphics Centurion XV, Warrenton, VA). Least squares means for treatments are reported. The experimental unit was the pen.

Results

Mean performance results for the studied period are shown in Table 1. The addition of SDP in diets fed during the first week post-weaning improved (p <0.05) ADG, ADFI and F:G.

Table 1. Mean performance parameters results by feeding period and dietary treatments.

Treatments	A	B	C	D
SDP	-	+	-	+
Vaccination	+	+	-	-
ADG (g) 0-7d	57.9^{a}	105.8^{b}	69.6^{a}	104.6^{b}
ADFI (g) 0-7d	83.7^{a}	111.5^{b}	86.1^{a}	107.3^{b}
F:G 0-7 d	1.48^{b}	1.08^{a}	1.33^{ab}	1.06^{a}
ADG (g) 7-14d	177.6	180.8	182.6	189.4
ADFI (g) 7-14d	221.8	245.6	224.5	249.1
F:G 7-14d	1.25	1.37	1.28	1.34

(a, b) Superscripts in the same row indicate statistically significant differences within main effect (p <0.05)

Conclusions and Discussion

Results suggest that supplementation of diets with SDP reduces the growth lag associated with weaning stress. This combined Mhyo-PCV2 vaccine had a very mild non-significant negative effect on growth during the first two weeks after weaning.

The data suggest that the inclusion of SDP, especially during the first week after weaning reduced the negative effects associated with weaning stress. Additional performance data and antibody titre development against these two pathogens used in the vaccine will be collected on these animals until slaughter time.

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Safety of the Suvaxyn® CSF marker vaccine in pregnant sows at different stages of gestation

B Alberca[1], S Juanola[1], L Garcia[1], M Mouriño[1], AVila[1], A Aldaz[2], A Urniza[1]
[1]*Zoetis Manufacturing & Research Spain, S.L.*, [2]*Zoetis Canada & La Region*, alicia.urniza@zoetis.com

Introduction

Classical swine fever (CSF) is one of the most devastating diseases to the pig industry and results in serious economic losses worldwide. Despite the availability and efficacy of life attenuated vaccines, these vaccines do not allow differentiation between infected and vaccinated animals (DIVA) by serological diagnosis. The inability of a country to prove the CSF-free status by serosurveillance due to vaccination, leads to restriction in the pig export. To overcome this problem, potent marker vaccines and accompanying diagnostic tools are required. One good candidate to substitute the present vaccines is the live CP7_E2alf vaccine, which proved to be very efficacious (1, 2, 3). Safety is one of the main concerns for live vaccines. The aim of the present study is to evaluate the safety of the CP7_E2alf vaccine (called Suvaxyn® CSF Marker vaccine) in pregnant sows at different phases of gestation.

Materials and Methods

Suvaxyn® CSF Marker vaccine contains the CP7_E2alf virus which is a genetically modified Bovine Viral Diarrhea virus expressing the E2 glycoprotein of CSFV strain Alfort 187 (4). Vaccine was produced by Zoetis Manufacturing & Research Spain, S.L. Animals were vaccinated with one dose of Suvaxyn® CSF Marker vaccine at the maximum antigenic concentration by intramuscular route.

Forty-one pregnant sows were allocated into six treatment groups as follows: Group 1: 2 control sows at 1st phase of gestation (0-38 days of gestation); Group 2: 2 control sows at 2nd phase of gestation (39-76 days of gestation); Group 3: 2 control sows at 3rd phase of gestation (77-114 days of gestation); Group 4: 10 sows at 1st phase of gestation vaccinated with Suvaxyn® CSF Marker vaccine; Group 5: 12 sows at 2nd phase of gestation vaccinated with Suvaxyn CSF Marker vaccine; Group 6: 13 sows at 3rd phase of gestation vaccinated with Suvaxyn® CSF Marker vaccine. Control sows were inoculated at the same time and route with PBS.

Sows were monitored for appearance of any systemic reactions just after inoculation, local reactions at the inoculation sites, rectal temperatures and reproductive parameters (parturition time, abortions, number of piglets born or piglets' health status…).

Blood samples were collected before vaccination and at the end of study from sows and newborn piglets in order to evaluate their serological status.

Results

None of the vaccinated sows presented local or systemic reactions after vaccination. Also, no relevant increase in body temperatures was measured after vaccination.

Vaccination at different phases of gestation had no adverse effect on the pregnancy. Reproductive parameters of the vaccinated sows were comparable to those of the control animals and no abnormalities attributable to the vaccine in the gestation or in newborn piglets were observed.

All the vaccinated sows seroconverted at the end of study and no transplacental transmission was demonstrated since newborn piglets did not present antibodies before ingestion of colostrums.

Conclusions and Discussion

Suvaxyn® CSF Marker vaccine proved to be safe when it is administrated to pregnant sows at different stages of gestation. Therefore, Suvaxyn® CSF Marker vaccine is a promising live marker vaccine candidate to replace the conventional vaccines in the eradication program for classical swine fever.

Acknowledgments

We would like to thank the laboratory technicians and the animal caretakers from Zoetis Manufacturing & Research Spain, S.L. involved in this work.

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Suvaxyn® CSF marker vaccine: Reversion to virulence and viral shedding studies in pigs

B Alberca[1], S Juanola[1], L Garcia[1], M Mouriño[1], AVila[1], A Aldaz[2], A Urniza[1]
[1]*Zoetis Manufacturing & Research Spain, S.L.*, [2]*Zoetis Canada & La Region*, alicia.urniza@zoetis.com

Introduction

Due to the vast economic consequences of classical swine fever (CSF) outbreaks, emergency vaccination plans are under discussion in European Union (EU) Member States. However, animals vaccinated with the conventional C-strain are subject to trade restrictions. To ease these restrictions, research efforts are directed at the design of new modified live marker vaccines. The efficacy and the safety profile of the marker vaccine CP7_E2alf has been demonstrated (1, 2, 3, 4).

Due to the fact that CP7_E2alf vaccine (called Suvaxyn® CSF Marker vaccine) is intended for use as a live vaccine, the absence of reversion to virulence and the transmission of vaccine virus were evaluated. The results of these studies are reported in this abstract.

Materials and Methods

Suvaxyn® CSF Marker vaccine contains the CP7_E2alf virus which is a genetically modified Bovine Viral Diarrhea virus expressing the E2 glycoprotein of CSFV strain Alfort 187 (5).

Study A. Reversion to virulence study after the administration of vaccinal strain of Suvaxyn® CSF Marker vaccine in pigs

Seventeen pigs were included in the study. In the first group (T01), seven pigs were inoculated with vaccine virus by intramuscular route. In the second group (T02), ten pigs were inoculated by oronasal route with 2 ml of the inoculum obtained from the first passage.

Animals were observed for any systemic reactions and clinical signs after inoculation. From days 2 to 7 upon inoculation, animals were bled in order to detect the presence of virus by titration and real-time RT-PCR.

Study B. Non-transmissibility (shedding) study after the administration of vaccinal strain of Suvaxyn® CSF Marker vaccine in pigs

Sixteen pigs were included in the study. Eight pigs were inoculated with vaccine virus by intramuscular route and eight contact controls were mixed 24 hours after inoculation. Animals were monitored for any systemic reactions and clinical signs after inoculation. Forty-five days after vaccination, all the pigs were euthanized and blood samples and tonsil samples were taken in order to detect antibodies against CSF (ELISA test) and the presence of viral genome (real-time RT-PCR) respectively.

Results

In both studies vaccine virus did not induce any systemic reactions and clinical signs after inoculation.

Concerning the reversion to virulence study, no virus was detected neither by titration (cell culture in SK cells) nor by CP7_E2alf specific real-time RT-PCR in the blood and plasma of the animals after the second passage.

Regarding viral shedding study, all the vaccinated animals seroconverted at the end of the study and no antibodies were found in contact controls. Moreover, no virus genome was detected by CP7_E2alf specific real-time RT-PCR in tonsils collected from contact controls.

Conclusions and Discussion

Vaccine strain did not revert to virulence during pig passage. In addition, no shedding or transmission of the vaccine virus was detected in any of the control animals. Therefore, it can be concluded that the spread of the Suvaxyn® CSF Marker vaccine is very unlikely.

Acknowledgments

We would like to thank the laboratory technicians and the animal caretakers from Zoetis Manufacturing & Research Spain, S.L. involved in this work.

The study was funded by the European Union's Seventh Framework Programme for Research (Grant No.: 227003 CP-FP).

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Investigations of the efficacy of an inactivated trivalent swine influenza virus vaccine against European porcine H1N2 viruses

M Schlegel[1], B Hundt[1], T Vissiennon[2], R Zell[3], R Dürrwald[1]

[1]*IDT Biologika GmbH, Am Pharmapark, D-06861 Dessau-Roßlau, Germany,* [2]*Tierpathologie Dr. Vissiennon, Bornasche Straße 81, D-04277 Leipzig, Germany,* [3]*Institut für Virologie und Antivirale Therapie, Universitätsklinikum Jena, Hans-Knöll-Str. 2, D-07745 Jena, Germany,* ralf.duerrwald@idt-biologika.de

Introduction

In the course of several reassortment events the novel swine influenza A virus subtype H1N2 emerged in Scotland in the 1990s and spread over Europe. Its lacking cross-reactivity to bivalent H1N1+H3N2 vaccines required the development of a new trivalent swine flu vaccine.

Materials and Methods

Such a vaccine was developed and licensed in 2010 under the trade names RESPIPORC FLU3 and GRIPOVAC3. This vaccine contains inactivated cell culture grown viruses of subtypes H1N1, H3N2, and H1N2. A substance from the carboxyvinylpolymers group was used as an adjuvant which provides a high safety because it is well tolerated by pigs. A major focus of investigation was the proof of efficacy against heterologous H1N2 field strains in experimental infection trials. Five challenge experiments were conducted on a total of 158 pigs. Three recently isolated field isolates of subtype H1N2 were used for the experiments. The strains were sequenced and analysed phylogenetically. Pigs were vaccinated twice. Vaccinated pigs displayed neutralizing and hemagglutination inhibiting antibodies as early as 7 days after the second vaccination. For proof of efficacy an aerosol method was used in which high doses of field viruses were sprayed by a generator.

Results

Vaccinated animals had almost no clinical symptoms after infection, whereas unvaccinated animals exhibited fever, dyspnea and sleepiness. The viral load in the lungs was significantly lower in vaccinated pigs compared to unvaccinated animals. Lung lesions typical for influenza were observed in the apical parts of medial and cranial lung lobes of the control animals, whereas vaccinated pigs showed no lesions. Moreover, histological investigations revealed a higher degree of inflammation in the unvaccinated pigs.

Conclusions

Vaccinated pigs were protected against all three H1N2 field strains used in the challenge trials.

Acknowledgements

Prof. Dr. Jochen Süss FLI Jena, Dr. B.-A. Schwarz LUA Sachsen Leipzig, Prof. Dr. Roland Zell IVAT Jena and Dr. Théophile Vissiennon Institute of Animal Pathology Leipzig.

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Immune responses against proliferative enteropathy in vaccinated and nonvaccinated pigs after feeding beta-glucan

P Reichel[1], J Soročinová [1], K Kovačocyová,[1] D Mudroňová [2], H Seidel[1], J Novotný[1], R Link[1], M Húska [1], V Macák [1]

[1]*Clinical Department for Swine,* [2]*Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice, Slovak Republic,* peter.reichel@uvlf.sk

Introduction

Porcine proliferative enteropathy is, along with salmonellosis and dysentery of swine, one of the most distinguished diarrheal diseases in pre-feed up and feed up period in swine (3). In present Europe, there are 34 – 67 % of swine and 88 – 100 % of rearings infected with Lawsonia intracellularis and following the latest evidence from 2011, prevalence of this disease is 12 – 50 % resp. 63 % in Slovakia (4). It is generally known that beta-glucans, like matters of natural origin, have potential immune-modulating effects (1). The aim of this study was to compare the results of of active non-specific immuno-modulation in form of feeding food additive containing beta-glucan to pregnant sows and consecutive specific immuno-modulation by vaccination of their sucklings.

Materials and methods

20 sows and their piglets (crossbreeds of Large White and Landrace) were included into the experiment. Sows were divided into two groups and sucklings to four groups (A, B, C, D) depending on intake of beta-glucan in their mothers and piglet vaccination against L. intracellularis (tab. 1).

Table 1. Dividing of experimental pigs

Group	Feeding	Sucklings	Vaccination
Experimental sows n = 10	Foodstuff + Imunol P	A n = 9	+
		B n = 9	-
Control sows n = 10	Foodstuff	C n = 9	-
		D n = 9	+

Experimental sows were fed foodstuff with additive containing of beta-glucan (IMUNOL P) in 5 % concentration from 14th day before parturition until weaning of piglets (28th day), control sows were fed only standard foodstuff. Sucklings were vaccinated with single dose of oral vaccine Enterisol® Ileitis (2 ml) by oral drencher one week before weaning. Collection of biological material was done in sows: 14 days before parturition (0th sampling), during parturition (1st), and during weaning of piglets (2nd). In sucklings the samples were collected: immediately after birth (0th), 14 (1st), 28 (2nd), 35 (3rd), and 60 days (4th) after the birth. Sample analysis: Phagocyte activity (FA) was analysed by commercial set Phagotest® (ORPEGEN Pharma, Germany). Identification of sub-populations of lymphocytes (CD4, CD8, CD4CD8, CD3, CD 21, CD4:CD8) in peripheral was performed by flow cytometry. For testing of oxidative burst of phagocytes (IMA) we used iodine-nitro-tetrazolium test (2). Statistics: evaluation by non-paired t-test and one-way analysis of variance ANOVA (Tukey's multiple comparison test; GraphPad Prism 5.

Results and discussion

Immune-modulating influence of food additive containing beta-glucan (IMUNOL P) was demonstrated in sows only partially in dynamics of immunologic profile indices, especially during parturition period, when significantly higher ($p < 0.05$) values of phagocyte activity were recorded compared with control group, while in the experimental groups of piglets (A, B) significantly higher values ($p < 0.05$, or $p < 0.01$) of FA, IMA at weaning period, or at post-vaccination period were recorded compared with the control groups of piglets (C, D). During the experiment, in all four groups of piglets we noticed decrease of IMA in first sampling and subsequent increase, when significant changes were in groups A, C a D (A: $p < 0.001$; C: $p < 0.01$; D: $p < 0.05$), while in group B we noticed no significance. Comparison of sub-population of lymphocytes showed statistically significant differences between groups only in case of double-positive CD4CD8 lymphocytes, whereas in piglets we noticed the same favourable trend of changes in observed sub-populations of lymphocytes in peripheral blood compared with the control groups.

Acknowledgements

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Survey on pig farmers' current knowledge on important aspects of good vaccination practices under field conditions in the Netherlands

F Vangroenweghe[1], L Broodcoorens[1], K Kelderman[1]
[1]*Elanco Animal Health Benelux, BU Swine & Poultry, Plantijn en Moretuslei 1A, 3018 Antwerp, Belgium*
vangroenweghefr@elanco.com

Introduction

Since 2007, stringent measures to reduce antibiotic reduction by 50% in food producing farm animals, including pigs, have been installed in the Netherlands. New targets have been imposed for the coming years, leading to a 70% reduction by 2015. Due to this antibiotic reduction, a major increase in the use of vaccinations against most currently present swine pathogens, such as *M. hyopneumoniae*, PRRSV and PCV-2, has been observed. To obtain maximal results from the applied vaccination strategies, the vaccine has to be handled with care from production over storage to application to the target animals under practical field conditions.

The aim of the current survey was to obtain data on the current knowledge and practices of pig farmers on the most important principles of the Good Vaccination Practices (GVP).

Materials and Methods

A Monkey Survey was designed with 8 short multiple choice questions on several aspects of vaccine storage, preparation, injection material and finally application of the vaccine to the animals. Fifty responses were collected during an event on intensive animal farming in Hardenberg (The Netherlands).

Table 1. Questions on good vaccination practice

1.	What is the optimal temperature to store vaccines?
2.	Are several persons involved in the vaccine administration?
3.	What is the needle strategy in piglet vaccination?
4.	What to do when vaccines get frozen in the refrigerator?
5.	Where in the neck are vaccines most optimally administered?
6.	What is the time interval to obtain vaccine at room temperature before vaccination of piglets?
7.	What needle specifications do you need for vaccination of piglets at 7 days of age?
8.	How long can half a bottle of vaccine be stored before the rest is used?

Results

Only 80% of the respondents know the optimal storage temperature (2-8°C) for swine vaccines. In 50% of the farms, 2 or more persons are involved in the vaccine administration protocol. According to 22% of the respondents, accidental freezing of vaccines during storage did not have any impact on subsequent immunization of the vaccinated animals. Seventy-eight percent were aware of potential damage of the antigens, which could affect the immunization strategy. Only 52% of the respondents could exactly point out the optimal injection location on the neck (1 finger behind the ear, 1 finger below the neck line) for intramuscular injection in piglets, whereas only 58% responded correctly to the ideal needle specifications (length 9 mm, diameter 0.8 mm) for vaccination of piglets at the age of 7 days. When asked for the optimal time interval to obtain room temperature for a vaccine to be suitable for injection, 76% responded 1 h was sufficient and only 18% chose the correct 5 h interval. Strategies on hygienic needle-management to omit pathogen transfer from one litter to another by needle change per litter were only known by 58% of the respondents. Five percent did only change needles when they were broken, blunt or remained into the pig following injection. Half of the respondents were working with hired farm help, which could increase the risk to make crucial mistake in relation to the basic principles of vaccination strategies. Finally, 62% of the respondents used a previous opened bottle of vaccine within 24 h, although 28% of them would use it during the next week.

Discussion

Good Vaccination Practices are crucial in obtaining optimal results for vaccine to protect animals from the specified diseases. From the present survey, it is clear that basic knowledge on several aspects of Good Vaccination Practice is still lacking under practical conditions. With the enormous expansion of farm size during the last decade, it is clear that more hired people are working on several critical task in swine farms nowadays. The need for regular training on Good Vaccination Practice seems crucial in order to improve the effectiveness of vaccination protocols.

Conclusions

In conclusion, basic knowledge on GVP has still room for major improvement in order to maximize efficacy of applied vaccines under Dutch field conditions.

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Vaccination against edema disease - a field trial

X Sidler[1], S Mattei[1], T Sydler[2], R Fricke[3], W Schmid[4], O Bastert[3], O Lüder[3], A Becker[3]
[1]*Department of Farm Animals, Division of Swine Medicine, Vetsuisse-Faculty Zurich;* [2]*Institute of Veterinary Pathology Vetsuisse-Faculty Zurich;* [3]*IDT Biologika GmbH, Dessau-Roßlau, Germany;* [4]*Achilles-vetclinic.ag, W. Schmid, Rossruti, Switzerland;* xsidler@vetclinics.uzh.ch

Introduction

Edema disease (ED), caused by shigatoxin 2e (Stx2e)-producing *Escherichia coli* (edema disease *E. coli*, EDEC), usually occurs in pigs shortly after weaning but is also seldom observed in older suckling piglets, in growers or in sows. Most of EDEC possess F18ab fimbriae. *E. coli* strains with fimbriae F4, F5, F6, F41 and F18ac are able to produce enterotoxins (heat-stable toxin a (STa) and b (STb), respectively) causing diarrhea, primarily in suckling and weaned piglets. Some *E. coli* strains carry both F18ab and F18ac fimbriae and produce enterotoxins as well as Stx2e.

Colonization with pathogenic *E. coli* requires the attachment of fimbriae to complementary receptors on the epithelium cells of the small intestine. It is speculated that feed induced changes of the receptors are involved in the reduced colonization by F18 positive *E. coli* in suckling piglets. Epithelial cell receptors for F4 or F18 are not present in every pig and these are resistant to infection. The receptor for F18 is controlled by a single locus on chromosome 6, close to the locus of stress susceptibility. The presence of F18 receptors is dominant over absence (1). Thus, strategies for ED prophylaxis are based on dietary management, genetic host selection or vaccination against fimbriae or toxins of EDEC.

The objective of the present study was to evaluate a new recombinant subunit vaccine ECOPORC SHIGA (an adsorbat vaccine containing a genetically modified Stx2e-antigen) (IDT Biologica GmbH, Dessau-Rosslau Germany) for its safety and efficacy in a case-control study in a Swiss farm with severe ED. ED was confirmed by necropsy of dead piglets and EDEC isolation. Also, mortality rate temporarily reached 25% despite antibiotic supplemented feed using Colistin and Amoxicillin in a dosage of 30 grams/100 kg body weight over 7 – 10 days.

Materials and Methods

336 randomly selected piglets of 31 litters were either vaccinated with 1 ml ECOPORC SHIGA (*i.m.)* at the age of 4 days (n=167) or served as placebo treated control animals (n=169) in two runs. Piglets were weaned at the age of 23 and 27 days in the first and second run, without antibiotic supplemented feed in the weaning period. Blood samples were collected twice at the beginning and at the end of the weaning period to examine seroconversion using an in-house serum neutralization test (SNT) (table 1). All dead piglets were necropsied and gut content was bacteriologically investigated.

Results

During the first run, neither adverse reactions to the vaccine nor the occurrence of ED were observed. In the second run, mortality due to edema disease dropped from 13.5% in the control group to 1.4% in the vaccinated group (table 1).

Table 1. Seroconversion and mortality rate (%) of piglets in the vaccinated and control groups.

		Vaccinated (n=167)	Control (n=169)
Run1	23 day pp	81.3*	0
	63 day pp	89.7*	3.2
	Mortality rate (%)	2.4	3.6
Run2	27 day pp	100*	13.2
	55 day pp	92.6*	7.7
	Mortality rate (%)	1.4*	13.5

*p<0.05 (Fisher`s exact-test)

Conclusions and Discussion

In both runs the number of pigs with neutralizing antibodies against Stx2e was significantly higher in the vaccinated group. Vaccination with ECOPORC SHIGA is well tolerated by 4 day old piglets. In the vaccine group a significant reduction in mortality rate was observed. At least, ECOPORC SHIGA is a new and very effective tool to prevent edema disease and to reduce antimicrobial substances in pig production.

Acknowledgments

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Effect of recombinant immunocastration vaccine in male swine production parameters

J Álvarez[1], D Siel[1], M Maino[2], S Vidal[1], L Sáenz[1],
[1] *Laboratory of Veterinary Vaccines VACCIVET,* [2] *Department of Animal Production Development.*
[1,2] *Faculty of Veterinary Medicine, University of Chile,* leosaenz@u.uchile.cl

Introduction

Surgical castration in pig production can decrease levels of aggressiveness of the animals and improve organoleptic characteristics of meat by decreasing levels of testosterone, androstenone and skatole (Walstraa et al., 1999; Ferro *et al.*, 2004). A new recombinant vaccine for immunocastration created in the Faculty of Veterinary Medicine, University of Chile has achieved these same effects, by blocking the hypothalamic-pituitary axis and subsequent secretion of Gonadotropin–releasing hormone (GnRH). It is also consistent with animal welfare avoiding the stress of managing the surgically castrated animals. To evaluate the response of this recombinant vaccine is necessary to obtain specific antibodies generated by the protein, in addition to assessing levels of testosterone and productive parameters in animals.

Materials and Methods

30 male pigs breed PIC (Pig Improvement Company) of 130 days and an average weight of 65 kg, were included in the experiment. Group A corresponds to 20 castrated animals and group B corresponds to 10 non-castrated animals vaccinated subcutaneously with 2 mL of recombinant vaccine (GnRX G/Q) in the area behind the ear (b). The animals were housed in exclusive pens under controlled environment conditions for evaluating feed conversion rate (FCR). For antibody and testosterone measurement, blood samples were collected from the jugular vein, immediately before the immunization (T_0), 21 days (T_1) y 35 days after the immunization (T_2). 36 days post immunization the animals were sacrificed. Indirect ELISA was performed to evaluate antibody response and competition ELISA to evaluate testosterone levels. Statistical analysis was made by analysis of variance (ANOVA, Statistics 17.0 software), comparing data among immunizated v/s non immunizated animals. A p-value ≤0,05 was considered indicative of a statistically significant difference.

Results

The average results for antibodies and testosterone levels determined by ELISA in both groups are shown in table 1. In table 2 is demonstrated an improvement in FCR in vaccine animals respected to surgically castrated pigs (p <0,05).

Conclusions and Discussion

Results in table 1 showns an increase of anti–GnRH IgG in vaccinated non–castrated pigs as compared to surgically castrated group and a decrease in serum levels of testosterone compared to boars. This results demonstrated the immunological effectiveness of the recombinant vaccine, inducing especific antibodies against GnRH.

Table 1. ELISA results of IgG and serum testosterone of barrows, boar and immunocastrated animals.

Antibodies	T_0	T_1	T_2
IgG (Abs 450 nm)			
A	0,361±0,03	0,317±0,03	0,339±0,4
B	0,354±0,01	0,479±0,01**	0,449±0,01
Testosterone (ng/mL)			
A	0,447±0,043	0,457±0,084	0,436±0,056
B	2,961±0,885	4,934±1,523**	2,169±0,989
C	5,6±0,78	6.2±1,3	7.7±0,03

* p ≤ 0.05 between groups. ** p ≤ 0.05 between days.

In Table 2 it can be seen a difference in FCA (8,59%) between immunocastrated and quirurgical castrated animals. There is not a difference in final weight and daily gain, while it can be seen a difference in feed intake of 16,45 kg for vaccinated animals.

Table 2. FCR results for quirurgical castrated and immunocastrated male pigs.

Groups	Initial Weight (Kg)	Final Weight (Kg)	Daily Gain (Kg)	Feed Intake (Kg/pig)	FCR
A	66,6	114,25	0,953	158,48	3,326
B	64,8	111,5	0,934	142,03 (-16,45)	3,04 (8,59%)

Acknowledgments

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Safety and serological response of combined administration Porcilis® ART DF & Porcilis® ColiClos

A Vela

Technical Services ARS Alendi, Zaragoza, Spain, avela@alendi.es

Introduction

In January 2013, a law obligatory for all countries of the European Union became effective for pregnant sows to be housed in groups from 4 weeks of pregnancy until farrowing. This situation causes several management challenges, including vaccination of sows for the passive immunisation of piglets by active immunisation of sows/gilts to reduce mortality and clinical signs caused by diseases such as: atrophic rhinitis, *E. coli* or *C.perfringens*. With this in mind, several studies have demonstrated the efficacy of Porcilis® ART-DF and Porcilis® Coliclos which both are administered at the end of the pregnancy period, when sows are group-housed.

The aim of the present report was to evaluate the safety and serological response of both simultaneous and separate use of Porcilis® ART-DF and Porcilis® Coliclos. (1) (2)

Materials and Methods

Forty (40) atrophic rhinitis free commercial gilts were selected and divided in two groups. They were nine months of age and had not been previously vaccinated against atrophic rhinitis and neonatal diarrhoea.

Group 1: 20 gilts simultaneously vaccinated with Porcilis® ART-DF + Porcilis® Coliclos, with both vaccines mixed in one syringe immediately prior to use.

Group 2: 20 gilts vaccinated with Porcilis® ART-DF and Porcilis® Coliclos, at two different sites with two separate syringes. Both groups received two doses, day 0 and the second one 28 days after. Animals were monitored for local and systemic side effects (6 hours and 24 hours post injection). Blood samples were taken at day 0 (T0), day 28 (T1) and day 43 (T2) and assayed to assess seroconversion for type 987P; K88AB; K88AC; K99 and LT, by Elisa-ECO and for TN-PM-DNT by serum neutralisation test. Statistical evaluation: the individual gilt was the statistical unit. The results were statistically processed with SPSS. Kolmogorov-Smirnov was used to determine normal distribution. Parametric (Paired samples t-test) and non-parametric tests (Wilcoxon) were used to calculate significant difference. Significance was set at 0.05.

Results

No local or systemic reactions were seen in either group post-vaccination. Rectal temperatures were not significantly different before and after vaccination (first vaccination p= 0.195, 2nd p= 0,804).

Post-vaccination serological titters for all *E. coli* antigens were statistically higher at T2 than T0 regardless of method of vaccination (Figure 1, 2).

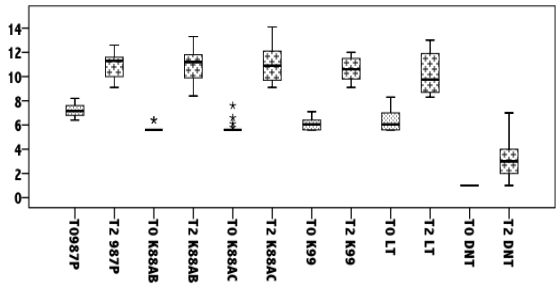

Figure 1. Mean antibody titters per antigen (Log_2), mixed

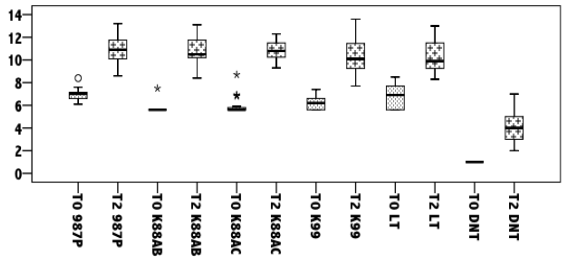

Figure2. Mean antibody titters per antigen (Log_2), two shots

Conclusions and Discussion

The results of this study demonstrate that simultaneous application of Porcilis® ART-DF and Porcilis® Coliclos with one syringe, in which both vaccines are mixed, increased antibodies for different adhesion factors of E. coli and seroconversion against DNT. In addition, no local or systemic reactions were seen.

Although combining Porcilis® ColiClos and Porcilis® ART-DF in one shot is off label use, the results support feasibility of this approach resulting in easier management of vaccinations of group housed sows.

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Evaluation of PRRSV inactivated vaccines against reproductive failure

I Onoda, T Waki, K Yamazaki, T Honda
The Chemo-Sero-Therapeutic Research Institute, onoda-isa@kaketsuken.or.jp

Introduction

PRRSV (porcine reproductive and respiratory syndrome virus) causes reproductive failure in sows, and respiratory disorders in pigs of all age. Attenuated and inactivated vaccines are used to control PRRS, but the effects of these vaccines are not clear. We studied the efficacy of two inactivated PRRSV vaccines against reproductive failure.

Materials and Methods

Vaccines. Two strains of PRRSV (YK09 and N44) were cultured in MA104 cells and inactivated with β-propiolactone (BPL). Vaccines were produced using BPL-inactivated virus and oil-in-water adjuvant. The YK09 vaccine contained 10^7.8 $TCID_{50}$/2 ml YK09 virus antigen, and the N44 vaccine contained 10^8.5 $TCID_{50}$/2 ml N44 virus antigen.

Challenge Virus. PRRSV (YK09 strain) was cultured with MA104 cells, and the supernatant was collected for use as a challenge virus. In a previous study we confirmed that the YK09 strain causes reproductive failure in sows.

Vaccine Evaluation in Pregnant Sows. Pregnant sows (PRRSV free) were intramuscularly administered 2 ml of either the YK09 or the N44 vaccine twice at four week intervals (approx. day 48 and 75 of gestation). Two weeks after the second vaccine administration (approx. day 90 of gestation) sows were intranasally administered PRRSV (YK09 strain, 5×10^5.0$TCID_{50}$). (Table 1)

Viremia was determined by real-time PCR. PRRSV specific antibody titer was determined by ELISA (IDEXX Laboratories, Inc., Westbrook, ME, USA). IFA and virus neutralization tests were also performed.

Table 1. Experimental design

Group	Number	Vaccine strain	Challenge strain
Control	4	-	YK09
YK09	4	YK09	YK09
N44	4	N44	YK09

Results

Mild anorexia and fever were observed after vaccination. Directly before the challenge increased ELISA and IFA antibodies were observed in vaccinated groups, however, neutralizing antibodies were not detected. Fever and anorexia were observed in all groups after the challenge. However, clinical signs were more severe in vaccinated groups, especially the N44 group. Sows farrowed at day 114 of gestation or earlier. In vaccinated groups the piglet survival rate was equal to the control group, and the duration of viremia was reduced. (Fig.1, Table 2)

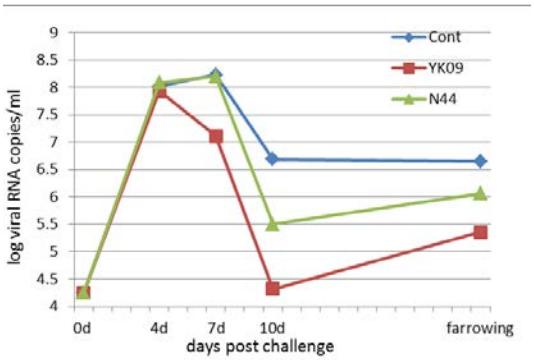

Figure 1. Viremia in pregnant sows

Table 2. Effects on reproduction

Group	Sow No.	Gestation Period (Days)	No. of piglets	
			Live	Stillborn
Control	1	112	10	5
	6	114	3	10
	8	114	13	2
	11	118	4	8
YK09	2	114	7	5
	4	109	5	7
	9	110	7	3
	12	108	8	5
N44	3	113	6	5
	5	109	7	3
	7	113	5	6
	10	115	6	8

Conclusions and Discussion

Although viremia reduction was seen, the vaccines were not effective against reproductive failure in pregnant sows. Clinical signs post challenge were more severe in vaccinated groups. This may be due to adverse effects from the vaccines. Further, as clinical signs in the N44 group were more severe than in the YK09 group, antibody-dependent-enhancement (ADE) may be induced by vaccination.

Parity effect on PCV2 and *Mycoplasma* vaccine performance in a multisite operation in Mexico

J Palacios[1], A Vega[1], R Espejo[1], A García Rendón[1]
[1]*Rancho Covadonga EdoMex.*, Juanmanuelpalacios7@gmail.com

Introduction

Maternal derived immunity (MDI) to PCV2 at high levels could reduce the serologic response and the vaccine performance in the production line, there is a sow effect in which the parity could influence it, in case of Mycoplasma vaccine the MDI effect is not relevant. This is particularly important in the design of early vaccination programs to both antigens. (1,2,3). The objective of the present study was to determine if the MDI from different parities could influence the PCV2/Mh performance vaccine.

Materials and Methods

360 just born piglets were selected from 60 sows from parties 1 to ≥5 (90 piglets by parity), after fostering, all were ear tagged and assigned to three treatments;T1. Mh vaccine at 7 days age and half dose PCV2 vaccine at 21 and 42 days age, T2. Mh/PCV2 combined vaccine at 7 and 21 days and T3. Mh vaccine at 7 days age and full dose PCV2 vaccine at 21 and 42 days age. Sows were bled before farrowing to determine Mh and PCV2 serologic response status, all samples were submitted to laboratory to perform Mh and PCV2 serological test using a commercial ELISA test (IDEXX Laboratories, Inc., Westbrook, ME, USA,and PCV2 Biocheck B.V.) . Pigs were bled and weight individually at 0, 21, 70,122 and 157 days. At slaughter lungs from 20 pigs were evaluated to define consolidation index. Weight and ADWG data were analyzed by variance analysis with the Tukey test, using the statistics software SPSS V. 15.0.

Results

Mean PCV2 ELISA S/P ratios are shown in Figure 1. by treatment and parity even the pigs from first parity react in a lower titre there were no statistically significant differences between treatments, At 156 days age full PCV2 doses react at a higher level than half dose group, in case of early vaccine reaction was similar to half doses but with more variation between parities. Table #1 show productive performance by treatment and parity there were no statistically differences at any stage between treatments.

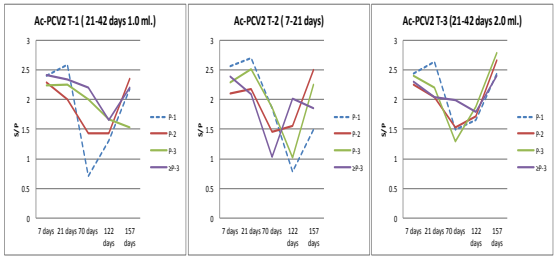

Figure.1. Serologic response to PCV2 in 3 treatments by parity.

Table1. Productive performance, mortality and slaughter lung lesions by treatment.

ADWG (Age days)			
0 -21 Kg. Mean (Std error)	0.237[a](0.005)	0.225[a](0.005)	0.227[a](0.005)
21-70 Kg. Mean (Std.error)	0.446[a](0.007)	0.447[a](0.007)	0.445[a](0.009)
70-122 Kg. Mean (Std. Error)	0.821[a](0.012)	0.804[a](0.01)	0.808[a](0.011)
122-157 Kg. Mean (Std.error)	0.840[a] (0.015)	0.858[a](0.017)	0.849[a](0.019)
21-157 Kg. Mean (Std. Error)	0.682[a](0.009)	0.680[a](0.007)	0.711[a](0.008)
ADWG 21-156 days age by parity			
Parity 1 Kg. Mean (Std error)	0.645[a](0.017)	0.641[a](0.017)	0.652[a](0.016)
Parity 2 Kg. Mean (Std error)	0.705[a](0.017)	0.688[a](0.014)	0.674[a](0.016)
Parity 3 Kg. Mean (Std error)	0.708[a](0.014)	0.704[a](0.013)	0.727[a](0.015)
Parity ≥ 4 Kg. Mean (Std error)	0.695[a](0.013)	0.689[a](0.015)	0.702[a](0.01)
Slaughter weight			
kg Mean (std error)	100.25[a](1.172)	99.75[a](1.209)	100.53[a](1.131)
Std. Dev	12.07	12.09	11.31
Var.Coeff. %	12.03	12.12	11.25
% pigs with ≥ 105 kg BW	41.26	40.17	36.87
Mortality and lung lesions			
Mortality % (Born-Finish)	9.9	15.8	14.0
Slaughter lung lesion %	2.3	2.5	2.6

Different superscripts in the same row indicate statiscally significant differences p≤0.05

Conclusions and Discussion

The three different treatments react in a similar form, MDI only affect the response in the pigs from first parity with no statistical differences in the later stage. Mortality and slaughter lesions were similar in all groups, Early Mh&PCV2 vaccination (T-2) increase it´s mortality but other parameters maintains similar to a convectional 21-42 vaccine scheme. There were no correlation between productive performance and serological response. Alternative to vaccinate in the farrowing house maintain a similar performance with less piglet management.

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Efficacy of the type I PRRSV MLV (UNISTRAIN®PRRS) in infected pigs farms with different PRRSV infection conditions

S Sunwoo[1], S Seo[2], S Ko[1], MH Kim[3], YS Lyoo[1]
[1]*College of Veterinary Medicine, Konkuk University, 120 Neungdong ro, Kwangjin-gu, Seoul, Korea, 143-170,*
[2]*CTCBIO inc., Bio Research Team, 450-34 Noha-ri, Paltan-myeon, Hwaseong-si, Gyeonggi-do, 445-913, Korea,*
[3]*Hipra Korea Inc. #1601, Jeongjail ro 177, Bundang-gu, Seongnam-si, Gyeonggi-do, Korea,* sunwoosy@empal.com

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a major problem of the pig industry in worldwide causing respiratory distress in piglets and reproductive disorders in sows. There are various strategies to reduce economic losses caused by PRRSV. One of the important purposes of those is improving serological defense mechanism and reducing actively circulating virus in affected pigs. In this study, commercial live attenuated type I PRRS vaccine was tested its efficacy under various infected type status of pigs. After vaccination, antibody titers and viremia in serum was measured for evaluation.

Materials and Methods

This study was performed in three farrow-finish farms which have different PRRSV status. Farm A and C had single infection with type I and II PRRSV, respectively. The farm B showed co-infection with both type I and II PRRSV. The piglets used for the experiment were 4 weeks old and the experimental groups consisted of 35 piglets of vaccine and 20 piglets of control. UNISTRAIN®PRRS (HIPRA, Spain) containing VP046 BIS strain; ($\geq 10^{3.5}$ TCID50) was administrated. Blood samples were collected at the prior to injection, 3wpi (weeks post injection, 7 weeks old), 6wpi (10 weeks old), 11wpi (16 weeks old), respectively. Antibody assay against PRRSV was performed with commercial PRRSV antibody test ELISA kit (3X) produced by IDEXX. Neutralizing antibodies in serum samples were also tested. The antigen detection method used PRRSV specific RT-PCR method recommended by QIA (KOREA).

Results

PRRSV specific immune response was monitored by using ELISA test. Pigs in farm A and B showed gradual increase of the PRRSV specific antibody until 11wpi and maintained higher antibody level (S/P ratio) compare to that of control pigs. In farm C animals showed similar antibody response between vaccinated and control pigs. Especially piglets in vulnerable age showed much higher positive rate of antibody to that of control group. The positive rate in 3wpi of farm A, B and C showed 82.8% (vaccinated) vs 72.2% (control), 85.7% vs 50% and 50% vs 15%, respectively. This higher antibody level considered as a very important factor to protect new infection while maternal derived antibodies are diminished.

Virus neutralization test showed more accurate efficacy index in vaccinated animals. Vaccinated pigs in all farms showed much higher VN titers after 3wpi compare to that of the control pigs. Table 1 showed VN titer in each group.

Antigen detection test showed that vaccinated pigs have lower viremia than control pigs. The vaccine reduced viremia not only the type I (EU) PRRSV affected pigs but also type II (NA) affected pigs (Table 2).

Table 1. VN titers of experimental groups after vaccination

Times		0wpi		3wpi		6wpi		11wpi	
Farm		**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
A	**Vac.**	3.24	2.3	2.74	2.7	4.5	3.3	4.38	2
	Con.	3.14	2.6	4.82	3.1	0.44	1.0	1.79	1.4
	P*	0.88		0.02		0.04		0.02	
B	**Vac.**	2.63	1.7	3.34	2.1	4.72	2.9	6.87	2.0
	Con.	2.45	2.1	2.94	1.9	1.25	1.2	4.72	2.0
	P	0.68		0.98		<0.001		0.003	
C	**Vac.**	4.18	2.4	4.26	1.9	6.82	1.6	7.51	1.1
	Con.	5.04	2.5	5.2	1.4	5.44	1.5	6.46	2.7
	P	0.17		0.03		0.001		0.08	

P* : P value

Table 2. PRRSV positive rate by type specific RT-PCR

Farm		Type	0wpi	3wpi	6wpi	11wpi
A	Vac	I	20	34.2	34.2	9.4
	Con	I	10	30	30	5.5
B	Vac	I	0	17.6	57.58	9.1
		II	0	0	0	21.2
		Total	0	17.6	57.58	30.3
	Con	I	5	25	55.5	22.2
		II	0	2.5	5.5	33.3
		Total	5	25	57.9	55.5
C	Vac	II	2.8	32.3	38.2	6.06
	Con	II	0	0	60	5.2

Conclusions and Discussion

The results showed that typeI MLV vaccination elicit steady and uniform immune response and higher antibody titer in ELISA test. In VN test, vaccinated group had significant higher titer than control, especially in highly susceptible period (3wpi~11wpi). In this study shows type I MLV is helpful for development of stabilized immunity level on PRRS infected farms . The antigen detection results suggested that vaccine reduced viremia of type I and typeII PRRSV. This results indicate that type I PRRSV vaccine is effective prevention method in those farms with PRRSVs.

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Efficacy of an experimental vaccine containing chimeric PCV1-2a virus as antigen in a challenge model using a mutant PCV2b strain

J Bubolz, S Dunn, J Johnson, C Lenz, G Nitzel, P Runnels, T Ricker, D Slade, L Taylor
Zoetis Veterinary Medicine Research and Development, Kalamazoo, MI, USA, gregory.p.nitzel@zoetis.com

Introduction

Porcine circovirus 2 (PCV2) is one of the most economically important pathogens affecting pigs worldwide. Although PCV2a was initially the predominant genotype, PCV2b has globally emerged as the most prevalent. Commercial vaccines are based on PCV2a but effective cross-protection against PCV2b has been widely demonstrated. In 2012 a new variant of PCV2b, termed mutant PCV2b (mPCV2b) was isolated in the US and later shown to be nearly identical to a strain previously found in China.[1] Initial concern that the new strain might be associated with lack of vaccine efficacy has decreased based on field experience and publication of data showing cross protection. This paper reports a further cross-protection study, using the chimeric PCV1-2a (cPCV1-2a) virus antigen which is used in several commercial vaccines.

Materials and Methods

Within a subset of a larger study, seronegative pigs were randomly allocated to groups of 10 at 23 to 30 days of age. One group was vaccinated intramuscularly with 2 mL of an experimental, sub potent, monovalent vaccine containing inactivated cPCV1-2a and adjuvant. The second group was administered 2 mL of a placebo vaccine, also containing adjuvant but no cPCV1-2a antigen. The day of vaccination was defined as day 0. Three weeks after vaccination (day 21) the animals were challenged with a mPCV2b strain (1 mL suspension by intramuscular injection, 2 mL intranasally). Blood samples were taken regularly before and after vaccination (days -2, 7, 14, 20, 28, 35 and 42) and checked for PCV2 antibody (ELISA) and PCV2 viremia (quantitative PCR). Fecal swabs were taken on days 20, 28, 35 and 42 and also assessed for PCV2 content by qPCR. Animals were humanely euthanized and necropsied 3 weeks after challenge (day 42) and 3 independent lymph nodes and tonsil were collected and assessed by immunohistochemistry (IHC) and for lymphoid lesions by histopathology. The primary variable for assessment of efficacy was considered to be viremia. Secondary variables were fecal shedding and histopathology. All *in vivo* work was conducted under Zoetis Animal Health IACUC-approved procedures and was in compliance with local, state and national regulations.

Results

All pre-challenge samples were negative for viremia. Viremia post-challenge was significantly reduced in magnitude 14 and 21 days after challenge with peak geometric mean viremia reduced from 384933 DNA copies per mL in controls to 314 in vaccinates. Results from day 20 are shown in Table 1.

Table 1. PCV2 viremia (LS Mean DNA copies/mL)

Study Day	20	28	35	42
Placebo	0	413	384933	50683
Vaccine	0	7	314*	65*

*Vaccinates significantly different than controls ($p \leq 0.0026$) after challenge

Results for fecal shedding (Table 2) mirrored those for viremia with and an approximate 1000-fold reduction in peak PCV2 shedding.

Table 2. Fecal shedding (LS Mean DNA copies/mL)

Study Day	20	28	35	42
Placebo	0	1007	199324	89204
Vaccine	0	881	1880**	65**

**Vaccinates significantly different than controls ($p \leq 0.0063$) after challenge

Histopathological lesions were generally low in both groups in this experimental model and differences were not statistically significant except for IHC ($p \leq 0.0108$), where 60% of placebo pigs showed the presence of PCV2 but none of the vaccinates. ELISA S/P ratios were significantly higher ($p \leq 0.0030$) in vaccinated animals immediately prior to challenge through Day 42.

Conclusions and Discussion

Vaccination using an experimental vaccine containing cPCV1-2a virus as antigen provided effective protection from challenge with the mPCV2 strain, as evidenced by reductions in viremia, fecal shedding and IHC at necropsy. Serology showed a marked anamnestic response following challenge, also indicating immune system recognition of the mPCV2b virus. The results confirm those of other studies indicating cross-protection between PCV2a and mPCV2b.

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Adjuvant selection for the development of a combined PCV2 and *M. hyopneumoniae* vaccine

J Bubolz, D Fredrickson, G Nitzel, V Rapp-Gabrielson, T Ricker, P Runnels, L Taylor
Zoetis Veterinary Medicine Research and Development, Kalamazoo, MI, USA, gregory.p.nitzel@zoetis.com

Introduction

Killed vaccines routinely contain adjuvants, which enhance the immune response to vaccination by influencing the way in which antigens are presented to the immune system. Different adjuvants, however, have unique properties, which can impact immune response and efficacy. The most appropriate adjuvant for one antigen may not be the optimal selection for another and this must be taken into account when formulating polyvalent vaccines. This abstract summarizes two studies undertaken to help guide adjuvant selection during the development of a ready-to-use, single dose, combination vaccine against PCV2 and *Mycoplasma hyopneumoniae* (M.hyo).

Materials and Methods

Three experimental bivalent vaccines were produced in unique adjuvant systems: T02, T03 and T04. Each vaccine contained identical quantities of inactivated chimeric PCV1-2a (cPCV1-2a) virus and M.hyo antigens. Separate challenge studies were conducted to assess efficacy of the PCV2 and M.hyo components in experimental bivalent vaccines. Results in Study 1 and Study 2 are subsets of larger studies.

In the PCV2 study (Study 1) piglets were randomly allocated to groups of 16 animals and each group was vaccinated with one of the experimental vaccines or saline control (T01) when piglets were approximately 3 weeks of age (day 0). Three weeks post-vaccination, pigs were challenged with virulent PCV2 and necropsied 21 days following challenge. Efficacy variables assessed were viremia (qPCR), shedding in fecal and nasal samples (qPCR), post-mortem lymphoid tissue pathology and PCV2 colonization (IHC).

In the M.hyo study (Study 2) piglets were similarly allocated to groups of 16, although 4 pigs were removed early for reasons unrelated to the study. Pigs were vaccinated at 3 weeks of age and challenged 3 weeks later. Necropsy was performed 4 weeks after challenge and the percentage of lung lesions was the primary outcome. Blood samples for serology were taken prior to vaccination, challenge and slaughter. All *in vivo* work was conducted under Zoetis Animal Health IACUC-approved procedures and was in compliance with local, state and national regulations.

Results

Results for PCV2 viremia are shown in Figure 1. All vaccine groups had significantly lower ($p \leq 0.0000$) PCV2 DNA copy numbers than T01 at all time points post-challenge. At days 28 and 35 T02 and T04 were significantly lower ($p \leq 0.0202$) than T03. All vaccines were significantly different ($p \leq 0.0059$) than T01 for shedding (fecal and nasal), lymphoid depletion and PCV2 colonization (IHC).

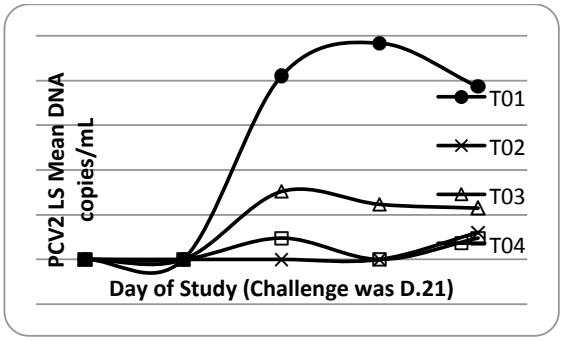

Figure 1. Viremia Results (PCV2 Quantitative PCR)

Results for M.hyo induced lung lesions in Study 2 are shown in Table 1. Due to the high variability of lung lesions and insufficient animal numbers, the overall treatment effect was not significant. Despite this, substantial numerical reductions were observed for T02 and T03 compared to T01. The T04 group did not appear to elicit any biologically meaningful reduction of lung lesions.

Table 1. Back transformed LSM Percent Lung Lesion[3]

Treatment	No. pigs	Percent lung lesions	P value v T01
T01	14	13.1	NA
T02	14	4.3	0.09
T03	16	4.7	0.10
T04	16	12.0	0.87

[3]Overall treatment effect not significant (p=0.1152)

Conclusions and Discussion

The results confirm that adjuvants may differ in their suitability for different antigens. Based on the above studies and others, the adjuvant used in T02 was selected as the most appropriate adjuvant for the development of the Fostera™ PCV MH vaccine.

Efficacy of the *M. hyopneumoniae* fraction of a combined PCV2-M. hyo vaccine in the presence of different amounts of chimeric PCV1-2a antigen

D Fredrickson, J Johnson, G Nitzel, V Rapp-Gabrielson, P Runnels, M Stephenson, L Taylor, J Toepfer
Zoetis Veterinary Medicine Research and Development, Kalamazoo, MI, USA VMRD, Zoetis, Kalamazoo, MI, gregory.p.nitzel@zoetis.com

Introduction

Vaccination against porcine circovirus 2 (PCV2) and *Mycoplasma hyopneumoniae* (M.hyo) is common practice in pig production. Fostera™ PCV MH (Zoetis) is a ready-to-use, bivalent vaccine designed to help protect pigs against both these diseases with a single dose administered from 3 weeks of age onwards. It contains inactivated chimeric PCV1-2a (cPCV1-2a) virus and cell-free M.hyo antigens. To confirm the efficacy of the M.hyo fraction, and the absence of interference from the PCV2 fraction, an M.hyo challenge study was conducted using experimental vaccines formulated with varying concentrations of cPCV1-2a antigen.

Materials and Methods

One hundred and fifty, healthy, M.hyo negative pigs were selected at 20-23 days of age (day 0) and randomly allocated to 5 treatments (n=30/treatment). Ten additional pigs were used as sentinel pigs to confirm freedom from disease. The pigs were housed in mixed treatment pens of up to 12 pigs per pen and vaccinated with 2 mL (IM) of test product at approximately 3 weeks of age (day 21). Treatments T02 to T04 were administered experimental Fostera PCV MH-like vaccines, all formulated with the same amount of the cell-free M.hyo antigen, but at a low potency to maximize the chance of detecting interference. The bivalent vaccines (as illustrated in Table 1) contained different amounts of cPCV1-2a antigen to assess potential interference. T01 contained only cPCV1-2a antigen (at T02 level) and acted as a negative control for the M.hyo challenge. T05 contained only M.hyo antigen. Three weeks after vaccination all pigs were challenged on 2 consecutive days by intra-tracheal infusion with 10 mL of a diluted lung homogenate containing live, virulent M.hyo. Prior to challenge the sentinel pigs were necropsied to confirm freedom from M.hyo. All other pigs were necropsied 28 days after challenge when lung lesions were scored, bronchoalveolar lavage fluid collected for M.hyo PCR and lung tissue taken for immunohistochemistry (IHC). Blood samples were collected for M.hyo antibody titers (IDEXX S/P ratio) on day 0, prior to challenge (day 19) and at necropsy (day 48 or 49). The primary variable for efficacy was lung lesions. All *in vivo* work was conducted under Zoetis Animal Health IACUC-approved procedures and was in compliance with local, state and national regulations.

Results

Prior to challenge, all NTX pigs were M.hyo negative by PCR and IHC. Lung lesion results are presented in Table 1. Compared to the negative controls (T01) treatments T02 to T05 demonstrated a significant reduction in least squares mean lung lesion score ($P \leq 0.0001$).

Table 1. Least squares mean percentage of total lung with lesions by treatment.

Treat-ment	N	Antigen content	Percent lung lesion	P vs. T01
Sentinel	10	None	0.03	
T01	30	High cPCV1-2a only	7.01	
T02	30	High cPCV1-2a/M.hyo	1.66	<0.0001
T03	29	Int. cPCV1-2a/M.hyo	1.11	<0.0001
T04	30	Low cPCV1-2a/M.hyo	2.33	<0.0001
T05	30	M.hyo only	1.67	<0.0001

Serology results are presented in Table 2. All pigs were M.hyo seronegative (S/P ratio <0.3) on day 0 and remained so prior to challenge, although groups T02 through T05 had significantly higher ($P<0.05$) IDEXX ELISA antibody titers than T01 at this time. At necropsy all pigs were seropositive for M.hyo with treatments T02 through T05 having a significantly higher ($P\leq0.0001$) antibody titers compared to T01. At necropsy 100% of challenged pigs in all treatment groups were M.hyo positive by PCR and ≥93.3 positive by IHC.

Table 2. M. hyo antibody titers (IDEXX S/P ratio)

Treatment	Day 0	Day 19	Day 48/49
T01	-0.012	-0.054	0.734
T02	-0.016	-0.042	1.115
T03	-0.009	-0.037	1.016
T04	-0.008	-0.036	1.104
T05	-0.012	-0.037	1.231

Conclusions and Discussion

Under conditions of this study all the M.hyo containing experimental vaccines were effective in reducing the percent of lung with lesions compared to the negative controls, confirming the efficacy of the cell-free M.hyo fraction used in Fostera PCV MH and its compatibility with the cPCV1-2a virus used to help protect against PCV2. Serology results confirmed that pre-challenge sero-conversion is not necessary for efficacy against M hyo.

Impact of Porcilis® PCV on production parameters in a Vietnamese swine farm

DM Nhat[1]; DD Phong[1]; PH Thanh[2]; PK Hoang[2]; R Jolie[3]
[1]MSD Animal Health, Vietnam; [2]Hoa hoi Farms, Vietnam;
[3]Merck Animal Health, Summit, NJ, USA rika.jolie@merck.com

Introduction

PCV2 remains one of the most important disease for domestic swine worldwide, causing significant economic losses to the pig industry.[1] PCV2 viremia may lead to growth performance reduction and lack of uniformity of slaughter pigs.[2] Results from experimental and field studies indicate that PCV2 vaccination is able to improve production parameters such as average daily gain (ADG), percentage of runts, body condition and carcass weight in PCV2 clinical and subclinical infection scenarios.[1]

The objective of this study was to evaluate the effect on various production parameters of Porcilis PCV, a one dose vaccine, in a Vietnamese farm.

Materials and Methods

The trial was conducted in Hoa Hoi Swine Farms in Ba Ria Vung Tau province, Vietnam. A total of 80 piglets originating from 20 sows, not vaccinated against PCV (4 piglets/sow) were assigned to either vaccinated or control group. Between 19 to 32 days of age (Phase 1) and 32 to 165 days of age, piglets were housed at GP and PS farm, respectively. At 19 days of age, piglets were vaccinated intramuscularly with 2 mL of Porcilis®PCV (batch number: A25501) and controls were injected with 2 mL saline. Each individual pig was weighed at 19, 25 (weaning), 70 and 165 days of age. A blood sample for PCV2 qPCR was collected at 70, 91 and 126 days of age. Morbidity, treatment rate, mortality and cull data were recorded and summarized between 25-70, 70-165 and 25-165 days of age. Average daily gain (ADG) was calculated for the same time periods. The same trial was repeated twice with 1 week apart.

Results

Although weight differences between vaccinated and control pigs were small up to 70 days of age, vaccinated pigs were 5.77 kg heavier than controls at 165 days of age and had a higher percent pigs >86 kg (Table 1). In addition, vaccinated pigs also had a higher ADG than controls between 70-165 days of age and over the whole observation period (Table 2).

Table 1. Distribution of weight (kg), % pigs per range.

	<65	65-75	76-85	86-95	>95
V	1.3	18.2	35.1	32.5	12.9
C	15.5	22.4	31.1	22.4	8.6

Table 2. Average daily gain (g) within age range (days).

	25-70	70-165	25-165
V	357	630	547
C	368	490	490

Morbidity and treatment rate was 77.3% and 22.7% in vaccinated and control pigs, respectively. In both groups, pneumonia and diarrhea were the primary cause of disease.

At any time, mortality was higher in controls than vaccinated pigs (Table 3) and was mainly the result of pneumonia.

Table 3. Dead and Cull (%) within age range (days)

	25-70	70-165	25-165
V	0	4	4
C	4	25	28

By 91 days of age, all pigs were viremic but the level of viremia in vaccinated pigs was minimal compared to the controls.

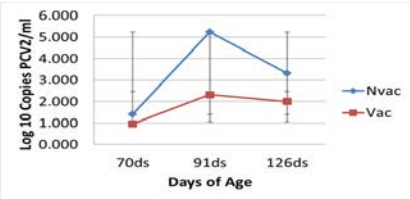

Figure 1. Viremia results, measured by Qpcr

Conclusions and Discussion

The results from this study clearly support that one dose of Porcilis PCV reduces morbidity, PCV2 viremia and mortality. In addition, vaccination positively impacts ADG and improves uniformity of the group by 165 days of age. In summary, vaccination with Porcilis PCV not only reduces reduces clinical signs and viremia, but also improves production parameters, resulting in increased return on investment.

Acknowledgments

The staff at Hoa Hoi Swine Farms for their assistance in this trial.

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Comparative field study of an intradermal *M. hyopneumoniae* vaccine Porcilis® M Hyo ID Once against an intramuscular competitor vaccine

S Chouet[1], E Cordeau[1], G Graur[1], R L'Helgoualch[1], C Trombani[1], D Roudaut[2], L Volant[2], M Rigaut[2]
[1] CATERCO, Changé, France [2] MSD, Beaucouzé, France
sylvie.chouet@cam.fr

Introduction

Different vaccination protocols are used worldwide against *Mycoplasma hyopneumoniae* (M.h) in pigs. They have in common the indication: reduction of lung lesions due to M.h infection.

The objective of this study was to compare two vaccination routes (intramuscular and intradermal) in a comparative field trial.

Materials and Methods

This multicentric, comparative, contemporary, controlled and randomized study was conducted on two consecutive batches in each of four farms in Western France to evaluate two M.h vaccines administered at 3 weeks of age : an intramuscular protocol (Stellamune® Mono Injection (Stel)), and an intradermal vaccination (Porcilis® M Hyo ID once (Porc) with IDAL® injector).

Among the farms, 4, 5 and 21 batch managements are represented, with 2 farms of 150 sows, 1 of 350 productive sows and 1 farm of 550 sows. The presence of M.h, PRRS, Influenza (SIV), and *Actinobacillus pleuropneumoniae* (App) was serologically tested on 10 pigs at the end of fattening in each farm. With respect to season and batch effects, all farms were found positive for M.h, 3 for SIV and App, and 1 for PRRS.

A total of 2882 piglets were randomized into 2 groups at the age of 21 days (Table 1). Groups were identified at inclusion with a tattoo. No changes were applied to the management; animals of the 2 groups were comingled.

At slaughter, a total of 2110 carcass weights were obtained, and 1063 lungs scored for lesions. Carcass weights were used to calculate an average daily weight gain (ADG). Pneumonia lesions were scored on a scale of 24[1]. Pneumonia scars and pleuritis were scored present or not.

ADG was tested with an ANOVA. The prevalence of lung lesions was analyzed with a Mantel-Haenszel, and the pneumonia scores by a non-parametric test.

Results

The results are summarized in Table 1.

Table 1. Growth and lung lesions

	Stel	Porc	p
ADG (g/d)	609	606	0.741
% lesion-free lungs	56.7	57.2	0.201
% mild pneumonia lungs (scores 0, 1 and 2)	84.3	79.8	0.363
% severe pneumonia lungs (score > 5)	7.1	8.2	0.894
Pneumonia score of all lungs	1.3	1.5	0.655
Pneumonia score of affected lungs only (score > 0)	3.1	3.4	0.047
Prevalence of pneumonia scars	3.4	4.8	0.953
Prevalence of pleuritis	2	1.9	0.265

The main criteria chosen for this study (ADG, pneumonia lesion prevalence) were not different between groups. The secondary criteria were also not different, with exception of the average score of pneumonia on affected lungs that was in favor of Stel, even if the median score was 2 in both groups.

Discussion

The farms and animals in this study had a high respiratory health status compared to typical French swine farms. Although neither ADG nor lung lesion scores were different between the vaccine groups, the farmers appreciated the ease and safety of intradermal vaccination with IDAL® injector and to not have to manage needles. Overall, IDAL makes vaccination of pigs easier and improves the quality of injections, resulting in better vaccination compliance.

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Field safety of a PRRS MLV vaccine administered once intramuscularly to pigs of 1 day of age

RG Ankenbauer, ML Keith, TL Martin
Zoetis Veterinary Medicine Research and Development, Kalamazoo, MI, USA Bob.Ankenbauer@Zoetis.com

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is a highly infectious RNA virus, which is endemic in almost all pig producing areas of the world. The optimal timing for PRRS vaccination in growing pigs depends on farm circumstances. In PRRSV negative sow farms vaccination is usually conducted after pigs have been weaned and moved to another site, to avoid introducing a live vaccine strain to a negative farm. On PRRSV positive sow farms, however, piglets are often vaccinated pre-weaning to give more time for immunity to develop before natural challenge occurs. In initial studies with Fostera™ PRRS (Zoetis) the vaccine was administered to piglets at 3 weeks of age. To provide veterinarians with maximum flexibility when designing vaccination protocols, further studies were conducted to show the safety and efficacy of Fostera PRRS when administered to 1 day old piglets, based on the principle that data generated in the youngest possible animals would be relevant to use at any age after this time. This paper describes a study to evaluate the safety of Fostera PRRS when administered to 1 day old pigs under field conditions.

Materials and Methods

Three hundred healthy 1 day old pigs from 30 sows on a 3,300 sow commercial farm were randomly allocated to 3 treatments. One hundred were given 2 mL IM of a placebo (sterile water) and 2 groups of 100 were given 2 mL IM of Fostera PRRS, using a different vaccine batch for each group. The pigs were not vaccinated against any other disease and had no symptoms of respiratory disease. Clinical observations were made and undesirable reactions such as depression, anorexia, dyspnea, diarrhea, and "other" were recorded for each pig within 5 hours following vaccination and 1 day post-vaccination (day 1). Injection sites were observed and palpated on days 1 and 8 post-vaccination. Designated site staff observed animals at least once daily and general health observations were recorded from day 2 through study closeout at day 21, with follow-up if abnormalities were observed. On days 8, 13 and 21 specific clinical observations, including depression, respiratory distress, cough, body condition and "other" were observed for all pigs.

Results

1. Clinical observations days 0 and 1 post-vaccination

The number of abnormal clinical observations was low in all groups (Table 1). Abnormal signs included diarrhea, anorexia and other. Observations of "other" were described as death, laid-on or lameness.

Table 1. Number of abnormal clinical observations at either day 0 (D0) or day 1(D1) following vaccination.

	Total abnormal		Diarrhea		Anorexia		Other	
	D0	D1	D0	D1	D0	D1	D0	D1
Placebo	0	4	0	0	0	1	0	3
Fostera PRRS(1)	1	2	0	2	0	0	1	0
Fostera PRRS(2)	0	5	0	4	0	0	0	1

2. Weekly abnormal clinical health observations

Forty eight animals were recorded with abnormal health observations during the weekly checks. These were evenly spread across the 3 treatments – 15 in the placebo group and 17 and 16 for the 2 vaccinated groups. The most common clinical signs were diarrhea, scrotal hernia and unthriftiness and there were no pigs with depression, coughing or signs of respiratory distress.

3. Injection site reactions

No injection site reactions were observed on day 1 post-vaccination. One placebo pig was observed with a minor injection site reaction on day 8. The reaction resolved by day 13.

4. General health observations

During the daily general health observations 72 pigs were observed with abnormal health conditions, evenly spread across the 3 treatments (Table 2).

Table 2. Number of animals with abnormal health observations during the daily health observations.

	Gastro-intestinal	Musculo-skeletal	Respiratory	Other
Placebo	8	0	1	16
Fostera PRRS(1)	8	2	0	13
Fostera PRRS(2)	9	1	1	17

Conclusions and Discussion

Field safety of Fostera PRRS was assessed in 1 day old commercial pigs in comparison with a placebo. The incidence of abnormal health events was low and not distinguishable between vaccinates and controls. The ability to administer Fostera PRRS at any time from 1 day of age provides veterinarians with flexibility when designing vaccination protocols.

Efficacy of a PRRS MLV vaccine administered at 1 day of age

RG Ankenbauer, ML Keith, TL Martin, LP Taylor
Zoetis Veterinary Medicine Research and Development, Kalamazoo, MI, USA Bob.Ankenbauer@Zoetis.com

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is a highly infectious RNA virus, which is endemic in almost all pig producing areas of the world. The optimal timing for PRRS vaccination in growing pigs depends on farm circumstances. In PRRSV negative sow farms vaccination is usually conducted after pigs have been weaned and moved to another site, to avoid introducing a live vaccine strain to a negative farm. On PRRSV positive sow farms, however, piglets are often vaccinated pre-weaning to give more time for immunity to develop before natural challenge occurs. In initial studies with Fostera™ PRRS (Zoetis) efficacy was demonstrated in piglets vaccinated at 3 weeks of age.[1] In order to provide veterinarians with maximum flexibility when designing vaccination protocols, further studies were conducted to show the safety and efficacy of Fostera PRRS when administered to 1 day old piglets, based on the principle that data generated in the youngest possible animals would be relevant to use at any age after this time. This paper describes a study designed to evaluate the efficacy of Fostera PRRS in 1 day old pigs using a virulent heterologous PRRS challenge strain (NADC20) 7 weeks after vaccination.

Materials and Methods

Forty-eight 1 day old pigs from PRRS negative sows were given either 2 mL IM of a placebo vaccine or 2 mL of Fostera PRRS at minimum potency. The trial pigs were not vaccinated against any other disease and were free of symptoms of respiratory disease. The pigs were weaned at 3 weeks of age and housed in pens of 8 until challenge at 7 weeks of age. Until challenge the 2 treatments were housed in separate rooms to avoid possible viral shed from PRRS vaccinates to control pigs. The day prior to challenge the pigs were re-housed in 1 room of 24 pens with 2 pigs of the same treatment per pen. Treatments were not co-mingled for challenge to avoid fighting and stress common in this age of pigs. All pigs were challenged (1.0 mL per nostril and 2 mL IM) at 7 weeks of age with a virulent PRRSV isolate NADC20 at 2.8 $\log_{10}TCID_{50}$/4 mL and were necropsied 10 days post-challenge. At necropsy, lung lesions were scored by visual assessment and manual palpation so that the percentage of consolidation for each lobe (left cranial, left middle, left caudal, right cranial, right middle, right caudal, and accessory) was recorded. Results are presented as back-transformed least squares means and were analyzed using a general linear mixed model. Clinical signs were monitored regularly throughout and blood samples were collected for testing using the IDEXX PRRS ELISA. All work was approved by the site Animal Care and Use Committee.

Results

The placebo pigs remained PRRSV negative until after challenge. Post-vaccination clinical signs and rectal temperatures were unremarkable. Following challenge a higher percent of placebo pigs showed clinical signs than Fostera PRRS vaccinated pigs (Figure 1). Mean body weights at weaning were similar (6.5 and 6.1 kg for placebo and PRRS vaccinated groups) but at challenge and necropsy the PRRS vaccinated pigs were significantly heavier ($P<0.05$) (13.7 vs. 17.5 kg and 19.7 vs. 23.7 kg). All pigs appeared healthy at challenge but the groups had been housed in different rooms.

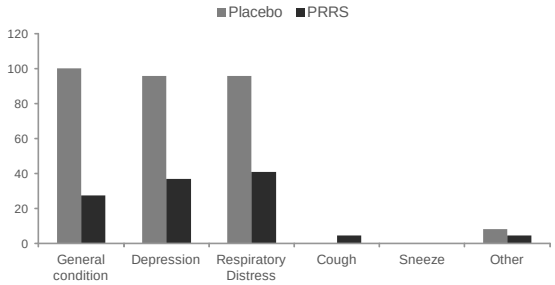

Figure 1. Percent of animals ever observed with clinical signs of any severity following challenge.

At necropsy the lung lesions in the Fostera PRRS vaccinated pigs were significantly lower ($P<0.0001$) than in the placebo vaccinated pigs (Table 1)

Table 1. Percent lung showing lesions

Placebo	43.9%
Fostera PRRS	0.7%

Conclusions and Discussion

The primary variable in determining efficacy for this study was percent lung lesions. Vaccination of seronegative piglets with Fostera PRRS, at 1 day of age, reduced lung lesions by 98%, confirming that neonatal pigs are immunologically capable of responding to vaccination with an MLV PRRS vaccine. Knowledge that Fostera PRRS can be used in piglets from 1 day of age provides veterinarians with maximum flexibility when integrating PRRS into their vaccination protocols.

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26-week duration of immunity of a PRRS MLV vaccine in 1 day of age pigs

RG Ankenbauer, ML Keith, TL Martin, LP Taylor
Zoetis Veterinary Medicine Research and Development, Kalamazoo, MI, USA Bob.Ankenbauer@Zoetis.com

Introduction

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Materials and Methods

Forty-eight 1 day old pigs from PRRSV negative sows were given either 2 mL IM of a placebo or 2 mL of Fostera PRRS at minimum potency. The trial pigs were not vaccinated against any other diseases and were free of symptoms of respiratory disease. The pigs were weaned at 3 weeks of age and housed in pens of 8 until challenge at 26 weeks of age. Until challenge the 2 treatments were housed in separate rooms to avoid possible viral shed from PRRS vaccinates to control pigs. The day prior to challenge the pigs were re-housed in 1 room of 24 pens with 2 pigs of the same treatment per pen. Treatments were not co-mingled for challenge to avoid fighting and stress common in this age of pigs. All pigs were challenged (1.0 mL per nostril and 2 mL IM) at 26 weeks of age with a virulent PRRSV isolate NADC20 at 2.1 $\log_{10}TCID_{50}$/4 mL and were necropsied 10 days post-challenge. At necropsy, lung lesions were scored by visual assessment and manual palpation so that the percentage of consolidation for each lobe (left cranial, left middle, left caudal, right cranial, right middle, right caudal, and accessory) was recorded. Results are presented as back-transformed least squares means and were analyzed using a general linear mixed model. Clinical signs were monitored regularly and blood samples were collected periodically for testing using the IDEXX PRRS ELISA. All work was approved by the site Animal Care and Use Committee.

Results

The anti-PRRS ELISA titers are shown in Table 1. The placebo pigs remained negative until after challenge.

Table 1. Anti-PRRS ELISA titers at various stages pre- and post-challenge at 182 days of age.

	Days of age				
	23	51	107	182	194
Placebo	0.000	0.004	0.018	0.024	1.654
Vaccinates	2.375	1.971	1.659	1.183	2.336

Post-vaccination clinical signs and rectal temperatures were unremarkable. Following challenge a numerically higher percentage of placebo pigs showed clinical signs than Fostera PRRS vaccinated pigs (41.7 v 12.5) but most pigs remained clinically normal. There were no significant differences in body weight between treatments, either at challenge or necropsy, 140.4 vs. 141.3 kg and 136.7 vs. 138.3 kg for placebo and PRRS vaccinates at the respective time points. At necropsy the lung lesions in the Fostera PRRS vaccinated pigs were significantly lower ($P<0.0001$) than in the placebo pigs.

Table 2. Percent lung showing lesions

Placebo	17.7%
Vaccinates	1.2%

Conclusions and Discussion

The primary variable in determining efficacy for this study was percent lung lesions. Despite the virulence of the challenge strain marked clinical signs are not expected in otherwise healthy older pigs. Vaccination of seronegative piglets with Fostera PRRS, at 1 day of age, reduced lung lesions by 93% and confirmed a duration of immunity of 26 weeks against a heterologous challenge. Knowledge that Fostera PRRS can be used in piglets from 1 day of age, and that vaccination at this age can provide immunity up to the typical age of slaughter for fattening pigs, provides veterinarians with maximum flexibility when integrating a PRRS vaccine into their vaccination protocols.

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Impact of vaccinating sows against Atrophic Rhinitis on the lesion score observed on the snouts of pigs

I Corrégé[1], A Hémonic[1], E Sallé[2],
[1] IFIP - French Institute for Pig and Pork Industry, Le Rheu, France, [2] MSD Santé Animale, rue Olivier de Serres - BP 171, 49071 Beaucouzé Cedex, France "2014-MS-0509", isabelle.correge@ifip.asso.fr

Introduction

Atophic Rhinitis can lead to reduced daily gain, poor body condition and variable growth, due to difficulty in eating. It may also increase the risk of infection by respiratory diseases. Vaccination of sows against Atrophic Rhinitis aims to protect piglets by passive immunity transmitted via the colostrum. The prevalence and intensity of Atrophic Rhinitis is often assessed by scoring nasal lesions (1). The objective of this study was to compare the rhinitis lesions scored on the snouts of pigs according the vaccine status of their mother.

Materials and Methods

Two batches (283 pigs) from one experimental farrow-to-finish farm were used. The farm has vaccinated against Atrophic Rhinitis for many years, and has a level of rhinitis lesions in the low to medium range.
Four groups of pigs were defined according to the vaccine status of the sows / gilt:

- Group 1: pigs born from unvaccinated gilts (n=30);
- Group 2: pigs born from unvaccinated sows (n=124);
- Group 3: pigs born from vaccinated gilts (n=33);
- Group 4: pigs born from vaccinated sows (n=96).

In the first batch, all gilts and sows were vaccinated, according to a classical vaccine program with Porcilis® AR-T DF: Gilts received 2 injections in quarantine (4 weeks apart), then all pregnant gilts and sows were also vaccinated 3 weeks before each farrowing. In the second batch, the gilts were never vaccinated, and the sows' vaccination was stopped before the two last farrowings.

The Atrophic Rhinitis lesions of all pigs born in the two batches were scored directly after slaughter (or if the pig died before getting to slaughter age). The severity of atrophic rhinitis lesions was scored on computer tomography images of snout sections obtained at the first upper premolars (figure 1). The lesion scores were graded by an experienced operator, according to the IFIP reference method (scored 0 to 20):each turbinate atrophy was scored 0 to 4 and the nasal septum deviation was scored 0, 1, 2 or 4 (2).
The average lesion scores in each group were calculated. For the statistical analysis we used the non-parametric test (Wilcoxon signed rank test) to compare the four groups and after to compare the groups two per two.

Results

25% of snouts had no Atrophic Rhinitis lesion for the vaccinated sows against 7% for the unvaccinated sows. The average lesion scores of pigs born from unvaccinated sows or gilts were higher than those born from vaccinated sows or gilts (see table 1). There was no difference between the lesion scores of pigs born from vaccinated gilts and those of pigs born from vaccinated sows. The average lesion scores of pigs born from unvaccinated gilts are significantly higher than those born from unvaccinated sows.

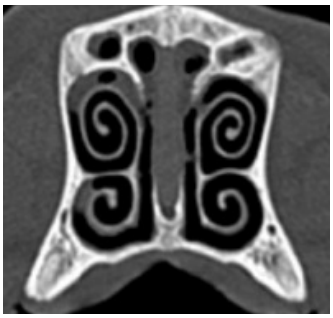

Figure 1. Computer tomography images on snout section obtained at the first upper premolars

Table 1. Average scores of Atrophic Rhinitis lesions for pigs born from unvaccinated or vaccinated sows or gilts.

Group	N	Mean	Statistics*
1)Pigs born from unvaccinated gilts	30	4.83	a
2)Pigs born from unvaccinated sows	124	3.84	b
3)Pigs born from vaccinated gilts	33	2.88	c
4)Pigs born from vaccinated sows	96	2.95	c

*Non-parametric test: Wilcoxon signed rank test

Conclusions and Discussion

Vaccination of gilts and sows against atrophic rhinitis transmits passive immunity to piglets via the colostrum. Stopping vaccination in a farm with a known risk of Atrophic Rhinitis increases the atrophic rhinitis lesions (groups 1 and 2 vs 3 and 4). Pigs born from gilts that have never been vaccinated have more severe lesions (group 1). Pigs from sows that received the first doses of vaccine, but did not receive the pre-farrowing dose(s) also had increased lesions compared to pigs from fully vaccinated sows (group 2 *vs.* group 4). This underlines the need to complete vaccination courses for maximal protection.

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Interference of maternal immunity with PCV2 vaccine in pigs

R Huerta[1,4], L Becerril[2], V Quintero[3,4], G Borbolla[3,4]
[1]Universidad Autónoma de Puebla, [2] Universidad Nacional Autónoma del Estado de México, [3]Universidad Nacional Autónoma de México, [4]The Scientific Partners. A swine-specialized group, rubenhuertac@live.com.mx..

Introduction

Porcine circovirus (PCV2) and its associated diseases caused losses of 17 to 40 kg per pig before vaccination[1]. Nowadays, although more controlled, the presence of this virus still represents a challenge for the porcine industry.

The widespread use of vaccination against PCV2 in piglets, pregnant sows (3), and the exposure of sows to vaccine and field virus have generated varying amounts of antibodies that are also transmitted to piglets via colostrum (4). These antibodies might interfere with the immune response in early-vaccinated pigs (< 21 d of age). The purpose of this study was to determine if maternal antibodies interfere with the immune response in pigs vaccinated with PCV2 at 3 weeks of age.

Materials and Methods

This study was conducted in a full-cycle pig farm, with 600 sows and a production flow of 280 ± 26 weaned piglets per week. The vaccination program against PCV2 consisted in one dose of Ingelvac Circoflex™ vaccine to piglets at 3 and 28 weeks of age. In sows, two groups of 14 animals each where one group was vaccinated and the other remain unvaccinated. Vaccine was applied at the week 13th of gestation.

Blood samples from sows were taken at week 16th of gestation, and their offspring at 3, 7, 10, 14, 18 and 22 weeks of each; with 5 pig in each age group. Indirect ELISA BioChek BV and real-time PCR were performed in all blood samples. A comparison of means was performed using an ANOVA test.

Results

In sows, humoral response was different (P < .01) among treatment groups (table 1). Meanwhile, there was no significant difference (P>0.01) for the amount of antibodies found in feeding pigs from vaccinated and not vaccinated sows (Table 1). In market pigs, viral load (copies/ml) was significantly different (P<0.01), with the highest load seen in pigs from unvaccinated sows compared with unvaccinated animals (29241 ± 4468 vs. 22308 ± 5778, respectively). A correlation of R^2 = 0.758 was found between vaccination of sows and the amount of antibodies produced (Figure 1). Vaccinated sows produced 19% more antibodies than unvaccinated animals. During the 25-weeks of this study, there were no clinical signs of any syndrome caused by PCV2.

Conclusions and Discussion

Vaccination with Circoflex™ generates a good immune response in pregnant sows, which does not interfere with the immune response of their litters when vaccinated at three weeks of age. Other studies reported that there is no interference with vaccination of females and piglets with PCV2 complete vaccine (5).

Table 1. Response to Circoflex™ PCV2 vaccination.

	Vaccinated Sows		No vaccinated sows	
Age weeks	Antibody	Genomic copies/ml	Antibody	Genomic copies/ml
3	1815	17800	2171	31700
7	674.5	20300	417.8	23550
10	186.8	19300	219.4	30600
14	602.2	24400	154.6	24950
18	1994.4	18900	1613.2	29000
22	2261	33150	2172	35650
3-22	1255.6	22308[a]	1124	29241[b]
Pregnant Sows	11103[a]	-	8987[b]	25200

[a,b] Different superscripts mean significant differences.

The piglets from vaccinated sows had a good response regardless of the level of maternal antibodies. Similarly, Opriessnig (4) found no interference of the vaccine in pigs with maternal antibodies against PCV2. Vaccination against PCV2 prevented the development of clinical signs, it could not prevent viremia but it reduced the viral load, like in other studies (2).

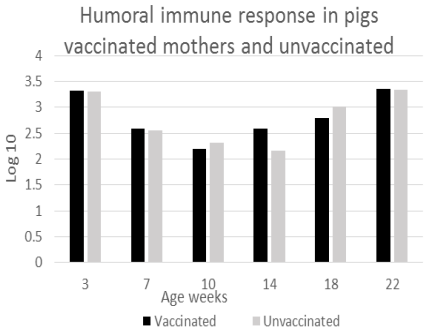

Figure 1. Immune response to vaccination.

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Field comparison of two commercial vaccination regimens for the control of *M. hyopneumoniae* and PCV2

C Moore[1], C Surprenant[2]
[1]*Moporc inc, Saint-Césaire, Canada,* [2]*F. Ménard inc, Ange-Gardien, Canada,* csurprenant@fmenard.com

Introduction

Vaccination is a widespread method to control enzootic pneumonia and PCVAD.[1] The objective of this trial was to assess the efficacy of RespiSure-ONE® given early in life (before 7 days of age) and Fostera PCV given at weaning in comparison to an Ingelvac MycoFLEX® and CircoFLEX® vaccination program given at weaning.

Materials and Methods

186 pigs were selected from a commercial sow farm which was positive and slightly active for *M. hyopneumoniae*and porcine circovirus type 2. The heaviest 2 males and 2 females were selected from each litter at processing and were randomly allocated to one of two treatment groups (Table 1). At arrival into the nursery (3 weeks of age) and finisher (10 weeks of age), the animals were randomly assigned to pens. Every treatment and gender was represented in all pens. These trial pigs were comingled with other pigs from the same sow farm and with pigs from a second sow farm. In total, 2000 pigs had been allocated equally to the treatments described in Table 1. Statistical analysis was conducted as a randomized complete block design with SAS® 9.2 (Cary, NC, USA) using a Linear Mixed Model Approach with 5% level of significance and limited to the subset of 186 pigs.

30 serum samples were collected from each treatment group on Day 3, 21, 63 and 162 and analysed to detect the antibody levels against *Mycoplasma hyopneumoniae* (DAKO Corporation, Carpenteria, USA) and against PCV2 (Biovet, St-Hyacinthe, Canada). 6 oral fluid samples were collected using pen ropes from each treatment group on Day 30, 55, 76, 111, 132, 162 and analysed to detect the presence of *Mycoplasma hyopneumoniae* and PCV2 using PCR (Biovet, St-Hyacinthe, Canada).

Table 1. Description of the treatment groups

Groups	Vaccination program
T1	RespiSure-ONE® at 3 days (processing) Fostera PCV® at 21 days (weaning)
T2	Ingelvac MycoFLEX® at 21 days (weaning) CircoFLEX® at 21 days (weaning)

Results and Discussion

There was no significant difference between groups for ADG wean to time of first marketing, ADG wean to slaughter, age at slaughter, % and prevalence of lung lesions and mortality.

Table 2. Production Parameters

Parameter	RespiSure-ONE Fostera PCV*	MycoFLEX CircoFLEX*
Weight at weaning (kg)	n = 95 6.29[a] ± 1.14 (4.00 – 9.00)	n = 91 6.30[a] ± 1.18 (3.60 – 9.10)
ADG wean to first marketing (kg)	n = 85 0.718[a] ± 0.06 (0.55– 0.89)	n = 83 0.714[a] ± 0.08 (0.44– 0.86)
ADG wean to slaughter (kg)	n = 67 0.800[a] ± 0.06 (0.67– 0.96)	n = 60 0.782[a] ± 0.07 (0.60– 0.93)
Age at slaughter (Days)	n = 79 191.47[a] ± 15.34 (172.00 – 222.00)	n = 81 193.71[a] ± 16.99 (171.00 – 223.00)

* LSMeans ± standard deviation (min to max range)

Table 3. Health Parameters

Parameter	RespiSure-ONE Fostera PCV	MycoFLEX CircoFLEX
Lung lesions %*	n = 77 0.86 (0.00 – 10.00)	n = 76 1.04 (0.00 – 15.00)
Prevalence of lung lesions (#) %	31 / 77 40.2[a]	31 / 76 41.1[a]
Mortality (#) %	9 / 95 9.6[a]	8 / 91 8.8[a]

*Geometric mean (min to max range)

63% of the animals sampled in T1 and 86% in T2 had seroconverted against *M. hyopneumoniae*on Day 162 whereas they were all negative on Day 63, except one pig. Oral fluids were all negative from collections Day 30 to 111 with more than 67% of the samples positive on Day 162. This is in line with the prevalence of lung lesions observed during the slaughter check. However, lung lesions were minimal with only 11 pigs showing lesions of 10%-15% lung affected.

37% of the sampled animals in T1 and 23% in T2 had seroconverted against PCV2 on Day 162 whereas they were all negative on Day 21, when the vaccination was administered. Oral fluid samples were all negative, except on day 55 for one sample in the T2 group. The low level of seroconversion and near absence of PCV2 virus suggests that the PCV2 vaccines were effective in the control of PCV2 circulation.

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Study of immunogenic effect of nanovaccine prototypes on transmissible gastroenteritis virus of swine

PV Mezhenniy, AA Volkov, SA Staroverov, SV Kozlov, IN Zhirkov
Saratov State Agrarian University named after N.I.Vavilov, Russia, zhircov@gmail.com

Introduction

Nanostructure design for targeted drug delivery [1], the invention of effective and safe drugs and the development of rapid-test methods for diagnosis of diseases of various etiologies [3] is of prior importance in modern veterinary medicine.

Besides, the research in the field of colloidal particles usage for constructing nano-modified vaccine is becoming of primary importance. A number of positive properties such as reducing the amount of antigen entering the body, the reduced time of immune response and direction of action [2] were shown.

Materials and Methods

We have designed the prototypes of nano-modified vaccine against transmissible gastroenteritis of swine (TGS) using colloidal particles of gold and selenium and the whole virus virions of TGS.

To conduct immunobiological research of obtained prototypes of nano-modified vaccine 4 groups of animals (guinea pigs), 9 species in each, were formed on the principle of analogues. Immunization of the animals was injected subdermally along the vertebral column twice within 10 days. Antigen solution of 1 ml of TGS was injected in the first group. Group II was treated with 1 ml of antigen conjugate of TGS with colloidal gold. Group III was injected with 1 ml of conjugate of antigen virus TGS with colloidal selenium. Group IV (control group) - 1 ml of normal saline solution. A blood sample to obtain serum was taken 10 days after the last injection for ELISA.

Determination of the concentration of serum interleukin was performed using a reagent kit for immunoassay determination of interleukins IL-1β, IL- 6 and INF-γ.

Results

The obtained during the experiments data show that the highest concentration of the interleukin INF-γ releases in the animals immunized with an antigen conjugated with colloidal selenium and gold particles and its concentration is 60,44 ± 9,91 pg / ml in relation to gold nanoparticles, and 48,17 ± 4,43 pg / ml relating to selenium . PTG- 44,17 ± 3,57 pg / ml, control - 34,15 ± 4,54 pg / ml.

In the study of the levels of interleukins IL-1β in experimental animals, we determined that the greatest increase in the concentration of interleukin IL-1β were observed in the group immunized with the antigen with the colloidal gold, which turned out to be 8,10 ± 0,74 pg / ml. In the group immunized with the antigen with colloidal selenium and a PTG antigen , the concentration of interleukin IL-1β turned out to be 6,58 ± 1,06 pg / ml 6,93 ± 0,91 pg / ml respectively. The animals of the control group had the concentration of interleukin IL-1β levels 4,93 ± 0,71 pg / ml.

In the study of the level of interleukin IL- 6 in experimental animals, there is a similar dynamics as with interleukin IL-1β.

Conclusion and Discussion

Immunization of laboratory animals with antigen of transmissible gastroenteritis virus of swine, conjugated with colloidal particles of gold and selenium stimulates production of interleukin in the organism of experimental animals. The obtained data show the increase of the cellular and humoral immune response to preventive immunization.

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Decrease of sow mortality rate using *C. novyi* vaccine (SUISENG®)

I Rodríguez-Ballarà[1], J Miranda[1], H Delgado[2], A Landa[3]
[1]*Technical services, HIPRA, Spain,* [2]*HIPRA MEXICO, Mexico.* [3] *Granja la Joya, Mexico.* isaac.rodriguez@hipra.com

Inroduction

Clostridium novyi (Cn) is an anaerobic, spore-forming, gram-positive rod and ubiquitous bacteria in swine farms[1]. Cn is a normal inhabitant of the posterior gut and liver of pigs[3]. However, during a disease outbreak, spores in the liver become vegetative and produce toxin. Cn has been associated with sudden death in sows[1]. A correct diagnosis is depending upon the appreciation of the gross pathologic appearance, and an understanding of the factors of laboratory outcomes. This diagnosis is highly dependent on time, since toxin production produce a rapid decomposition of the carcass. Therefore diagnosis of Cn infection in sow is usually missed[2]. Three avenues have been proposed for control of Cn infection[2]. First is to prevent predisposing liver disease. Second is the use of antibiotics. Third is through vaccines[3,4].

Company located in Mexico had a historical high index of sow mortality. The objective of the study was to implement a *Clostridium novyi* vaccination (SUISENG® HIPRA) to diminish this mortality.

Materials and Method

The study was carried out in a farm with 3000 sows in Puebla state. An increase of sow mortality was reported during 2011 and systematic necropsies were performed in all sudden death sows. Clinical signs observed were submandibular edema, pulmonar edema, and serosanguineous exudate in the respiratory tract, so clinical signs compatible with a clostridial septicemia in adult animal [1].

In 2011, sows were vaccinated with a commercial coli vaccine which does not include Cn α toxine. In order to improve the situation of the sudden deaths sows they switched to SUISENG® in December 2011. SUISENG® is a polivalent vaccine with two main indications, prevention of neonatal diarrhoea and, prevention of Sudden Death caused by Cn infection of adult animals. Sows were vaccinated at 30 days before farrowing and Gilts vaccinated and revaccinated gilts 60 and 30 days respectively.

Results

After the SUISENG® implementation, sow mortality decreased significantly 4.6%. There were statistical differences between the 2011 and 2012 annual sow mortality rate (p<0, 05, t-test for independent samples).

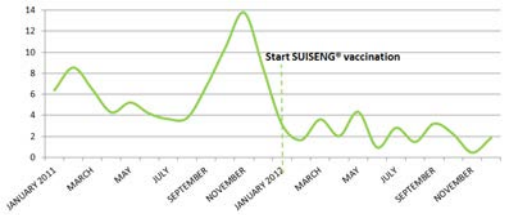

Figure 1. Evolution of the average percentage of sow mortality rate by month during 2011 and 2012.

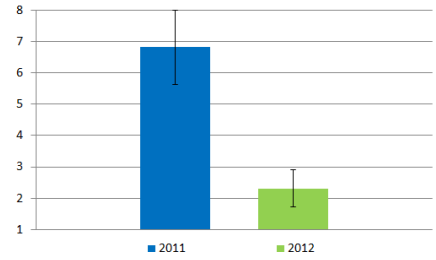

Figure 2. Annual mean percentage of sow mortality rate (±SEM) at 2011 and 2012.

Conclusions and Discussion

The appearance of anatomopahologic findings suggested that the increase of sow mortality during 2011 was linked with a Clostridia infection. Laboratory diagnostic was not able to be performed, since in the labs of the area the clostridia isolation was not available. Furthermore the culture of Cn is difficult and positive culture does not indicate that this is the cause of the problem, since Cn is a normal habitant of different tissues of the animal[2,3]. Decrease of mortality after implementing SUISENG® vaccination was significant. Therefore the connection among decrease of mortality and the vaccine change indicate that Cn would be the main responsable of the sow mortality, since Cn is the only antigen in SUISENG® indicated for sows. Besides the efficacy of Cn inactivated vaccine to control sow mortality outbreaks has been demonstrated. Therefore vaccination with inactivated Cn vaccines is a primary measure to prevent high sow mortality rates in farms[3,4]. Otherwise Cn laboratorial diagnostic should be improved in order to help in the differential diagnostic of sow mortality causes.

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Comparative study of different *E. coli-clostridium* vaccines by measuring antibody levels of *E. coli* virulence factors

S Bel[1], A Luengo[1], V Robles[1,] R Santamaria[2], M Jimenez[2], R Menjón[2]
Technical Services Cooperativa Ganadera de Caspe, Zaragoza, Spain[1],
Technical Service MSD Animal Health, Spain[2], ruth.menjon.ruiz@merck.com

Introduction

Enterotoxigenic *E.coli* associated with neonatal diarrhea may possess one or more F4 (K88), F5(K99), F6(987p) and F41(2) fimbriae, with K88 having the highest prevalence (1). Adequate immunization of sows is fundamental for providing piglets with effective protection against this disease via the colostrum (2)(3). The purpose of this study was to evaluate and compare the safety and efficacy of the main *E.coli* vaccines available in Spain by comparing the levels of specific antibodies against the main *E.coli* virulence factors in gilts vaccinated with Porcilis®ColiClos and other commercial vaccines with the same indication (vaccine A,B).

Materials and Methods

The trial was conducted in a farm with 3,000 sows in northeast Spain operating a two-phase production system. Sixty-one individually identified primiparous sows, not already vaccinated against *E.coli*, were randomly allocated to one of 4 groups. Prior to farrowing, three of the groups were vaccinated with a different commercially available product according to each manufacturer's instructions. The fourth group was maintained as an unvaccinated control.

The groups, identified by the product used, were: Vaccine A (Ginseng adjuvant); Vaccine B (aluminum hydroxide adjuvant); Porcilis®ColiClos and Control.

Blood samples were collected from the sows at the time of the first vaccine dose and 2 weeks after the second dose to determine antibody titers against specific antigens of *E. coli* containing vaccines with a specific ELISA test (internal MSD AH test).

Safety was assessed by taking the sows' body temperatures at 6 and 24 hours post-vaccination, from observations of any adverse systemic and/or local reactions. Efficacy was evaluated from antibody titers measured in each group. The Linear Method (GLM: program SPSS 15.0) was used for the statistical analysis.

Results

Safety: No sow stopped eating during the trial, there was one abortion in the Porcilis®ColiClos group. At 24 hours post-vaccination, one sow in each Vaccine A and B groups had a temperature >39,5ºC.

Efficacy: post-vaccination antibody levels are summarized in Graph 1. There were significant differences (P<0.05) between Porcilis®ColiClos and the others groups in 987p, highly significant differences vs control in K88ab, significant differences (P<0.05) versus vaccine A and control in K88ac, highly significant differences (p<0.001) vs Vaccine A and control in K88ab, highly significant differences between Porcilis®ColiClos and the other groups (p<0.001) in LT antigen (Table1).

Table 1. Mean antibody titers per group (Log2)

	987p	K88ab	K88ac	K99	LT
Vaccine A Ginseng	6.8[b]	8.4	9.3[b]	7.3[b]	5.6[b]
Vaccine B AlOH	8.2[b]	9.9	10.5	8.0	6.0[b]
Porcilis®Coliclos	10.1[a]	10.9[a]	10.7[a]	9.9[a]	9.3[a]
Control	5.9[b]	3.3[b]	4.1[b]	4.8[b]	4.9[b]

[a,b]: values with different superscripts represent statistically significant differences (p<0.05).

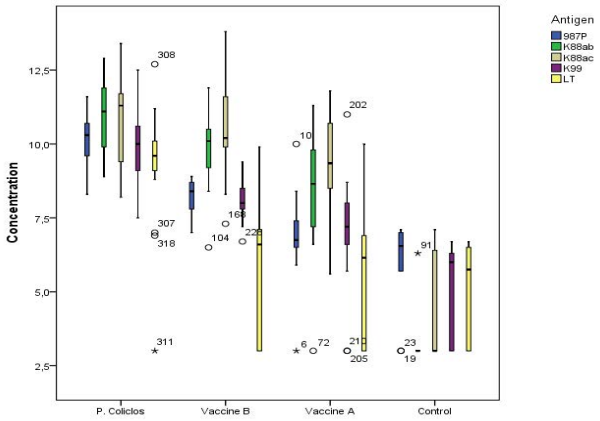

Figure 1. Mean antibody titers per group (Log_2)

Conclusions and Discussion

Porcilis®ColiClos was shown to be safe, and induced high and more homogeneous titers against every *E.coli* antigen. Porcilis®ColiClos performed significantly better than Vaccines A and B and control.

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Decreased viral load of Porcine Circovirus after vaccination with Circumvent PCV® in one commercial farm in the Midwest region of Brazil

TF Cruz[1], RSYamatogi[1], EH Okuda[2], C Feronato[3], BMFPP Marques[3], T Oliveira[3], MH Tsunemi[4], JP Araujo Jr[1]
[1]*Department of Microbiology and Immunology, Biosciences Institute, Univ. Estadual Paulista (UNESP), Botucatu, SP,* [2]*Suinocultura Asa Alimentos, Brasília, DF,* [3]*MSD Saúde Animal, São Paulo, SP,* [4]*Department of Biostatistics, Biosciences Institute, Univ. Estadual Paulista (UNESP), Botucatu, SP,* tfcruz@yahoo.com.br

Introduction

The objective of this study was to evaluate the use of Circumvent® PCV (MSD Animal Health) vaccine through viral load quantification by qPCR in whole blood of vaccinated and control animals and measurement of performance parameters in one PCV2 positive commercial farm in the Midwest region of Brazil.

Materials and Methods

Blood samples were collected from one commercial farm (8000 sows) situated in the Midwest region of Brazil, PCV2 positive and non-vaccinated for PCV2. Blood samples were collected from 65 (n = 39), 115 (n = 20), 135 (n = 20) and 159 (n = 20) day old piglets. The animals in this first group were not vaccinated against PCV2 and were the control group. Vaccination against PCV2 (Circumvent PCV®, MSD Animal Health) was introduced in the farm, according to the manufacturer's vaccination schedule. Eight months after vaccination, a second group of piglets was sampled at 65 (n = 41), 115 (n = 20), 135 (n = 20) and 195 (n = 20) days of age. The extracted DNA was analyzed for PCV by qPCR (2). Seventy-five positive samples for PCV were tested for PCV1 by qPCR (3). The performance parameters of control lot (n = 10) and vaccinated lot (n = 10) in finishing phase were recorded. Each lot (n = 1200) had 12,000 animals in total. The median viral load determined for each age was compared between vaccinated and control groups by Mann-Whitney test with independent samples; $P < 0.05$ was considered statistically significant.

Results

After vaccination, there was a decrease in the number of piglets positive for PCV (Figure 1). The reduction of viral load in whole blood for the vaccinated group compared with the control group was statistically significant (65 days ($p < 0.001$), 115 days ($p = 0.001$), 135 days ($p = 0.001$) and 159 days ($p < 0.001$). All samples analyzed were negative for PCV1 by qPCR. The performance parameters are shown in Table 1. The cost of medicament per animal was 37.56% higher for the control group when compared to the vaccinated group.

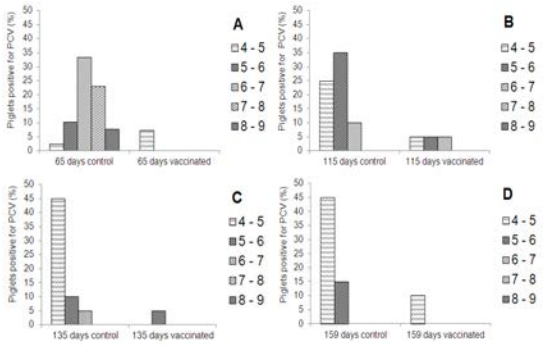

Figure 1. Percentage of piglets positive for PCV in control and vaccinated groups. Ages of 65 (A), 115 (B), 135 (C) and 159 (D) days. Viral load (log_{10} DNA copies/ml blood) in the legend

Table 1. The performance parameters of the control and vaccinated lots

Parameters	Control[1]	Vaccinated[1]
Age (days)	159.82 ± 2.88	159.08 ± 5.18
Average slaughter weight (kg)	104.29 ± 2.61	105.06 ± 7.64
Mortality rate (%)	3.93 ± 0.67	3.07 ± 1.31
Daily gain (kg/day)	849.90 ± 24.66	876.50 ± 42.53
Feed conversion	2.64 ± 0.09	2.61 ± 0.06

[1]Mean ± standard deviation

Conclusions and Discussion

The results support vaccine efficacy through reduced PCV viremia, reduced medication cost and improved performance parameters. Similar results have been reported in studies under field conditions with Circumvent® PCV vaccine in the United States (1, 4).

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Effect of an autogenous vaccine of *C. perfringens* type A on pigs performance under field conditions

J Mendoza[1], F Gonzalez[2], A Ruiz[1,] D Fuentes[2], JA Tobar[2]
[1]*Departamento de Patología y Med. Preventiva, Facultad de Cs. Veterinarias, Universidad de Concepción, Chile ,* [2] *Laboratorio Virbac-Centrovet, Chile,* felipe.gonzalez@centrovet.com

Introduction

Clostridium perfringens type A is a bacterium which is part of the normal flora at the gastrointestinal tract in pigs. However, it also has been described as an agent responsible of diarrhea in piglets (1). The main lesions produced are mild enterocolitis and intestinal villous atrophy (2), which causes a delay in piglet growth and susceptibility to other diseases.

The aim of this study was to evaluate the effect of an autogenous vaccine applied in sows on productive performance of their piglets under field conditions on a farm of Chile.

Materials and methods

The study was conducted on a commercial farm which showed enteric problems with a positive diagnosis to *Clostridium perfringens* type A as responsible agent. The farm has 3850 sows, segregated weaning, management AI/AO and three sites production system. For the effects of the study, three weeks of production were evaluated, where each week was a replicate of the study.

Three hundred thirty sows were selected on the 3 weeks of production and randomly divided into two groups: (A) sows treated with autogenous vaccine (n = 167) and (B) sows not treated with the autogenous vaccine (n = 163). The total number of animals used in the study for the productive parameters evaluation was 1745 piglets from sows of group A and 1741 piglets of sows of group B.

The vaccination program consisted in the administration of one dose of autogenous vaccine in adult sows (five weeks before farrowing) and two doses in gilts (5 and 3 weeks before farrowing). The proportion of sows and gilts were similar for both groups at every week of production.

To measure the impact of the implementation of the autogenous vaccine, piglet's weight at farrowing and weaning was recorded, and the average weight of both treated groups was compared. Additionally, the piglet's efficiency at weaning was determined based on the expected performance of the animals according to their age, as indicated by the genetic company. Finally, piglet's mortality was recorded during the lactation period.

To establish statistical differences Chi square and non-parametric Mann-Whitney test were used.

Results

Piglets from both experimental groups had similar weight at farrowing and no statistical differences between groups were found at that age. On the other hand, as shown in Table 1, piglets originated from vaccinated sows were bigger at weaning and also had a superior productive performance than piglets from non-vaccinated sows, both parameters showing statistically significant differences (p value of 0.022 and 0.032, respectively). Additionally, the mortality rate of piglets from vaccinated sows was lower than the offspring of non-vaccinated sows, however, this difference was not statistically significant (p = 0.51).

Table 1. Productive performance of piglets at weaning, of both experimental groups, in the different replicates.

	Weight (Kg)		Piglet's efficiency (%)		Mortality rate (%)	
	A	**B**	**A**	**B**	**A**	**B**
Replicate 1	6.99	6.52	104.9	97.83	11.34	14.77
Replicate 2	6.79	6.27	104.6	96.56	10.99	14.44
Replicate 3	7.26	7.25	108.2	108.05	10.86	12.06

A: Group of sows treated with autogenous vaccine. B: Control group

Conclusions and Discussion

The administration of an autogenous vaccine against *Clostridium perfringens* type A in sows, produced an impact on the productive performance of their offspring, which was observed in the 3 replicates of the study, and was statistically significant for the weaning weight and piglet´s efficiency. Regarding mortality, a clear tendency to decrease was observed in the 3 replicates of the offspring of vaccinated sows, but was not possible to establish statistically significant differences. The results of this study clearly show the positive effect of the use of the autogenous vaccine, which can be economically important for large-scale production, especially if it is sustained over time.

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Immunoprotector potential of cellular vaccine formulations developed from *L. interrogans* ballum in swiss

JC Fernández [1], W Jirón [2], N Batista [3], JF Infante[3]
[1]*National Center of Biological Safety, Playa, Havana. Cuba.* [2]*Veterinary Medicine School, León, Nicaragua.*
[3] *Finlay Institute. La Lisa. Havana. Cuba,* julioc@orasen.co.cu

Introduction

The currently available antileptospiral commercial vaccines are killed, whole-cell vaccines, with or without adjuvant that include in their formulations serovars of common circulation in the region selected for their application[1]. In Cuba from the 90′s, it has been administered a trivalent vaccine (vax- SPIRAL®) conformed by the serovars: Canicola, Copenhageni and Pomona[2]. The effectiveness and safety of this product to control and to diminish the lethality of swiss leptospirosis have been confirmed thoroughly[3]. However, in the last years they have been changes in the immune epidemiological situation of leptospirosis in Cuba and the serovar Ballum has become one of common circulations and incidences together with Canicola, Pomona, and Copenhageni. Studies carried out in the Golden/ Syrian hamsters (Mesocricetus auratus) biomodel have determined that the immunization with vax-SPIRAL® only protects in a range of 50-60% against the lethal challenge with Ballum virulent strains, which presupposes that with this vaccine the protection levels required to avoid the development of the leptospirosis infection are not reached. The objective of the current study was to develop killed vaccines with isolates of *Leptospira interrogans* serovar Ballum and determine their eficacy (protection and immunogenicity) against Ballum and heterologous serovars included in vax-SPIRAL®. Two monovalent formulations developed with two highly virulent strains were evaluated (FoBa and FoBb).

Materials and Methods

Ballum monovalent vaccines were developed with clinical strains isolated from Cuba and Nicaragua using production methodology developed for vax- SPIRAL®2, with slight modifications. For the vaccine trial, three groups, each composed of 20 female adult pigs were used. A group 1 was inoculated with a formulation derived from strain FoBa, the group 2 with a formulation derived from strain FoBb and the third group was inoculated with PBS as a control. Animals were each administered (via intramuscular) a series of two inoculations, 6 weeks apart. Before each inoculation, serum samples were taken from animals in the same vaccine group; these were pooled and tested for the presence of anti-leptospiral antibodies (against Canicola, Icterrohaemorragiae, Pomona and Ballum) using ELISA. After the immunization schedule was conducted the challenge trial. For this last trial was divided each group in four groups of 5 animals and challenge with virulent strains of main serovars of epidemic swiss importance: Canicola, Icterrohaemorragiae, Pomona and Ballum. Post-mortem examinations were performed and the liver and kidneys were harvested for in order to prove Koch's postulates.

Results

The results showed that both formulations generated a protection of 100% against the Ballum lethal infection together to high levels of IgG antibodies and were efficient in the elimination of homologous carrier state but not heterologous carrier states. Pigs vaccinated with the experimental vaccines (FoBa and FoBb) and challenged with Ballum showed no clinical signs of leptospirosis and there was no post-challenge mortality. These animals were also culture-negative with no gross or histological pathological lesion. The IgG response against Ballum was revealed in 70% of the animals immunized with FoBa and in 80% of the group immunized with FoBb. After applying the second dose, the values of IgG generated by both monovalent vaccines were very significant regarding the values obtained after the first dose. Also 100% of the animals in the non-immunized group (control group) were positive for leptospirosis and displayed the greatest morbidity with 100% mortality. Non mortalities were observed in vaccinated groups with monovalent vaccine and challenged with Canicola and Icterrohaemorrhagiae but was observed leptospiras in kidney and liver biopsys. Instead severe clinical signs were observed in 80 to 100% of individuals between 8 to 20 days post- challenge for both vaccinated groups challenged with Pomona.

Conclusions

Both FoBa and FoBb vaccines were protective against Ballum with high IgG titers, absence of clinical signs and dead, and absence of leptospires in kidney of sacrificed animals. Besides this new formulations were effective against Canicola and Icterohaemorragiae lethal infection but no eliminated carrier state and no protection was observed against infection with serovar Pomona. These results suggest that a new formulation with serovar Ballum and Pomona in high cell concentration with low cell concentrations of Canicola and Icterohaemorragiae will control effectively swiss leptospirosis in a new epidemic scenario.

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Investigation about the presence of common microorganisms of swine to be considered among potential Xenotransplantation-associated infectious risks for human recipients: Report from 9 pigsties (Southern part of Belgium)

JP Dehoux, P Gianello
University of Louvain, Faculty of Medicine, Experimental Surgery Unit, Brussels, Belgium, jean-paul.dehoux@uclouvain.be

Introduction

In xenotransplantation, there is the unique potential risk for the transmission of both known and unknown zoonotic infectious agents of animal origin into human recipients and into the wider human population. Thus, the term ‘‘xenosis’’ was coined to reflect both the unique epidemiology of infection of source animals used for xenotransplantation and experience with immunocompromised patients that indicates that novel pathogens may emerge as a cause of infection, including organisms not normally associated with human disease. The degree of risk is unknown in the absence of clinical trials. The development of surveillance and safety programs for clinical trials in xenotransplantation is guided by a ‘‘Precautionary Principle’’, with the deployment of appropriate screening procedures and assays for source animals and xenograft recipients even in the absence of data suggesting infectious risk. WHO has provided series of guidance documents related to xenotransplantation.

In order to develop some pigsties as potential safe pig breeders to xenotransplantation, it was crucial to have an idea about the presence of the common microorganisms of swine to be considered among potential causes of infection in immunocompromised swine and / or human xenograft recipients.

Materials and Methods

Nine “normal” pigsties from the southern part of Belgium were investigated regarding the list of the common porcine microorganisms (Bacteria, parasites and fungi) (1).

Samples from nostrils, throat, lungs, feces, urine, skin, hair and sera were taken in 9 pigs (1 per pigsty) for diagnosis. In total thirty di fferent bacteria, parasites and fungus were investigated according to these different sites.

Results

The main microorganisms found in the different organs are shown in Table 1.

Table 1. Results on samples collected from 9 pigs

Nostril	Throat	Lungs	Faeces	Urine	Skin	Hair	Sera
Actinobacillus spp -	Corynebacterium spp +	Bordetella bronchiseptica -	Campylobacter spp +	Corynebacterium spp -	E.coli spp -	Microsporum spp -	Chlamydia Ag -
Bordetella bronchiseptica -	Haemophilus spp -	Klebsiella spp -	Clostridium difficile +	E.coli spp -	Staphylococcus spp +	Scopulariopsis spp +	Brucella suis -
Corynebacterium spp +++	Shigella spp -	Nocardia spp -	E.coli spp +++	Leptospira interrogans -	Streptococcus spp +	Trichophyton spp -	
Haemophilus spp -	Staphylococcus spp +	Pasteurella spp -	Pseudomonas spp +	Legionella spp -	Microsporum spp -	Candida albicans +	
Klebsiella spp +	Streptococcus spp ++	Shigella spp -	Salmonella spp -		Trichophyton spp -		
Nocardia spp -		Staphylococcus spp +	Shigella -		Scopulariopsis spp +		
Pasteurella spp -		Mycoplasma hyopneumonia -	Yersinia spp -				
Shigella spp -		Streptococcus spp -	Ascaris -				
Staphylococcus spp +++			Cryptosporidium -				
Streptococcus spp +++			Echinococcus -				
Aspergillus -			Isospora spp -				
Candida -			Strongles +				
Cryptococcus -							
Chlamydophila psittaci -							

+++ (numerous); ++ (a few); + (rare); - (negative)

Conclusions and Discussion

The data suggests that the majority of the different microorganisms listed by the WHO with a potential risk in xenotransplantation were absent in the 9 pigsties investigated in the southern part of Belgium. Only the more common bacteria (*Corynebacterium*, *Staphylococcus*, *Streptococcus* and *E. Coli*) were found in a large prevalence. These results indicate that the level of health standard is already good in these “normal” pigsties and that the potential pathogens could be managed with a specific pathogen free facility.

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Health status on small pig farms in Slovenia

I Golinar Oven[1], M Štukelj[1]
[1]*Veterinary Faculty, Institute for health care of pigs, Gerbičeva 60, 1000 Ljubljana, Slovenia,*
irena.golinaroven@vf.uni-lj.si

Introduction

Most pig farms in Slovenia are small-sized. We have 3888 farms with 1 to 20 breeding pigs, 231 farms with 21 to 50 breeding pigs, 46 farms with 51 to 100 breeding pigs, 8 farms with 101 to 200 breeding pigs, 2 farms with 501 to 1000 breeding pigs and only 3 farms with more than 1000 breeding pigs.

In Slovenia systematic monitoring is implemented only for classical swine fever and Aujeszky's disease. The health status for other diseases on small pig farms is mostly unknown.

The objective of this study was serological survey of reproductive and respiratory syndrome (PRRS), salmonella, *Actinobacillus pleuropneumoniae* (APP) and leptospirosis at small farrow-to-finish pig farms in Slovenia. After completed serological testings we had proposed farmers appropriate control measures.

Materials and Methods

The study was conducted during 2012 and 2013 on 16 small one-site pig farms with 34 to 79 breeding animals, free of classical swine fever and Aujeszky's disease. At the beginning of the research pigs from 14 small farms were vaccinated against *Mycoplasma hyopneumoniae*, pigs at 4 farms were vaccinated against porcine circovirus diseases and porcine parvovirosis, pigs from 3 farms were vaccinated against atrophic rhinitis, pigs from 2 farms were vaccinated against PRRS and pigs from 1 farm were vaccinated against *Erysipelothrix rhusiopathiae*.

1206 serum samples of breeding animals and fatteners were tested with IDEXX PRRS ELISA (HerdChek X3, IDEXX Laboratories Westbrook, Maine, USA). 365 samples (from 6 small farms) were screened with one step RT-PCR (Qiagen, Germany) and specific primers for detection of EU/NA PRRSV in highly conserved region of ORF 7 (1). 166 serum samples were tested with Swine Salmonella Antibody Test Kit (IDEXX) and CHEKIT*APP-ApxIV (IDEXX). 130 serum samples of sows were assayed for leptospirosis antibody using a microscopic agglutination test (MAT).

Results

Antibodies against PRRSV were detected in 74.9% of serum samples of breeding animals and in fatteners in 48.5%. By RT-PCR, PRRSV was detected in 17.5% of serum samples. The prevalence of serum samples with salmonella antibodies in breeding animals was 21% and in fatteners 5.8%. The prevalence against APP was 92.6% in breeding animals and 45.8% in fatteners. Three farms were serologically positive to leptospirosis (serovar hardjo, serovar grippotyphosa).

Conclusions and Discussion

Slovenia was free of PRRS before joining EU in 2004 (2). Only few years later the detected percentage of sero-positive herds was 44.8%, as indicated by the data of a study on antibody prevalence in 194 herds in 2010 (3). In our study 11 small pig farms had antibodies against PRRS and PRRSV was detected in breeding animals in 74.9% of serum samples and in fatteners in 48.5%. We suggested to farms free of PRRS to continue with biosecurity practices and to 11 farms with PRRS we had suggested biosecurity measures and herd closure. One farm quit with vaccination against PRRS, because vaccine strain was different from farm isolate and the health status of animals did not improve with vaccination.

Almost all breeding animals had antibodies against APP, though clinical signs were not present. The ApxIV ELISA does not differentiate serotypes of APP. The previous survey made at large farms showed that in Slovenia pathogen serotypes are present (4).

Seroprevalence of salmonella in Slovenia is low. From 2006 to 2008 we had started the salmonella control of fatteners at one large farm. In 2008 the level of samples with optical density (OD) % equal or greater than 20% was 24.8% (5). At small farms OD 20% in fatteners was only 5.8%. Comparison of the seroprevalence between large and small farms shows that the number of positive fatteners is higher at the large farms.

3 small pig farms had antibodies against leptospirosis. All tree farms had started with treatment prescribed by their veterinarian. We had additionally proposed strict biosecurity and rodent control programs instigated in and around the farm complex.

By monitoring and enforcing measures against various diseases we could improve the wellbeing of these animals, decrease the necessity of medications and other pharmaceutical additives which would result in a more economically sound husbandry and consequently safer food for consumers.

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Surveillance of *B. hyodysenteriae* in Swiss pig herds

J Peter Egli[1], Y Masserey[1], P Scheer[1], M Harisberger[1], X Sidler[2], H Nathues[3], F Zeeh[1,3]
[1] *SUISAG Pig Health Service,* [2] *Vetsuisse Faculty Zurich, Division of Swine Medicine,* [3] *Clinic for Swine, Vetsuisse Faculty Berne, University of Berne; Switzerland,* friederike.zeeh@vetsuisse.unibe.ch

Introduction

Brachyspira (*B.*) *hyodysenteriae* is the causative agent of Swine Dysentery (SD), which has a large impact on pig production due to illness, mortality and reduced performance (1). In Switzerland the Pig Health Service (PHS) used to monitor SD in its member herds by clinical observation, sample analysis in cases of SD suspicion and epidemiological tracing-back. Sanitation was obligatory only for nucleus herds.

In January 2014, a new guideline, which is mandatory for member herds, was established. Herds positive for *B. hyodysenteriae* have to eradicate the pathogen. Epidemiological tracing forward and backward has to be performed (3). Aim of the new guideline is a sustainable eradication of *B. hyodysenteriae* at least in the member herds. This shall lead to improved health and pig performance, but to a reduction of antibiotics too.

Materials and Methods

The guideline describes the procedure in a suspicion case, diagnosis and measures, eradication and control after the eradication in a confirmed *B. hyodysenteriae* infection.

If specific signs of SD are observed, then the PHS has to be informed immediately. Rectal fecal samples of at least 6 (fattening farm) to 10 (breeding farm) pigs must be taken within 2 working days. Pig sale is restricted by the PHS, until results of sample analysis are available. Samples are analyzed by culture and/or PCR.

If all samples tested negative, restrictions are lifted. If there is at least one positive result, then the farm gets the status "infected with *B. hyodysenteriae* (I B hyo)" which means that its pig trade is restricted, market, price for growers is reduced (approx. -7$/pig) and an eradication has to be performed within one year.

The eradication has to take place in the warm summer months and is based on partial depopulation in suitable breeding farms or total depopulation in every farm type. Professional rodent control, cleaning and disinfection of pens, equipment and slurry are essential parts of the eradication. The eradication is planned for every farm individually, has to be approved by the PHS and is checked during the running sanitation. After a partial depopulation, a surveillance time of 6 months with sampling every 2 months follows. A herd with total depopulation is monitored clinically and with samples in cases of diarrhea.

In parallel, possible sources and potential spread of the infection are evaluated. All herds that received pigs from an infected nucleus herd in the 3 months prior to the positive test have to be clinically examined and samples must be analyzed (at least 10 pigs in multipliers and at least 6 pigs in fattening farms). Multiplying herds that had purchased replacements additional 3 months before the ban of the source herd are clinically examined and tested if diarrhea occurs. If a multiplier herd tests positive, on the one hand all supplying nucleus herds from the last 12 months are examined and tested if necessary. On the other hand, all fattening farms that purchased pigs in the 3 months prior to the positive test result must be examined and at least 6 pigs are tested. Pig trade is restricted until test results are available. If a fattening herd tests positive, then 10 pigs of every source herd currently placed in the positive herd are tested.

If the test results are all negative for *B. hyodysenteriae*, then the temporary restrictions are lifted. If the samples show positive results, then the herd is declared positive, gets the status "I B hyo" and is handled according to the eradication procedure described above.

Results

At the time of the abstract submission, no new infection had occurred since the introduction of the new guideline. With the experience made with other eradication programs (4) and with the new approach to *B. hyodysenteriae* infections and sanitations, a transient increase in *B. hyodysenteriae* detection is expected. Clarification of contact herds requires time and man power. Analysis costs will increase and will be paid by the PHS and the farmer.

Success of the surveillance procedure and the eradication should be reevaluated on a yearly basis.

Conclusions and Discussion

The new guideline shall improve health, minimize the spread of the infectionand reduce use of antibiotics in pigs, which has to be reevaluated. Withdrawals of members are possible. The sample size is debatable. It is not calculated based on the actual size of the group at risk but represents a compromise between feasibility, costs and diagnostic validity (2). Further research is needed, especially in the field of *B. hyodysenteriae* detection in subclinically infected herds.

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Evaluating the disease surveillance for dysentery and progressive atrophic rhinitis in Swiss swine breeding herds using scenario tree modelling

A Hillebrand[1], S Rossteuscher[2], W Zimmermann[1], P Scheer[2], H Nathues[1], G Schüpbach[3], C Nathues[3]
[1]*Swine Clinic, University of Berne* [2]*Swiss Pig Health Service, Berne,* [3]*Veterinary Public Health Institute, University of Berne, Switzerland* christina.nathues@vetsuisse.unibe.ch

Introduction

The Swiss pig population enjoys a favorable health situation [1]. To further promote this, the Swiss Pig Health Service (PHS) conducts a surveillance program in affiliated herds: Sow herds assigned to the highest PHS-health and hygiene status have to comply with strict hygiene regulations and consistently provide evidence that they are free from progressive atrophic rhinitis (PAR) and swine dysentery (SD). Closed multiplier herds are examined overall 4 times a year including laboratory testing for both diseases, each twice a year. Besides, 4 so called "mixed fattenings" are arranged yearly: Pigs from different herds are mixed following a predefined protocol and checked for clinical symptoms during fattening and at slaughter. This intensive surveillance implies high effort and costs while questions about its sensitivity i.e. effectiveness to detect an infection in a herd arose, especially after a PAR outbreak in 2011 [2].

Materials and Methods

The sensitivity of the PAR and SD surveillance was assessed using "scenario tree modelling" (STM) [3]. Herein, the pathway necessary to lead to a certain event (detection of an infection in a herd) is plotted. A probability is then assigned to each step within the "tree" and all values are multiplied to obtain the result. Trees were created for all surveillance components: clinical surveillance (CS) by PHS and farmer; active sampling (AS) (cultural & PCR examination of nasal swabs from 2x10 pigs for toxigenic *Pasteurella multocida* / fecal swabs from 4 and 6 pigs for *Brachyspira hyodysenteriae*); 4 mixed fattenings (MF). Data for model parameters / distributions were obtained from literature and expert poll. Combining the component sensitivities (CoSe) as indicated in [3], the total sensitivity (ToSe) of PAR and SD surveillance at herd level over one year was calculated with 50.000 iterations in @risk®. Furthermore, the average time between infection of a herd and its detection was estimated using the formula:

$$TD = CoSe_1 * 0.5\ I + (1\text{-}CoSe_1) * CoSe_2 * 1.5\ I + (1\text{-}CoSe_1) * (1\text{-}CoSe_2) * CoSe_3 * 2.5\ I \ldots$$

TD = time to detection; I = average time interval in months between surveillance components

Results

The sensitivities for PAR and SD are given in table 1&2. The TD was 3.6 months for SD and 2.2 for PAR. Total costs were 793 and 695 €/herd/year for SD and PAR.

Tables 1&2. CoSe and ToSe of SD and PAR surveillance

Component sensitivities SD	5%ile	**Median**	95%ile
CS by farmer	4.5	**13.0**	24.2
CS by PHS	6.3	**17.9**	32.5
AS 6 animals	52.7	**73.5**	88.0
AS 4 animals	39.3	**58.7**	75.6
MF (exam. by PHS)	6.0	**12.7**	23.5
MF (exam. by farmer only)	3.2	**7.1**	14.0
Total sensitivity SD*	**87.2**	**95.7**	**98.9**

* accounting for 3 MF examined by farmer per year

Component sensitivities PAR	5%ile	**Median**	95%ile
CS by farmer	17.6	**28.9**	39.4
CS by PHS	18.0	**29.6**	40.3
AS 10 animals	56.6	**81.7**	95.1
MF (exam. by PHS)	24.4	**38.6**	53.7
MF (exam. by farmer only)	13.7	**21.7**	32.5
Total sensitivity PAR*	**98.2**	**99.8**	**99.9**

* accounting for 2 AS and 3 MF examined by farmer per year

Conclusions and Discussion

The total sensitivities for both diseases over one year were high. Nevertheless, the time to detection could be too long in herds with frequent pig trade, as experienced in the recent PAR outbreak. Results indicate that the CoSe of mixed fattenings did not contribute much to the ToSe whereas that of AS was always highest. E.g., if MF were abandoned and nasal swabs from 4 pigs at all 4 visits taken, the ToSe for PAR would be 99.9% at a reduced TD of 1.9 months and costs of 405 €/h./y. To significantly reduce the TD for SD to 2.5 months at an increased ToSe of 99.6% without MF, sampling of 4x6 animals would be required, at the costs of higher expenses of 938 €/h./y. The method of STM is a well suitable tool to assess the effectiveness of a surveillance system. Since all components can be modified, all kinds of alternative scenarios can be easily calculated to optimize surveillance sensitivity, time to detection or costs.

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An outbreak of PRRSV in Switzerland after import of virus-containing boar semen

C Nathues[1], S Bruhn[2], D Suter[2], H Nathues[3], G Schüpbach-Regula[1], B Thür[4], L Perler[2]
[1]*Veterinary Public Health Institute, University of Berne,* [2]*Federal Veterinary Office, Liebefeld,* [3]*Swine Clinic, University of Berne* [2]*Swiss Pig Health Service, Berne,* [4]*Institute for Virology and Immunology, Mittelhäusern, Switzerland* christina.nathues@vetsuisse.unibe.ch

Introduction

Switzerland (CH) has traditionally been free of porcine reproductive and respiratory syndrome virus (PRRSV). While the import of living pigs was always restricted via very small import quota, there were no such restrictions for boar semen import from member states of the European Union. As a consequence, imports of boar semen from PRRSV infected European countries like Germany, France or Austria increased significantly over the last few years. Since the risk of PRRSV introduction was unknown, a risk assessment was conducted in 2011/2012 (1). Results indicated that there was a substantial risk of PRRSV introduction into CH via imported boar semen. Before risk mitigating measures could be implemented, in November 2012 an outbreak of PRRSV occurred in Swiss sow holdings after the use of imported boar semen.

Materials and Methods

The infection was detected after a Swiss breeding company was informed by a German boar stud that they had detected PRRSV (genotype 1) in one boar with recent semen export to Switzerland. The company supplied a growing number of Swiss breeding herds with semen from that stud and accounted for > 95% of all semen imports. The stud was considered PRRSV-negative and did a biweekly monitoring (alternately via PCR on semen / PCR & ELISA on serum from a sample of boars). After detection of the infection, available aliquots from semen collections of that day were tested for PRRSV, and serum samples from all boars examined for PRRSV. After the Swiss Veterinary offices were informed, extensive investigations in Swiss sow herds were initiated: Based on the importers' records, 26 sow herds that had received semen from the infected stud after its last negative routine monitoring as well as 62 herds with contacts to these sow herds were identified, put under movement restrictions and examined consecutively: All sows in each farm were tested via ELISA & RT-qPCR. Meanwhile, as precautionary measure, the importer initiated immediate slaughter and testing of 59 sows that had lately been inseminated with semen from boars already known as positive. All contact farms remained under restrictions for at least 21 days after the first testing and were re-investigated serologically in January 2013.

Results

Eight boars from which semen had been sent to CH after the last routine monitoring were positive: In 7 cases antibodies & virus were detected in serum, in one case only antibodies; in only one boar virus was detected in semen. In total, 2 "high risk" boars were identified that had already been positive on the day of their last semen collection for CH. Their semen had been sent to 5 Swiss sow holdings. From the 59 slaughtered sows, 5 from 3 different herds were virus-positive. In one case the herd had only received 2 semen doses, whereas no virus was found in another herd that had used 35 doses from the same boar and semen collection. Examinations of all sows in the contact herds revealed that in one of the positive herds the virus had already spread. All 1900 pigs in the herd were slaughtered / culled. In the remaining contact herds, no further infections were detected, and confirmatory examinations 3 weeks later were all negative. In January 2013 all restrictions were abolished. CH was PRRSV-free again.

Conclusions and Discussion

The events prove that the import of semen from non-PRRSV-free countries, even from negative studs, poses a considerable risk of PRRSV introduction. It shows that monitoring protocols in boar studs are often insufficient to timely detect an infection in a stud. Especially the use of semen does not seem suitable to monitor disease freedom. Furthermore, this outbreak shows that an infection of sows / herds via PRRSV-containing semen is possible even with a very low number of semen doses. The fact that the outbreak was eradicated successfully was mainly due to the high disease awareness and initiative of the importer. First actions were already taken just on suspicion, and this is a rare example of an outbreak that was controlled before any clinical diagnosis or laboratory confirmation of a single case in the country had been made. Despite this, its economic implications were huge. In order to minimize the risk of a new introduction of PRRSV into CH in the future, new guidelines for the import of fresh boar semen were developed. These contain obligatory testing of each boar – semen and serum, PCR and ELISA – at the day of each semen collection for CH. Results have to be submitted to the Federal Veterinary Offices before use of the semen, and the semen may only be used if all test results are negative. Sow herds are put under animal movement restrictions until a sample taken at minimum 28 days after the last insemination with imported semen is negative.

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Lung scoring program as a monitoring tool in Asia

R Krejci[1], R Muñoz[2]
[1]Ceva, Libourne, France, [2]Ceva Asia Pacific, Malaysia, roman.krejci@ceva.com

Introduction

Ceva Lung Program (CLP) offers the methodology which assists in evaluating the presence, incidence and impact of *M. hyopneumoniae(M. hyo)* and *Actinobacillus pleuropneumoniae (A.p)* infections using adapted lung scoring of slaughter pigs.

Materials and methods

In total 1774 plucks of pigs originated from 19 farms were examined for the presence and extension of lung lesions. CLP consists of the modified Madec[1] method for scoring enzootic pneumonia (EP)-like lesions and modified SPES[2] method, for scoring A.p –like lesions. Bronchopneumonia (BP), scars in the predilection parts of lungs and cranio-ventral pleurisy (CP) is considered as suggestive for previous *M. hyo* (eventually complicated by other pathogens) infection. BP is scored from 1-4 per each lobe and weighed according Christensen 1999[2]. Dorso-caudal pleurisy (DP) (scores 2, 3 and 4 in original SPES) is an indicator of previous *A.p* infection.

Results

EP-like lesions: Pigs of all farms were affected by BP. Of all pigs 88% had in some extent BP lesions. The incidence varied from 80- 100%. The percentage of the affected lung parenchyma in lungs with BP ranged between 10-20%. It showed some correlation with the incidence (Fig 1).

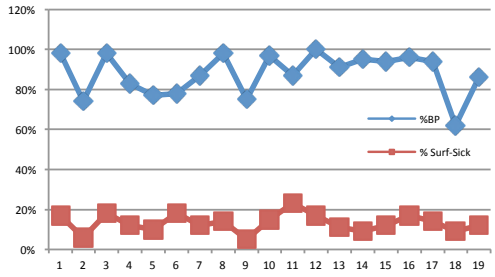

Figure 1. Relation between the incidence (%BP lungs) and average extension of EP-like lesions in lungs with BP (% Surface/sick)

The scars were found from 3 to 45 %, indicating high variability in the onset of the mass infections between the farms.

A.p- like lesions: Pigs from all farms had some extension of DP lesions suggestive for previous pleuropneumonia. The average incidence was 22%, ranging between 0 to 59%.

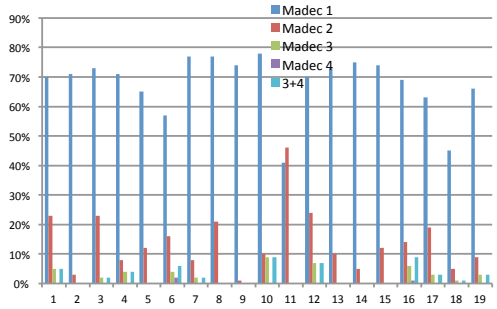

Figure 2. Prevalence of Madec lesions by score

The distribution of APPI values showed minimum = 0.31, maximal value 1.59 and average 0.57

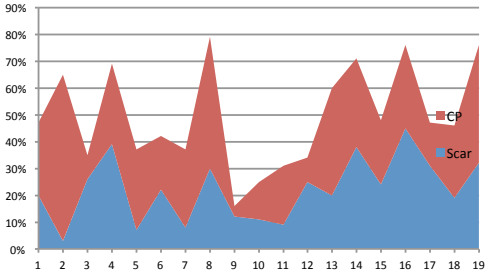

Figure 3. CP incidence and Scar lesions

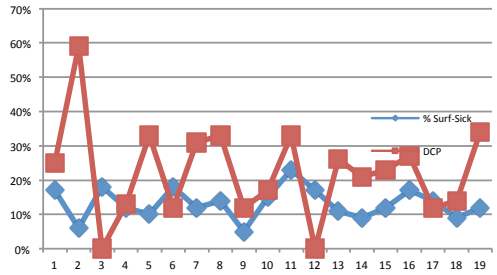

Figure 4. Relation between affected surface of Pneumonic lungs and DP lesions

Conclusions

The average prevalence and extension of lesions attributed to EP is high in Vietnam & Philippines with high variability among farms. There is no correlation between EP-like lesions and A.p–like lesions regarding the incidence or severity.

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Biochemical characteristics of *Y. pseudotuberculosis* isolated from Swedish wild boars (*Sus scrofa*)

A Sannö[1], S Thisted Lambertz[2], M Jacobson[1]
[1]*Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden,*
[2]*Department of Science, National Food Agency, Uppsala, Sweden* magdalen.jacobson@slu.se

Introduction

The Swedish wild boar (*Sus scrofa*) population is increasing with an annual cull for 2013 estimated to almost 100 000 animals. The risk for contacts with domestic swine, both direct and indirect through rodents and faeces left in fields with growing crops, are increasing. Further, the meat is now becoming more accessible to the public [1]. In a Swedish baseline study on domestic pigs at slaughter, *Yersinia enterocolitica* have been detected [2], However *Y. pseudotuberculosis* was not detected in that study. With a confirmed presence of *Y. pseudotuberculosis* in the Swedish wild boar population, this situation might change, and eventually cause concern for the public health [3]. This study aims to characterize strains of *Y. pseudotuberculosis* isolated from wild boars.

Materiasl and Methods

Samples consisting of tonsils, ileocaecal lymph nodes and faeces were collected from 88 free-living Swedish wild boars. Eight isolates of the human pathogen *Yersinia pseudotuberculosis*, originating from five different individuals, were obtained from a total of 319 samples.

The samples were cultivated overnight in Buffered Peptone Water (BPW) in +30°C, spread on cefsulodin-irgasan-novobiocin (CIN) agar plates and then further incubated overnight before colonies were analyzed by real-time PCR for detection of the chromosomally located *ail* gene encoding attachment and invasion of the pathogen in humans [4]. The BPW from PCR positive samples were cultivated again on CIN-agar to obtain pure isolates of *Y. pseudotuberculosis*. All characteristic isolates were thereafter subjected to PCR analysis to confirm the presence of the *ail*-gene. The isolates were subjected to further biochemical typing including API 20E, cultivation on Congo Red –Magnesium Oxalate (CR-MOX) agar plates, serotyping [5] and antimicrobial resistance pattern investigations[1]. [1]*In accordance with the accredited method at NVI (National Veterinary Institute, Uppsala).*

Results

All isolates were serotyped as O:1 and typed as *Y. pseudotuberculosis* using API 20E. All isolates were resistant to spiramycin and oxacillin, while three were sensitive and five were intermediate to tetracycline. The isolates were sensitive to all other antibiotics tested. Six isolates were positive on CR-MOX indicating presence of the virulence plasmid (see table 1).

Table 1. Sample type, weight and sex of sampled animals and the results from the biochemical testing of eight isolates of *Y. pseudotuberculosis*

Sample type	Weight/ sex	PCR	API 20E (code) [ID certainty%]	CR-MOX[b]	Serotype
Tonsil	15kg/F	pos *ail*	Y. pseudotuber. (1014152)[99,9]	5+	O:1
Tonsil	34kg/F	pos *ail*	Y. pseudotuber. (1014152) [99,9]	4+	O:1
Ileoca-ecal lkn	15kg/M	pos *ail*	Y. pseudotuber. (1014112) [99,8]	5+	O:1
Tonsil	66kg/M	pos *ail*	Y. pseudotuber. (1014112) [99,8]	Neg	O:1
Tonsil	39kg/M[a]	pos *ail*	Y. pseudotuber. (1014112) [99,8]	5+	O:1
Tonsil	---[a]	pos *ail*	Y. pseudotuber. (1014112) [99,8]	Neg	O:1
Tonsil	---[a]	pos *ail*	Y. pseudotuber. (1014112) [99,8]	3+	O:1
Tonsil	---[a]	pos *ail*	Y. pseudotuber. (1014112) [99,8]	4+	O:1

[a]Samples derived from the same individual
[b] Positivity graded on a scale 1-5

Conclusions and Discussion

The presence of zoonotic bacteria in the wild boar population poses a direct threat to public health but it is also of concern for domestic swine producers and the food industry. Rodents and migratory birds have been shown to carry different species of *Yersinia* as well as *Salmonella* spp. [1]. This implies that the pathogens present in the wild boar population easily can make their way into the swine barns and spread within the food chain. It is therefore important to further characterize these pathogens and also store them for future investigations, especially in conjunction with foodborne outbreaks.

Further ongoing studies focuses on the development and evaluation of methods for molecular epidemiological research on food-borne pathogens found in wild boars.

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Herd level factors associated with European H1N1 and H1N2 influenza virus infections in fattening pigs

C Fablet[1], G Simon[1], V Dorenlor[1], F Eono[1], E Eveno[1], O Bourry[1], S Gorin[1], S Quéguiner[1], F Madec[1], N Rose[1]
[1] *Agence Nationale de Sécurité Sanitaire (Anses), B.P. 53, 22440 Ploufragan, France, olivier.bourry@anses.fr*

Introduction

Swine influenza viruses (SIVs) are widespread in pig populations throughout a large part of the world [1]. Despite concerns regarding the huge impact of influenza infection in pigs and the possible transmission of SIVs to humans, few studies have investigated the risk factors for such virus infection in pigs. We therefore used data and sera from a cross-sectional study for respiratory diseases carried out from 2006 to 2008 in 125 herds located in western France to assess herd-level factors related to farm characteristics, management practices, housing and indoor climate associated with European SIV H1N1 or H1N2 infections.

Materials and Methods

In each herd serum samples from 15 fattening pigs were tested for antibodies against European SIVs H1N1 or H1N2 by haemagglutination inhibition [2]. Data related to herd characteristics, biosecurity, management and housing conditions were collected by questionnaire during a farm visit. Climatic conditions in the post-weaning and fattening rooms, where the sampled pigs were housed, were measured over 20 hours. An animal was considered positive for a given SIV subtype when the HI antibody titre was 20 or more [3]. However the outcome was defined at the batch-level for each SIV subtype. A batch was classified as positive for the virus subtype giving the highest frequency of sero-positive animals. In the case of positivity for more than one subtype, individual titres, i.e. at the pig level, were considered so as to take into account possible serological cross-reactions. When at least one animal had an equal or higher positive titre against the second subtype than against the first subtype, the infection with the second subtype was also considered as positive; the batch was then considered positive for both subtypes [3]. Factors associated with H1N1 or H1N2 sero-positive status of the herd were identified by logistic regressions for binary outcome (PROC LOGISTIC, SAS, 2001).

Results

Sixty percent [51.2%-68.8%] of the herds were considered positive for H1N1 SIV and 57.6% [48.8%-66.4%] were considered positive for H1N2 SIV. Thirty five percent [26.7%-43.7%] of the herds were positive for both SIV subtypes. For both subtypes, the odds for a herd to be SIV sero-positive increased if there were more than two pig herds in the vicinity (OR=3.2, 95% Confidence Interval (95% CI): 1.4-7.6 and OR=3.5, 95% CI: 1.5-8.1 for H1N1 and H1N2 respectively). Other factors were specifically associated with either H1N1 or H1N2 SIV infections. The odds for a herd to be H1N1 sero-positive were significantly increased by having a large number of pigs per pen in the post-weaning room (OR=3.2, 95% CI: 1.2-8.6), temperature setpoints below 25°C (OR=2.6, 95% CI: 1.1-6.4) and below 24°C (OR=2.6, 95% CI: 1.1-6.1) for the heating device in the farrowing room and the ventilation controller, respectively. Moving the pigs to the fattening facility via a room housing older pigs also significantly increased the odds of being H1N1 sero-positive (OR=3.3, 95% CI: 1.1-9.6). A H1N2 sero-positive status was associated with a brief down period in the farrowing room (OR=2.6, 95% CI: 1.1-6.3), high pig density in the post-weaning pens (OR=2.9, 95% CI: 1.2-7.0), large-sized fattening room (OR=2.5, 95% CI: 1.1-5.9), lack of all-in all-out management in the fattening room (OR=2.4, 95% CI: 1.0-5.8) and a temperature range of less than 5°C controlling ventilation in the fattening facilities (OR=3.2, 95% CI: 1.4-7.4).

Conclusions and Discussion

Four main groups of herd level factors were highly related to external and internal biosecurity: herd location, herd management, hygiene and building characteristics and husbandry. Hence, recommended measures aimed at a better control of SIV infections would include proper biosecurity measures to minimize the risk of virus introduction, management practices minimizing direct and indirect contacts between animals from different batches and within batches whilst providing the pigs with favorable climatic conditions. All these factors should be considered together when designing and implementing herd health management programmes.

Acknowledgments

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Seroprevalence of *L. intracellularis* in Korea 2012-2013

HK Seo, JH Park, SW Lee, HJ Chae, JM Kim, HP Hwang, ES Kim, SJ Sung, CS Shin, BJ Cho
Boehringer-Ingelheim Vetmedica Korea, hkseo@seo.boehringer-ingelheim.com

Introduction

Porcine Proliferative Enteropathy (PPE) is a economically devastating intestinal diseases for pig production which is caused by *Lawsonia intracellularis* and has a negative impact on performance parameters especially feed conversion.

Because the use of antibiotic feed additives as a growth promoter is a big concern for human health the Korean government has banned the use of antibiotic feed additives in July 2011. Since then, the incidence of the digestive disorders has been increasing.

The objective of this study was to investigate the seroprevalence and seroepidemiology of ileitis after ban of antibiotic feed additives in Korea.

Materials and Methods

From August 2012 to August 2013, a total of 4,400 serum samples were collected from 218 farms nationwide. Two different local laboratories conducted the testing. The samples were tested with a blocking ELISA (bioScreen Ileitis Antibody ELISA, bioScreen, Germany) according to the manufacturer's instructions.

Results

Of the total 4,400 samples, 1,447 (33%) samples were tested positive. Lab A examined 2,615 samples and 799(31%) were tested as positive and Lab B 1,785 samples, 648(36%) positive (Table 1)

Table 1. ELISA results of the samples tested .

	No. of farms	No. of samples	Positive samples	Positive %
Lab A	132	2,615	799	31%
Lab B	86	1,785	648	36%
Total	218	4,400	1,447	33%

For gilts 63% of the samples were tested positive while for the sows the seroprevalence was 88 %. In pigs, only 5% of the samples were tested positive at the age of 50 days, 30% at the age of 100 days, 49% at 120 days, 67% at 150 days and 67 % in 170 day old pigs (Figure 1). In all tested farms at least one sample was tested positive or a herd level the prevalence of 100 %.

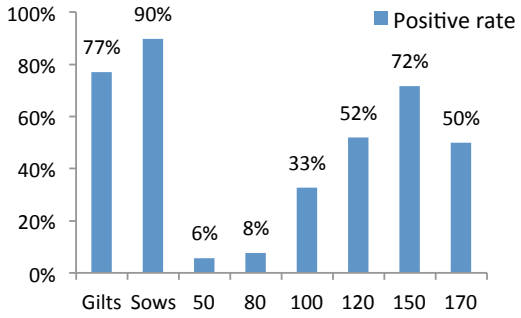

Figure 1. Percentage pigs seropositive against PPE at various age groups

Conclusions and Discussion

The two different labs had comparable results showing that one-third of the pigs are positive for antibodies against ileitis. Most of the sows had been exposed to *Lawsonia intracellularis* before testing. Most of farms were seropositive at 100 days of age which means that pigs get infected at the end of nursery or start of the grower stage. With an increase in age, the percentage of seropositives increases. Clinically, all tested farms had ileitis problems especially at the end of finishing where almost two-thirds were exposed to the pathogen.

When compared to a previous study[3] the results of this trail indicate that the withdrawal of antibiotic growth promoters led to an earlier time of infection and a higher seroprevalence at the end of finishing which suggests that the impact of ileitis has become more important.

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Prevalence of respiratory pathogens on 40 Dutch pig farms measured by oral fluid testing

GJR Groenland[1],
de Heus Ede, The Netherlands, g.j.r.groenland@hotmail.com

Introduction

PRDC is often seen on conventional Dutch pig farms (1), and diagnostics and monitoring are often complex and expensive. Vets and farmers are constantly looking for more accurate and cheaper diagnostic methods, which preferably have more welfare and are more user-friendly for man and pig. In the last few years Oral Fluid (OF) testing technology using ropes has been advanced and is fulfilling a lot of these requirements. Until now only a few reports with field data using this technique have been published (2).
The objective of this investigation was to get more insight in the infection dynamics involved on Dutch pig farms with PRDC problems by using rope collection with PCR testing.

Materials and Methods

In the period of September 2009 to September 2011, 32 Dutch pig farms with respiratory problems were investigated. Age groups of pigs with respiratory symptoms were sampled in multiple pens using OF/rope testing as described by Prickett (2), with 2 alterations: cotton rope was used and 5 cc physiologic saline was added to make collection easier. In general 2 ropes in 1 compartment with coughing pigs were used for diagnostics. In total 156 samples were analyzed from 40 samplings with 59, 42, 13, 21 and 21 samples for the age groups 3-8, 8-10, 10-12, 12-16 and >16 weeks of age respectively. The samples were analyzed by a multiplex PCR (IVD-GmbH lab Hannover (D) comprising PCR on PRRS, PCV2, Mycoplasma hyopneumoniae, APP and Influenza) and reported as negative or positive for the respective pathogen.

Results

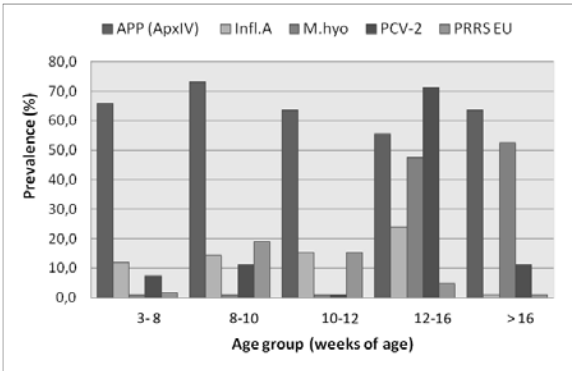

Figure 1. Prevalence of respiratory pathogens in Oral Fluid samples, for the different age groups

The prevalence of the lung pathogen for the different age groups is presented in figure 1. Pigs from 10 weeks of age or older are pigs from fattening units. APP had the highest prevalence in almost all the age groups with a minimum prevalence of 55% in the group 12-16 week old pigs. In 3-8 week old pigs 11,4 % of the samples were already positive for Influenza increasing to 23,8 % in the 12-16 week old group. Mycoplasma was hardly found in piglets younger than 10 weeks of age (1%). In the fattening period the prevalence for Mycoplasma increased to 47% or more in both groups older than 12 weeks of age. The PCV2 virus is most often found in the age group of 12-16 weeks (71,4% prevalence) and for the PRRS type 1 virus the highest prevalence (19%) was found in piglets around 8 weeks of age.

Conclusions and Discussion

Based on these results on Dutch pig farms with PRDC problems the following can be summarized: APP has a high prevalence in all the age groups. The test can detect 15 different APP strains (3), but not all are pathogenic. In the Netherlands type 2 and 9 are the most prevalent strains (4). In the nursery Influenza and PRRS are the most prevalent pathogens. More than 10% of the samples are already found positive for Influenza which is in line with other reports (5,6,7). With serology this infection is difficult to diagnose in these age groups due to maternal immunity. PRRS type 1 virus has the highest prevalence in piglets around 8 weeks of age. PRRS type 2 was hardly found (data not shown). The prevalence for Mycoplasma for the different age groups shows that Mycoplasma doesn't play a major role in the nursery but is primarily starting in the beginning of the fattening period, since it was not found in piglets younger than 10 weeks of age, followed by the far higher prevalence of above 47% for both groups older than 12 weeks of age. The PCV2 virus is most often found in the age group 12-16 weeks. But also in younger age groups there is some circulation. These OF results, sampled using pen detection with ropes, are in line with other recent Dutch results found with trachea-bronchial swab technique (8).

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Isolation and identification of a novel swine influenza virus subtype H1N2 in México

JH Lara P, R Cortes F, F Castro P, R Echeveste G, B Lozano D, D Sarfati M, E Soto P, F Quezada M
Laboratorio Avi-Mex, S. A. de C. V., México D.F., horacio.lara@avimex.com.mx

Introduction

Swine Influenza (SI) is a highly contagious disease capable to reach morbidity rates of up to 100% and a mortality rate of around 1%. SI virus (SIV) is genetically unstable and suffers antigenic variations. Antigenic drift involves the gradual accumulation of subtle genomic mutations, especially in the HA and/or NA genes, which in turn result in antigenic variation as a result of variants in an immune population. Antigenic shift is a major genome change occurring when a cell is infected simultaneously with two different SIV; recombination occurs and could potentially end up in 256 genetically new viruses (2, 3, 4, and 5).

The objective of this paper was to identify a group of SIV isolates suspected of being a new unreported subtype in México.

Materials and Methods

A total of eight SI viruses isolated between 2010 and 2012 (all originated from different states of the country) were selected (1). A Multiplex RT-PCR test for the M region (which is highly conserved among SIV strains and allows its identification) was used. Then, the obtained products were sequenced for the regions N, NA, NP and H for subtypes H1N1 and H3N2 and analyzed with the Vector NTI advance v11.5.1: Geneious Basic v5.0 program. The aminoacid sequences were compared among them and among an international reference of human and swine influenza viruses, including the 2009 pandemic H1N1 virus.

Results

Three isolates (37.5%) were identified as H1N1 and five isolates (62.5%) as HXN2 by RT-PCR. Then, the obtained products of the HXN2 isolates were sequenced for regions NS, M, NA (N1- N2), NP and H (H3 – H1). Results indicated that the five viruses belong to the subtype H1N2.

We also found that four of the H1N2 viruses showed a high genetic relation with recent US swine strains for segments NS, NA (subtypes H1N2 and H3N2); with human and swine viruses for the HA segment (subtypes H1N1 and H1N2) and with the pH1N1-2009 human virus for the M and NP segments. The fifth H1N2 SIV isolate is genetically similar to other H1N2 SIV's at segments NS, NA and HA, but showed a very high homology with a H1N1 SIV at segments M and NP.

One of the H1N1 SIV isolates was genetically different to the other two, and show a high homology in N, NA, NP to a H1N2 SIV, except at HA (that looks more like human strains) and at NA.

Conclusions and Discussion

Our results indicate the relevance of a robust diagnostic protocol in order to detect different SIV subtypes since the usual PCR primers could not detect well all SIV subtypes isolated from field samples (as been previously reported by FAO/OIE researchers for SIV and HI) (6).

This is the first report of the isolation and identification of a SIV subtype H1N2 in Mexico.

It is important to continue with field research in order to determine the productive impact of this new SIV subtype and to investigate further to find a possible genetic relation of this virus with others already present in Mexico and the world.

Note: Isolates identified as H1N2 in this work were officially reported to the Direction for Epidemiology and Risk Analysis (DEAR), and delivered to the CPA of SAGARPA on April 25, 2012, as the official Mexican regulations indicate.

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Antibiotic use in French pig farms: Indications and therapeutic strategies

A Hémonic[1], C Chauvin[2], I Corrégé[1]
[1]*IFIP - French Institute for Pig and Pork Industry, Le Rheu, France,* [2]*ANSES - French Agency for Food, Environmental and Occupational Health & Safety, Ploufragan, France,* anne.hemonic@ifip.asso.fr

Introduction

The French EcoAntibio 2017 Plan (1) aims to reduce antibiotic use in veterinary medicine by 25 % in 5 years. The aim of this study is to review the main reasons for antimicrobial treatments in pig farms. These data will help to develop strategies for reducing antibiotic use.

Materials and Methods

The study is based on collected data from the INAPORC Panel consisting of 169 pig farms representative of French production (2). For each antimicrobial they bought in 2010, farmers described, during a phone call, their antimicrobial usage pattern, as category of animals treated and indications of treatment. The indications of treatment were prioritized according to the percentage of concerned farms and the amounts of antibiotics required in the corresponding age group, expressed in number of Animal Daily Dose / animal (ADD/a) and number of Animal Course Dose / animal (ACD/a), as recommended by the European Medicines Agency (3).

Results

In sows, antibiotic treatments for urogenital infections (like cystitis or Leptospirosis) dominated, as they were mentioned by 71 % of farms and represented 65 % of ADD/a and 61 % of ACD/a (Table 1). They were mostly treated with Tetracyclines (**21** % of farms concerned, 42 % of ADD/a and *29* % of ACD/a) or Trimethoprim-Sulfonamides (**18** % - 26 % - *22* %). Penicillins were used in more farms (**41** %) but represented less quantities of antibiotics (12 % - *19* %) as they mostly concerned individual treatments. In suckling piglets, antibiotic treatments for digestive and locomotor disorders dominated. The first-line antibiotics were Penicillins for locomotor problems (**41** % - 61 % - *64* %) and Colistin for digestive disorders (**41** % - 43 % - *30* %), followed, in this last case, by Fluoroquinolones (**33** % - 15 % - *22* %). For all these treatments in suckling piglets, the injectable route was widely used. In post-weaning pigs, digestive disorders were the major indication of treatment. They were mostly treated with Colistin (**82** % - 53 % - *66* %), by oral forms. Respiratory diseases, the second indication of treatment, were mainly treated with Tetracyclines (**30** % - 47 % - 57 %), also orally. In fattening pigs, respiratory and digestive diseases also dominated. Tetracyclines (**36** % - 38 % - *51* %, Macrolides (**18** % - 28 % - *12* %) or Trimethoprim-Sulfonamides (**8** % - 22 % - *20* %) were used orally for respiratory treatments, like Colistin (**16** % - 42 % - *54* %) and Macrolides (**20** % - 34 % - *20* %) for digestive treatments.

Table 1. Indications of treatment by age group: part of nDD/a, nCD/a and farms concerned

	Sow	Suckling piglet	Post-weaning pig	Fattening pig
	% farms*	% farms	% farms	% farms
	% nDD/a – nCD/a	% nDD/a – nCD/a	% nDD/a – nCD/a	% nDD/a – nCD/a
Digestive	31	68	89	38
	7 - 6	53 - 35	69 - 69	40 - 34
Respiratory	24	6	46	63
	8 - 6	1 - 3	17 - 16	45 - 49
Locomotor	28	58	29	36
	2 - 3	33 - 49	2 - 2	1 - 2
Systemic	53	16	34	19
	7 - 12	8 - 8	8 - 8	8 - 6
Urogenital	71	-	-	-
	65 -61	-	-	-
Udder	27	-	-	-
	2 - 4	-	-	-
Skin	10	1	5	5
	0 - 0	0 - 0	1 - 1	2 - 4
Nervous	0	0	7	2
	0 - 0	0 - 0	1 - 1	0 - 0
Mortality	0	2	2	1
	0 - 0	2 - 3	1 - 1	1 - 1
Don't know	8	6	12	8
	10 - 6	2 - 2	3 - 3	3 - 2

*Percentages of farms involved is greater than 100 because a farmer could cite several indications of treatment for one antimicrobial

Conclusions and discussion

Approaches to reduce antibiotic use, which had already been undertaken in France since 2010 (4), should go on targeting in priority these main reasons for treatment.

Acknowledgments

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Anti-*T. Gondii* antibodies IgG and IgM in heavy pigs reared in Northern Italy

E Giacomini[1], M Lazzaro[1], S Giovannini[1] , N Ferrari [1], P Razzini[2], A Costa[2], E Pozio[3], P Pasquali[3], L Alborali[1]
[1]Istituto Zooprofilattico Sperimentale Lombardia Emilia Romagna, Brescia, Italy; [2]Azienda sanitario locale di Mantova; [3]Istituto Superiore di Sanità, giovanni.alborali@izsler.it

Introduction

Toxoplasmosis, a disease affecting all warm blooded animals including humans, is caused by the protozoon *Toxoplasma gondii*. The disease, is a serious public health problem and pork is considered an important source of infection for humans. The aim of this study was to estimate the *T. gondii* seroprevalence in heavy pigs reared in high containment level farms of Northern Italy and slaughtered at 9 months of age.

Materials and Methods

We randomly selected 240 herds from 2010 to 2013 at the slaughterhouse (Table 1). From each herd, blood samples were randomly collected from 20 heavy pigs. *T. gondii* specific IgG and IgM antibodies were detected by two commercial competitive ELISA ID Screen Toxoplasmosis Indirect Multi-Species (ID VET innovative diagnostics). The ELISA results were interpreted according to the manufacturer's instruction as: negative, inconclusive (borderline), positive, and positive/acute infection. When at least one blood sample per batch resulted as "positive" or as "positive/acute" by the ELISA, the whole batch was considered as "positive".

Table1. Herds sampled per years

Year of study	N° herds analized
2010	60
2011	60
2012	60
2013	60

Results

Anti T. gondii antibodies were detected in 178 (3,70%) out of 4800 pigs (Table 2). According to the manufacturer's instruction, 43 (17,5%) of 240 herds resulted positive (Ph1) and 5 (2,1%) herds resulted "positive with acute infection". In 31 (12,9%) herds, positive animals ranged between 1 and 2, in 4 (1,7%) between 3 and 5, and in 8 (3,3%) >5 (Ph 2)

Table2. Results of animals

N° of animals sampled	N° of animals positive	N° positive animals with I.A	N° of animals negative
4800	178 (3,7%)	21(0,4%)	4622 (96,3%)

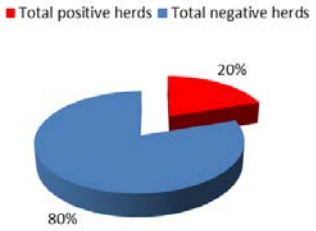

Figure 1. Results of herds

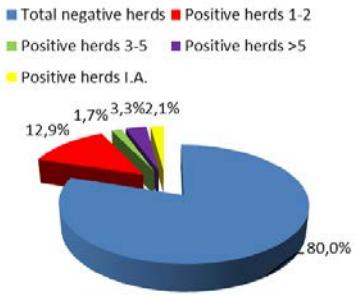

Figure 2. Positive animals in herds with different range

Conclusions and Discussion

The results of the present study show that the *T. gondii* seroprevalence in heavy pigs reared in Northern Italy was relatively high, even if the acute infection occurs only sporadically. The collection of data on feed, environmental and management conditions at the herd level will be useful to evaluate the risks of *T. gondii* transmission, to improve the integrated measures and reduce the seroprevalence in the heavy pig production of Northern Italy.

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Impact of the use of Ceftiofur on the emergence of *Salmonella* resistant to cephalosporins in four conventional pig farms

[1]K Cameron-Veas, [2]MA Moreno, [3]L Fraile, [1]L Garcia-Migura
[1] *Centre de Reserca en SanitatAnimal (CReSA), Barcelona, Spain.* [2]*Centro de Vigilancia Sanitaria Veterinaria, Universidad Complutense de Madrid,* [3]*Animal Production Department, Lleida, Spain,* lourdes.migura@cresa.uab.cat

Introduction

The use of ceftiofur is licensed for treatment of systemic bacterial infections in pig production. The worrisome of extended spectrum cephalosporinases (ESC) producing *Enterobacteriaceae* entering the food chain have raised the debate on the use of this type of antimicrobials for animal husbandry (Jorgensen et al., 2007). This study was performed to evaluate if the treatment with ceftiofur is a risk factor for the emergence of ESC producing *Salmonella* during the rearing period in four conventional farms and asses if these resistant bacteria can enter the food chain.

Materials and Methods

This study was carried out in four farms belonging to a large farm integration system in Spain. In each farm, a total of 70 seven-day-old piglets were divided in two groups; control (n=30) and group treated (n=40) with ceftiofur (Naxcel®, Zoetis). Animals were fed under a standard nutritional program set by the company that included the use of amoxicillin, apramycin, tiamulin and oxytetracicline in a prophylactic way during the nursery period (21-70 days of age). Fecal swabs were taken from piglets before treatment with ceftiofur (aprox. 7 days-old), 48 hours and 7 days post-treatment. A final sample was performed before the animals departed to the slaughterhouse (180 days of life). *Salmonella* isolation was performed according to ISO 6579:2002.

Results

A total of 39 *Salmonella* strains were recovered from three of the farms. One of the farms was negative for the presence of *Salmonella*. Three ESC producing *Salmonella strains* were recovered from seven-day-old piglets before receiving any type of medication. One extra isolate was recovered from one animal previously positive for ESC producing *Salmonella* after treatment. However, ESC producing *Salmonella* could not be detected during the rest of the study period. By the finishing time, all samples were negative for cephalosporin resistant *Salmonella* in this farm. This is a relevant observation since different antimicrobials were used in a prophylactic way during the nursery period, including amoxicillin. Moreover, it was not observed any increase in the percentage of samples positive for cephalosporin resistant *Salmonella* 48 hours post-treatment within the treated group.

Conclusions and Discussion

The ceftiofur treatment did not increase the presence of cephalosporin resistant *Salmonella* over the course of treatment in any of the studied farms. This preliminary study suggests that it is not necessary to implement additional control measures focused on reducing the load of cephalosporin resistant *Salmonella* of pig origin.

Acknowledgments

This work was supported by project AGL2011-28836 from the Ministerio de Economia y Competitividad of Spain.

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Occurrence of Cysticercosis in pigs at slaughterhouses of São Paulo State, Brazil

HMS Almeida[1], GAM Rossi[1], IRH Gatto[1], ACG Siqueira[1], LF Ribeiro[1], MF Garnica[1], MEF Oliveira[1], AMC Vidal-Martins[2], LG Oliveira[3]

[1] *Department of Preventive Veterinary Medicine and Animal Reproduction, College of Agricultural and Veterinary Sciences, São Paulo State University (UNESP), Jaboticabal, São Paulo, Brazil* [2]*Department of Veterinary Medicine, College of Animal Science and Food Engineering, University of São Paulo (USP), Pirassununga, São Paulo, Brazil* [3] *Department of Veterinary Clinic and Surgery, College of Agricultural and Veterinary Sciences, São Paulo State University (UNESP), Jaboticabal, São Paulo, Brazil,* luis.guilherme@fcav.unesp.br

Introduction

The pig cystycercosis-taeniasis complex is a zoonosis caused by *Taenia solium* that infects humans as definitive hosts and swines and humans as intermediate hosts. The disease is important for causing economic losses in slaughterhouse due to carcass condemnation or the cost of treatment when a mild infection occurs. It is also a public health issue due to the treatment cost of the infected humans. For the disease's prevention some measures are recommended, such as: basic sanitation, sanitary inspection of swine meat or use of Good Agricultural Practices (GAP) during animal husbandry. The present study aimed to evaluate the occurrence of pig cysticercosis during 2008 to 2013 at pig's slaughterhouses registered in the São Paulo State Sanitary Inspection System, Brazil.

Materials and Methods

The data reports from 29 pig's slaughterhouse registered and supervised by the São Paulo State Sanitary Inspection System (SISP) were analyzed. Those reports had informations about alive and calcified cysticercosis occurrence in carcasses from January 2008 to June 2013.

Results

In the period from 2008 to 2013, 1.305.723 pigs were slaughtered, which 125 (0.009%) had cysticercus in the carcass. From those, 116 (92.89%) were calcified, in other words, were not infective, while 9 (7.20%) were viable, as demonstrated in Table 1.

Table 1. Swine cysticercosis occurrence from 2008 to 2013 in 29 pig's slaughterhouse at São Paulo State, Brazil.

Year	Animals (n)	Occurrence (%)	Live (%)	Calcified (%)
2008	169.071	0.005	40	60
2009	175.913	0	0	0
2010	225.991	0.01	9.1	90.9
2011	282.541	0.008	4	96
2012	303.180	0.01	0	100
2013	149.027	0.001	50	50
Total	**1.305.723**	**0.009**	**7.2**	**92.8**

Conclusions and Discussion

The observed occurrence of swine cysticercosis in that period was under the aceptable limit (3%) estabilished by FAO (1986) to developing countries. Falavigna-Guilherme et al. (2006) did not observed the occurrence of swine cysticercosis in 1,406 animals slaughtered at Paraná State, Brazil. These data evidence the low occurrence of swine cysticercosis in the brazilian pig herd, demonstrating preventive measures are been efficiently used to avoid the occurrence of the disease. According to Talamini (2005), the brazilian swine meat supply chain has been suffering modifications over the last decades, changing from extensive husbandry to an integrated production system. In Brazil, the main comercial swine breeders use intesive production system, with proper technical assitance and owners with basic education or higher education level (EMBRAPA, 2013). In addition biosecurity and profilatic measures are been widely adopted by those breeders.

It can be concluded that a low occurrence of swine cysticercosis was observed pig's slaughterhouse of São Paulo State, suggesting that the disease can be erradicated from this state, reducing economical losses in the pig production and consequently the impacts in public health.

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Third and fourth generation cephalosporines : Use cut by 3 between 2009 and 2012 by French pig vets

S Chouet[1], F Voisin[2], JY Jouglar[3], M Liber[4], P Le Coz[5]
[1]*SELAS les Ondines, Change, France,* [2]*ZOOPOLE développement, Ploufragan , France,* [3]*AFMVP, ENVT, Toulouse, France,* [4]*AVPO, Landivisiau, France,* [5]*SNGTV, Paris, France,* sylvie.chouet@cam.fr

Introduction

French vets paid attention in 2010 to the rise in human medecin of Methicillin-resistant *Staphylococcus Aureus* and Extended **Spectrum Beta-Lactamases producing bacteria**[1]. Last generation cephalosporines were identified as the first target because of there strategic role in human health, even if all classes of antibiotics are eligible.

The french pig practitioners represented by there technical organisations (AFMVP, AVPO, SNGTV), agreed not to wait for a regulation, and made a consensus to volontarily limit and frame the prescription of cephalosporines, specially those of 3rd and 4th generation (C3G/C4G). The practitioners also agreed to communicate there cephalosporine prescription datas to an independant organization in order to show they respected the position taken.

Framed prescription consensus

A total of 89 vets returned a consensus engagement form. This represents 60,1% of the praticioners.

Cephalosporin dispenses between 2009 and 2012

On a voluntary basis, the dispenses of 33 practices housing 110 vets (92 full-time equivalent practitioners) were collected for the period from 2009 to 2012. This corresponds to the dispenses of 74,3% of the french pig vets. Table 1 summarizes the datas.

As a whole, the dispenses of C3G/C4G per pig vet had a 71% drop between 2009 and 2012. The biggest step down are 46% between 2010 and 2011 followed by 45% between 2011 and 2012. In terms of corporal weight, this represents a 73% cut in the C3G/C4G dispenses between 2009 and 2012. During this period, the ceftiofur based proprietary drug dispenses had a 73% drop between 2009 and 2012 (respectively 1,2 and 0,3 kg of activ principle per full-time equivalent practitioner). Regarding cefquinome based proprietary drug dispenses, they fell from 61% between (69 and 41 g of activ principle per full-time equivalent practitioner respectively in 2009 and 2012). This represents a 40% fall of the live weight treated.

Table 1. Cephalosporines dispenses and corresponding Pig Exposure.

	2009	2010	2011	2012
Number of full-time equivalent practitioners	76	73	80	92
Cefquinome (g)	69	66	45	41
Ceftiofur (kg)	1,20	1,16	0,63	0,33
Live-weight treated (T)	431	418	212	115
French Production (10^6 carcass equivalent T) [2, 3]	2,05	2,31	2,07	2,03
Pig Exposure	0.211	0.180	0.102	0.056

Conclusions and Discussion

The response and collection rates are high for a voluntary process. The reported fall of C3G/C4G dispenses between 2009 and 2012 is consistent with the 73,3% decrease of live weight treated and the 62,1% decrease of pig exposure reported by the French Agency for Veterinary Medicinal Products between 2010 and 2012[4]. This represents a two third cut in the exposure of pigs to last generation cephalosporines. This is the result of a clear engagement in response to clear arguments, messages from technical associations, and discussions with the other pig professionals.

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Gastrointestinal parasites of zoonotic potential semitechnified swine farms in Cundinamarca-Colombia

A Pulido-Villamarín[1], MF Mendoza-Gómez[1], A Barbosa-Buitrago[2], R Cubillos-Azcárate[3]
[1]*Unidad de Investigaciones Agropecuarias (UNIDIA), Departament of Microbiology, Faculty of Sciences, Pontificia Universidad Javeriana, Bogotá, COLOMBIA.*. [2]*MV, MSc (C) Universidad El Bosque.* [3]*MV, Faculty of Animal Sciences, Universidad de Ciencias Aplicadas y Ambientales (UDCA),* adriana.pulido@javeriana.edu.co

Introduction

The presence of gastrointestinal parasites (GIP) is an economic limiting factor in hog production in the U.S. it is estimated that the economic losses could rise to 155 million dollars annually (1); although, many efforts to control and prevent them are committed, these conditions are still a serious problem and are realted to respiratory problems, the most common diseases in worldwide swine productions systems (3). These GIP not only affect economically, but are also involved in processes that cause zoonotic public health problems (2). Therefore, the objective of this study was to determine the presence of endoparasites with zoonotic potential in swine and humans in two semi-technical production farms located in the countryside of Ubaque and Tena (Cundinamarca, Colombia).

Materials and Methods

Three samplings serial with 15-day intervals were performed and were obtained from a total of 60 of faecal samples (in group and individual) for different age groups as follows: sows (n = 17), gilts (n = 7) , boars (n = 3), piglets (n = 5), pre-fattening (n = 16) fattening (n = 12). Humans who agreed consent to enter the study, only authorized one sampling and a total of 15 samples were obtained (7 men, 5 women and 3 children). The faecal samples were evaluated by direct analysis, with the qualitative flotation technique, qualitative sedimentation technique and staining with modified Ziehl-Neelsen (4). Data were analyzed using descriptive statistics.

Results

On average, the obtained results showed a variety of parasites inside the swine age groups: *Entamoeba coli (40%), Endolimax nana (35%), Iodamoeba bütschlii (25%) y Balantidium coli (5%).* The parasites observed in humans were: *Entamoeba coli* (50%), *Balantidium coli* (5%), *Iodamoeba bütschlii* (20%), *Blastocystis hominis* (5%) *y Entamoeba histolytica/ dispar* (20%).

Conclusions and Discussion

Probably, the presence of these parasites in both species is maybe due to the poor sanitation levels, and kept the question if contamination of water font is involved in the parasitical processes. The presence of parasites as *Balantidium coli, Iodamoeba bütschlii* and *Entamoeba coli* in swine and humans suggests a possible rotation of parasites species among hosts.

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Nitric oxide participate in the shooting of sexual behavior in gilt

JM Ramírez Orduña[1], BL Zavalza Valdez[1], R Ramírez-Orduña[1], C Arevalo-Alvarez[1], R Cepeda-Palacios[1].
[1]*Autonomous University of Baja California Sur. e-mail: jramirez@uabcs.mx Km.5.5 Road South La Paz-Los Cabos, Baja California Sur. C.P.23080,* jramirez@uabcs.mx

Introduction

Recent studies show that estrogens have an acute effect on the production of nitric oxide (NO) , stimulating the activity of NO synthase (NOS) , and that the biological effects of steroid hormones is due to elevated levels of mRNA, Ceccatelli et al (1996) reported an association between increased expression of mRNA levels and levels of estradiol (E_2) 17 - β , these authors showed that the expression of neuronal NOS is increased in the ventromedial hypothalamic nucleus in rats ovx treated with 17-β estradiol. These data suggest that 17-β estradiol increases NO production by inducing the expression and / or increased enzyme activity of the NOS. Additionally, results obtained in our laboratory show in vivo the involvement of cGMP as a complementary route used by E_2 in shooting of sexual behavior (non-genomic pathway) in the brain of the sow, these data support the hypothesis that through NO-cGMP -PKG is involved in sexual behavior induced by oestradiol, and suggest the possibility that in the case of the sow, the induced sexual behavior may be triggered in a complementary manner by stimulating PKG via nitric oxide. With this background, we evaluated the involvement of NO in the induction and maintenance of sexual behavior by E_2 in the sow.

Materials and Methods

Animals and general procedure**.** 36 pubertal gilts were ovx and implanted into the right lateral ventricle (RLV), according to the atlas of Bernadette, et al, 1997. 72h after implantation treatments were supplied intracerebroventricularly (ICV) and 24h later the sexual behavior of sows was evaluated three times (24 h, 36 and 48, according to the methodology described by Ramirez- Orduña, et al., (2004). The ratio of immobility (CI) was calculated and the proceptivity. Free estradiol (E_2l; 16 μg, maximum effective dose administered ICV, reported by Ramirez- Orduña, 1994), was dissolved in propylene glycol (100 μl), and NG-nitrto-l-arginine methtylester (L-NAME) NOS inhibitor was dissolved in 100 μl of saline solution, and administered 15 min after the E_2l. At the end of the experiment the animals were sacrificed and the precision of the implant was verified. Data were analyzed with analysis of variance Kruskal-Wallis and Wilcoxon-Mann Whitney test (Siegel and Castellan, 1988).

Experiment I. For this experiment twelve animals randomly divided into two groups were used:

Table 1. Used treatments for the selection of the L-NAME dose and evaluation of non-specific effects.

Groups	Treatments	N
1	L-NAME vehicle (saline, 100 µl* l) + Saline (100 µl).	6
2	L-NAME (100nmol / 100 µl of saline solution) + saline solution (100 µl)	6

Experiment II. Twenty four sows randomly divided into four groups were used:

Table 2. Used treatments for the evaluation of the effect of ONS inhibitor, L-NAME on female sexual behavior induced by E_2l in sows previously ovx.

Groups	Treatments	N
1	Vehicle of free E; 100µl + vehicle of L-NAME 100µl;)	6
2	Vehicle of free E; 100µl + L-NAME (5,000µg /100µl)	6
3	Free E (16µg/ 100µl) + vehicle of L-NAME (100µl)	6
4	Free E (16µg /100µl) + L-NAME (5,000µg/100µl)	6

Results

Exp. I Treatment with L-NAME did not affect the CI nor the proceptivity of sows compared with the control group, so that the dose and route of administration proven was selected.

Exp. II. The results are shown in table 3

Table 3. Ratio of immobility in sows induced to the estrus with free E2 and treated with the inhibitor of the nitric oxide synthase L-NAME.

	HOUR		
Groups	**24**	**36**	**48**
1	20 ± 6^a	15 ± 8^a	17 ± 7^a
2	15 ± 12^a	26 ± 20^a	30 ± 21^a
3	28 ± 11^a	66 ± 15^b	68 ± 23^b
4	48 ± 11^b	63 ± 13^b	58 ± 15^b

Literals indicate difference between rows ($p<0,05$).

Conclusions and Discussion

Estrogen stimulated immobility behavior in ovx sows 24 hrs after the ICV infusion ($p < 0.01$), effect persisted for the next 24 h. The administration of L-NAME decreased the immobilization behavior triggered by estrogen (time 0, $P < 0.01$)). No effects on the h12 and 24 ($p > 0.05$) were observed. The results show in vivo the involvement of NO in the firing of sexual behavior, but not in the maintenance of this conduct, it is probably a complementary route used by E_2 in their interaction with E_2 receptors located in the nucleus of nerve cells in the CNS. The mechanism regulating estrous behavior is complex in nature.

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The influence of environmental temperatures on farrowing rates and litter sizes in South African pig breeding units

L Janse van Rensburg[1], BT Spencer[2]
[1] Delpen Building, Department of Agriculture, Forestry and Fisheries, Pretoria, South Africa; [2] Onderstepoort Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa, LeanaJvR@daff.gov.za

Introduction

The reproductive performance of pigs is the main determinant of the profit farmers make in pig production. This study was undertaken to describe whether periods of high environmental temperature have an effect on the farrowing rate, litter sizes and number of stillbirths, on commercial breeding units in South Africa.

Materials and Methods

Data was collected from four commercial breeding units with good records on a weekly basis from December 2010 to August 2012. This data included the number of sows mated, number of sows farrowed and the number of piglets born alive, as well as the number of stillbirths. Note was also taken of whether environmental temperature control mechanisms were employed. Temperature data from weather stations within 100km of the breeding units was obtained from the South African Weather Service.

Results and Discussion

Similar to the findings by other authors (1,2), in all four breeding units evaluated in this study, it was observed that the trend was for the farrowing rate to decrease as the environmental temperatures increased around the time of mating.

Table 1. Farrowing rates for different breeding units

Breeding unit	Mild temperatures	Severe temperatures	Difference
1	92.9%	90.5%	-2.5%
2	93.5%	87.9%	-5.6%
3	91%	87.6%	-3.4%
4	90.7%	90.1%	-0.6%

It can be seen from table 1 that the results were similar to those found in Kenya (1), with an economically significant decrease in farrowing rate following matings during severe average temperatures (>30°C) when compared to the farrowing rate following matings during mild average temperatures (<22°C).

The observations of this study with regard the trends of litter sizes were similar to those by other authors (1,3), following mating and early gestation with high ambient temperature. When mating occurred at higher temperatures the resultant litter size was marginally decreased in the breeding units that did not employ environmental temperature control, but was unaffected in the breeding units which did. However, it did not have a significant influence on the average number of piglets born alive.

It was observed in all four breeding units that the trend was for the average number of piglets born alive to increase as the environmental temperature around the time of farrowing increased.

The trend in three of the four breeding units was for the percentage of stillbirths per litter to decrease with increasing temperature around the time of farrowing. This could possibly be attributed to improved viability of the piglets at higher temperatures.

Conclusion

On all breeding units an economically significant decrease in farrowing rate following matings during severe average temperatures (>30°C) when compared to the farrowing rate following matings during mild average temperatures (<22°C) was observed. When mating occurred at higher temperatures the resultant litter size was marginally decreased in the breeding units that did not employ environmental temperature control, but was unaffected in the breeding units which did. In all four breeding units the trend was for the average number of piglets born alive to increase as the environmental temperature around the time of farrowing increased and the trend in three of the four breeding units was for the percentage of stillbirths per litter to decrease with increasing temperature around the time of farrowing. Environmental temperature control did not negate this effect, but the breeding units employing the environmental temperature control did show higher average farrowing rates overall.

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Reproductive performance during first and second parities of gilts synchronized with Altrenogest

A Alzina[1], P Chimal[1], JL Velasco[2], J Segura[1], M Álvarez[1]
[1]*Facultad de Medicina Veterinaria y Zootecnia UADY,* [2]*Laboratorios Virbac México S.A. de C.V.*alzina@uady.mx

Introduction

Variation in the number of sows inseminated per group brings about problems associated with management, because of the over- or sub- utilization in weaning and fattening facilities. One alternative to reduce that variation is the use of a hormonal product. Therefore, the objective of this study was to evaluate the use of a progestagen to synchronize estrous in replacement gilts and how it affects their reproductive performance during first and second parity.

Materiasl and Methods

The study was carried out in a farm with capacity for 3800 sows, fed commercial diets. The farm was seropositive to PRRSV. To synchronize the oestrus a progestagen was applied to five groups of at least 70 gilts each. The batches loading was planned to include female replacements in those groups were the number of farrowings were low to complete the number of females served. Females of each group were given orally a 20 mg/day dose of Altrenogest, (Virbagest®, Virbac, France), during 18 days. At the end of treatment, the females were moved to the service area for oestrous detection and insemination. Only data of four groups were used at second parity due to health problems in one of the five initial groups. Data was analyzed using descriptive statistics.

Results

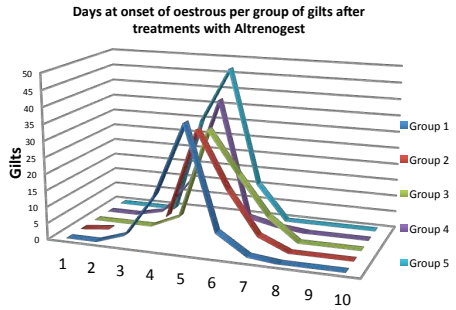

Figure 1. Response of gilts to Altrenogest treatment.

Table 1. Reproductive performance during first and second parity of gilts treated with Altrenogest.

Gilts treated (n)	AI 1	Farrowings 1	FR 1	LS 1	PBA 1	PBD 1	Mom 1
394	379	357	94.2	12.17	11.79	0.22	0.16
SD				0.44	0.43	0.05	0.04
	AI 2	Farrowings 2	FR 2	LS 2	PBA 2	PBD 2	Mom 2
	261	249	95.4	11.8	11.4	0.21	0.18
SD				0.32	0.31	0.03	0.04

AI *Artificial insemination*, **FR** *Fertility rate from service to farrowing*; **LS** Average litter size; **PBA** *Piglets born alive;* **SB** *Stillborn*, **Mom** mummified, **SD** *Standard Deviation.*

Conclusions and Discussion

At the end of the treatment, 96.2% of the gilts showed oestrous behavior and were inseminated before 7 days (Figure 1), which indicates the efficacy of Altronogest to synchronize estrous in gilts as reported in other studies (Davis, *et al.* 1985; Dimitrov, *et al.* 2010). As a result of grouped farrowings and weanlings, piglets are expected to be more homogeneous in age. Fertility rate from service to farrowing and other reproductive traits here studied did not show adverse effects at first or second parities (Table 1). In conclusion the application of Altrenogest on gilts may help to reduce the variation in batch production, without affecting reproductive traits.

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Relationship of different birth weight categories with weaning weight and average daily gain in litters of hyper-prolific sows during lactation

AU Rendón[1], GR Martínez[1], LM Herradora[1], SM Alonso[2]
[1]*Departamento. de Medicina y Zootecnia de Cerdos. Facultad de Medicina Veterinaria y Zootecnia. Universidad Nacional Autónoma de México.* [2] *Departamento de Producción Agrícola y Animal, Universidad Autónoma Metropolitana-X*, bagheera259@hotmail.com

Introduction

Piglet survival is of mayor importance when working with hyper-prolific sow lines where relevant piglet survival genetic correlations have been found in relation to litter size, and within-litter variation of birth[1] and weaning[2] weight. Weight and its dispersion become highly significant considering that the introduction of hyper-prolific sows to increase the number of newborn piglets requires a specific evaluation of efficiency in the complete production cycle[3]. Therefore, it is most relevant to acknowledge the proportion and performance of low-weight piglets (LWP) in existent pig farms to search for viable economic strategies that make these animals productive and financially profitable.

Materials and Methods

Data was taken of piglets born from 32 litters of F1, first, and second parity sows (L-LW) from a hyper-prolific genetic line in a commercial farm in the state of Veracruz, México. A data record was registered for every sow and each delivery, considering sow identification, parity date, parity number, total piglets born (TPB), individual piglet birth weight (BW), weaning weight (WW), days of lactation (DL), and average daily weight gain during lactation (ADG). The mean birth weight and the standard deviation were calculated and 3 weight categories were established: low weight (LW) under 986 g, mean weight (MW) between 987 and 1562 g, and high weight (HW) over 1567 g. During lactation the nipples that each piglet sucked were determined and classified as front (FRO), medial (MED) and inguinal (ING). WW and ADG were measured. For each of the variables evaluated a variance analysis was done with a random model. Likewise, a correlation of the TPB with BW and ADG was performed.

Results

The weight of 417 piglets was determined, 405 born alive, 135 piglets from the first parity and 148 from the second one. A TPB average of 13.59 was obtained; 14.2 in the first parity and 13.02 in the second. The LW percentage was 16.78 %. A negative correlation was found between TPB with BW and ADG (R^2=0.102; P<0.001).

The weaning weight, lactation length and average weight gain according to the piglet born weight category are shown in table 1.

Table 1. Average WW (kg), DL and ADG for each birth weight category.

Category	N	WW (kg)	D.L.	ADG (kg)
LW	49	4.92 a	21.86a	0.181a
MW	279	5.96 b	21.60a	0.211b
HW	49	6.93 c	21.43a	0.240c

Different letters in each column show difference (P<0.001).
N=number of piglets

Altogether, no difference was found in WW and ADG among the nipple categories (P>0.01) and in the LW and HW (P>0.01) piglets. However, a difference was found for ADG in MW between FRO (0.218 kg) with MED (0.214) and ING (.201) ING (P<0.05); for WW there was a difference for MW between MED (6.62 Kg) and FRO (6.33) and ING (5.8 Kg) nipples.

Conclusion and Discussion

The increase in litter size caused larger weight dispersion and a higher number of piglets with low weight. Being born in a low weight results in a reduced weaning weight and a lower average daily gain. The nipple position has no effect upon the performance of low- birth- weight piglets.

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Possible repeating rates of sows inseminated week by week in Northern parts of Northern hemisphere

M Sviben

[1]*Freelance consultant, Siget 22B, HR-10020 Zagreb, Republic of Croatia,* marijan.sviben@zg.t-com.hr

Introduction

In 23 EU member states in 2005 60.7% of all sows were in 2.4% of herds having 200 and more sows, 542.5 sows per herd on an average. In so large piggeries the weaning on Thursday and the insemination of sows through the week broadened not only in Europe (1). The farrowing rates observed in Danish farms week by week were published (2) but the trend of fluctuations of the farrowing rates through economic year was not presented by the parabola derived from some kind of polynomial. No one published the trend of fluctuations of the repeating rates of sows inseminated after the weaning. Suchlike rate is the first magnitude needed for the algorithm of calculating the numbers of services required for continuous and even number of farrowings (3).

Materials and Methods

Ordinates measured in the Figure 4[2] presenting the farrowing rates observed in the herd 11 from 280 till 332 weeks (2) were the material in this research through which the repeating rates for the months of economic year were established as the differences between 1.00 and the farrowing rates for the month. With so observed magnitudes the trend of fluctuations of the repeating rates from the beginning of September till the end of August was expressed by the equation $Y_C = 0.10585 + 0.00902\ X_C + 0.00234\ X_C^2 - 0.00021\ X_C^3$, where $X_C = (X - 182.5) / 30.4167$ and X = 1 for September 1st (Figure 1.). Possible repeating rates in weeks through calendar year were established finding the magnitudes of Y_C with X_C for January 4th i.e. the day 126 and for 7 days more until August 30th i.e. the day 364 and after September 6th i.e. day 6 and for 7 days more until December 27th i.e. day 118. To be more acceptable the repeating rates were multiplied by 100 and expressed as the percentages of services in a week.

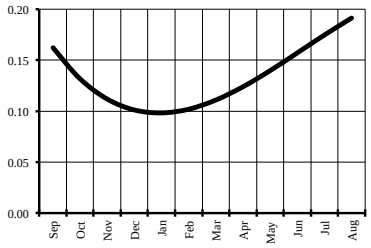

Figure 1. The trend of fluctuations of possible repeating rates of sows inseminated through economic year in northern parts of northern hemisphere

Results

Table 1. Possible repeating rates (PRR) in weeks through calendar year in northern parts of northern hemisphere

Week	Day (X)	PRR (Y_C %)	Week	Day (X)	PRR (Y_C %)
1	126	9.73	27	308	16.81
2	133	9.82	28	315	17.22
3	140	9.84	29	322	17.62
4	147	9.88	30	329	18.01
5	154	9.96	31	336	18.40
6	161	10.07	32	343	18.77
7	168	10.21	33	350	19.14
8	175	10.38	34	357	19.50
9	182	10.57	35	364	19.83
10	189	10.79	36	6	17.33
11	196	11.03	37	13	16.46
12	203	11.29	38	20	15.65
13	210	11.58	39	27	14.89
14	217	11.88	40	34	14.20
15	224	12.20	41	41	13.57
16	231	12.53	42	48	12.99
17	238	12.88	43	55	12.46
18	245	13.24	44	62	11.99
19	252	13.62	45	69	11.57
20	259	14.00	46	76	11.20
21	266	14.39	47	83	10.87
22	273	14.79	48	90	10.60
23	280	15.19	49	97	10.36
24	287	15.59	50	104	10.18
25	294	16.00	51	111	10.03
26	301	16.41	52	118	9.92

Conclusions and Discussion

The trend of fluctuations of the repeating rates of sows from beginning of autumn till the end of summer can be expressed by the polynomial. It is possible to find out the magnitude of possible repeating rate of sows inseminated during any week of calendar year. Starting with possible repeating rate the pigman can see how many services of repeaters and gilts he should perform through certain period to get desired number of farrowings.

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Effect of supplementation with arginine in primiparous sows on fetal and placental development at 35 and 70 days of pregnancy

Y López [2], D Contreras[1], H Jiménez[1, 2], C Mejía[1, 2], G Valdez[1], M Espinosa[1], A Vargas[2].
[1]*CENID Fisiología INIFAP*, [2]*FES Cuautitlán UNAM*. dcaro2003@gmail.com

Introduction

During the first half of pregnancy there is a critical placental growth (4), to support higher demand of nutrient supply to the growing fetuses (3,4). In the sow, 30 to 50% of embryos do not complete their development to full term (2), which affects their reproductive performance. The objective of this study was to analyze the physiologic effect of dietary supplementation with arginine in gilts, on placental and fetal development at 35 and 70 d of pregnancy.

Materials and Methods

The study was carried out in Querétaro, México (20º 42'N; 100º 01'W). Fourteen gilts (145 kg body weight; 259 d of age) were used. All animals were estrous synchronized and artificially inseminated (AI). After AI, gilts were offered 2.5 kg of a commercial ration (12% CP), and were distributed randomly into two groups: Arginine (HCL-Arginine, 1% added in the ration), and Control (2% of L-Alanine). Furthermore, each group was distributed by pregnancy age at slaughter into two groups: 35 and 70 d (35 d: Arginine (n=4), Control (n=3); 70 d: Arginine (n=3), Control (n=4)). When gilts were slaughtered, the reproductive tract was collected and processed to obtain the following variables: ovaries total weight (OTW), number of corpora lutea (NCL), weight of each corpus luteum (MWCL), weight of full uterus (WFU), length of full uterus (LFU), diameter of full uterus (DFU), placenta mean weight (PMW), fetus length (FeL), fetus weight (FeW), fetus diameter (FeD), number of fetuses (NFe), number of viable fetuses (NVFe), litter weight (LW) and the ratio of number of viable fetuses per CL (rVFe:CL). Data were analyzed by ANOVA for a completely randomized design, using GLM procedure. Some variable data were previously transformed to squared root (NCL), or to arcsine squared root (rVFe:CL).

Results

There was not effect of treatment for any of the variables analyzed at both pregnancy ages: 35 or 70 d (P>0.05). The results are shown in Table 1.

Conclusions and Discussion

Data from the present study did not show any effect of arginine treatment on reproductive performance of gilts, which disagree with data previously reported. Supplementation with 1% of HCL-arginine from 30 d of pregnancy (2) or from day 14 to 28 (1) had beneficial effects on litter size; however, when 0.8% HCL-arginine was administered during the first 25 d after AI, detrimental effects on reproductive performance were observed (4). According to these authors, arginine administration at the beginning of pregnancy may interfere with ovulation rate and reduce CL number and progesterone concentration, which in turn, in addition to a possible effect via excessive nitric oxide production, is detrimental for embryo/fetus survival, even though placental angiogenesis is enhanced (4). In conclusion, data from the present study indicate that supplementation with 1% arginine, starting just after AI, had no significant effect on reproductive performance of gilts at 35 and 70 d of pregnancy; ongoing studies are being conducted to determine if other variables at the molecular and structural level are affected by arginine supplementation.

Acknowledgments

Table 1. Mean and Standard Error of the Mean of the variables analyzed at 35 and 70 days of pregnancy.

	35 days**			70 days**		
Variable*	Control	Arg.	SEM	Control	Arg.	SEM
OTW, g	19.6	20.5	2.2	22.1	21.6	1.1
NCL	19.0	16.7	1.8	17.7	19.2	1.6
MWCL,g	0.4	0.5	0.4	0.6	0.6	0.04
WFU, kg	5.2	5.2	0.8	20.3	16.7	4.3
LFU, cm	122	110	10.1	132	158	15.3
DFU, cm	8.0	8.6	0.3	14.9	16.2	1.0
PMW, g	48	66	7.7	286	280	31.2
FeW, g	4.4	4.6	0.4	287	272	14.8
FeL, cm	3.7	3.7	0.1	17.6	17.0	0.5
FeD, cm	1.7	1.7	0.03	5.6	5.6	0.1
NFe	16.7	15.3	1.5	12.7	14.0	2.0
NVFe	16.0	15.3	1.1	12.3	12.7	2.4
LW***	70	70	6.1	3.5	3.4	0.5
rVFe:CL	0.8	0.9	0.1	0.7	0.7	0.1

*Acronyms, as described in materials and methods. **No significant effects of treatment were detected for any variable at both pregnancy ages (P>0.05). ***Units of measure are g (day 35) and kg (day 70).

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Reproduction disorders in a French farm: A field case

G Friocourt[1], E Sallé[2], M Collell[3]
[1] *Selvet, groupe vétérinaire Chêne Vert Conseil, Loudéac, France,* [2] *MSD Santé Animale, Beaucouzé, France,*
[3]*MSD Animal Health, Summit, NJ, USA* g.friocourt@chenevertconseil.com

Introduction

In 2012, differences of productivity between French farms were linked to prolificacy (43%), losses of life-born piglets (30%), duration of lactation (14%) and Weaning-to-Fertilization-Interval (13%). Lack of productivity results in farms is therefore considered multifactorial [1].

Materials and Methods

The study was done in a good health status farrow-to-finish farm (250 sows), using 7 batches management and weaning 4week old piglets every Wednesday. The gilts were purchased at 7 weeks of pregnancy.
The lack of productivity was observed through a low and instable fertility rate (82.5% average, ranging from 65% to 90%) and low prolificacy. Estrus was checked twice daily with a boar, and 2 AI per sow were performed 24/36h and 48h after the beginning of estrus, with a few 3rd AI on demand. Weaning-to-Estrus-Interval was low, and estrus was easy to detect, but of short duration. Return to estrus was either cyclic or not.
As all the sanitary hypotheses were inconclusive, other approaches were proposed:
1) monitoring of ovulation moment
As Weaning-to-Oestrus-Interval was low (5.2d), a transcutaneous ovarian ultrasonography was performed on 20 sows on the morning and afternoon of the Monday following weaning (convex probe 3.5-5 MHz, Exago®, ECM) to assess a cause of early ovulation
The gestating status was checked by ultrasonography 4 weeks later.
2) Statistical Analysis
All statistical analysis, both quantitative and qualitative, was performed with R®.

Results

Among 20 sows observed on Monday morning (4d after weaning), 5% were already in luteal phase, 25% had recently ovulated, 10% were ovulating and 60% did not ovulate yet. Seventeen (17) of these sows were also observed in the afternoon, with 4 other sows: 5% were already in luteal phase (same sow), 43% had recently ovulated, 19% were ovulating and 33% did not ovulate yet (Figure 1). None of these sows was in anestrus nor presented ovarian anomalies.

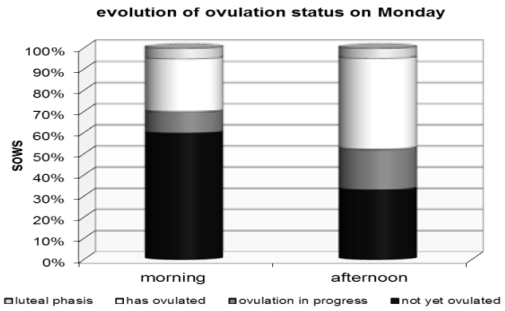

Figure 1. Ovarian status of the sows on Monday morning and afternoon.

Such early ovulations, in a farm with good but short estrus, lead the farmer to change the AI protocol to a new one, e.g. weaning on Thursday with AI at estrus detection and every 12 hours.
These modifications were quickly followed by an increase of 5.2% of the fertility rate (Table 2).

Table 2. Fertility rate (except gilts and repeat breeders)- a,b Khi2 p=0.01

Batches	Number of sows	Fertility rate
Classical AI protocol	996	85.0% [a]
New AI protocol	397	90.2% [b]

The change in AI protocol also impacted the overall prolificacy of the sows (+0.3TotalBorn/litter).

Conclusions and Discussion

Productivity troubles in sow herds have multiple causes. Although ovulation has been observed to occur at 70% of the duration of estrus [2], recent studies suggest that this is not so obvious anymore [3]. The use of ovarian ultrasonography isn't easy in a daily practice, but can be of huge interest in complex cases. In this case, modification of the AI protocol following the ovarian observation was quickly followed by increase of fertility and prolificacy.
Other hypotheses were also pointed out in this farm and are described in another abstract.

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Testing for a genetic association with the heart lesions of market hogs that died during transport to an abattoir in Ontario, Canada

K Zurbrigg[1], T van Dreumel[2], R Friendship[1], M Rothschild[3], E Kim[3], T O'Sullivan[1]
[1]*Department of Population Medicine, University of Guelph, Ontario, Canada* [2]*Pathology Consulting, Guelph, Ontario, Canada, Ontario,* [3]*Department of Animal Science, Iowa State University,* tosulliv@uoguelph.ca

Introduction

Market hogs that die during transportation to the abattoir are an economic loss and a welfare concern for the swine industry. In 2010, the average annual rate of in-transit loss (ITL) for Ontario's three federally inspected abattoirs was 0.07% (1). Weekly rates at each plant were similar (1).

Hogs that die during transportation are sent to rendering and rarely examined for cause of death. Rates of ITL increase during hot weather and as such the cause of death for these hogs is assumed to be heat stress. A pilot study performed by two of the authors determined that the majority of hogs that die in-transit to an Ontario abattoir have pre-existing cardiac abnormalities. The hog cardiac lesions are comparable to hypertrophic cardiomyopathy (HCM), a genetic heart disease recognized in humans, and some breeds of cats and dogs. HCM is an important cause of sudden death, arrhythmias and heart failure (2). The objectives of this study were to examine and characterize hog heart lesions and investigate if there was genetic differences between hogs that die in transit and those that did not.

Materials and Methods

Between May 2012 and September 2013, ITL hogs from an Ontario abattoir were sent to the Animal Health Laboratory, University of Guelph for post mortem examination of the carcass. A standardized protocol was developed and followed for the examination and sampling of the carcasses and hearts. Hearts were also randomly collected from the processing line of the abattoir to serve as controls for comparison (non-ITL). A 0.5cm^3 tissue sample from the mid-left ventricle was removed and frozen prior to the remaining heart being preserved in formalin. Total heart weight (THW), left ventricle plus septum (LV+S) and right ventricle (RV) weights were recorded for ITL and non-ITL hearts and the weights compared using the Two Sample T-Test in Statistix 8 (Analytical Software, Tallahassee, FL). Frozen heart tissue samples from ITL and non-ITL hearts were submitted for genotyping to Geneseek (Lincoln, Nebraska) using the Illumina 60K SNP chip. Genotypes were analyzed using PLINK1.07 (pngu.mgh.harvard.edu/Purcell/plink/) for genome-wide associations with ITL hogs.

Results

Post-mortems were completed on 93 ITL hogs. Complete data on the heart weight and gross and histological heart lesions of 83 ITL and 67 non-ITL hogs were collected and are presented in Table 1.

Gross hypertrophy of the left or right ventricle was noted in 77/83 (93%) of the ITL hogs and (5/67) 7% of non-ITL hearts. Histologic lesions such as cellular disarray, medial hyperplasia and perivascular fibrosis of the intramural coronary arteries or fibrosis of the endocardium, myocardium or interstitium was noted in 63/87 (76%) of the ITL hogs and 51/67 (76%) of non-ITL hearts.

Table 1. Comparison of the average heart weights (g) between ITL and non-ITL hogs

Type	THW [SD]	LV+S [SD]	RV [SD]	LV+S/RV [SD]
ITL n=83	442.0* [±66.4]	275.0* [±43.7]	98.5* [±21.4]	2.9 [±0.5]
Non-ITL n=67	368.8 [±37.9]	243.0 [±25.6]	83.0 [±13.3]	2.9 [±0.4]

*$P<0.05$

38 ITL and 34 non-ITL heart tissue samples were submitted for genotyping. In a genome-wide association test that compared the sequences of ITL hogs to non-ITL hogs, more than 10 regions of interest (40 gene associations) were found across the genome. Regions on chromosome 2 and 4 contain genes previously associated with HCM in humans.

Conclusions and Discussion

Pre-existing cardiac lesions were associated with death of hogs during transportation. The heart lesions are similar to HCM in humans, both grossly and histologically. While the genetic analyses are preliminary, it appears that the HCM-like hog heart lesions may have a genetic association. Further samples are being collected.

Acknowledgments

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Comparison of three different flow cytometry protocols to assess extended boar sperm viability

E Taberner[1], G Althouse[1]

[1] *Department of Clinical Studies- New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA,* gca@vet.upenn.edu

Introduction

Flow cytometry (FCM) has become an important methodology in evaluating various aspects of spermatozoa, making it a vital tool in veterinary andrology laboratories for not just routine assessments but for diagnostic and forensic work. Changes in boar sperm plasma membrane integrity have been evaluated in order to determine semen quality before artificial insemination or after semen cryopreservation (1,2).

Today, several fluorochromes and protocols are available for assessing sperm plasma membrane integrity. They can be used alone or in combination with other fluorophores which allow for evaluating multiple functional characteristics of the sperm.

Viable sperm can be distinguished by staining with propidium iodide (PI), a non-permeable DNA-specific dye, alone or in association with the membrane-permeable DNA-specific SYBR14 stain (3). Also, PI can be used in association with fluorescein isothiocyanate-conjugated peanut (*Arachis hypogea*) agglutinin (FITC-PNA) to simultaneously assess the plasma membrane integrity and acrosome integrity, respectively (4).

The aim of the present study was to compare the ability of different flow cytometry protocols to assess sperm viability at two different incubation times.

Materials and Methods

Extended semen samples (n=36) were obtained from commercial boar studs. All samples were packaged to maintain a temperature of 17ºC for shipment to the Reference Andrology Laboratory (New Bolton Center, Kennett Square, PA) within 24h post-collection.

Sperm plasma membrane integrity was assessed by either PI single staining, dual staining with SYBR14/PI, or a dual staining FITC-PNA/PI after incubation at 37 ºC for 30 and 120 min. Both PI and SYBR14 dyes were used according to the protocol described by Garner et al. (3). The FITC-PNA/PI protocol was performed using the procedure described by Nagy et al.(4).

Samples were analyzed in a FACScan flow cytometer (BD Biosciences) equipped with a 488-nm argon excitation laser. The flow cytometer was validated for each fluorochrome assay before the start of the experiment. An ANOVA with repeated measures was used to detect differences in viability between tested protocols. If significant ($P<0.05$), Bonferroni (SPSS, Armonk, NY) post-test was used to compare the different protocols.

Results

Mean sperm viability percentages are shown in Table 1. Significant ($P<0.05$) differences were observed between protocols at both time periods. Similar differences in sperm viability were found within time, with PI>SYBR14/PI>FITC-PNA/PI.

Table 1. Percentages (mean ± SEM) of viable spermatozoa as assessed by individual and dual staining after 30 and 120 min incubation at 37º C.

Protocol	Time	
	30 min	120 min
PI	81.32±0.82[a]	83.60±0.92[a]
SYBR14/PI	76.26±1.18[b]	75.88±1.20[b]
FITC-PNA/PI	70.75±1.16[c]	72.01±1.15[c]

(a,b,c) Superscripts indicate statistically significant differences within time ($p \leq 0.05$)

Conclusions and Discussion

Our study showed variability in percentages of viable sperm depending upon protocol used. The FITC-PNA/PI showed lower sperm viability than the other protocols assessed (PI or SYBR14/PI). This protocol assesses both plasma membrane integrity and acrosome status. Given that the acrosome reaction is essential for sperm penetration through the zona pellucida, the percentage of viable acrosome-intact spermatozoa provided by this protocol seems most beneficial when evaluating semen quality. The SYBR14 fluorochrome stains the nuclei of living cells and, in combination with PI, is useful to identifying sperm cells from non-sperm particulates (4). The PI-alone protocol seems the least selective in determining viability given its lack of discrimination versus the remaining protocols. Similar decreases in sperm viability were observed within 30-min and 120-min incubation at 37ºC. In conclusion, the FITC-PNA/PI combination seems to provide a more robust functional evaluation of boar sperm in extended semen, regardless of 30-min or 120-min incubation.

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P.433

An outbreak of Tiamulin + Narasin poisoning in swine

A Palomo
Setna Nutrición S.A.U.- InVivo NSA . PI Santa Ana c/El Clavo, 1 Madrid (Spain), antoniopalomo@setna.com

Introduction

An outbreak of antagonism poisoning with narasin and tiamulin in swine is described. A total of 156 lactating sows died over a period of two weeks after being drink tiamulin and eat feed ration a accidentally contaminated with narasin .

The farm have an swine dysentery acute case on sows and decided to treatment with pleutomutilin on water at 60 mg/l or 8 mg/kg of life weight.

Materials and Methods

A farm with 3.000 sows open cycle had a swine dysentery outbreak and Vet decide a tiamulin oral treatment during one week .

On second day, start died lactation sows on acute form previous neurological clinical signs (posterior paresis , weakness, depression, ataxia, progressing to lateral recumbency), anorexia, lethargy and respiratory distress (1,2) .

Body temperature stay in normal ranges .

We stopped the water treatment and take the feed for analytical study of micotoxins, antibiotics antagonist, serum and faeces.

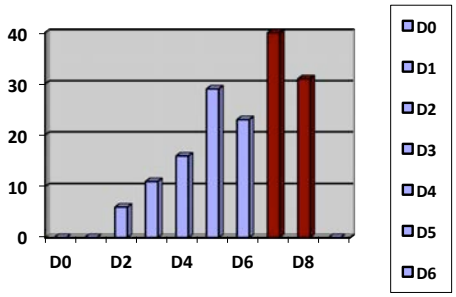


Table 1. Sows dead by day

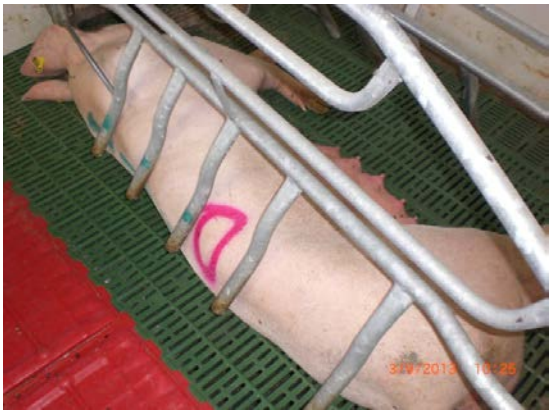
Results

At necropsy of 20 sows , we had lesions on heart , kidney , urinary vesicle , skeletal muscles and digestive system .

The feed samples revealed levels of 85 to 110 ppm of narasin. At this moment we studied the feeding production process and determined the sow feed contamination with poultry premixes than included narasin at 100 ppm .

Feed samples were negative to micotoxins , microbiological contaminations and others carboxylic ionophores (monensin , salinomycin and lasalocid).

Serum samples were negative to PRRSV.



Conclusions and Discussion

It appears that ionophore narasin was accidentally included in the sow lactation feed at a feed mill where poultry feed is also processed. The toxicity of ionophores antibiotics with pleuromutilin antagonis . is believed to be due to interference with the Na:K transport pump across cellular membranes, in particular of striated muscle fibres. This leads to increased intracellular calcium concentrations with consequent calcification and swelling of mitochondria, swelling of the sarcoplasmic or endoplasmic reticulum, cell membrane damage and eventual cell death .

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Effect of sodium butyrate on incidence and severity of post-weaning diarrhea in piglets

TC Reis[1], G Mariscal[2], MJ Guerrero[1], A Aguilera[1], K Escobar[1], MG Bernal[1]
[1]*Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, Qro, México,*
[2]*CENID Fisiología, INIFAP, Ajuchitlán, Qro, México,* tercia@uaq.mx

Introduction

Weaning is a critical time in the life of piglet with a high susceptibility to malnutrition and post-weaning diarrhea syndrome (1). The incidence of diarrhea has increased trough the world after the ban of antibiotics in animal nutrition. Many studies have demonstrated the effectiveness to decrease the occurrence of diarrhea in the post-weaning period by reducing dietary protein level and including several additives to the diet replacing antibiotics (2). Butyric acid is the main source of energy to epithelial cells of the ileum and large intestine; and it is produced by the fermentation of carbohydrates in the gut (3). Sodium butyrate, a butyric acid source, has been used to improve intestinal health (3). The aim of this work has shown how the presence or absence of sodium butyrate in a low crude protein (CP) diet affects the incidence and severity of diarrhea during the first three weeks after weaning.

Materials and Methods

89 piglets, weaned at 20.5±1.1 days of age weighing 7.1±0.5 kg were used. Immediately after weaning, the piglets were placed in 18 elevated pens equipped each one with six manual feeding spaces and a nipple water dispenser. Three diets were evaluated: a high crude protein diet (HCPab, 20% CP) with antibiotic and without butyrate; a low crude protein diet (LCPbut, 17% CP) with 0.2% of sodium butyrate and without antibiotic, and a low crude protein diet (LCP, 17% CP) without sodium butyrate neither antibiotic. Diets and water were available ad libitum. Fecal consistency was visually examined daily for 21 days after weaning to determine the incidence (ID) and severity (SD) of diarrhea. A fecal score was used to determine the SD, following scoring criteria: 0 (normal), 1 (soft feces), 2 (mild diarrhea), or 3 (severe diarrhea) (2). The mean fecal consistency was calculated for each experimental group based on the measured fecal scores. The ID was calculated based on the mean proportion of days that diarrhea was observed relative to the total experimental period. Data were analyzed as a completely randomized design. The experimental unit was the pen; means comparison was performed by Duncan's test, using the GLM procedure of SAS.

Results

The results do not show an effect of the diet on the incidence of diarrhea (Table 1). However, the diarrhea severity at the first week post-weaning was lower ($P < 0.05$) in animals fed with the diet added with butyrate (LPCbut) than in piglets fed with the LPC diet (without butyrate neither antibiotic). Animals fed with HPCab diet, had a severity diarrhea score intermediate to the others piglets. In the second and third weeks post-weaning and total experimental period, the presence and severity of diarrhea were similar among treatments.

Table 1. Incidence (ID) and severity (SD) of diarrhea.

Variable	Diet			SEM
	HCPab	LCPbut	LCP	
ID (days)				
Week 1	2.8	2.7	3.2	0.25
Week 2	5.2	5.0	5.0	0.22
Week 3	3.8	4.7	3.8	0.30
Total period	11.8	12.3	12.0	0.48
SD				
Week 1	0.5ab	0.4b	0.7a	0.03
Week 2	1.0	0.9	0.9	0.04
Week 3	0.4	0.6	0.5	0.05
Total period	0.6	0.6	0.7	0.03

(a, b) Superscripts indicate statistically significant differences within main effect ($p \leq 0.05$)

Conclusions and Discussion

Post-weaning diarrhea is associated with the stress of weaning, once all the experimental animals showed diarrhea, even this was moderated. Diarrhea is a deficit in the absorption of water and nutrients, and butyrate improves the absorption of electrolytes and reduces the incidence of diarrhea (4). This could explain why the presence of sodium butyrate in the diet had a positive effect in severity of diarrhea at the first week post-weaning, when the villus integrity is more affected (5). Butyric acid is the main source of energy for most cells of the colon and terminal ileum (3). However, at the total period of the experiment the addition of butyric acid had no effect on ID or SD. These results justify additional research on butyrate utilization in piglet's diets.

Acknowledgments

CONACYT for the financial support to this study.

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Selected metabolic indices changes after probiotics administration

R Link [1], P Reichel[1], J Novotný [1], M Linková [2]
[1] *University of Veterinary Medicine and Pharmacy, Clinic for Swine, Komenského 73, 041 81 Košice, Slovakia*
[2] *PJ Šafarik University, Faculty of Medicine, tr. SNP 1, 040 11 Košice, Slovak Republic*, robert.link@uvlf.sk

Introduction

Great potential in prevention of diseases of young animals has been associated with probiotics. The following species of the genus Bacillus are used as probiotics the most frequently: coagulans, subtilis, clausii, cereus, toyoi (4). B. licheniformis is also used to improve the health of pigs.
The aim of this study was to determine the effect of probiotic bacteria B. subtilis and B. licheniformis on selected parameters of haematological and protein profile in suckling piglets.

Materiasl and Methods

Eighteen newborn piglets were included in the experiment. They came from two litters, every litter was divided into the experimental group and the control group.
The experiment lasted five weeks, from the birth until weaning. The experimental piglets (n = 9) received the probiotic preparation BioPlus 2B, which consisted of equal proportions of B. licheniformis and B. subtilis. Each experimental piglet received 3.2 x 10(7) B. licheniformis and B. subtilis. No probiotics were administered to the control piglets (n = 9).
During the trial, we determined selected parameters of haematological and protein profile in the serum of piglets. Blood samples were collected on 0, 7, 14, 21, 28 and 35 day of the experiment. Statistical data of groups were processed by t-test.

Results and Discussion

Haematocrit (Hc) remained within the reference range in both groups throughout the experiment. Haemoglobin level was significantly higher in the experimental group at the 4th sampling (Table 1).
Leukocytes were within the standard range, they ranged from 10 to 16 x 10(9)/L in both groups.
The level of total proteins in the experimental piglets showed a slight increase during the first two weeks. Significant intergroup differences were recorded on 14th and 28th day, 78.6 vs. 71.5, resp. 62.5 vs. 56.7 g in L ($P < 0.05$). Albumin levels in the experimental group increased gradually from the lowest level at 0-sampling to the highest at the final one. Significant intergroup difference in albumin level was observed at the final sampling. The level of immunoglobulins (TIg) in the experimental group was steady throughout the experiment. Immunoglobulins in control piglets decreased at the 1st sampling which resulted in a significant difference between the groups.

Table 1. Haematological profile

	Day 0	Day 28	Day 35
Er–E, T/L	4.4±0.7	6.7±0.52	7.2±0.48
Er–C, T/L	4.7±0.5	6.7±0.22	6.9±0.22
Hc–E,L/L	0.3±0.04	0.38±0.01^{b}	0.39±0.03^{b}
Hc–C,L/L	0.3±0.04	0.35±0.02^{b}	0.34±0.03^{b}
Hb–E,g/dL	7.8±1.3	10.4±0.6^{b}	10.7±1.07
Hb–C,g/dL	8.8±1.2	9.6±0.85^{b}	9.8±1.05

Er – erythrocytes, Hc – haematocrit, Hb – haemoglobin, E – experimental group, C – control group, ^{b} $P < 0.05$, T/L – 10(12)/L

Our results resemble those obtained in the study (1), which reported a significant increase in Hc and haemoglobin concentration in germ-free newborn piglets which were given Lactobacillus casei for ten days.
Total proteins in our experimental group increased. B. licheniformis and B. subtilis produce many enzymes, including the most important proteases, amylase and lipases. Stimulation of IgA and IgM production after probiotic administration was also described in the study (2).

Acknowledgement

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Influence of bio-active peptides from FPP* on fattening pig performance

W Depondt[1], D Smulders[1], A Kanora[1]
[1]*Huvepharma NV, Antwerp, Belgium,* *FPP: fermented potato protein, wouter.depondt@huvepharma.com

Introduction

Previous research demonstrated a positive effect of Lianol®, a complementary feed based on fermented potato protein, on plasma insulin-like growth factor-1 (IGF-1) levels (IPVS 2010). There is considerable circumstantial evidence that the actions of growth hormone on protein accretion in skeletal muscle and other lean tissues are mediated by IGF-1 (1).
This trial investigates the effect of this new feedstuff on lean meat content and performance in fattening pigs.

Materials and Methods

This research is a summary of three consecutive well-controlled field trials performed in Belgium. The animals originate from Large White x Landrace sows and a Belgian Piétrain boar. Each trial consisted of 720 animals equally distributed between a control and a Lianol® group. The animals were housed in the same compartment. The diets were equally formulated. The diet of the Lianol® group was supplemented with 300 grams Lianol® Solapro/mT for pigs from 40kg until slaughter.
The daily gain, feed conversion (FCR) and lean meat percentage on the carcass was evaluated.

Results

In the first trial the lean meat percentage increases from 59.36 to 60.56% in the control and Lianol® group respectively. In the second trial, this percentage increased from 59.62 to 60.43% in the control and Lianol® group respectively. In the third trial, no lean meat data was gathered.
As a consequence of the higher lean meat percentage, the FCR improved by 5.0%, 4.7% and 7.4% in the first, second and third trial respectively due to the supplementation of the feed with Lianol®.

Table 1. Fattening performance relative compared to the control group

	Control group	Lianol® groups		
		Trial 1	Trial 2	Trial 3
Average daily gain	100%	102.3%	104.3%	102.2%
FCR	100%	105.0%	104.7%	107.4
Lean meat percentage	100%	102.0%	101.4%	/

Conclusions and Discussion

The supplementation of fattening feed with Lianol® Solapro from of 40kg body weight improved lean meat percentage by about 1% and FCR by 5 to 7% in fattening pigs.

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Influence of bio-active peptides from FPP* on post weaning performance in piglets

W Depondt[1], D Smulders[1], C Wu[2], J Chiang[2], A Kanora[1]
[1]Huvepharma NV, Antwerp, Belgium, wouter.depondt@huvepharma.com[2]Huvepharma Taiwan, 12F.-1, No.190, Sec. 2, Zhongxing Rd., Xindian Dist., New Taipei City 23146 Taiwan, *FPP: fermented potato protein

Introduction

Previous research demonstrated a positive effect of Lianol®, a complementary feed based on fermented potato protein, on plasma insulin-like growth factor-1 (IGF-1) levels (IPVS 2010). Previous research (1,2) reported a positive interaction between IGF-1 levels and growth performance.
This trial investigates the effect of this new complementary feed on piglet performance during the first 2 weeks post weaning.

Materials and Methods

48 weaning piglets were housed in the same compartment in 12 pens of 4 piglets each. Half of the pens were attributed to the control group and the other half to the Lianol® treatment. The feed of the treated group was supplemented by 1.5 kg Lianol® Solapro per ton of feed during the trial period. All piglets were a (Large White x Landrace) x Duroc cross. The trial started at weaning; when the piglets had an average age 24 days. The trials lasted for 2 weeks.

Results

The animals in the control group weigh 5.88 ± 0.2 kg and the animals in the Lianol® group weigh 5.88 ± 0.1 kg at weaning (day 24 of life).
At 2 weeks post weaning, the animals in the control group reached a weight of 8.33 ± 0.3 kg whereas the Lianol® group was 8.70 ± 0.2 kg; this is an extra weight of 370 grams in the supplemented group. The average daily feed intake increase from 241.5 grams to 267.9 grams in the control and Lianol® group respectively. The FCR improved by 5 points from 1.38 in the control to 1.33 in the Lianol® treatment. No mortalities were observed in this trial.
The performance data is summarized in table 1.

Table 1. Post-weaning piglet performance

	Control group	Lianol® group
Number of piglets	24	24
Weaning weight (kg); day 24	5.88 ± 0.2	5.88 ± 0.1
Weight day 38 (kg)	8.33 ± 0.3	8.70 ± 0.2
Daily feed intake (g/d)	241.5	267.9
FCR	1.38	1.33
Mortality (%)	0	0

Conclusions and Discussion

Supplementation with Lianol® Solapro during the first 2 weeks post weaning improved piglet performance. The weight at 2 weeks post weaning increases by 370 grams. The average daily feed intake was 26.4grams higher and the feed conversion improved from 1.38 in the control to 1.33 in the Lianol® group.

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Efficacy of an innovative food to reduce neonatal losses in piglets and increase pre-weaning growth

F Voisin[1], M Dia[1], H Gabillet[2], E Pagot[1]
[1] *ZOOPOLE développement – CTPA, Ploufragan, France,* [2]*EARLYPIG, Saint-Grégoire, France*
florian.voisin@zoopole.asso.fr

Introduction

Pre-weaning mortality is numerically the biggest source of piglets losses in pig production in Brittany in 2012 (13,3% losses)[1]. The same is observed worldwide with 12,7 to 19,7% losses[2] and is thought to be mostly linked to initiation problems of lactation[3]. This randomized field trial was designed to evaluate the effect of an innovative food distributed to piglets between birth and 10 days of life on pre-weaning losses and growth performance.

Materials and Methods

In a conventional French farm, litters from 35 sows were randomized at birth: 17 in the Earlystart group, 18 in the Control group, i.e. respectively 214 and 213 cross-bred piglets individually identified at birth (D0). Piglets were left under their mothers. In the Earlystart group, each litter received daily 200g of a food (EARLYSTART® gel) during the ten first days of life. In the Control group no food was given to the piglets. Cross-fostering was permitted after inclusion within the same group and taken into account in the calculation of litter's average daily weight gain (AWDG). No other food was distributed to the piglets before weaning. The animals were individually weighed at D0, D11 and D21 (weaning). The amount of feed intake was measured daily for each litter in the Earlystart group. All mortalities were recorded. Statistics used were an ANOVA to test the ADWG of the litters and a Mantel-Haenszel test for mortality.

Results

The two groups were not different at D0 in terms of piglet individual weight, parity of sows, piglets gender distribution, nor litter size.

Table 1. Pre-Weaning Growth and Mortality results

	Mortality (%)	ADWG D0-D11 (g/d)	ADWG D0-D21 ± SD (g/d)
Earlystart	7,5	163,6	166,5 ± 36,15
Control	14,6	146,2	141,9 ± 45,6
p	0,026	0,185	0,04

Pre-weaning D0-D21 growth and mortality are significantly in favour of the Earlystart group. The results are summarized in table 1, ADWG being presented per piglet. As shown in figure 1, most losses occurred during the 3 first days of life.

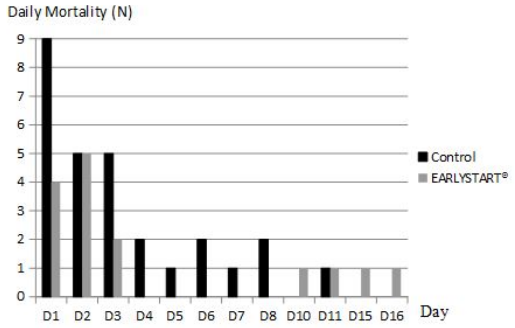

Figure 1. Mortality per study day

Conclusions and Discussion

The reduction of nearly 50% of neonatal mortality rate in the Earlystart group represents a real progress. The rate of losses in the control group is higher than the average rate of losses in pig farms in Brittany[1]. The improvement of litter's ADWG between D0 and D21 is remarkable because the piglets did not receive a feed at 14 days of life, so the increase in growth performance is only associated with the consumption of feed distributed between D0 to D10. It is not linked to a greater consumption of the first age feed due to an early education by the provision of solid feed.

This study shows the zootechnical efficacy of the tested feed distributed in complement to maternal milk. It would be interesting to study until slaughter the performances of piglets receiving this innovative product.

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Erratum : We appologize for two errors in the paper published in AFMVP 2013 congress : litter size were not different at inclusion, ADWG D0-D11 were inverted. This was corrected here.

P.439

Maternal and nursery dietary vitamin D concentrations altered tissue mRNA expression

LA Rortvedt-Amundson[1], TD Crenshaw[1]
[1]Department of Animal Sciences University of Wisconsin-Madison, Madison, WI, lrortvedt@wisc.edu

Introduction

An abrupt increase in hypovitaminosis D diagnosis of swine lameness and mortality cases were reported over the past 5 yr (1). During the same period, kyphosis, an idiopathic disease that causes abnormal spinal curvature, was associated with feeding diets devoid of supplemental vitamin D (D). Pigs produced by sows fed no supplemental D or minimum levels (325 IU D/kg diet), developed kyphosis and had reduced bone mineral density (BMD) (2). Maternal dietary D concentrations were implicated as a pre-disposing factor for skeletal tissue abnormalities in the young pigs.

This experiment was designed to study potential carryover effects of varied levels of maternal dietary D fed to sows on pig bone traits and tissue gene expression. Pig BMD responses to nursery diets were dependent upon maternal dietary D concentration (3). Gene expression data associated with D homeostasis are reported herein to further delineate the potential maternal carryover effects on pig bone traits.

Materials and Methods

In 2 trials, gilts were fed 1 of 3 diets (n = 6, 8, or 9 gilts/treatment, respectively) with 0, 325, or 1750 IU D/kg from breeding through lactation. At weaning (23 ± 2 d), pigs within a litter were assigned to pens and fed an adjustment diet with no supplemental D for 1 wk. Then for 4 wk, pigs were fed 1 of 4 nursery diets (arranged as a 2 X 2 factorial) with 0 (-D) or 280 (+D) IU D/kg, each with 95% (95P) or 120% (120P) of the P requirement (Table 1). Pigs were killed prior to colostrum consumption at birth (n = 23), weaning (n = 22), and a subsample at the end of the nursery (n = 88) for tissue collection. The cortex of the kidney (Kid), the duodenum (Int), and sections of the right third metatarsal (Bn) were collected immediately and snap frozen in liquid nitrogen. Tissue extraction and RT-qPCR methods were used to measure mRNA expression (4). HPRT1 was used for Kid and Int and the average of ribosomal S15 and S18 was used for Bn as housekeeping genes.

mRNA expression of the following genes were analyzed in the tissues indicated: vitamin D 24-hydroxylase (CYP24 in Kid, Int), vitamin D 1α-hydroxylase (CYP27 in Kid), vitamin D receptor (VDR in Kid, Int, Bn), receptor activator of nuclear factor kappa-B (RANKL in Bn), osteocalcin (OTC in Bn), fibroblast growth factor 23 (FGF23 in Bn), osteoprotegerin (OPG in Bn), and transient receptor potential vanilloid type 6 (TRPV6 in Int).

Results

Differences in tissue mRNA expression of genes due to diet were not detected ($P > 0.05$) unless specifically stated.

At birth, pigs produced by sows fed 1750 IU D had increased Bn mRNA expression of OTC. At weaning pigs produced by sows fed 325 or 1750 IU D had increased mRNA expression of Bn OTC, Int and Kid CYP24, Int TRV6, and decreased mRNA expression of Bn OPG.

Differences in tissue mRNA expression due to nursery diets were detected, but carryover effects of maternal diets were not detected.

Kidney mRNA expression of CYP24 increased in pigs fed +D diets.

Unexpectedly, Int mRNA expression of CYP24 increased in pigs fed –D and 120P diets, but decreased if fed +D and 120P diets.

Bone mRNA expression of FGF23 increased in pigs fed both +D and 120P diets, but no interaction between D and P was detected.

Conclusions and Discussion

The dose response to maternal dietary D concentrations in pig Int and Kid mRNA expression of CYP24 at weaning reflected a potential for increased degradation of D. The unexpected differences in Int mRNA expression of CYP24 infers a potential regulatory role for intestinal tissue beyond the primary role of renal tissue for the degradation of excess D.

The diet-induced changes in bone mRNA expression of FGF23 at the end of the nursery are consistent with a direct role for this newly discovered hormone in D and P homeostasis.

The absence of maternal by nursery diet interactions in tissue mRNA expression infers either regulation of these specific signals at the protein level or other primary signals are involved. These results stimulate the need to better understand the role of D in controlling bone growth.

Table 1. Dietary Composition

Treatment	D_3, IU/kg	Ca, %	P, %
-D95P	0	0.70	0.57
-D120P	0	0.70	0.72
+D95P	280	0.70	0.57
+D120P	280	0.70	0.72

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Effect of Zn levels in diets for gilts and their relation to morphological indicators

Y De Loera-Ortega[1], A Cruz[2], B Rosales[2], A Alvarado[2], I López[2], M Vega[2]; A García-Contreras[2]
[1]*Facultad de Estudios Superiores-Cuautitlán. UNAM;* [2]*Laboratorio de Imagenología, UAM-Xochimilco.*
adelfa@correo.xoc.uam.mx

Introduction

Zn is important for all life forms and promotes a plethora of physiological and biochemical functions, integral to growth, skeletal development, dermal health, wound healing and reproduction[1]. During the reproductive period of the sow, voluntary feed intake is often insufficient to meet nutrient requirements for maintenance and lactation, leading to mobilization of fat and protein reserves[2]. This has caused an increase in live weight and maintenance requirement at any given age, reduction in voluntary food intake, making more difficult to meet nutrient requirements from commercial feeds at times of high metabolic demand such as lactation, as well as reduced body fat reserves that act as a buffer against negative energy balance at such times. The aim of this study was to investigate the effects of a Zn level in different morphological indicators the gilts.

Materials and Methods

Twenty-one gilts 4-months-old (York-Landrace breed crossbred) individually housed were allotted to three treatments, with seven replicates. One group was fed the basal diet (BD) (BD: T_1=35ppm of Zn), T_2=T_1+100ppm Zn and T_3=T_1+150ppm Zn. The BD was balanced according to FEDNA (2006); including Zn proteinate source feed and water were available *ad libitum*. Body measurements (BM) of gilts were taken weekly: body weight (BW;Kg); daily gain (DG;g); body length (BL;cm); Cross height (CH;cm); thorax width (TW;cm); vulva width (VW;cm); length vulvar (LV;cm); vagina-cervix penetration length (VCPL;cm); backfat (BP_2;mm). The vagina-cervix penetration length (VCPL;cm) was measured using a graduate catheter[3]. The data were analyzed using the MIXED model procedures of SAS, and a procedure of correlation.

Results

The incorporation of Zn in the diet did not show differences in the values of the body measurements (P>0.05). Although differences (P<0.05) between treatments were observed in the BP_2, and there was no Treatment*Time interaction (P>0.66). BW did not differ between treatments (P>0.75) during the six weeks of measurement (Treatment*Time interaction: P>0.79). The correlation between BW and BM was higher than r=0.54 to 0.83 (P<0.0001). VCPL and TW being the lowest and highest r.

Table 1. Least squares means of the effect of the level of Zn in the morphometric measurements of gilts

	Body measurements of gilts						
	DG	BL	CH	TW	VW	LV	VCPL
	g	cm					
T1	810	89.12	64.12	93.12	2.33	3.00	23.59
T2	849	88.57	64.96	93.29	2.43	3.02	25.17
T3	787	87.95	62.21	95.08	2.30	3.06	23.10
P=F	0.844	0.864	0.132	0.440	0.497	0.893	0.391

	Backfat (P2, mm)					
Treatments	Average	Time (weeks)* mm				
	mm	1	2	3	4	5
T1	13.73 [a]	09.66	10.83	14.16	16.00	18.00
T2	11.54 [b]	07.43	08.57	12.14	13.43	16.14
T3	13.57 [a]	07.71	11.14	15.00	16.57	17.43
BW		65.38	70.73	76.57	82.39	88.63
EEM	0.405	1.02				
P=F	0.05	[a]0.66				

T1= BD with 50ppm Zn; T2=BD+50ppm ZnP; T3=BD+100ppm ZnP. *P = F: Effect of Time <0.0001;[a] Interaction Treatment * Time 0.66. n= 35 gilts

Conclusions and Discussion

The inclusion of 50 or 100ppm Zn in the BD of gilts did not improve weight gain, as neither morphometric related reproductive characteristics. BP_2 showed that 100ppm of Zn is reduced by at least 2mm. Hahn and Baker[3] suggest that Zn is related to voluntary intake, although in this study no effect of treatment was observed. Zn is critical to good hoof health in many species, Berger[4] indicate that pigs develop skin lesions over the extremities which is a common sign of zinc deprivation, however in the gilts not existed evidence of skin damage or hooves. It was observed that the V-CPL shows a medium development similar to those reported by Martin-Rillo *et al.*[5]; Taracco and Kirkwood[6], this measure had a significant positive correlation with long dorsal and live weight of the pig.

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Effects of probiotics on the utilisation different fibre feedstuffs by weaning pigs

EO Akinfala, ST Ogundeji, AOK Adesehinwa
Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria, oakinfala@yahoo.com

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Introduction

Fibre feed stuffs such as palm kernel cake (PKC), brewers' dried grain (BDG) and wheat offal (WO) are current being used by pig farmers in South western Nigeria as basal ingredients in the diets of all classes of pigs. There had been study by Akinfala and Macauly, (2013) on the utilisation of some of these fibre feedstuffs by weaning/growing pigs. The need to improve the utilisation of these feedstuffs stimulated this study on the effects of probiotics on their utilisation..

Materiasl and Methods

A total of Eighteen (18) weaner pigs (Large White × Hampshire) of average weight (6.17 ± 0.44kg) were randomly distributed into 6 experimental diets of 3 animals per diet with each animal serving as a replicate. Six diets were formulated; diets 1, 3 and 5 contained 25% each of brewers dried grain (BDG), wheat offal (WO) and palm kernel cake (PKC) respectively while a commercial probiotics was added at recommended rate of 2.5ml/kg feed into diets 2, 4 and 6 which had the same formulation as 1, 3 and 5 respectively. The bacteria in the probiotics include *Lactobacillus spp*, *Bacillus spp* and *Saccharomyces spp*. The fixed ingredients which constituted 75% in each of the 6 experimental diets formulated in this study were made up of 45% maize, 15% groundnut cake, 10% soybean meal, 2% fishmeal, 2.25% bone meal, 0.5% salt and 0.25% vitamins/minerals premix. Growth trail which lasted 70 days was carried out. The design was completely randomized design. The animals were fed 5% of their body weight on daily basis and water was supplied *ad libitum* to the animals during the experiment.

Results

The result from growth performance is represented in Table below. There were no significant differences ($P \geq 0.05$) across the six treatments for final weight (FW), Average daily weight gain (ADWG), feed conversion ratio (FCR) and Average daily feed intake (ADFI). The final weight ranged from (17.67 – 24.17kg) with highest value occurring with pigs on diet 3 while the lowest value occurred with pigs on diet 1. The highest value for ADWG was found with pigs on diet 3 followed by those on diet 6 while the least was found with pigs on diet 1. The ADFI was highest in diet 3 and the lowest in diet 1. The FCR was best in diet 3 (2.14). Result from economics of production showed no significant difference ($p \geq 0.05$) across the six treatments for cost of feed /Kg. Diets 4 and 6 had the highest cost of £ 0.35 while diet 1 had the least cost (£0.31). There was also no significant difference ($P \geq 0.05$) in feed cost/kg gain. The feed cost/kg gain was highest in diet 1 and lowest in diet 3.

Conclusion

It can be concluded from this study that the inclusion of probiotics had positive effects (though not significantly ($p>0.05$) on PKC and BDG on growth of weaning pigs while it had negative effect on WO on growth of weaning pigs. The economics of production showed that the inclusion of probiotics in this study increased cost of feeding in all the diets, while cost of feed/kg gain of the animals was better in diet 1

Table 1. Performance and Economy of Production of Experimental Animals Fed Experimental Diets

	DIETS						
PARAMETERS	**1**	**2**	**3**	**4**	**5**	**6**	**SEM ±**
Initial weight (Kg)	6.17	7.17	7.00	6.50	6.33	6.17	0.44
Final weight (Kg)	17.67	20.00	24.17	22.17	21.17	22.50	1.44
Average daily weight gain (kg)	0.160	0.178	0.238	0.218	0.206	0.227	0.02
Average daily feed intake (kg)	0.4562	0.453	0.518	0.502	0.488	0.503	0.08
Feed conversion ratio (FCR)	.93[a]	2.53[ab]	2.14[a]	2.30[b]	2.39[b]	2.23[b]	0.03
Cost of feed/Kg (£)	0.31	0.34	0.32	0.35	0.32	0.35	0.01
Cost of feed/kg gain (£)	0.89	0.85	0.68	0.81	0.75	0.77	0.02

Diet 1 : 25% Brewer's dried grain without probiotics diet 2: 25%Brewer's dried grain with Probiotics
Diet 3 : 25% Wheat offal without probiotics diet 4: 25% Wheat offal with Probiotics
Diet 5 : 25% palm kernel cake without probiotics diet 6: 25% Palm kernel cake with Probiotics

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Sow feeding program and litter size

G Borbolla[1,3], G Velázquez[1], E Alvarez[2], A Jiménez[2], A Herrera[2,3], M Aguilar[2]
[1] *Department of Swine Medicine and Animal Husbandry. School of Veterinary Medicine. National University of Mexico.*
[2]*Granja El Mirasol, Jalisco.* [3]*The Scientific Partners. A swine-specialized group,* borbolla@unam.mx

Introduction

During the last decade feeding the pregnant sows has become a challenge for producers due to the ever-changing ratio muscle:fat in the high prolific sow (2). Efficient feeding programs for gestating sows are important in order to obtain a proper body condition at farrowing. Milk production, reproductive ability and lifespan performance are affected by an efficient nutritional and feeding strategy. Therefore, the objective of the present study was to evaluate the effect of different feeding strategies during pregnancy in litter size of gilts.

Materials and Methods

Two hundred and forty gilts were randomly assigned to 3 different feeding allowances of a 3000 kcal-diet during gestation. Feeding programs were as follows: 1) 2.1 kg of feed from 0 – 114 d. (n=80) 2) 2.1 kg of feed until day 90, followed by 2.6 until d 114 (n=80); and 3) 2.6 kg from 0 – 35d, 2.1 kg from day 35 – 90, and 2.6 kg from 90 to 114 d (n=80). Sows were fed once a day, and had ad libitum access to water. At farrowing piglets were individually weight. Data were analyzed using ANOVA.

Results

Mean litter size using different feeding programs in gilts are shown in table 1.

Table 1. Effect of different feeding programs during pregnancy in litter size and litter weight of gilts.

Treatment	Litter size	Piglet weight
Feeding program	Piglets	Kg.
1	12.77[a]	1.39
2	12.76[a]	1.43
3	13.07[b]	1.35

1) 2.1kg /day from 0 -114d of pregnancy (n=80).
2) 2.1kg/day until day 90l, followed by 2.6kg/day until day 114 of pregnancy (n=80).
3) 2.6kg/day (Day 0 to 35), 2.1kg/day (Day 35 to 90) and 2.6kg/day (Day 91 to farrowing) (n=80).
[a, b] P<0.05

Gilts fed with feeding program 3 showed a larger (P<0.05) litter size compared to gilts fed with feeding programs 1 and 2 (Table 1). Piglet weight did not show a difference (P>0.05), among any of the feeding programs in gilts (Table 1).

Conclusions and Discussion

Increasing maternal feed intake (2.6 kg/day) from day 0 to day 35 of pregnancy had a positive effect in litter size compared with a feeding program that is more restricted at the beginning of the pregnancy. These results agree with Musser (4), who showed that increasing the feed intake amount in pregnant sows during early gestation affected the milieu on both the maternal and the conceptus sides of the placenta, mainly observing an increase in the IGF-1 and Urea N concentrations in plasma, essential metabolites of pregnancy. It could be that the increase in the feed intake in early pregnancy does not restrict the gilt in her growth and neither the availability of nutrients for the embryos during the pre-implantation period, as it might happen in the restricted feeding programs in treatments 1 and 2. Furthermore, the increase in nutrient provision during last stage of pregnancy provides larger amounts of nutrients (amino acids and energy), for the sows which have to replenish the body reserves that are being directed to fetus and mammary gland growth having a beneficial effect in viability and survivability of piglets (1,3). On the other hand, applying a single phase feeding program in pregnant sows can lead to overfeeding during early gestation resulting in higher costs; and to underfeed during late gestation which leads sows to start lactation in a severe catabolic state, with detrimental consequences for the future reproductive performance.

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The effect of mineral supplementation on performance of post-weaning piglets in Thailand

P Poolperm, P Jirawattanapong, P Nilsuwan
Department of Farm Resources and Production Medicine, Faculty of Veterinary Medicine, Kasetsart University, Kamphangsean, Nakornpathom, Thailand, fvetpap@ku.ac.th

Introduction

Performance of post-weaning piglets is determined by survivability, growth rate and feed efficiency. Feed additive has been used in nursery diets to improve those performances. Mineral supplemented and algae extracted has been recently documented (1) to improve active glucose transporter, a process of glycogen accumulation, in muscle of piglets. (2). The objective of this study was to study the effect of Weaner Advantage®, mineral supplemented with algae extracted, on performance of post-weaning piglets using survivability, growth rate and feed efficiency.

Materials and Methods

A total of 618 post-weaning pigs (5 weeks of age) were randomly selected into control (n=280) and treatment (n=338) groups. The control (C) pigs were fed conventional feed, broken rice-soy bean based, and the treatment (T) pigs were fed feed supplemented with Weaner Advantage® (Alltech Biotechnology, Thailand) at 2kg/ton for the whole nursery period. The C group had 2 replicates, each contained 156 and 124 pigs. The T group also had 2 replicates, each contained 160 and 178 pigs. Feed consumption and weight of pigs were recorded, then average daily gain (ADG), feed conversion ratio (FCR) and feed intake (FI) were calculated. The results were statistically analyzed using Student's t-test.

Results

The results showed in table 1. Feed intake, weight in and weight out of pigs in this study were the same in both groups. However, the percentage of losses were trended to be lower in T group compared to that of C group (0.88±0.35 VS. 1.77±0.22, p=0.09). ADG and FCR were significantly better in treatment group than the control (379.92±1.03g VS. 357.78±5.46g and 1.535 VS. 1.64, respectively, p<0.05).

Table 1 performance of post-weaning piglets on survivability, growth rate, feed intake and feed efficiency.

N	Control (n=280)	Treatment (n=338)	p-value
ADG (g)	357.78±5.46	379.92±1.03	0.03
FCR	1.64±0.0	1.535±0.02	0.02
%loss	1.765±0.22	0.875±0.35	0.09
FI (kg/d)	0.585±0.01	0.58±0.01	NS
days	43.485±3.72	41.84±1.12	0.61
weight in	7.13±0.59	6.855±0.25	0.61
Weight out	22.86±0.98	22.81±0.24	0.95

Conclusions and Discussion

In the present study, nursery pigs in treatment group with Weaner Advantage® supplemented improved ADG and FCR (p<0.05). Mineral supplemented feed improved growth performance in nursery pigs.

Acknowledgments

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Effect of the essential oil of *S. terebinthifolius* Raddi (Brazilian red pepper) on growth performance and intestinal histology of weanling pigs

FD Gois[1], PL Cairo[1], LMRodrigues[2], CD Fernades[2], LF Rocha[2], C Andrade[3], LB Costa[3]
[1]*Department of Agricultural and Environmental Sciences – UESC,* [2]*Department of Animal Science - UFLA,* [3]*School of Agricultural Sciences and Veterinary Medicine – PUCPR, Brazil,* batista.leandro@pucpr.br

Introduction

Numerous studies have been conducted in order to identify alternative feed additives to antimicrobial growth promoters. The use of commercial additives derived from plants, including essential oil of *Schinus terebinthifolius* Raddi, have shown similar effect on growth performance of broilers compared to animals fed with antimicrobial (1). However, in pigs, no studies were performed. Thus, the objective of this study was to evaluate the effects of the essential oil of *Schinus terebinthifolius* Raddi on growth performance and intestinal histology of weanling pigs.

Materials and Methods

Ninety 21-d weaned male pigs (5.6 ± 0.78 kg BW) were used in a randomized complete block design experiment with six treatments, six replications per treatment and three animals per experimental unit (pen). The treatments were basal diet (BD) with 120 mg/kg of chloro-hydroxyquinoline (ANT), and BD with 0, 500, 1,000 or 1,500 mg/kg of the essential oil. The majority composition of essential oil used was 41.01% of δ–carene, 14.4% of phellandrene, 12.36% of limonene and 10.36% of α–pinene. The body weight (BW), average daily gain (ADG), average daily feed intake (ADFI) and feed to gain ratio (F:G) were calculated from day 1-35 of the experiment. At the end of the experimental period, one animal from each pen was slaughtered to evaluate the intestinal histology: villus height (VH), crypt depth (CD) and villous density (VD). Data were submitted to analysis of variance using the "R" 3.00 for Windows (2). When ANOVA was significant ($p < 0.05$), differences between treatments means were compared using Tukey test ($p < 0.05$).

Results

The data are shown in Table 1. No effects ($p > 0.05$) of the treatments were observed on the performance. For intestinal histology, 500 mg/kg of essential oil increased ($p < 0.05$) the VD on duodenum compared to ANT, 1,000 and 1,500 mg/kg.

Conclusions and Discussion

Performance data from the present study corroborate with some researches (1, 3, 4), no affecting animal performance with essential oil in the diet. The increase in VD of duodenum suggests positive effect in digestion and absorption of nutrients (5), however without affecting animal performance.

Therefore, essential oil of *Schinus terebinthifolius* Raddi can be used as replacement for antimicrobial in weanling pig diets without affecting the pigs' growth performance and intestinal histology.

Table 1. The growth performance and intestinal histology of pigs fed with antimicrobial or different levels of essential oil (*Schinus terebinthifolius* Raddi) for 35 days post-weaning

Item[1]	Growth performance				
	BW, kg		ADG,	ADFI,	F:G
	d 0	d 35	g	g	
ANT	5.64	14.85	321.23	497.82	1.71
0	5.65	16.99	311.56	557.29	1.80
500	5.66	16.30	345.53	543.14	1.72
1,000	5.65	14.83	308.65	478.88	1.61
1,500	5.65	16.66	323.79	538.84	1.90
SEM[2]	-	0.08	34.88	103.90	0.001
P value	-	0.10	0.36	0.14	0.16

Item[1]	Intestinal histology					
	Duodenum, µm			Jejunum, µm		
	VH	CD	VD[3]	VH	CD	VD
ANT	226	106	25[c]	271	121	33
0	221	96	34[ab]	236	102	32
500	221	96	39[a]	229	111	29
1,000	247	107	18[c]	251	120	27
1,500	233	101	26[bc]	235	106	37
SEM[2]	57.19	7.05	0.81	69.18	11.62	2.55
P value	0.83	0.56	<.05	0.56	0.39	0.31

[1]ANT=Antimicrobial; 0; 500; 1,000 and 1,500 mg/kg of the essential oil of *Schinus terebinthifolius* Raddi.
[2]The standard error of the mean.
[3]Within a column, means without a common superscript difffer ($p \leq 0.05$)

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Effect of vitamin and mineral feed supplementation "Volstar" on blood biochemical parameters of piglets

IA Pomeshchikov, SA Staroverov, AA Volkov, SV Kozlov, IN Zhirkov
Saratov State Agrarian University named after N.I.Vavilov, Russia, zhircov@gmail.com

Introduction

The basis of high productivity of animals and resistance to environmental agents is well balanced ration (nutrients, trace-elements, vitamins).

Lack of vitamins and minerals negatively affects on the digestive processes and gastrointestinal homeostasis of animals. Piglets are very sensitive to changes in the composition of their diets. Any modification of the intestinal micro flora content results in strengthening of fermentation and putrefaction processes. The latter may cause gastroenteritis and damage other digestive organs such as liver, which participates in the detoxification of alien substances.

Materials and Methods

60 healthy “Large White” piglets aging of 3 months were participated in trials. The average weight of animals was 26-27 kg. Two experimental and one control groups of 20 animals in each were formed.

We tested our new preparation named “Volstar” which is vitamin and mineral feed supplementation. It was used orally with drinking water once a day for 5 days. “VolStar” was administered in the following dosages:

1. Animals of the first experimental group were given the preparation orally in a daily dosage of 0.5 ml per 10 kg of body weight.
2. Animals of the second experimental group were given the preparation in a daily dose of 1 ml per 10 kg body weight.
3. Control animals were injected the liquid preparation "Nitamin" (Nita-Farm inc.) at the rate of 1 ml per 10 kg bodyweight.

Trials were lasted 30 days. The effectiveness of the drugs was evaluated using the clinical and laboratory methods. The most important index of evaluating we believed the weight gain in every group.

Results

The experiment found that after 30 days from the start of the experiment was a significant increase in total protein in piglets of all groups, mainly due to the albumin fraction. This indicates increased absorption of nutrients feed. This fact is confirmed by the increase in the concentration of glucose in the blood serum of pigs, which also indicates the normalization of carbohydrate metabolism in animals. At the same time the most intense dynamics of changes in these indicators noted in the second experimental group of animals. Safety of pigs in the experimental groups was 100%. The highest average daily gain of pigs observed in the second experimental group of piglets. The high efficiency of the developed water-soluble vitamin and mineral feed supplement «Volstar " due to the selection of optimal solvents provide high bioavailability by creating micellar system . In particular, there is the work of scientists, which is well established that the most effective drugs are in micellar or colloidal systems.

Conclusions and Discussion

The use of vitamin and mineral supplements "Volstar" at a dosage of 0.5-1 ml per 10 kg body weight contributes to the normalization of metabolism in piglets.

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The peroxide value of soy bean oil after heating for 36 hours

A Boonsoongnern[1,2,3], P Poolperm[3]
[1] *Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand,* [2] *Center of Excellence on Agricultural Biotechnology: (AG-BIO/PERDO-CHE), Bangkok 10900, Thailand,* [3]*Department of Farm Resources and Production Medicine Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand.* fvetpap@ku.ac.th

Introduction

Approximately 65-70% of the total pig production cost in Thailand is directly related to feed cost. Usually, energy is the most expensive nutrient in feed formulation. Oil, either vegetable or animal source, is used for energy in feed formulation to improve feed efficiency (4). Soybean, rice bran, coconut, and palm oil are commonly used in feed mills. Since the price of oil has been increased, oil-by products used in human food industry was introduced into homemade feed mills. Used oil is well documented in increasing levels of peroxidation values (1) intestinal oxidative stress (5). In general, quality of oil could be classified by various parameters. The purpose of this study was to measure the peroxide value (PV) in soy bean oil induced oxidization using heat and oxygenated at various time points.

Materials and Methods

A total of 1.8 litres of food grade soy bean oil was poured into a 3.0 litre beaker and then heated to 100-110º C on a hot plate. Oxygen was continuously supplied through the oil during the heating process (2 bar per min). The oil was heated for the first 15 hrs, and then stop heating for 6 hrs, re-heat again for 15 hrs, and stop for 8 hrs, and re-heat for 6 hrs. Thirteen samples were collected, 150 ml each, every 3 hrs of heating period in 250 Duran® bottles and kept at 4º C until assay. All samples were duplicated and measured using titration technique (2).

Result

The PV of heated oil showed in Figure 1. A substantial increasing of PV from the start point (0 hrs) through its peak at 24 hrs of heating, then PV has decreased dramatically through the end of study (36 hrs of heating).

Conclusions and Discussion

The results revealed that the process of heating and oxygenation through the oil could induce oxidization of the oil and increase peroxidation products. The PV was increased only in the first 24 hrs of heating and delining afterward. According to the PV results, it could be pointed out that PV is not an appropriate parameter to be used for classify between good and bad oils. Fortunately for the farmer, other lipid peroxidation can be indentified using several methods; [p-anisidine value (AnV), thiobarbituric acid reactive substance concentration (TBARS), hexanal concentration, 4-hydroxy nonenal concentration (HNE), and 2,4-decadienal (DDE)] and 2 predictive tests [active oxygen method stability (AOM) and oxidative stability index (OSI) (3). Thus, the study concluded that PV is not a good parameter to classify oil for good or bad quality.

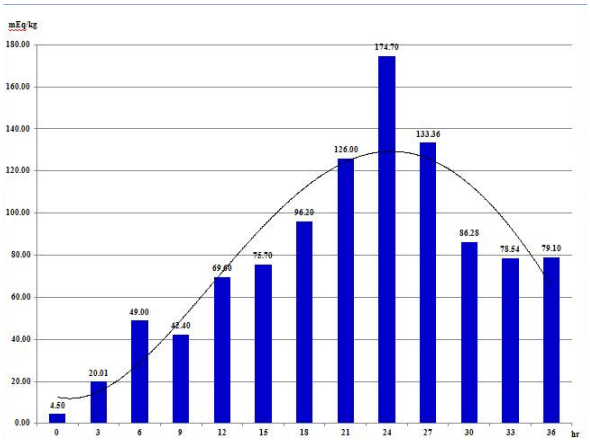

Figure 1. Mean of PV on the consequence time (from 0 to 36 hrs).

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A survey of quality of palm oil and soy bean oil used in feed in Thai pig farms

A Boonsoongnern[1,2,3], N Ratanavanichrojn[3], P Jirawattanapong[3], P Poolperm[3*]
[1] *Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand,* [2] *Center of Excellence on Agricultural Biotechnology: (AG-BIO/PERDO-CHE), Bangkok 10900, Thailand,* [3]*Department of Farm Resources and Production Medicine Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand.* fvetpap@ku.ac.th

Introduction

Oil is commonly added to pig feed as an energy source to improve feed efficiency (1). Oil, either vegetable or animal source, is the most important source for energy in feed formulation. In Thailand, soybean, rice bran, coconut, and palm oil are commonly used in feed mills. Crude oil is less expensive than refined oil. However, quality of both types of oil cannot be classified by farmers. In general, classification of oil quality could be used various parameters, such as peroxide value (PV), iodine value (IV), free fatty acid (FFA) and moisture. The objective of the study was to survey quality of palm oil and soy bean oil, both crude and refined oil, generally used in feed in Thai pig farms.

Materials and Methods

Palm oil (n=12) and soy bean oil (n=9) were collected from pig farms during February and March 2013. All samples were categorized into 2 types, crude and refined oil. Each oil sample was collected in a 500 ml glass sterile bottle, kept in an ice box during transport to the laboratory and stored at 4°C until assay. The samples were measured for levels of pH, peroxide value, iodine value, free fatty acid, moisture and impurity by a commercial laboratory facility (Thai Vegetable Oil Public Company). The results were presented in terms of average number using both a descriptive and statistical analysis. In addition, all parameters in crude and refined oils were analyzed using the t-test analysis program by Graphpad prism5.

Results

All samples revealed several parameters as mean ± SEM. Crude palm oil had significantly higher free fatty acid than refined palm oil. On the other hand, it showed a peroxide value significantly lower than refined palm oil. The results also showed a different trend in iodine value in palm oil, pH and free fatty acid in soy bean oil (Table 1 and 2).

Conclusions and Discussion

According to the results, palm oil should be identified as crude or refined grades using peroxide values, iodine values and free fatty acid parameters. In addition, soy bean oil may be classified as crude or refined grades using pH and free fatty acid as the refinement process can reduce 1.0-1.5 % of FFA (2). Recommendation; the results should be utilized on Thai pig farms to evaluate the different qualities of palm and soy bean oil as there is a substantial difference in oil prices.

Table 1. The differentiation between crude and refined palm oils were analyzed using the t-test technique (the data reported as mean ± SEM)

Parameters	Crude palm oil (n=6)	Refined palm oil (n=6)	P-value
pH	6.28 ± 0.64	6.72 ± 0.84	0.418
Peroxide value (mEq/kg)	4.73 ± 1.62	8.59 ± 1.62	0.005
Iodine value	34.83 ± 22.69	56.80 ± 0.98	0.059
% Free fatty acid	2.29 ± 1.76	0.29 ± 0.07	0.033
% Moisture	0.08 ± 0.03	0.07 ± 0.02	0.383
% Impurity	0.09 ± 0.16	0.02 ± 0.01	0.302

Table 2. The differentiation between crude and refined soy bean oils were analyzed using the t-test technique (the data reported as mean ± SEM)

Parameter	Crude soy bean oil (n=4)	Refined soy bean oil (n=5)	P-value
pH	6.951 ± 0.20	6.11 ± 0.32	0.075
Peroxide value (mEq/kg)	10.45 ± 1.49	18.23 ± 5.65	0.272
Iodine value	108.5 ± 12.32	114.8 ± 10.28	0.704
% Free fatty acid	1.71 ± 0.23	1.24 ± 0.09	0.076
% Moisture	0.13 ± 0.04	0.09 ± 0.01	0.389
% Impurity	0.06 ± 0.02	0.00	ND

ND = non detectable

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An outbreak of alkaloids feed poisoning in fattening pigs

A Palomo[(1)], A. López[(2)], C Quintana[(2)]
(1)SETNA NUTRICIÓN S.A.U.- InVivo NSA . PI Santa Ana c/El Clavo, 1 Madrid (Spain). AGRONSELLA(2)
antoniopalomo@setna.com

Introduction

An outbreak of lupin alkaloids in growing and finishing pigs is described. A total of 309 fattening pigs died over a period of one week after being eat new feed formulation included lupins with high level of alkaloids (1). The acute outbreaks involved six different fattening units at the same time when served the growing and finishing feed with 5 and 10 % of lupins .

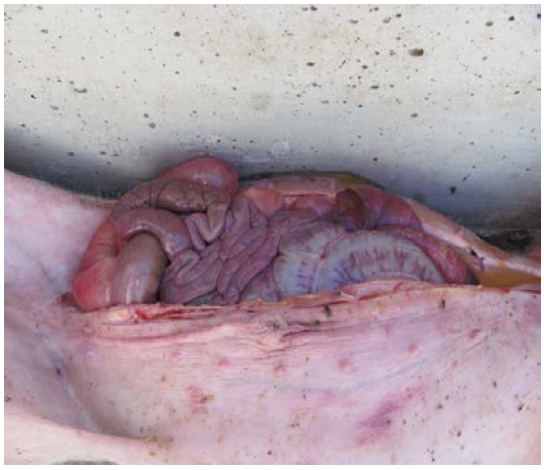
Materials and Methods

Eight fattening units with 10.250 pigs involved in different sites . The same feed were served between two days on full or partial empty storage pits.
On second day , start died pigs on acute form . The body temperature stay in normal ranges . Pigs push away feed , high level of vomiting pigs , digestive disorders , swollen , anorexic , lethargic and prostrate . At necropsy we observed vascular congestion on stomach , large and small intestine .
At second day we decided close the feeders and take out and served other feed formulation without lupins . The previous problem feed formulations without lupins and we suspected .
We studied the feeds at different levels , included feed and raw materials : NIR – nutrients levels , microbiological study , micotoxins , anti-nutritional factors on lupins and soya . At the same time we studied de feed mill process , protocols and traceability .

Results

Feed samples were negative to micotoxins, microbiological contaminations and nutritional levels staid in normal ranges . We study and discovered the different origin of lupins (Australian and national) . The second didn´t genetic selection to levels of alkaloids. The lupins are the first vegetable protein for pig nutrition.

The feed samples revealed by HPLC levels of 5.880 to 9.100 ppm of alkaloids in lupins (0,58-0,91%) . The maximum tolerance levels described are 0,2 % (1) .



Conclusions and Discussion

We concluded that the origin of pigs mortality were directly relation with high levels of alkaloids in complete feed and the inclusion of lupins with antinutritional factor had the first implication .

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The effects of the phytobiotics (Enviva EO 101) on the health and performance of weaned pigs

S Radulović [1], R Marković [1], D Jakic Dimić [2], B Kureljušić[2], D Šefer[1]
[1]*Faculty of Veterinary Medicine, University of Belgrade, Department of Nutrition and Botany,* [2] *Scientific Veterinary Institute of Serbia, Belgrade,* stamen.radulovic@gmail.com

Introduction

Weaning is a stressful period characterized by slow growth, reduced feed intake and diarrhea. Increased bacterial resistance to antibiotics have led the European Union to completely ban the use of antibiotics as a feed additive from January 1st, year 2006. These policy changes have led the feed industry to propose an alternative supstances to control post weaning disorders. Therefore the basic aim of this study was to investigate the effects of diet with the addition of phitobiotic, as natural growth promoters, on the health and performance of pigs, which could contribute to a better understanding of the effectiveness of their actions.

Materials and Methods

A total of 24 pigs, F1 generation (♀Landrace x ♂Large White), were weaned at 35 d of age and divided into two groups (control K and experimental O-I) with 12 individuals in each with equal sex ratio. The experiment lasted 40 days and body weight gain, daily feed consumption and feed eficiency (feed:gain ratio) were observed 20. and 40. d of the experiment. The control group received a diet of standard raw and chemical composition while the experimental group O-I received an identical diet as a control but with the adition of phytobiotic (Enviva EO 101, (cinnamaldehyde and thymol), 100 g/ton of feed, in accordance with the manufacturer's recommendations).

Results

During the trial there were no detected disorders of health conditions or manifestation of clinical signs of diseases of animals. Feed concumption observed for the entire feeding period (d 1- 40) was higher in experimental compared to control group. At the end of experiment (d 40), compared to the control group, pigs of experimental group achieved higher body weight as shown in Table 1, but the differences did not reach statistical significance (p>0,05). Adition of phytobiotic, observed for the entire feeding period had a positive effect on feed eficiency for pigs of the experimental group (2.06) compared to the control group (2.11).

Conclusions and Discussion

Wenk (2003) reported that herbs, spices and their extracts can stimulate appetite and endogenous secretions such as enzymes or have antimicrobial, coccidiostatic or anthelmintic activities in monogastric animals. The consequence of this may be a better utilization and absorption of nutrients, or the stimulation of the immune system. This applies especially to critical phases of an animal's production cycle characterized by high susceptibility to digestive disorders, such as the weaning of pigs. Zitterl-Eglseer et al. (2008) reported a significant improvement in digestibility of dry matter and crude protein as a result of the addition of carvacrol and thymol in the diet for weaned pigs. Results obtained by the authors Pengfei Li et al. (2012) have pointed out the positive impact of the use of phytobiotic on the average daily gain of pigs compared to the control group. In presented work addition of phytobiotic as a natural growth promoter resulted in better pigs performances compared to control group suggesting that addition of phytobiotic has its nutritional, medical and economic justification. The application of phytobiotics to animal nutrition is at on relatively early stage of implementation and will require further research input.

Table 1. Body weight *of pigs during the experiment, [kg]

DAYS OF TRIAL	GROUP	
	K	O-I
1.	8.53±2.20	8.82±1.40
20.	13.20±4.04	13.51±2.82
40.	25.32±6.31	27.19±4.77

* The value is expressed as $\overline{X}$ ± Sd

Acknowledgments

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The frequency of leg injuries after mixing gestating sows

LU Hansen, MF Nielsen
Danish Pig Research Centre, Axeltorv 3, DK 1609 Copenhagen V, Denmark, luh@lf.dk

Introduction

Sow lameness is a major cause of sow mortality due to euthanization and culling[1] Lameness indicates animal welfare problems, something which is of growing public concern. With the implementation of the EU legislation on January 1st, 2013, all gestating sows in Denmark are group-housed.

Leg problems were identified as the most common cause of sow mortality on 37 Danish farms[2]. Causes of lameness are multifactorial and often develop in the gestation unit[3]. Lameness can be influenced by flooring[4].

The aim of this study was first to investigate whether "soft flooring" reduced the frequency of medical treatments due to leg injuries after mixing, and secondly to examine whether mated gilts and 2nd parity sows have more medical treatments due to leg problems than older sows.

Materials and Methods

A total of 1,722 mated gilts and sows (Landrace × Yorkshire) in one herd with group housing from service until farrowing were included in the study.

Three types of flooring in the activity area were included: concrete floor, rubber floor and DUO-plastic modules. The latter two types are examples of "soft floor".

The sows were housed in static groups of 90 and fed in electronic sow feeding stations. The sows were examined[5] for leg problems in group-housing pens on the day of moving to the gestation pen, one week after moving and before farrowing.

Data consist of counts and therefore a Chi-Square test was used. Dichotomous variables (+/- lameness) were analysed using linear regression.

Results

Sows in the pen with DUO-plastic modules had a significantly higher frequency ($p<0.03$) of medical treatments compared to sows on concrete and rubber floor (Table 1). Mated gilts and 2nd parity sows had a higher frequency of treatments compared to older sows (Table 2). There was a higher frequency of treatments the first two weeks after mixing compared to later in gestation (Table 3).

Conclusions and Discussion

The results show that floor type has an impact on the frequency of treatments related to leg problems in the gestation pen. Particularly the young sows seem to develop leg problems possibly due to establishment of the hierarchy of the group.

Sows with leg problems/lameness were moved to a hospital pen and treated. Averagely 10% of the sows were moved to a hospital pen – the highest number among sows in the pen with DUO-plastic.

Table 1. Effect of flooring on treatments due to lameness

Floor type	Concrete	Rubber	DUO
Number	613	516	593
Treatments	128	124	160
% sows treated	15.2[a]	18.2[ab]	20.6[b]

(a, b) Superscripts indicate statistically significant differences within main effect (p <0.03)

Table 2. Distribution of treatments due to lameness on parity (P) 1-8 (% of sows in parity groups)

	Floor type		
Parity	Concrete	Rubber	DUO
P1 – P2	38.1	42.6	48.9
P3 – P8	25.6	24.9	29.6

Table 3. Distribution of treatments (number/day) on parity P1-P8 and days after mixing

Floor type	Concrete		Rubber		DUO	
Parity	*1-2*	*3-8*	*1-2*	*3-8*	*1-2*	*3-8*
0-14 days	3.4	1.8	2.9	1.9	4.1	2.3
13-60 days	1.7	0.6	1.8	0.5	2.0	0.7
60-77 days	1.0	0.5	1.1	0.4	1.0	0.6

Rubber floor and DUO-plastic did not reduce the frequency of medical treatments due to leg injuries and more knowledge on floor type or floor surface is needed to prevent leg problems among loose-housed sows. Further research into mixing strategies may provide more information on why young sows are at high risk of developing leg problems.

Acknowledgements

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Simulator to assess the economic impact of differences in pig farm technical performances

A Aubry[1], I Corrégé[1], B Badouard[1], Y Salaun[1], T Vila[2], F Joisel[2]
[1]*IFIP – Institut du porc, Le Rheu, France;* [2]*MERIAL S.A.S., Lyon, France,* alexia.aubry@ifip.asso.fr

Introduction

In field conditions, many decisions have to be evaluated, aiming to solve dysfunctions or to improve technical efficiency. They have a financial impact, which is very complex to estimate, especially when reproduction is involved. A simulator has been developed to assess the economic impact of changes in reproductive performance and/or in growth performance in the post-weaning and/or the fattening stages, between two stabilized situations.

Materials and Methods

Input data are technical-economic items for breeding herds and wean-to-finish phase provided by French technical and economic databases: *Gestion Technique des Troupeaux de Truies* (GTTT) and *Gestion Technico-Economique* (GTE). Descriptive data of the farm as the numbers of rooms and economic parameters as prices of feeds and selling prices of pigs are also considered.

For reproductive parameters, it is stated that farrowing units are fully used. Thus, reproduction, as far as sow culling and replacement are managed to reach this goal, is considered as managed with some consequence on the average number of present sows.

In the post-weaning and fattening stages, the calculation depends on changes in weights, mortality and feed conversion ratio of pigs. If feed efficiency is unknown, the simulator offers to estimate it, using a modeled value from growth (daily weight gain), based on various consumption profiles (feed restriction *vs ad libitum* patterns). In any way, it is supposed that no change is occurring regarding structural descriptors (barn capacities, sow herd organization etc.).

Results

The simulator has to estimate a cluster of technical items to perform the economic simulation: the numbers of present sows, the number of required replacement gilts and the number of culled sows (based on the conception rate and other reproduction criteria, including the farmer's choices as the number of unfertile estrus accepted before culling the sow), the number of pigs produced and the global feed consumption. The levels of inputs, outputs and gross margin are assessed using all these items as well as prices of feed and pork.

The gross margin difference (in €) between the two situations (both situations being assumed to stay stable) can be expressed with regards to different units depending on the case: per the farm in total, per sow per year, per pig produced or per kilogram produced. To explain the overall difference, the results from intermediate calculations are also available.

Concluisions and Discussion

While the back-office simulation model incorporates some interactions between criteria, most of the input parameters are considered independent enough to let the user choose their value.

This simulator is a quick and easy tool that provides a first estimate of the economic impact of changes in technical performances (1).

The simulator is available as a web tool and can be freely used online (www.ifip.asso.fr).

Reference

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Composting swine mortality: Costs of tools and accessories

A Vargas[1,2], ME Trujillo[2], JJ Nava[2], LB Reyes[1], A Ciprián[1], E Hernández[1], E García[1], S Mendoza[1]
[1]*Facultad de Estudios Superiores Cuautitlán-UNAM,* [2]*Facultad de Medicina Veterinaria y Zootecnia- UNAM, México.*
cachemira3@yahoo.com.mx *PASPA scholarship. Grants: PAPIIT ITE218711-3 and CONS-23.*

Introduction

Composting sheep mortalities was very important in the survival and development of the Canadian sheep industry (4), so this degrading system can be adopted by swine industry worldwide. Disposal of organic residues to be composted offer an environmental friendly alternative but first must demonstrate its financial viability to adopt it as a part of the biosecurity plan. The type of composting system generate variability in the costs, and for that reason, the investment of financial resources is never the same. In developed countries, the mayor cost involved in carcass composting is the acquisition and handling of machinery (2), but in developing countries, the main costs are: man power (5) and consumed water (3), however, the additional costs must be investigated.

Materials and Methods

We were obtained the cost of the accessories needed for the development of the process of composting mortality in a one-site operation of 135 breeding sows property of UNAM (6), where the composting of the organic matter was established since 2008. Additionally, the prices for the accessories were investigated and multiplied by the number of units needed to perform the activities involved in the composting process. The total cost of each set of accessories was divided by their lifetime. The mean rate of exchange (13.10) between Mexican peso / American dollar was used. Finally, to obtain the cost (tools and accessories) per metric ton of compost produced, the annual output was used.

Results

The list of accessories and equipment and the total and annual costs is showed in Table 1. The highest percentage of the costs obtained were 66.60% for the analytical equipment in comparison with the tools and protective clothing (21.35 and 12.04 %, respectively). Dividing the annual cost of the entire equipment: US$139.44, between the compost output: 18 metric tons, generate the cost per metric ton: US$7.75

Table 1. Total and annual costs for equipment

Accessories	Total (US$)	Annual cost (US$)
Plastic container (200 litres)	19,1	1,91
Plastic hose with spray gun	30,5	6,11
Brushes	9,2	9,20
Garden rake (metalic)	7,6	1,53
Stainless steel shovels	22,9	4,58
Trolley (metalic)	64,9	6,49
Protective mask	6,1	3,05
Protective gloves (plastic)	13,7	13,74
Thermometers	114,5	22,90
Hygrometer	26,7	8,91
pH meter	152,7	30,53
Electrical conductivity meter	152,7	30,53
TOTAL	**620,61**	**139,44**

Conclusions and Discussion

The investment in tools and accessories is reduced in comparison with the man power cost (without machinery) obtained of US$43.66 per metric ton (5), and with the obtained cost for composting weaners and suckling pigs of US$8.54, and US$4.88, respectively (2). The costs of the equipment to monitor the degrading process is the highest, but, the use of it allows us to monitor properly the physicochemical changes that take place inside the piles of material composted, which is necessary to perform management changes.

The exact knowledge of the costs allows the investment of financial resources to adopt the composting process in-site in order to strengthen the biosecurity and so avoid the spread of pathogens into the environment (1).

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Economic impact of Mexican pork trade

G Gómez[1], S Rebollar[1], J Hernández[1], A Rebollar[1], H Velázquez[1], FE Martínez[2]
[1]*Centro Universitario UAEM Temascaltepec,* [2]*Instituto de Ciencias Agropecuarias y Rurales de la UAEM* ,
gomte61@yahoo.com

Introduction

The changes in international trade of pork in Mexico after trade liberalization in 1988 and the Free Trade Agreement (NAFTA) in 1994 have been huge. On the one hand, imports grew from 31.0 thousand tonnes in 1988 to 761.2 in 2012, an increase of 2351.9% making Mexico the fifth biggest importer behind only Japan, Russia, Hong Kong and China[4]. 90% of Mexico´s imports come from USA. On the other hand, exports began in 1990 with 5.0 thousand tonnes and in 2012 reached 71.3 tonnes, an increase of 13,848.7%. 67% of Mexico´s exports go to Japan. Therefore, in 2012 net imports of Mexico were 689.9 thousand tonnes.

The objective of the present study was to calculate the economic impact of free pork trade has had, obtaining the functions of supply and demand during pork trade liberalization and calculating benefits and losses to consumers and producers.

Materials and Methods

The demand and supply functions were obtained according to Kawaguchi[1] *et al*., based on the price elasticities of supply and demand[2], the market price and the quantities of pork consumed and produced in a given year[4]. All prices were deflected with the consumer prices index (IPC) 2010.

$\beta_{mt} = \varepsilon_p (y_{mt}/p_{mt})$ where: β is the slope of the function of demand or supply

ε is the price elasticity of demand or supply *y* are the quantities consumed or produced and*p* are the prices of selling and buying pork

Results

At the start of trade liberalization pork and so far (2013), the price in the U.S. (P*u*,) has been lower than in Mexico (P*m*) so that the magnitude of the cost of transport U.S. pork to Mexico (t) determines the occurrence of trade between them. Imports from the U.S. to Mexico, caused a right displacement of the supply curve in Mexico (Fig. 1 blue line) reducing domestic prices. Assuming that it would have not been changes in the size of the herd due to changes in price, then the total income of all mexican hog producers decreased by $ 26.4 billion over the 25 years of free trade or $1.06 per kg of live hog. Whereas the transmission of wholesale prices to retail consumer is inelastic in Mexico[2] benefit for consumers was $ 12.4 billion only on this period.

Conclusions and Discussion

Pork free trade reduced prices for mexican hog producers and consumers. Producers income decreased but per capita consumption grew up from 11.4 kg a 16.4 kg.

The asymmetric price transmission may be due to an oligopolistic market where retailers have tended to pass more rapidly price increases to consumers, whilst it has taken longer for consumer prices to adjust to producer prices when the latter has decreased .[3]

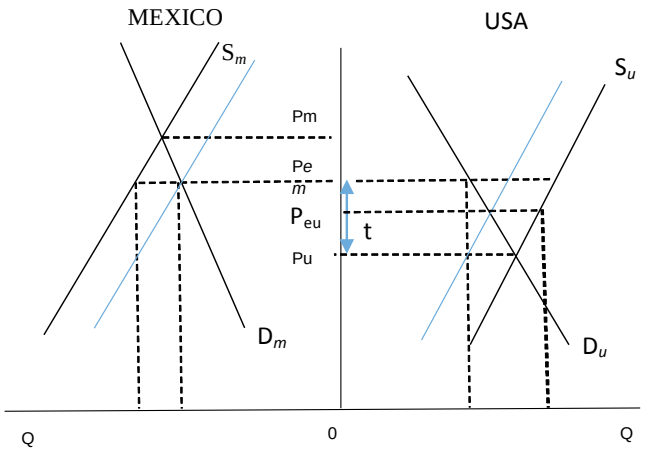

Figure 1. Supply and demand curves of pork in USA and Mexico

Acknowledgments

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Individual Pig Care (IPC) program is able to monitor and evaluate health status in pigs from intensively immunized gilts

T Tucci[1], M Roveri[1], E Tecli[2], A Manso[3], A Dereu[4], P Doncecchi[4], C Piñeiro[3], J Morales[3]
[1]*Swine Vet Practitioner, Italy;* [2]*Zoetis, Italy;* [3]*PigCHAMP Pro Europa, Spain;* [4]*Zoetis – EuAFME, France,*
carlos.pineiro@pigchamp-pro.com

Introduction

Percentage of piglet survival during lactation is lower for primiparous than for multiparous sows litters. One cause of this might be related to a lower immune transmission via colostrum, because immune system of gilts is not completely adapted to the farm-endemic pathogens (1, 2). A strategy to solve this problem might be to stimulate the immune system through vaccination.
The aim of this study was to evaluate the effect of intensive immunization of gilts by monitoring health status of their progeny using the Individual Pig Care (IPC) program.

Materials and Methods

The experiment was conducted in a farrow-to-finish commercial farm in Italy. A total of 21 sows (14 gilts and 7 multiparous sows) were used and managed as follows: 7 gilts (GILT) and 7 multiparous sows (SOW) under usual vaccination program (Aujeszky, PRRS, Erysipela, Parvovirus); and 7 gilts (H-GILT) were more intensively immunized by adding Circovirus and colibacillosis vaccines (twice for each vaccine during pregnancy).
After weaning (21 days of age), 216 pigs were distributed in 24 pens of 9 pigs each (8 pens per treatment). Pens of every treatment were randomized within every room. Health status of pigs was evaluated in nursery and growing-finishing periods using the IPC program. According to the IPC guidelines, sick pigs were scored and symptoms were quantified according to the severity (A-mild signs of disease; B-medium; C-serious and D-dying) and type of disease (digestive, respiratory, lameness, nervous, biting or other). Clinical signs and mortality were monitored from weaning to slaughter at 270 days of age (about 160 kg BW). Data were analysed as binary variables using the glimmix procedure of SAS (v 9.2).

Results

Percentage of losses (deaths and removed pigs due to sickness) tended to be higher (P=0.08) in GILT group than in the other groups in the growing-fattening phase (GILTS 0.9%, H-GILTS 10.1% and SOWS 8.2%), with no significant differences between H-GILTS and SOWS groups. At the slaughtering, presence of lung lesions was also higher (P=0.03) in the GILT group than in the other groups (table 1).

Table 1. Percentage of pneumonia lesions and of presence of pleuritis at the slaughtering.

	Pneumonia	Pleuritis
GILT	45.1[a]	74.5[a]
H-GILT	19.4[b]	51.6[b]
SOW	33.3[ab]	44.4[b]
SEM[1]	*0.070*	*0.068*
Probability[2]	*	**

[1]Standard Error of Mean; [2]Probability: *, P<0.05; **P<0.01

Clinical signs daily assessment showed that the H-GILT group presented lower incidence of disease than the other groups, and the disease outbreaks were more moderate. The figure 1 shows the health index, which is the daily percentage of healthy pigs in GILT and H-GILT groups during the total period.

Figure 1. Percentage of healthy pigs by time in GILT (upper) and H-GILT (lower) groups.

Conclusions

We conclude that an intensive immunization of gilts decreased the incidence of disease and reduced the lung lesions of their progeny at slaughtering in a conventional farm. The IPC program provides accurate clinical data information obtained in real-time.

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Lifetime preweaning performance of sows in four farms of Yucatan, Mexico

JE Ek-Mex[1], JC Segura-Correa[1], A Alzina-López[1], L Batista-Garcia[2], J Rodríguez-Pacheco[3]

[1]*Faculty of Veterinary Medicine and Zootechny, University of Yucatan, Mexico,*
[2] *Boehringer-Ingelheim Vetmedica, USA,* [3] *Adviser in swine production software.* jeemvz@hotmail.com

Introduction

The lifetime performance of sows plays an important role in the profitability and efficiency of swine production systems. One way to increase the profitability of farms is to increase lifetime piglets born alive (LBA) and lifetime number of piglets weaned (LPW). The aim of this study was to determine the effect of some non-genetic factors, on LBA and LPW in four pig farms in the tropics of Mexico.

Materials and Methods

Data from four commercial pig farms, representative of the central area of Yucatan Mexico, were used. Information from 2007 to 2011 to estimate LBA and LPW of sows were obtained from the production records kept in the Pigchamp program. In this study, only the data of the culled sows were used. LBA and LPW were calculated as the sum of the number of piglets born alive and weaned from the first to the last farrowing. Cross-fostering of piglets was carried out the first two days after birth in all farms, and sows were fed commercial diets. The statistical model to explain LBA and LPW included the fixed effects of farm, year of first farrowing (YF1), season of first farrowing (SF1), number of piglets born alive at first farrowing (NBA1) and age at first farrowing (AF1), simple interactions and the error term.

Resulted and Discussion

Table 1 shows the results of sows by farm, YF1, SF1, NBA1 and AF1 for LBA and LPW. Farm 3 had the highest LBA and LPW. Farm differences may partly be due to differences between farms in the length of productive life and the number of parity at removal (1). The primiparous sows with small NBA1 got the lowest LBA and LPW. Gilts that farrowed at early age had the highest LBA and LPW. Only the interaction between YF1 and SFI was significant. Similar results have been reported by other authors (2,3,4).

Table 1. Least squares means and standard errors by main factor for lifetime piglets born alive (LBA) and lifetime number of piglets weaned (LBW) in four farms in Yucatan, Mexico

Factors	N	LBA	LPW
Farm 1	5,391	46.7±0.4^{b}	46.2±0.38^{b}
Farm 2	720	45.5±0.9^{b}	43.1±0.86^{c}
Farm 3	391	51.0±1.0^{a}	48.6±1.08^{a}
Farm 4	1,024	40.9±0.9^{c}	36.4±0.8^{d}
SF1 Dry	2,819	48.9±0.5^{a}	46.1±0.56^{a}
SF1 Rainy	2,703	44.2±0.5^{b}	41.8±0.54^{b}
SF1 Windy	2,004	45.0±0.6^{b}	42.7±0.59^{b}
NBA1 ≤8	1,584	39.3±0.6^{c}	41.8±0.62^{c}
NBA1 9–12	2,711	46.2±0.5^{b}	43.7±0.53^{b}
NBA1 ≥13	3,211	52.6±0.5^{a}	45.1±0.53^{a}
AF1 ≤330	1,760	46.2±0.7^{ab}	43.3±0.69^{a}
AF1 331–347	3,363	46.9±0.6^{a}	44.1±0.62^{a}
AF1 ≥348	2,403	45.0±0.4^{b}	43.3±0.46^{a}

^{a,b,c,d}Means with different letters by factor and column are statistical different (P<0.05)

In conclusion difference was found between farms, YF1 and SF1 for LBA and LPW. The sows with small litter size had less LBA and LPW. Sows farrowed for the first time at a young age had highest LBA and LPW. Sows with large NBA1 and early AF1 should be accompanied by better management and care at farrowing.

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Antibiotic benchmarking for prairie swine centre

H Gauvreau[1], L Whittington[2]
[1]Consulting Veterinarian, Warman Veterinary Services, Saskatoon, SK, Canada,
[2] Prairie Swine Centre, Saskatoon, SK, Canada, helen.thoday@usask.ca

Introduction

Antibiotic benchmarking is a new tool being used in Canadian swine herds relative to other regions of the world. (1) The benchmarking process analyzes information gathered at the farm using an accepted model and reports the results back to the producer. The calculated measure determined by the benchmarking process provides an objective measure for the producer and veterinarian to track overall usage, discuss dose and timing and provides an opportunity for improved management. It is critical that the method used is well understood and consistent to allow for results to be comparable. (2) Several methods have been used in other countries including: animal daily dose/1000 animal days (ADD/1000 animal days), ADD/animal year, ADD/100 animal days and mg active substance/kg's of biomass. (3,4) Each method uses its own specified time period for calculation as well as its own ADD tables. It will be important moving forward as a Canadian industry that both the calculation method and dose charts used are standard to allow for results to be both comparable and of the maximum benefit to the industry.

Materials and Methods

The Prairie Swine Centre (PSC) is a 300 sow high health farrow-to-finish swine unit located near Saskatoon, Saskatchewan, Canada. It is a swine production research institute affiliated with the University of Saskatchewan with a mandate to provide Canadian producers with emerging technologies and information. Individual animal treatment and feed medication records for a representative three month period were collected for analysis. The ABCheck calculator (3) developed at the University of Gent, Belgium was used to calculate standardized antibiotic usage. This is reported as "animal daily dose" per 1000 animals days or animal year. Four stages of production animals were identified; sow-gilts-boars, suckling piglets, nursery pigs (wean-30 kg), and grow-finishers (fatteners). The following treatment information was entered into data fields; stage of production, how product was administered (injection, feed medication etc.), antibiotic administered, dosage, average weight of animal, number of animals treated and days treated.

Results

Table 1 shows the data presented by phase of production rather than a composite overall herd calculation as the value in the exercise is identifying areas for future observation and potential management change. It also illustrates how different the numbers will look depending on the method of calculation chosen

Table 1. Treatment incidence by production area in animal daily dose per 1000 animal days and animal daily dose per animal year.

	ADD/1000 animal days	ADD/ animal year
Breeding Herd	175	64
Suckling Piglets	180	66
Nursery	240	88
Grow/Finish	216	79

Conclusions and Discussion

Antibiotic benchmarking is a relatively new aspect of production management in Canada. To date only a few farms have worked through the benchmarking process so no comparisons or conclusions can be made on usage levels at this time other than there is variation between farms The PSC appears to be at a low level; antibiotic usage is limited to parenteral injections and the stage 1 nursery diet. No water medication is used.

Antimicrobial use evaluations made through the benchmarking process allow for objective on-farm decision making and improved veterinary oversight. Through this project the PSC re-evaluated some of its treatment protocols both in product choice and dose to make more prudent on farm antimicrobial decisions.

The value of the exercise is to understand the process and leading the industry in a standard direction. It not only highlights the need for a standard method but also standard definitions for weaned pigs, fatteners and standardized ADD tables. As an industry just starting into this process we can learn from systems in place in other countries to work toward a harmonized "Canadian" solution for antibiotic benchmarking.

Acknowledgments

Dr. Gail Cunningham, technical services veterinarian, Boehringer Ingelheim, Canada

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P.458

Cost comparison between use of commercial mixing feed in relation with self-produced mixing feed, in farrow to market pig farms

E Pérez[1], R Trueta[2], C López[2]
[1]*Departamento de Medicina y Zootecnia de Cerdos FMVZ-UNAM,* [2]*Departamento de Economía, Administración y Desarrollo Rural FMVZ-UNAM,* emilioepsilon@yahoo.com.mx

Introductión

In order to increase benefits, farmers have to adopt decisions that are not always simple, one of them has to do with self-producing or buying commercial feed depending on their physical (cost per kg of food) and economical efficiency (cost per live weigh kg sold). Therefore it is important to shed light in to this subject, since it represents 70-80% of total cost.

Materials and Methods

A sample of 208 farms from 8 states of Mexico were selected from the "Padrón Nacional Ganadero" (the largest in the country) and interviewed in the 2012 SICEC poll (1). The selection was randomly made in each of the following four stratums: 50 to 100 sows, 101 to 200, 201 to 500 and 501 or more sows. Out of that sample, 132 farms were selected for congruent information. Finally, after applying information filters to remove farms with less than 50 sows, a group of 92 farms from the states of Jalisco, Guanajuato, Aguascalientes, Tlaxcala and Veracruz were studied.

To compare the costs of feed, farms were divided into two groups according to the origin of the feed: self-produced (milling and mixing) or commercial. The variables compared were: average cost of kg of feed throughout the entire production cycle (fc), and the cost of production per kg live pig sold (fc/kg) excluding all inputs except feed. Costs are in Mexican pesos of 2012.

Comparison of means was performed using ANOVA with SPSS19 ® software

Results

In farms using commercial feed, the fc is cheaper ($p=0.017$) than the cost of self-produce feed (Table 1). In the case of fc/kg, the latter have an average fc/kg of $17.06, that is cheaper by $1.3 pesos to farms using commercial feed (Table 2). However no significant difference was found between the groups ($p=0.43$).

Conclusions and Discussion

The results show that the fc of self-produced feed is significantly more expensive; however, their fc/kg is cheaper all do there is no significant difference for the two groups. The food efficiency being responsible for the cheaper live kg sold due to a better feed conversion and because diets are formulated based on the specific requirements of each farm.

Table 1. Average cost per kg of feed (fc)[1]

Feed	N	Media	Std Error
Commercial	33	5.54	0.13
Self-produced	59	5.94	0.10
Total	92	5.80	0.08

1. Groups are different ($p<0.05$)

Table2. . Cost feed per kg live pig sold (fc/kgs)

Feed	N	Media	Std Error
Commercial	26	18.36	1.28
Self-produced	52	17.06	0.90
Total	78	17. 49	0.74

Another factor contributing to these result might be the inability of some farms to buy large volumes of grain enabling them to obtain better prices.

The above results are an example of how SICEC provides valuable evidence for decision making at the farm level and also for public policy. Therefore contributing to the achievement of competitiveness of pig production.(2,3)

Acknowledgments

Coordinación General de Ganadería de la SAGARPA

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Analysis of sow mortality among breeding sows in Spanish pig herds

A Palomo , JM García , R Gómez
Setna Nutrición S.A.U.- InVivo NSA . PI Santa Ana c/El Clavo, 1 Madrid (Spain), antoniopalomo@setna.com

Introduction

The current article analyses mortality in breeding sows without sacrifice among a field work undertaken during the last five years (2009-2013) in Spanish pig farms.
Sow death direct economic losses are estimated around 285 and 350 $, associated to the higher replacement costs and opportunity costs (1,5,8). By having available weaned sows, gilts and occasional sows, we could reach our targets (9). Sows dead are non predictable and broken the weekly farrowing organization and, in consequence, the whole farm management (3,7).

Materials and Methods

Sow mortality has been studied for five years (from January 2009 to December 2013) using the record keeping Spanish farm´s database.
In the trial study, we collected a total of 196.852 breeding sows records from nine different Spanish regions .
Breeding sows are from seven different genetic background. Herd size goes from 145 to 5000 sows, with an average of 658 sows for farm.

Results

The current study performed in Spain reported an annual mortality sow rate of 7.26 ± 4.12 % . Rate sow mortality increase 1 % from periods 2001-05 to 2009-13 We have to emphasize that we found a wide range between farms from 1.78 % to 18.7 % sow mortality rate and increase on summer time (July , August and September).
As following, we reproduce the field trial data grouped by death origin and stage of productive cycle.

Parity	1º	2º	3º	4º	5º	6º+	x-
Sudden Death	8.10	4.20	2.40	2.20	1.60	1.25	**19,75**
Metabolic Problems	5.40	3,80	1.35	1.40	1,50	1,90	**15,35**
Skeleton disorders	5.20	4.10	1.40	0.30	0.45	2,05	**13,50**
Gastric Ulcers	2.40	2.10	1.50	0.20	0.10	0,10	**6,40**
Urinary infections	1.70	1.30	1.00	0.40	0.40	0.80	**5,60**
Digestive disorders	2.00	1.20	1.10	0.30	0.10	0.0	**4,70**
Respiratory disorders	1.40	1.10	1.00	0.00	0.00	0.00	**3,50**
Accidents	1.20	0.80	0.50	0.40	0.10	0.20	**3,20**
Farrowing disturbance	1,70	0.50	0.20	0.00	0.00	0,10	**2,50**
Don´t know	8.10	5.60	3.20	2.50	2.30	3.80	**25,50**
Mean rate	**37.20**	**24.70**	**13.65**	**7.70**	**6.55**	**10.20**	**100**

Conclusions and Discussion

The five years trial reported annual average rates of 7.26 ± 4.12%, corresponding to similar rates than in other European countries , Canada and USA , and we think than the sow mortality are the global pig production problem around the world . The most common causes of known deaths in Spanish farms are: sudden death, metabolic and skeleton disorders, gastric ulcers and urinary infections. The first five causes represent the 60,60 % of the total sow deaths and the 81,34% of the known deaths. In addition, over 35.10 % of sow deaths are concentrated on sudden death (19.75%) and metabolic problems (15.35%).The third important cause to take into account are skeleton disorders (13.50%), follows very close by metabolic problems and far away to gastric ulcers (6.40%) and urinary infections (5.60 %). We wish to emphasize on the fact that a quart of the deaths were from unknown causes. Related to the number of reproductive cycle, to detach that 2 of every 3 death sows of the survey are from first and second parity (61.90 % - 37.20 % first and 24.70 % second) .

Acknowledgments

The authors like to thank to all swine veterinarians and producers involve in this study around the Spanish swine industry .

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The volume and economic efficiency of the weaner production with equal number of pens but different number of piglets weaned per litter

M Sviben[1], P Gnjidić[2]
[1]*Freelance consultant, Siget 22B, HR-10020 Zagreb, Republic of Croatia,* [2]*Biotim KG, Aleja Matice Hrvatske 37, HR-32270 Županja, Republic of Croatia,* marijan.sviben@zg.t-com.hr

Introduction

The number of weaners produced in a year is the product of the number of litters achieved through the year and the number of piglets weaned per litter. Latter averages varied during some period and they differ in different areas at the same time. Investors may be interested in the quantity of products and in the indices of economic efficiency of production with equal number of pens but different numbers of piglets weaned per litter, since they have to estimate the possibility of paying the sum of money invested into the means for the work with the interest. This report should give wanted information to experts who perform the investment and the investors themselves.

Materials and Methods

The data in the farm in Darda (during the periods:1, 2 - Croatia), published by Ferić (1990), on the farm in Nova Topola (3 – Bosnia and Herzegovina), registered through the consultative work – and on the Dubravica (4 - Croatia) as well as on the farm in Malo Kneževo (5 - Croatia)annually reported by the Croatian Agricultural Agency, made the material in this research. The numbers of weaned piglets a year on an average were connected with the means of the numbers of weaners per litter. Economic efficiency of the use of pens was measured by the ratio of the production volume and the number of pens (E_P = PV/n_P). There were 360 farrowing pens at each farm. To explain the differences between the production volumes and between the indices of economic efficiency the numbers of shifts/pen/year were used.

Results

Results of calculations are presented in Table 1.

Table 1. The production volumes, numbers of weaned piglets per litter, indices of economic efficiency and numbers of shifts per farrowing pen a year at the farms 1, 2, 3, 4, 5

Farm	Period	PV	Weaner per litter	E_P	Shifts per pen a year
2	1974-76	42,538	7.867	118.2	15.01
3	1974-79	37,015.3	7.913	102.8	12.99
1	1971-73	35,704	8.054	99.18	12.31
4	1995-99	35,546	8.915	98.74	11.07
5	2010-12	34,163	11.216	94.90	8.46

Conclusions and Discussion

The production volume and the index of economic efficiency of the use of pens were greatest in the farm 2, where the number of weaned piglets per litter was least, since mean number of shifts/pen/year was 15.01 or 77,42% greater than at the farm 5, were during 2010-2012 pigmen achieved 8.46 shifts/pen/year using hyperprolific PIC sows to get 42.57% more piglets weaned per litter than at the farm 2 through 1974-1976. Because of various difficulties managers at the farm Darda from 1977 decreased the number of shifts/pen/year to 12 approximately, as it had been earlier at the same farm (1). At the farm 3 almost 13 shifts/pen/year was registered through 1974-1979. When the pigmen faced the problems during the globalisation crisis, it was recommended the number of shifts/pen/year to be increased from 8.7 to 13.1 (2). It happened on the contrary. Using hyperprolific sows and getting the average of 8.20 shifts/pen/year through 2007-2011 at the farm in Bratina, Croatia, it was achieved 32.81% less production volume and 30.92% less index of economic efficiency than it would be with 12.17 shift/pen/year (3).

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Analysis of sow mortality among breeding sows in spanish pig herds

A Palomo, JM García, R Gómez

SETNA NUTRICIÓN S.A.U.- InVivo NSA . PI Santa Ana c/El Clavo, 1 Madrid (Spain). antoniopalomo@setna.com

Introduction

The current article analyses mortality in breeding sows without sacrifice among a field work undertaken during the last five years (2009-2013) in Spanish pig farms.

Sow death direct economic losses are estimated around 285 and 350 $, associated to the higher replacement costs and opportunity costs (1,5,8). By having available weaned sows, gilts and occasional sows, we could reach our targets (9). Sows death was no predictable and broke the weekly farrowing organization and , in consequence , the whole farm management (3,7) .

Materials and Methods

Sow mortality has been studied for five years (from January 2009 to December 2013) using the record keeping Spanish farm´s database.

In the trial study, we collected a total of 196.852 breeding sows records from nine different Spanish regions .

Breeding sows were from seven different genetic backgrounds . Herd size goes from 145 to 5000 sows, with an average of 658 sows per farm.

Results

The current study performed in Spain reported an annual mortality sow rate of 7.26 ± 4.12 % . Rate sow mortality increase 1 % from periods 2001-05 to 2009-13

We have to emphasize that we found a wide range between farms from 1.78 % to 18.7 % sow mortality rate and increase on summer time (July , August and September).

As following, we reproduce the field trial data grouped by death origin and stage of productive cycle.

Parity	1º	2º	3º	4º	5º	6º+	x-
Sudden Death	8.10	4.20	2.40	2.20	1.60	1.25	**19,75**
Metabolic Problems	5.40	3,80	1.35	1.40	1,50	1,90	**15,35**
Skeleton disorders	5.20	4.10	1.40	0.30	0.45	2,05	**13,50**
Gastric Ulcers	2.40	2.10	1.50	0.20	0.10	0,10	**6,40**
Urinary infections	1.70	1.30	1.00	0.40	0.40	0.80	**5,60**
Digestive disorders	2.00	1.20	1.10	0.30	0.10	0.0	**4,70**
Respiratory disorders	1.40	1.10	1.00	0.00	0.00	0.00	**3,50**
Accidents	1.20	0.80	0.50	0.40	0.10	0.20	**3,20**
Farrowing disturbance	1,70	0.50	0.20	0.00	0.00	0,10	**2,50**
Don´t know	8.10	5.60	3.20	2.50	2.30	3.80	**25,50**
Mean rate	**37.20**	**24.70**	**13.65**	**7.70**	**6.55**	**10.20**	**100**

Conclusions and Discussion

The five-year trial reported annual average rates of 7.26 ± 4.12% , corresponding to similar rates than in other European countries , Canada and USA , and we think than the sow mortality is the global pig production problem around the world . The most common causes of known deaths in Spanish farms are: sudden death, metabolic and skeleton disorders, gastric ulcers and urinary infections . The first five causes represent the 60,60 % of the total sow deaths and the 81,34% of the known deaths. In addition, over 35.10 % of sow deaths are concentrated on sudden death (19.75%) and metabolic problems (15.35%).The third important cause to take into account are skeleton disorders (13.50%), followed very close by metabolic problems and far away to gastric ulcers (6.40%) and urinary infections (5.60 %) . We would like to emphasize on the fact that a quart of the deaths were from unknown causes. Related to the number of reproductive cycle, to detach that 2 of every 3 death sows of the survey are from first and second parity (61.90 % -37.20 % first and 24.70 % second)

Acknowledgments

The authors would like to thank to all swine veterinarians and producers involved in this study about the Spanish swine industry .

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Treatment compliance and traceability by use of the new ETIC® electronic recording and injecting device, associated to electronic identification of pigs

R Galofré[1], G Guardia[1], M Espona[1],R Segundo[1], J Sanmartín
Optimal Pork Production Pig Advisory Group. Lerida, Spain, r.segundo@oppgroup.com

Introduction

Traceability of medications and other veterinary treatments has become an increasing concern in livestock farming because of its potential implications on residues in meat for human consumption. Withdrawal time, use restrictions for antibiotics, hormones and other drugs, has become a major concern for the pig industry.

Manual recording of injectable treatments is; time consuming, subject to miss interpretation, tampering, paper loss, and frequently hard to verify.

Electronic identification of sows, is already a established production method specially in farms that use Electronic Sow Feeding Stations (ESFS). The possibility of relating treatments to individual pigs using electronic ear tag, plus, automatically recording injectable treatments, to a pre registered product, which can also be connected to software, that provides complementary information, opens new possibilities not only for treatment compliance but also for the traceability of treated animals.

Materials and Methods

An ETIC® electronic device was developed by Optimal Pork Production, for the recording of injectable treatments. It was designed to fit a Prima marc vaccinator (Prima Tech, Kenansville, NC, USA), paint spray container.

The design was evol…pe 1 to prototype 2, to ensure; a) ease of us…ment at the end of the injecting process (8 …rage capacity), c) no movable parts, d) …ual confirmation of treatment, e) easy ea…ater tightness, g) long lasting rechargeable …), h) and easy down load of recorded info…h).

Results

Various trials invol…reatment of sows in loose group gestatio…re carried out in large scale commercial … These trials were established to determ…sability of the ETIC®

as compared to a standard vaccination, by experienced stockmen and veterinarians.

The procedure being: 1. Record the product to be injected, 2.Read ear tag (includes visual and sound confirmation), 3. Inject product (includes visual and sound confirmation), pass on to another animal and repeat procedure. 4. Download information to PC. The device also records time of treatments. From these trials, many parameters and design aspects were adjusted.

Conclusions and Discussion

All trials concluded with 100% electronic recording of treated animals.

A summarized over all evaluation by users was:

1. Reading ear tags before injecting, somewhat slows down the speed of the injecting process as compared to not recording.
2. After some practice, the completion of injectable treatment (including reading) was considered easy and practical, by most users.
3. The automatic download of the information was considered very practical, for record keeping purposes.

The ETIC® device, can be adapted to various types of Primamarc vaccinators, including bottle mounted vaccinators. It can also record number of treated piglets in a …ear tag at weaning). It can also be …

…

However evolutions and interconnections … are under development.

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Determination of QTLs for fresh traits in Duroc by means of genome-wide association study (GWAS)

G Ramis[1], E Hanenberg[2], S Wijga[2], JM Herrero-Medrano[2], G Usero[3], J Corchero[4], JJ Quereda[1], L Calvo-Adiego[5], AI Rodríguez[5], FJ Pallarés[6], MB Linares[7], MD Garrido[7], A Muñoz[1,3]

[1]*Dep. Producción Animal. Universidad de Murcia. Spain,*[2] *TOPIGS Research center IPG B.V., The Netherlands,* [3]*TOPIGS Ibérica, Spain,* [4]*TOPIGS International., The Netherlands,*[5]*Dep. I+D+i Incarlopsa, Spain,* [6]*Dep. Anatomía y Anatomía Patológica Comparadas. Universidad de Murcia, Spain,* [7]*Dep Tecnología de los Alimentos. Universidad de Murcia, Spain.* guiramis@um.es

Introduction

The production of cured ham in Spain represents a very important economic issue in the frame of pork production. The main focus of genetic selection up to the date was related to numeric production. Today, in a "genetic on demand" program the meat quality has increased its importance for costumers. Today the genetic improvement requires of new strategies, including markers assisted selection (MAS). Here we described several Quantitative Trait Loci (QTLs) for fresh meat traits in a Spanish Duroc genetic line.

Materials and Methods

Information from cured hams was available from 248 animals (all TOPIGS Duroc gilts mated with TOPIGS Duroc boars). The parameters studies were: Test daily gain during test (TGR), Ultrasound back fat (BFE), Ultrasound loin depth (LDE), Ultrasound IMF (IMF_LMS), Loin weight (WT_LOIN), weight ham (WT_HAM), Intramuscular fat (IMF), Marbling (MARBLN), Conductivity (CONDUCT), Japanese color (JSCD1), Minolta L value (MINL) Minolta A value (MINA) and Minolta B value (MINB). The phenotypic measurements for TGR, BFE, LDE, IMF_LMS were obtained in the farm, assessed the last three using an ALOKA ultrasound system.

On the other hand, to perform the GWAS analysis, DNA from 244 animals was isolated and every animal was genotyped using Illumina 60K SNPs chip at GeneSeek (USA). The relation among SNPs markers and productive traits was establishing using! a Bayesian variable selection model. There were only consider those regions containing at least two significant SNPs (significance level of Bayes Factor>10) within 1Mb.

Results

One hundred and one regions containing at least two significant SNPs (BF>10) within 1Mb were identified for 11 traits in the chromosomes 6, 7, 12, 13, 14 and 15. Among them, eight regions, explaining more than 2% of genetic variation, showed significant relation with different traits. Region 1 (SSC6~66) and region 2 (SSC6~142) were significant for MARBLN and BFE, respectively. For region 3 (SSC7~120), 4 (SSC12~10) and 5 (SSC13~189) evidence was found for genes IMF or marbling. The region 6 (SSC14~5) was related with TGR, and the region 7 (SSC14~123) and 8 (SSC15~152) with BFE..

Table 2. QTLs with a large effect (>2% of σ_g^2), position on chromosome (SSC), start and end (in Mb), and number of significant SNPs (BF>10).!

QTL	SSC	Start	End	Gen. Var explained (%)	No. SNPs	trait
1	6	65.8	66.1	2.9	4	MARBLN
2	6	134.3	137.0	3.5	4	BFE
3	7	118.7	123.2	4.5	8	IMF_LMS
4	12	10.	10.5	3.9	3	MARBLN
5	13	188.7	190.4	6.6	19	IMF
6	14	4.6	4.9	2.5	3	TGR
7	14	121.3	125.6	3.1	12	BFE
8	15	151.4	154.1	2.5	8	BFE

Conclusions and Discussion

Regions 1 (SSC6~66) and 2 (SSC6~142) are both described in literature for IMF, including candidate genes[1]. No genes were described in literature in the region 6 (SSC14~5). Maybe this region for TGR should be considered as "false-positive" (3 SNPs only).!Region 2 was only significant for BFE, but in literature this region is also linked to IMF[2]. The correlation of region 7 (SSC14~123) and 8 (SSC15~152) are both described in literature for backfat[2] in Duroc. The regions 3, 4 and 5 had been previously related with genes influencing backfat, but not for IMF or MARBLN.

Some of these regions have also showed correlation with cured ham traits. In example, region 5 (SSC13~189Mb) was also found for Sensorial IMF and region 8 (SSC14~123Mb) for brightness of fat and loin (LNB, FTB).

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Determination of QTLs for cured ham traits in Duroc by means of genome-wide association study (GWAS)

G Ramis[1], E Hanenberg[2], N Duijvesteijn[2], JM Herrero-Medrano[2], G Usero[3], J Corchero[4], JJ Quereda[1], L Calvo-Adiego[5], AI Rodríguez[5], FJ Pallarés[6], MB Linares[7], MD Garrido[7], A Muñoz[1, 3]

[1]*Dep. Producción Animal. Universidad de Murcia. Spain,* [2] *TOPIGS Research center IPG B.V., The Netherlands,* [3]*TOPIGS Ibérica, Spain,* [4]*TOPIGS International, The Netherlands,*[5]*Dep. I+D+i Incarlopsa, Spain,* [6]*Dep. Anatomía y Anatomía Patológica Comparadas. Universidad de Murcia, Spain,* [7]*Dep Tecnología de los Alimentos. Universidad de Murcia, Spain,* guiramis@um.es

Introduction

The production of cured ham in Spain represents a very important economic issue in the frame of pork production. The main focus of genetic selection up to the date was related to numeric production. Today, in a "genetic on demand" program the meat quality has increased its importance for costumers. Today the genetic improvement requires of new strategies, including markers assisted selection (MAS). Here we described several Quantitative Trait Loci (QTLs) for cured ham traits in a Spanish Duroc genetic line.

Materials and Methods

Information from cured hams was available from 249 animals (all TOPIGS Duroc gilts mated with TOPIGS Duroc boars). The parameters studies were: Fat brightness (FTB), Intramuscular fat (IMF), Lean brightness (LNB), Uniformity of color (UNC) and Color intensity (INC), Cured smell intensity (CSI), taste to raw meaty (RWM), Salty taste (SAL), Cured taste (CUR), Hardness (HDN), Chewiness (CWN), Mellowness (MLN), Juiciness (JUN), Total acceptance (ACP), Slice texture average (SLA) and Slice texture standard deviation (SLD). The phenotypic information related to cured ham sensorial meat quality traits was obtained from the Dep. of Food Technology from the University of Murcia (Spain) by means of a sensorial analysis panel, including 2 male and 6 female and it was done in accordance with the ISO 4121 (2003).

On the other hand, DNA from 244 animals was isolated and every animal was genotyped using Illumina 60K SNPs chip at GeneSeek (USA). The relation between SNPs markers and productive traits was establishing using a Bayesian variable selection model. It was only consider those regions containing at least two significant SNPs (significance level of Bayes Factor>10) within 1Mb.

Results

One-hundred and four regions containing at least two significant SNPs (BF>10) within 1Mb were identified for 14 traits. Only 7 QTL regions showed large effects explaining more than 2% of the genetic variation, for ACP, IMF, MLN and HDN. The chromosome, position and number of significant (BF>10) SNPs in the region are shown in table 1. On chromosome (SSC)3, a region showing large effects on several traits together was identified. Five regions showed a significant effect on more than one trait (Table 2). The highest number of effects were found for the trait SAL (29 QTL with BF>10).

Table 3. QTLs with a large effect (>2% of σ_g^2), position on chromosome (SSC), start and end (in Mb), and number of significant SNPs (BF>10).

QTL	SSC	Start	End	Gen. Var explained (%)	No. SNPs	trait
1	3	13	15	5.42	9	ACP
2	3	16	17	2.88	5	MLN
3	3	17	18	3.23	4	HDN
4	3	17	18	2.23	5	ACP
5	12	46	48	2.50	23	IMF
6	13	189	190	5.57	16	IMF
7	16	63	67	4.33	10	ACP

Conclusions and Discussion

On the tip of SSC3, Soma et al. (2010) described an effect on meat color in Duroc measured as Minolta values. On SSC12, it has been identified an effect on IMF and marbling score covering the same interval in a Chinese Large-White X Minzhu intercross[1]. On SSC13, an effect in purebred Spanish Duroc on meat colour Minolta A (redness) of the ham muscle glutaeus medius have been detected[2]. Moreover, Soma et al. (2010)[3] describe an effect in a Japanese Duroc population on IMF in this region (130-195Mb). None of the effect found for SLA trait showed a meaningful overlap with large effects found for the underlying traits. This emphasizes the need to work with detailed phenotypes if the goal is to pinpoint specific region in the genome even with large effects and the underlying functional mutations.

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Sensory quality of dry-cured ham slices from an improved genetic line of Spanish Duroc

MB Linares[1], MD Garrido[1], P Díaz[1], MC Espinosa[1], G Ramis[2], JM Herrero-Medrano[3], JJ Quereda[2], G Usero[4], J Corchero[5], A Muñoz[2,4]

[1]*Dep Tecnología de los Alimentos. Universidad de Murcia, Spain* [2]*Dep. Producción Animal. Universidad de Murcia. Spain,* [3] *TOPIGS Research center IPG B.V., The Netherlands,* [4]*TOPIGS Ibérica, Spain,* [5]*TOPIGS International, The Netherlands.* blinares@um.es

Introduction

Dry-cured ham is one of the most traditional products in the Mediterranean area with extraordinary sensory and nutritional characteristics associated. The sensory quality of dry-cured ham results from the interactions between the characteristics of the fresh matter and the biochemical changes occurring during the processing[1]. For example, the excessive softness and pastiness are two of the main texture problems in dry-cured ham[1] and both are associated with high proteolysis activity and high-temperature during the last month of ripening. Sensory analysis permits assessing organoleptics properties which are directly related with quality perception by consumers[2]. The objective of this work was to evaluate the dry-cured ham sensory quality of a genetic TOPIGS Duroc sire line.

Materials and Methods

A total of 252 samples of commercial dry-cured ham were analyzed. The hams from white pigs female (Duroc) had a minimum curing period of 14 months. The ripening process was same in all samples. Ham packs (240 g/12 slices/2 mm thick) under protective atmosphere (80% CO_2 y 20% N_2) were stored in refrigeration at 4 ºC until analysis. Samples for panelists corresponded to the *Biceps femoris* muscle measuring approximately 2.5 x 7 cm. The packs were opened 15 min before to start sensory test carried out by a trained panel consisted of 7 people (2 men and 5 women) from the Food Technology, Nutrition and Food Science Department. For sensory test, an unstructured scale of 10 cm [0 (low intensity) to 10 (high intensity)] was used[3]. The appearance parameters [marbling, colour homogeneity, red intensity] were analyzed in a single slice on white background. For Odour, Flavour [odour intensity, cured odour intensity, raw meat, saltiness, spicy and cured meat] and Texture attributes [hardness, chewiness, pastiness and juiciness] two samples were analyzed per panelist.

Results

The results for different attributes are shown in Table 1. The slices had a half marbling and an intense red colour, homogeneous, although sometimes differences were observed between the internal and external zone. The odour intensity was high, mainly characterized by a cured meat odour. The cured meat flavour was highly valued by panellists. Regarding to texture analysis, the hams showed intermediate hardness and chewiness and were similar to those reported by other authors in white pig[4]. The ham slices were not pasty but slightly-moderate juiciness.

!

Table 1. Mean values and standard deviations of the sensory attributes.

	Attribute n=1616	m ± sd
Appearance	Marbling	5.3 ± 1.4
	Colour homogeneity	6.6 ± 1.1
	Red intensity	6.9 ± 0.9
Odour	Odour intensity	7.1 ± 0.9
	Cured odour intensity	6.9 ± 1.0
Flavour	Raw meat	0.3 ± 0.5
	Saltiness	4.7 ± 0.8
	Spicy	0.1 ± 0.3
	Cured meat	6.0 ± 1.0
Texture	Hardness	5.0 ± 1.1
	Chewiness	5.0 ± 0.6
	Pastiness	0.4 ± 0.7
	Juiciness	5.3 ± 0.7
	Overall aceptability	6.6 ± 1.2

Conclusions and Discussion

The flavour intensity seem to be the most important features in determining acceptability in dry-cured ham, being an intense cured meat flavour, the most important attribute for the ham quality[5]. The overall assessment of the hams was higher than average (6.00-7.00) in 40.1% of cases and only 26.2% received scores below 6.00 (data not showed).

This sensorial attibbutes showed enought variability to be taken into account into a genetic improvement program "on demand".

Acknowledgments

The results described in this communication was funded by CDTI "PROCADECO" (nº IDI 20090377); developed by Incarlopsa, Grupo TOPIGS Ibérica, the University of Murcia and TOPIGS Research Center IPG.

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Sound attributes of vocalizations of pigs exposed to diverse distress conditions

IA Nääs[1], FR Caldara[1], M Moi[1], L Foppa[1], RKS Santos[1], RG Garcia[1], GB Moura[1], C Eyng[1], MS Amadori[1], B Bazzo[1]
[1]*College of Agricultural Science, Department of Animal Sciences, UFGD, Brazil* fabianacaldara@ufgd.edu.br

Introduction

Animals' vocalization is the expression of a particular state, being an important tool for the assessment of animal welfare. Vocalizations emitted may assist understanding animals' living quality when exposed to different distress. This study aimed to assess the distinction of different stressful stimuli for pigs through their vocalization.

Materials and Methods

Castrated males (150) 100 day old were randomly divided into five pens. In alternate days, the animals were subjected to different stress situations: thirst, hunger and heat stress. For the control treatment, the animals were kept in a comfortable situation. Acoustic signals were recorded every 30 minutes during an uninterrupted period of three hours, totaling six samples for each stress situation. The signals were digitized at a frequency of up to 44,100 Hz, for a period of 3 minutes. The audios were analyzed using Praat®5.1.19 software, and the attributes generated were: signal energy (SE) (Pa^2 * s), minimum (MaA) and maximum amplitude (MiA) (Pa), Pitch frequency (PF) (Hz), sound intensity (SI) (dB) and four formants (F1, F2, F3 and F4) (Hz). Data were subjected to analysis of variance and Tukey test ($p < 0.05$).

Results

From each sequential band of noise it was formed a sonogram (Figure 1) with the acoustic features.

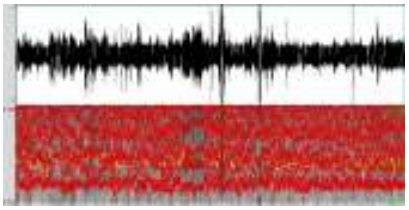

Figure 1. Sonogram of vocalizations of pigs, dotted red are the formants; yellow line the sound intensity and dotted blue the Pitch frequency.

Except for formants 1 and 3, all sound attributes evaluated showed significant differences between the distress to which animals were subjected. Nevertheless, they behaved in a different manner (Table 1). The SE from the swine vocalization is significantly higher in animals subjected to heat stress, followed by the condition of hunger. The MaA and MiA of vocalizations were not different between pigs subjected to the condition of heat, hunger or comfort. Thus, were not considered a good indicator for distress differentiation. The SI did not allow the differentiation of thirsty distress from the comfortable situation, but it was higher in animals subjected to heat stress and starvation. Therefore, when associated to the attribute SE, it can be a good indicator of conditions of heat stress or starvation. The PF was higher in vocalization of pigs subjected to heat stress and comfort condition. Thereby, this attribute may help to differentiate the thirsty distress from the comfort, and heat stress, and hunger, for which other attributes were not efficient. The formants 2 and 4 only allowed the differentiation between hunger and in comfort.

Table 1. Average of the signal energy (SE), the maximum amplitude (MaA) and minimum (MiA), sound intensity (SI), Pitch frequency (PF) and formants (F1, F2, F3 and F4) of vocalizations of pigs submitted the stress and welfare (W).

Attributes	Thirsty	Heat	Hunger	W
SE	5.31c	17.35[a]	12.02b	7.48c
MaA	1.71b	1.93[a]	1.90ab	1.75ab
MiA	1.64b	1.82[a]	1.84a	1.68ab
SI	77.75b	83.47[a]	81.78a	75.76b
PF	267.96b	325.40[a]	185.92c	310.13[a]
F1	1058.46	1084.33	1068.83	1082.28
F2	2116.46ab	2151.69[a]	2152.02a	2094.52b
F3	3210.95	3249.31	3250.97	3221.35
F4	4232.47ab	4269.43ab	4275.74a	4218.20b

In lines, means followed by different letters differ significantly by the Tukey test ($p < 0.05$).

Conclusions and Discussion

Cordeiro et al. (2009) found that SE increases according to the stress which the animal is subjected. Marx et al. (2003) evaluating pigs during castration found that the discomfort associated with vocalizations can be identified and characterized through the SE. The sound attributes of swine vocalization vary in a different manner depending on the stressful stimuli, being an efficient tool to differentiate the distress.

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Data mining vocalization to estimate stress conditions of pigs

IA Nääs[1], FR Caldara[1], M Moi[1], RK S Santos[1], L Foppa[1], RG Garcia[1], ICL Almeida Paz[1], MS Amadori[1], GB Moura[1], C Eyng[1]

[1]*College of Agricultural Science, Department of Animal Sciences, UFGD, Brazil* fabianacaldara@ufgd.edu.br

Introduction

The vocalization is an objective and noninvasive tool which enables to identify evidence of a lack of welfare from distinct vocal patterns (Nääs et al., 2008). Marx et al. (2003) identified different parameters of energy emission, frequency and duration of vocalization in pigs. The technique of data mining has provided favorable results in the discovery of knowledge in the field of animal production. The aim of this study was to identify differences in the pattern of pig vocalization according to different stress exposures using data mining.

Materials and Methods

Castrated males (150) 100 days old were randomly divided into five pens. The animals were subjected to a different kind of distress: thirst, hunger and heat stress and the comfort condition (control). Acoustic signals were recorded every 30 minutes, totaling six samples for each condition. The signals were digitized at a frequency of up to 44,100 Hz, for a period of 3 minutes. The audios were analyzed using Praat®5.1.19 software, and the attributes generated were: signal energy (SE) (Pa^2 * s), minimum (MaA) and maximum amplitude (MiA) (Pa), Pitch frequency (PF) (Hz), sound intensity (SI) (dB) and four formants (F1, F2, F3 and F4) (Hz). Data were processed to determine the stress conditions using the software WEKA® 3.5 with the J48 algorithm, considering the cross-validation samples with 10% (10-fold cross-validation), and the goal attribute was the stress condition.

Results

The Decision Tree (Figure 1) obtained by J48 algorithm had an accuracy of 77, 66% and Kappa statistic of 0.62. The most important attribute for classification of acoustic stress conditions was the sound intensity (root node). For IS less than or equal to 73.87 dB, there is an indication that the animals were in the comfort while for 73.87 <IS <80.18 Hz it is assumed that they are thirsty. When IS> 80.18 dB it was necessary to use the frequency of Pitch in order to classify the sound. When FP> 276.71 Hz there is an indication that the animals are in heat stress. When 276.71≥ PF ≥ 212.87 Hz animals were hungry, and when PF> 212.87 Hz it was also necessary to verify the Formant 1. When Formant 1> 1066.4 Hz, there is an indication that the animals are thirsty, and when Formant 1 ≤ 1066.4 Hz it indicates the pigs are hungry.

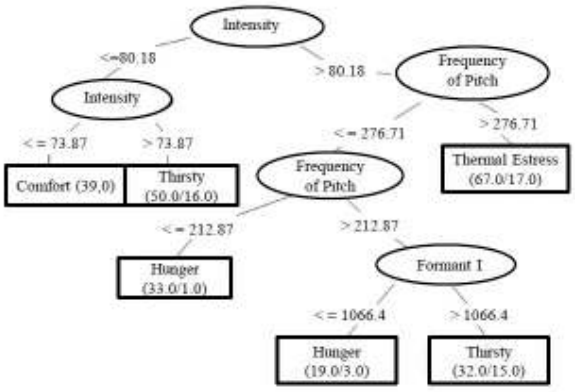

Figure 1. Classification of stress conditions using the J48 algorithm

Conclusions and Discussion

Piglets respond with screams of high intensity sound of pain (Marx et al., 2003) which enables the identification of sound for just one sound attribute and easily identifiable by the human ear. Sampaio et al. (2007) showed that the noise emitted by pigs under thermal discomfort premises, tends to grow with heat stress. Similar results were found in this study, when it was found that the frequency of pitch, which determines the loudness of the sound is one way to identify heat stress.
The identification of hunger and thirst using Formant 1 is also reported by Düpjan et al. (2008), which indicated that these expressions are distinct from others related to different social distress or fear and frustration. However, several authors showed the difficulty of classifying these distress conditions, since these are mixed up and need more than one attribute, or yet the combination of them, to identify more precisely the type of stress (Marx et al., 2003).

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Conditioning of pigs for oral administration of drugs and biologicals *(Taenia. solium)*

N Villalobos[1], M Sánchez[1], E Chávez[2], LF Rodarte[3], AS de Aluja[1]
[1]Depto. de Patología FMVZ-UNAM., [2]INIFAP Palo Alto, [3]Depto. de Etología FMVZ-UNAM, rodarte@unam.mx

Introduction

Behaviorism (also known as conditioning) and the social learning theory are important approaches to study the learning processes. The former relies on observable, measurable behaviors that can be recorded. In classical conditioning, the study of animal learning is based on the response to a previously unknown stimulus, which is then repeatedly associated to a response-triggering one, in a three-step process: before, during, and after conditioning. Animals learn in the same way as human beings: they react before certain environmental features they find pleasant, painful, or menacing. The behavior of a hungry animal is more sensitive to the effect of food rewards than to those unrelated to feeding. Research activities involving pigs as test animals require a number of procedures for vaccination, inoculation, and drug application by different routes of administration. This is a source of stress for the animal and of risk for the practitioner or caretaker. This study is aimed to condition animals to willingly accepting oral inoculation of Taenia solium eggs.

Materiasl and Methods

Forty-nine 7-week-old female pigs were distributed into seven homogeneous, hierarchically well-defined groups. Seven days were allowed to accustom the animals constant monitoring and pen cleansing. Conditioning started on day 7, consisting in offering the animals a tuna-bread-mixture croquette, covered with commercial pig meal, every other day until 11 repetitions were completed, at day 30. At this moment, the challenge with *T. solium* eggs started.

The time needed for each pig to accept and swallow the croquette when individually offered was measured (Figure1).

Results and Discussion

After the first conditioning sessions, the subordinate animals in each group displayed a mistrustful behavior, and were reluctant to eat. The time measured for each group was longer than 30 min, since the higher-hierarchy pigs did not allow the subordinate animals to approach the food, and it was difficult to make sure that each animal ate the corresponding doses. After the fourth session, 90% of the animals were approaching with no signs of fear, and willingly ate the croquette. After the fifth session, the time measured for each group to accept and swallow the croquette was less than 3 min. The use of this procedure is suggested in studies requiring oral treatment, vaccination, and/or inoculation of pigs, where its use is expected both to promote animal welfare and to reduce the risk of accidents during the handling of pigs.

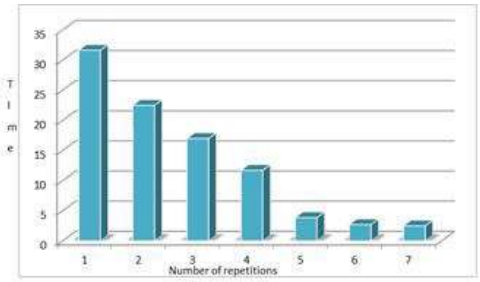

Figure 1 Graphical representation of the average time spent in each repetition

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How does rubber flooring in farrowing pens for loose housed sows affect their lying behavior and time spent lying down?

A-Ch Olsson[1], N Winter[1], C-J Ehlorsson[2], J Botermans[1], C Bergsten[1], J Svendsen[1]
[1]*Swedish University of Agricultural Sciences, Department of Biosystems and Technology (BT), Alnarp, Sweden,*
[2]*Swedish Animal Health Service, Sweden, hyocon@gmail.com*

Introduction

Solid concrete floors in the lying area are most often used in Swedish farrowing pens for loose housed sows. The amount of bedding and the concrete floor quality greatly influence the occurrence of injuries and the comfort and behavior of the sow and her offspring (1,2). The use of bedding material has been decreasing with the development of intensive pig production systems (3). The objective of the present study was to assess whether the use of rubber flooring in the farrowing pen would benefit piglet and sow welfare. The occurrence of leg and claw injuries on the piglets was recorded and is reported elsewhere (4). In the present study, sow lying behavior was studied, and the influence of environmental temperature was determined. In addition, the durability of the rubber floorings was evaluated.

Materials and Methods

The study was made in a commercial sow herd. The farrowing pens (2.2 x 3.0 m) for loose housed sows had a solid concrete lying area (4 m^2) and a plastic slatted dunging area (2.6 m^2). For each of 5 farrowing batches, the sows in 2 pens with concrete flooring (due to video problems, a total of 9 pens with concrete only were studied) and 2 pens with rubber flooring (total of 10 pens, where 3 had a rubber coating [Procoat], 4 had a rubber mat [KraiburgA] and 3 a second type of rubber matting [KraiburgB]) were studied. - Sow behavioral observations were made using continuous, 24 h, video recordings (MSH-video client) at 1 and 3 weeks after farrowing, respectively. The following was recorded: sow location in the pen and the time spent lying on the side, on the abdomen, for sitting, standing/walking, and for lying down. Pen temperature (Tiny tag data logger) was measured twice per hour. Statistics using software in SAS version 9.3 (PROC GLM) were applied.!

Results

The rubber coating was spackled on the floor; it did not last in spite of several efforts. The 2 types of mats were durable and lasted well during the trial period.

In all, at 1 week after farrowing the total lying time for the sows was on average 87% of the 24 h period while at 3 weeks the time was 82%. When lying there was a trend, that the "rubber pen" sows more often chose to lie on the solid rubber area, as compared to the sows in "concrete pens" (Table 1). There were large individual variations between sows as to lying position, lying location, lying down time and as to how the environmental temperature affected lying time on the slatted floor area.! The assumption that sows could be more sensitive to higher temperatures when on the rubber floor, due to less thermal conductivity, could not be disregarded. However, the temperature during the observation period did not vary enough to confirm this.

Table 1. Sow lying location (%) in pens with rubber flooring and concrete flooring, respectively

	1 week			3 weeks		
	Concrete	Rubber	p-value	Concrete	Rubber	p-value
Lying- solid area	65.6	75.7	*0.35*	63.0	74.7	*0.14*
Lying- transition area	11.1	9.5	*0.67*	15.2	6.4	*0.02**
Lying- slatted area	23.3	14.8	*0.38*	21.8	18.9	*0.71*

Conclusions and Discussion

We consider that the large individual variations in sow behavior in this study are a sign that sows, when housed loose in farrowing pens, could determine their comfort and use this option. This could not have been expressed by a crated sow. There was a trend that the sows preferred the solid rubber area compared to the solid concrete area when lying. If, for welfare reasons, a softer, non-abrasive floor is required, or if the existing concrete floor is of poor quality and increases the risk of injuries, then rubber flooring is an option. There are now rubber mats on the market which can sustain the weight and behavioral activity of sows.

Acknowledgments

Partnership Alnarp supported this project.

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Taping the fore knee of piglets reduces skin abrasion injuries

J Botermans, P Olsson, E Järlesäter, A-Ch Olsson, J Svendsen
Swedish University of Agricultural Sciences, Department of Biosystems and Technology (BT), Alnarp, Sweden,
hyocon@gmail.con

Introduction

Skin abrasion injuries on the forelimb of piglets early in life are very common (1,2,3,5). They develop as a result of direct contact between the piglet and the floor and almost all vital and active piglets develop them. Skin abrasion injuries may cause lameness and may contribute to disturbances in the pig´s normal gait; in addition, they may serve as a means of entry of infection. The forelimb abrasion injuries occur most often immediately below the carpus or extending from the carpus down to the metacarpus. - The objective of the present study was to determine if taping the fore knees immediately below the carpus shortly after birth would reduce skin abrasion injuries on piglets in commercial farms, and thus benefit pig welfare.

Materials and Methods

The study was in 3 commercial herds (A, 38 litters; B, 14 litters; C, 16 litters). Equal numbers of litters in each herd were taped and non-taped, respectively. Piglets were taped after they were all dry, within the first day of birth, using a well adhering tape, Leucoplast. The tape (2.5 cm in width) was cut in 2 cm long pieces which were placed dorsally, immediately below the carpus (Fig. 1), not around the knee in order not to disturb normal blood circulation. Production statistics (sow identity, No. liveborn, No. deaths during suckling, No. treatments and causes) were continuously recorded in the herd. At the age of 7 days (range 5-10 days) skin abrasions on the 2 fore knees of each piglet were recorded and measured using a ruler.

Statistics: Each litter was one statistical unit. The average diameter of the wounds was calculated per litter. An one-sided GLM-test was performed in SAS.

Figure 1. Shows how the tape was placed at the fore knee

Results

For some few of the pigs, the tape lasted to the recording of the skin abrasions, but in most cases the tape was gone by this time.- The results from the 3 herds are summarized in Table 1. The size of the skin abrasions was significantly smaller on pigs which had been taped. There were no significant differences in the number of pigs treated for arthritis/polyarthritis.

Table 1. Average wound diameter and treatment for joint inflammation in pigs with or without tape on their front legs

	Control	Tape
No. Litters	34	34
No. of piglets	394	405
Piglets/litter	11.6	11.9
Average wound diameter (mm)	8.7[a]	5.9[b]
No. pigs treated for joint infl.	52	43

(a, b) Superscripts indicate statistically significant differences within main effect ($p \leq 0.001$)

Conclusions and Discussion

The pigs were examined for skin abrasion injuries at 5-10 days of age; at this time the prevalence of abrasion injuries is high (3,5). Abrasion injuries on the fore knee are enhanced when the piglets fight for a teat and suckle. In addition to problems with floor quality and/or insufficient bedding material, knee injuries are aggravated when the sow milk supply is inadequate. - In weak born piglets these skin abrasions are usually insignificant.

Abrasion injuries of piglets in farrowing pens with concrete flooring may be reduced by using careful bedding routines around farrowing (4) and by improving floor quality (3,5). Taping of the fore knee is an additional option to reduce skin abrasion injuries and thus increases piglet welfare. There were no effects of taping on the number of piglets treated for joint ill.

Acknowledgments

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Effect of hypokinesia in sows during pregnancy period on cortisol and acute phase proteins level in the piglets in early postnatal period

M Kulok[1], K Wojtas[1], M Porowski[2], Z Pejsak[3], R Kołacz[1]

[1]*Wroclaw University of Environmental and Life Sciences, Department of Environmental Hygiene and Animal Welfare, Chelmonskiego Str. 38C, 51-630 Wroclaw,* [2]*Veterinary Clinic, ul. Kościuszki 1, 62-010 Pobiedziska,* [3]*Department of Swine Diseases, National Veterinary Reserch Institute, Partyzantów 57, 24-100 Pulawy,* kolacz@gmail.com

Introduction

Keeping pregnant sows in pens that restrict movement (gestation crates) is a common practice in many countries but at the same time a significant stress factor for those animals (1,4). Severe stress of sow during pregnancy period can cause prenatal stress and significantly reduce welfare and health of piglets (2). The aim of the research is to examine the impact of movement restriction of sows during pregnancy period on piglets welfare, measured by plasma cortisol and acute phase proteins concentration.

Materials and Methods

The experiment was conducted at two farms that use individual and group housing system. Two research groups were established:

Free movement group (FM): Pregnant sows in this group were kept in group pens in a number of 10 animals in a pen and an area of 2.25 m^2 per animal. Sows in this group had a possibility to move freely. Before birth sows were moved to individual pens with a possibility of movement. Piglets stayed with sows up to 28th day of life.

Movement restriction group (MR): Pregnant sows in this group were kept in individual pens of an area of 1.3 m^2. Movement of these animals was limited only to the possibility of getting up and lying down. Before birth sows were moved to individual pens without possibility of movement. Piglets stayed with sows up to 28th day of life.

Blood samples were collected from piglets in all groups at 3th, 7th and 21th day of life.

Laboratory tests:

a) Cortisol level was determined by commercial ELISA kit, Diagnostic Systems Laboratories, Inc. USA.

b) Acute phase protein, haptoglobin, serum amyloid A and C-reactive protein level was determined by commercial ELISA kit, Tridelta Company Ltd. Ireland. For determination of both cortisol and acute phase proteins Bio Tek Power Wave analyzer was used.

Results

Cortisol in saliva, haptoglobin and C-reactive protein concentration is shown in Table 1. Cortisol level was significantly higher at the movement restriction group (MR) at first 3 days of the experiment. Haptoglobin level was significantly higher at movement restriction group up to 7th day of the experiment. C-reactive protein was significantly higher at movement restriction group at first 3 days of the experiment. Although amyloid A concentration fluctuated at the level of 53.81-149.13 mg/ml, but no statistically significant changes have been observed.

Conclusions and Discussion

Previous studies have shown that keeping sows in pens that restrict movement cause stress reaction in sows (1,4). Results of presented reseach shows that also piglets can be afected by this type of housing. Increased levels of cortisol, haptoglobine and C-reactive protein, specialy in first days of life sugest that prenatal stress ocured (3). Stress in such an early stage of life does not only questiones welfare of these animals but can also cause health problems.

Table 1. Cortisol, haptoglobin and C-reactive protein level of piglets.

Parameter	Day	MF (n=40)	MR (n=40)
cortisol	3	10.38^{a}	11.37^{a}
	7	2.15	2.29
	21	2.2	2.06
haptoglobin	3	0.51^{A}	0.56^{A}
	7	0.57^{a}	0.61^{a}
	21	0.68	0.7
C-reactive protein	3	14.58^{A}	15.76^{A}
	7	14.13	13.85
	21	13.52	13.04

Superscripts indicate: (A) highly significant differences ($p \leq 0.01$) and (a) statistically significant differences ($p \leq 0.05$) in columns.

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Serum enzyme activity of pigs

J Novotný [1], P Reichel [1], G Kováč [2], R Link[1], H Seidel[1], M Húska[1], V Macák[1], K Kovačocyová[1]
1Clinic for swine, University of Veterinary Medicine and Pharmacy, 041 81 Košice,2 Clinic for ruminants, University of Veterinary Medicine and Pharmacy, 041 81 Košice, Komenského 73, Slovak Republic jaroslav.novotny@uvlf.sk

Introduction

Measuring serum biochemical parameters of farm animals can provide important information on health and metabolism (1) and is a practical diagnostic tool for assessing pathological conditions in the live animal or for monitoring the health status of groups of animals (2). The detection of enzymes in serum by their catalytic activity as a reporter of tissue damage is a cornerstone of medical laboratory analyses (11). The aim of this experimental work was to analyze and compare serum activities of AST, ALT, ALP, LDH, CK, and GGT of six different production categories of pigs.

Materials and Methods

Thirty six clinically healthy pigs (Landrace) from one pig herd located in Eastern Slovakia were divided to six categories based on the different age and production phase. Six pregnant sows 1 week before farrowing; six lactating sows 1 week after farrowing; six weaned sows 1 week after weaning (artificially inseminated); six sucking piglets aged 14-21 days; six weaned pigs 2 weeks after weaning; and six fattening pigs 2 weeks prior to slaughter were selected. The housing and feeding of all production categories was up to standard. Blood was collected from vena cava cranialis (sows and fattening pigs), and sinus ophtalmicus (piglets and weaned pigs). The enzyme activities of ALT, AST, ALP, CK, GGT and LDH were determined by the spectrophotometric method using commercial diagnostic tests (Enzyline®) on the automatic biochemical analyser ALIZE (Lisabio, France). The significance (P) of differences in the means of corresponding variables was evaluated by one way analysis of variance (ANOVA). The significance of differences between the groups using Tukey's Multiple Comparisons Test was evaluated at the same time.

Results, Conclusions and Discussion

Statistical evaluation showed significant differences between examined categories of pigs in serum AST activity ($P < 0.05$; $P < 0.01$; $P < 0.001$), serum ALT activity ($P < 0.05$; $P < 0.01$), serum ALP activity ($P < 0.01$), and serum LDH activity ($P < 0.05$; $P < 0.001$). On the other hand serum CK and GGT activities weren't statistically influenced. These results show that activity of serum enzymes is diverse in different production categories of pigs. Reference values should be established specifically for every category of pigs. It results from different level of metabolism, growth and development of animal, different stages of gestation and many another factors which create differences between categories of pigs. Our study confirm previous conclusions (4) that age is determining for blood reference intervals and those reference values should be determined by each laboratory, taking into account the age of subjects, the sample size and methods of analysis.

Table 1 Mean activities (x ± SD) of enzymes in sows

	pregnant sows	lactating sows	weaned sows
AST	$0.52\pm0.10^{1,4}$	$0.55\pm0.16^{2,B}$	$0.72\pm0.18^{A,a}$
ALT	1.20 ± 0.27	$0.84\pm0.10^{A,a,b}$	1.42 ± 0.39^{A}
ALP	$1.85\pm0.48^{1,5}$	$1.90\pm0.24^{2,6}$	$2.70\pm1.07^{3,7}$
LDH	$9.71\pm1.9^{1,c,3,d}$	$16.57\pm5.3^{a,c,e}$	$13.52\pm1.07^{2,4}$
CK	10.75 ± 3.82	15.28 ± 5.11	13.45 ± 8.06
GGT	0.47 ± 1.17	0.56 ± 0.11	0.63 ± 0.14

Table 2 Mean activities (x ± SD) of enzymes in pigs

	sucking piglets	weaned pigs	fattening pigs
AST	$1.05\pm0.13^{1,2,3,A}$	$0.99\pm0.11^{4,a}$	$0.64\pm0.10^{3,B}$
ALT	1.34 ± 0.11	1.20 ± 0.22^{a}	1.26 ± 0.08^{b}
ALP	$20.72\pm3.4^{5,6,7,8}$	$22.90\pm7.4^{1,2,3,4}$	$6.73\pm1.33^{4,8}$
LDH	$23.1\pm4.7^{1,2,a,b}$	$22.5\pm2.3^{3,e,4,f}$	$16.47\pm1.8^{b,d,f}$
CK	8.06 ± 2.27	9.21 ± 1.45	12.52 ± 4.21
GGT	0.47 ± 0.21	0.68 ± 0.20	0.59 ± 0.15

Explanations: Results with the same superscripts within a row differ significantly at $P < 0.05$ (a; b; c; d; e; f); $P < 0.01$ (A; B); $P < 0.001$ (1; 2; 3; 4; 5; 6; 7; 8)

Acknowledgments

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Experiences with piglet castration under isoflurane anesthesia or injection anesthesia (ketamine, azaperone) in Switzerland

A Enz[1],!G. Schuepbach-Regula[2], E Fuschini[3], E Buergi[1], X Sidler[1]
[1]*Department for Farm Animals, Division of Swine Medicine, Vetsuisse Faculty Zurich,* [2]*Veterinary Public Health Institute (VPHI), Vetsuisse Faculty Bern,* [3]*SUISAG, Swine Health Service, Sempach,* xsidler@vetclinics.uzh.ch

Introduction

Surgical castration of male piglets without anesthesia conflicts with animal welfare, ethics best practice and with the Swiss law. Therefore, research for alternatives is needed. Vaccination against gonadotropin-releasing factor (GnRF) and finishing intact boars are two promising alternatives to surgical castration. Sperm sexing is not ready for implementation in the near future.
!In Switzerland, anesthesia and prolonged analgesia have been required by law for piglet castration since January 2010. Approximately 1.3 million male piglets per year are castrated under general anesthesia either with isoflurane and additional preoperative prolonged pain management with NSAIAs by the farmers themselves or with a combination of ketamine, azaperone and butorphanole or meloxicam by veterinarians. Anesthesia with isoflurane requires the acquisition of an anesthesia machine and special training for the farmers.
The objectives of this study were a description of the practical implementation of the painless castration under isoflurane anesthesia with assessment of animal welfare, workplace safety and expenditure of time (1).

Materials and Methods

100 randomly selected farms using castration under isoflurane anesthesia and 30 farms using injectionable anesthetics were visited. Visits took place at days, when at least 10 piglets were castrated. The farmer was instructed to castrate piglets as usually. Anesthesia quality was assessed by a 4 level graduation. 1: no movement and vocalization at castration (optimal anesthesia); 2: 1-2 movements (unconscious reflexes); 3: several movements, weak vocalization (inadequate anesthesia); 4: strong movement and vocalization (very painful).

Results

Anesthesia with isoflurane

44% of the visited farmers used analgesics during anesthesia or less than 10 minutes before castration. 86% of the piglets were castrated without moving or vocalizing and 18% showed stronger bleeding tendency after castration. The mortality rate was less than 0.1%. According to the farmers, 37% of piglets were awake within less than 2 minutes and 62% within 2-5 minutes after the end of inhalation anesthesia. 22% of the swine farmers reported having headache themselves or experienzing dizziness during or after castration work. The isoflurane level on 2 farms was above the Swiss safety limits (10 ppm), measured by a portable monitor (ChemExpress TM Personal Monitor, Assay Technology, AT Labs, Livermore, CA, USA). The expenditure of time was with 4.3 minutes distinctly above the time necessary without anesthesia.

Anesthesia with ketamine, azaperone

66% of the piglets showed no movements during castration under injection anesthesia and 17% had excitations during recovery from anesthesia. After 48 minutes half of the piglets were in sternal position and after 112 minutes half of them showed coordinated movements. Body temperature decreased by 3.1°C until 60 minutes after castration, especially small piglets reached critical temperature levels. 38% of the piglets showed strong bleeding after castration. Wound healing was inconspicuously according to 82% of the farmers. 83% of the farmers reported piglet losses, especially at the beginning of the anesthesia period.

Conclusions and Discussion

Piglet castration under isoflurane anesthesia or injection anesthesia is well implemented in Switzerland. Most of the visited farmers gave a positive feedback, especially for isoflurane. However, anesthesia exhausts may be inhaled also by the users. Moreover, it was criticized by most producers that the piglets are significantly more stressed while pushing them in a supine position into the anesthesia mask. The anesthesia with ketamine and azaperone showed very long recovery times, which may be improved by using butorphanole. Isoflurane anesthesia as well as the anesthesia with ketamine/azaperone led to a higher bleeding tendency compared to castration without anesthesia.

Acknowledgments

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Characterization of Fluroquinolone resistant *A. pleuropneumoniae* isolates in Korea

S-J Yun, A Kim, M-J Chae, J Kim, S-J Joh, B-Y Jung, B-J So
Animal and Plant Quarantine Agency, Anyang, Republic of Korea, paru33@korea.kr

Introduction

*Actinobacillus pleuropneumoni*ae is the etiologic agent of porcine contagious pleuropneumonia, a lethal respiratory infectious disease (1). The use of antimicrobials is effective measure for the control of pig pleuropneumonia outbreaks. Nevertheless, increasing levels of acquired resistance to antimicrobials have been reported worldwide. In this study, we investigated antimicrobial susceptibilities of *A. pleuropneumoniae* field isolates and mutations in quinolone resistance-determining regions (QRDRs) associated with fluroquinolone resistance in Korea.

Materials and Methods

In total, 124 isolates of *A. pleuropneumoniae* were collected from diseased pigs during 2007-2012 in farms and slaughter houses. The antimicrobial susceptibilities of the isolates were determined by a microdilution method with commercial BOPO6F Sensititre 96-well microtiter plates (Trek Diagnostic Systems, Inc.) using Veterinary Fastidious Medium (VFM; Trek Diagnostic Systems, Inc.). Tested antimicrobials were as follows: ampicillin, ceftiofur, chlortetracycline, clindamycin, danofloxacin, enrofloxacin, florfenicol, gentamicin, neomycin, oxytetracycline, penicillin, spectinomycin, sulfadimethoxine, tiamulin, tilmicosin, tulathromycin and tylosin. *A. pleuropneumoniae* ATCC 27090 were used as quality control. To identify the QRDR mutations in fluroquinolone resistant isolates, the *gyrA, gyrB, parC* and *par E* genes were amplified and sequenced using the previously published primers (2).

Results

All of *A. pleuropneumoniae* isolates were susceptible to ceftiofur. A low level of resistance was observed toward ampicillin (14.5%), enrofloxacin (3.3%), florfenicol(12.5%), penicillin (11.2%) and tilmicosin (9.9%). However, we observed a high level of resistance to chlortetracycline (49.3%), tetracycline (82.9%), clindamycin (48.7%) and danofloxacin (51.3%). Most of resistant isolates of danofloxacin and enrofloxacin were found to carry at mutations in the QRDRs. Overall, two substitutions in *gyrA* (S83F, G81A), four substitions in *parC* (D89K, D84N, S85R, L208F) and two substitutions in *parE* (D479E, L489F) were identified.

Table 1. Mutations in QRDRs of fluroquinolone resistant isolates

Isolates	Serotype	Danofloxacin MIC (μg/ml)	Enrofloxacin MIC (μg/ml)	Mutation			
				gyrA	*gyrB*	*parC*	*parE*
08-1	2	1	0.5	S83F			D479E
08-2	2	1	1	S83F			D479E
08-4	5	>1	1	S83F		E89K	
08-6	2	1	1	S83F			D479E
08-11	5	>1	1	S83F		E89K	
08-33	2	1	1	S83F			L489F
08-49	2	>1	1	G81A, S83F		D84N	
08-50	2	1	1	S83F			D479E
09-5	2	>1	1	S83Y		E89K	D479E
09-8	2	1	1	S83F			D479E
10-13	2	1	1	S83F		S85R L208F	D479E
11-26	1	1	1	S83F		S85R L208F	D479E
11-28	1	1	1	S83F			D479E
11-29	1	1	1	S83F			D479E
08-10	5	>1	2	S83F			D479E
08-20	5	>1	2	S83F			D479E

Conclusions

The aim of this study was to determine the antimicrobial susceptibilities of *A. pleuropneumoniae* isolates and the mechanisms for fluroquinolone resistance in Korea. *A. pleuropneumoniae* isolates were highly resistant to tetracycline, danofloxacin, chlortetracycline and clindamycin. Especially, we reported that the fluoroquinolone resistance of *A. pleuropneumoniae* was mediated by several mutations of the QRDRs.

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Frequency of *A. pleuropneumoniae* serotypes in Brazil

BLP Costa[1]; ATR Costa [2]; EMMS Pereira [2]; R Reis, [2] AM Moreno[1]
Laboratory of Swine Health - Department of Preventive Veterinary Medicine- FMVZ/USP
[2]IPEVE – Instituto de Pesquisas Veterinárias Especializadas - Rua Esmeralda, 786, Prado, CEP 30.410.080, Belo Horizonte - MG, Brasil, bcosta@usp.br

Introduction

Porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae* ("App") is among the most economically significant contagious respiratory diseases causing losses to pig farms worldwide (1). In Brazil, it is a re-emerging disease. Within its two biotypes, at least 15 serotypes have been identified to date (2). The prevalence of serotypes varies according to location, although under permanent change. Porcine pleuropneumonia has been successfully controlled through vaccination. However, identifying those serotypes involved in surges is essential, as vaccine immunogenicity occurs through epitopes of capsular polysaccharides, which differentiate serotypes and avoid cross protection (3). Accordingly, the purpose of this study is to identify the prevalence of *A. pleuropneumoniae* serotypes in cases of porcine pleuropneumonia in Brazil.

Materials and Methods

A total of 234 records of App positive isolations were analyzed at IPEVE laboratory in the period from January 2003 to march 2011, from samples collected in the South, Southeast and Midwest regions of Brazil. These samples have been isolated from lungs with lesions of acute or chronic pleuropneumonia. Swabs were collected from these lungs, then plated on MacConkey medium, aerobically incubated and placed on sheep blood agar at 4% with beta hemolytic *Staphylococcus aureus*. Then, incubated in 10% of CO_2 for 24-72 hrs at 37ºC ± 1ºC. The following biochemical tests were conducted: NAD (Nicotinamide Adenine Dinucleotide) necessity for growing, haemolysis, CAMP test, catalase, oxidase, urease and mannitol as Quinn et al. (1994) (4). After biochemical confirmation, the samples were subject to immunodiffusion in agarosis gel with serum against serotypes 1, 2, 3, 4, 5a, 5b, 6, 7, 8, 9, 10, 11,12, 15 for final identification.

Results

The serotyping results are shown in table 1. In this research, serotype 3 was the most common (24.8%) and serotypes 5, 5a and 5b showed low frequency (8.9%). Serotype 15 was the second most frequent (20.9%), having been identified and tested from 2008 (1).

Conclusions and Discussion

The frequency of isolates of *Actinobacillus pleuropneumoniae* have increased over the years. Based on the results obtained we can conclude that the swine pleuropneumonia remains an important rspiratory disease in Brazil. The diversity of common serotypes throughout the years demonstates the difficulties of control through common vaccines that does not contemplate all the frequent serotypes. The largest number of isolates from the year 2006 can be explained probably due to the increase in viral diseases of livestock as Porcine Circovirus type 2 (PCV2), which affected on the porcine immune response to a pleuropneumonia vaccine. The low recurrence of serotype 5 indicates that the herd has been less affected, considering that it is among the most virulent serotypes (1).

Table 1. App serotype frequency from January 2003 to March 2011.

Sero-type	'03	'04	'05	'06	'07	'08	'09	'10	Jan-Mar 2011	Total	%
1	1	2	1	-	-	-	-	-	-	**4**	**1,7**
2	-	-	1	-	-	-	-	-	-	**1**	**0,4**
3	-	1	9	13	28	7	-	-	-	**58**	**24,8**
4	1	-	-	-	-	-	-	-	-	**1**	**0,4**
5	-	-	-	-	-	5	-	-	-	**5**	**2,1**
5a	-	-	-	-	-	-	-	7	1	**8**	**3,4**
5b	-	-	-	-	-	3	3	2	-	**8**	**3,4**
6	1	1	-	-	-	-	1	-	-	**3**	**1,3**
7	-	-	4	10	-	1	7	4	1	**27**	**11,5**
8	-	-	-	10	6	14	4	-	-	**34**	**14,5**
9	-	-	-	-	-	-	-	-	-	**-**	**-**
10	1	2	2	2	2	-	3	1	-	**13**	**5,6**
11	-	-	-	-	-	-	-	-	-	**-**	**-**
12	-	-	-	-	-	-	-	-	-	**-**	**-**
13	-	-	-	-	-	-	-	-	-	**-**	**-**
14	-	-	-	-	-	-	-	-	-	**-**	**-**
15	-	-	-	-	-	8	19	14	8	**49**	**20,9**
Non-serotype-specific	1	3	4	4	2	4	1	2	2	**23**	**9,8**
Total	**5**	**9**	**21**	**39**	**38**	**42**	**38**	**30**	**12**	**234**	**100**

Acknowledgments

IPEVE, Belo Horizonte, MG, Brazil. College of Veterinary Medicine, Université de Montréal, Quebec, Canada. CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

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Isolation of *A. pleuropneumoniae* from piglets to one at four week old tonsils in comercial farm conditions

M Vargas[1], S Mendoza[2], M Mendoza[2], R Huerta[4], V Quintero,[2]
[1] *Private practice;* [2] *Departament of Biological Science, Facultad de Estudios Superiores Cuautitlan, UNAM,*
[3]*FMVZ Benemérita Universidad Autónoma de Puebla,* patologiavictor@hotmail.com

Introduction

The Porcine Contagious pleuropneumonia is a worldwide disease and is caused by *Actinobacillus pleuropneumoniae*, coccobacillary Gram positive bacterium that produces lung and pleural injury through capsular APx lipoproteins and Apx toxins, causing fibrino-necrotic pleuropneumonia. The pigs were infected by aerosols and direct contact of mothers or carrier pigs. The aim of this study was to perform the isolation of A. pleuropneumoniae from tonsils macerated of pigs with 1 to 4 week-old from a commercial pig farm to determine the prevalence of infection and week-old pigs that are detected early colonized.

Materials and Methods

The study was carried out in a 2000 sow, farrow-to-finish herd positive from infection with App. Four groups with 15 pigs from the 1 at 4 weeks age old was selected from early mortality, necropsied and tonsil was aseptic recovering and was keep frozen at -20°C until planting.

The mash tonsils using a Tenbroeck and PBS solution was performed. The supernatant was centrifuged at 3,000 rpm and was grown on blood agar media with *Staphilococcus aureus* strain and BHI agar enriched with NAD media.

Bacterial growth was purified and identified with primary and secondary biochemical tests.

Results

Isolation of *Actinobacillus pleuropneumoniae* was achieved from the first week of age, with 26% of positive samples, in the case of positivity in the 2nd, 3rd and 4th week was 20%, 33% and 46% respectively. The prevalence of pigs colonized by *A. pleuropneumoniae* within 4 weeks of age was 31.6%. (Fig. 1)

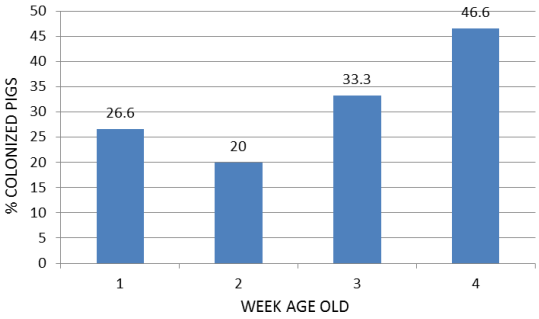

Figure 1. Average of 1 - 4 week-age old *Actinobacillus pleuropneumoniae* colonized pigs

Discussion

It was determined that *A. pleuropneumoniae* is able to colonize the pigs from the first week of age, consistent with Marsteller and Fenwick [1] that indicates this possibility when pigs are infected motherhood. These results suggest that although inconclusive usefulness of early weaning as a possibility of removing *A. pleuropneumoniae* is not possible. According to Muirhead and Alexander [2] not as premature colonization unfolded, so that the possibility of establishing weaning at least 21 days of lactation without risk of transmission of infection. The procedure of tonsillar tissue was macerated a suitable procedure for the isolation of *A. pleuropneumoniae.*

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Genotypic characterization of *B. bronchiseptica* strains from Brazil

MR Felizardo[1], RM Senaga[1], TA Coutinho[1], TSP Ferreira[1], MC Dutra[1], AM Moreno[1]
[1] *Laboratory of Swine Health - Department of Preventive Veterinary Medicine- FMVZ/USP*
São Paulo, SP/ Brazil, morenoam@usp.br

Introduction

Bordetella bronchiseptica is one of the etiological agents of atrophic rhinitis and pneumonia in swine. Although economic losses generated by these respiratory disorders are widely recognized, the impact of this pathogen on herd health is frequently underestimated (1). Knowledge of the epidemiology of *B. bronchiseptica* infection is crucial for the adoption of effective measures for control and prevention of infection in pigs and in this sense, molecular epidemiology studies are of great relevance. The aim of the present study was characterize *B. bronchiseptica* strains isolated in pneumonia and rhinitis cases from Brazilian swine using pulsed field electrophoresis (PFGE).

Materials and Methods

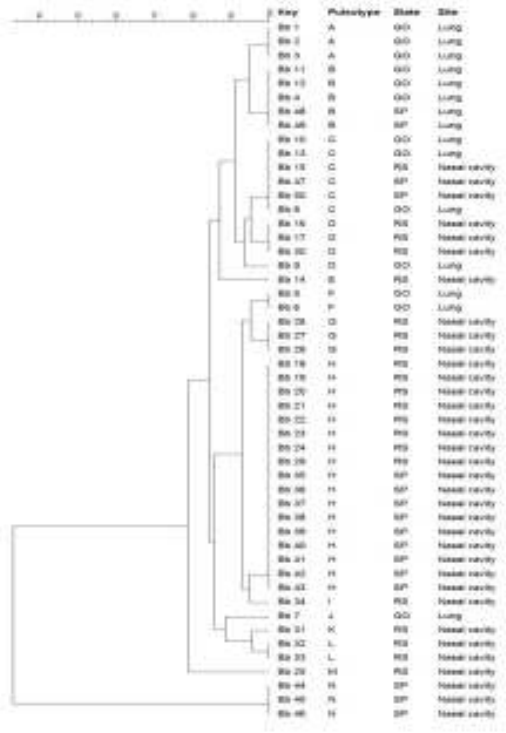
Were evaluated a total of 50 strains of *B. bronchiseptica* from pneumonia and rhinitis cases in 39 Brazilian swine herds. All isolates were previously characterized by biochemical tests and the identification was confirmed using PCR to *fla* gene as described previously (2). For PFGE, the strains were grown in brain heart infusion at 37o C overnight. Then they are submitted to preparation of plugs that after were clivated with the restriction enzymes *Xba*I (3). The DNA fragments were separated by PFGE using a CHEF DR III system (Bio-Rad); for both enzymes the cycle was at 14°C, 6 V cm –1, and pulsed time ramp from 2 to 20 s for 20 h. The gel was stained with of Sybr-safe and documented in the Gel Doc XR equipment.

Results

All 50 strains were characterized by PCR as *B. bronchiseptica* and typed using PFGE. Genotyping strains using PFGE results in 14 pulsotypes identified as A to N and illustrated in Figure 1. The most part of strains associated with pneumonia were clustered in pulsotypes A, B, C and F. The strains present a tendency to be clustered according herd and state of origin. The discriminatory index was 0.85.

Conclusions and Discussion

The *B. bronchiseptica* infections are frequent in brazilian swine herds, besides the large use of vaccines against the agent. In the present study were evaluated *B. bronchiseptica* strains from different herds and states of Brazil, but in some cases the PFGE with *Xba*I enzyme was not able to discriminate the strains from different states of origin as can be observed in pulsotype B, C or H. The results obtained indicates a tendency to cluster the isolates from lung and discriminate strains isolates from nasal cavity. Besides the relatively low discriminatory index (0.85), PFGE shown to be a usefull tool to genotype *B. bronchiseptica* strains.



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Minimum inhibitory concentration patterns of *Brachyspira* species isolated in 2013 from swine herds with history of clinical colitis in Brazil

FA Vannucci, MR Henriques, KCP Reis, LEM Bouillet, WV Guimaraes, DL Santos, LF Santos, JL Santos
Microvet – Microbiologia Veterinaria Especial, Vicosa, MG, Brazil, fvannucci@microvet.com.br

Introduction

Brachyspira species are the etiologic agent of swine dysentery (*B. hyodysenteriae*) and spirochetal colitis (*B.*

growing-finishing pigs. This fastidious bacteria species requires anaerobic atmosphere and specific media for cultivation (1). These features has significantly limited the diagnostic and research in this area, especially in developing countries. The objective of this study was to determine the *in vitro* susceptibility patterns of *B. hyodysenteriae* and *B. pilosicoli* isolated from swine herds against commonly used antimicrobial using broth dilution method.

Materials and Methods

During 2013 five *Brachyspira* strains (four *B. hyodysenteriae* and one *B. pilosicoli*) were isolated from swine herds located in five States of Brazil which represent the most important regions of swine production. Antimicrobial susceptibilities were tested using broth dilution method to commonly used antimicrobials including tiamulin hydrogen fumarate (Tia), valnemulin hydrochloride (Val), doxycycline hyclate (Dox), tylvalosin tartrate (Tylv), lincomycin hydrochloride (Lin) and tylosin tartrate (Tyl) (2).

A 500 ul of BHI/ supplemented with 10% fetal bovine serum containing approximately 10^6 *Brachyspira* organism/ml was added into each well of a 48-well plate coated with a panel of antimicrobials according to the manufacturer (VetMIC™ Brachy panels, SVA Sweden). The plates were incubated in agitation at 37°C for 4 days. An internal growth control was used to confirm the purity of each isolate (3).

The minimum inhibitory concentration (MIC) was identified as the lowest concentration of the tested antimicrobial which was able to inhibit the visible growth.

Results

Based on the breakpoints proposed by Pringle et al (2012), the general patterns of the four *B. hyodysenteriae* isolates showed high MICs for lincomycin and tylosin. An intermediary level of resistance was observed for tylvalosin and low for doxycycline. The MICs patterns for tiamulin and valnemulin vary according to the state and region. Finally, the single *B. pilosicoli* isolate showed high MICs for the majority of the antimicrobials, except for doxycycline. Interestingly, this isolate showed the lower MIC for doxycycline (0.25) compared with all samples tested. The results are summarized in Table 1.

State/Species	Tia	Val	Dox	Tylv	Lin	Tyl
ATCC/Bh	<0.06	<0.03	0.5	2	1	8
MG/Bh	0.25	0.063	1	2	16	>128
SP/Bh	4	1	1	4	32	>128
SC/Bh	>8	>4	1	2	>64	>128
RS/Bh	8	4	2	>32	>64	>128
RS/Bp	>8	>4	0.25	>32	32	>128

ATCC: Type Strain
Bh: *Brachyspira hyodysenteriae*; Bp: *Brachyspira pilosicoli.*
States: Minas Gerais (MG), São Paulo (SP), Santa Catarina (SC) and Rio Grande do Sul (RS).

Table 1. Results of broth dilution MICs in µg/ml.

Conclusions and Discussion

The results showed a consistent pattern of resistance for lincomycin and tylosin. In contrast, all the MICs were low for doxycycline, including the *B. pilosicoli* isolate. Regarding the MICs for tiamulin and valnemulin, the isolates from two States located on the southeast of Brazil (MG and SP) exhibited low to intermediary resistance. However, the isolates from the two States located in the south (SC and RS) of the country were more resistant to these two antimicrobials. Further studies with a higher number of *B. hyodysenteriae* isolate may help to characterize this observation. Additionally, the molecular characterization of these isolates associated with the MICs patterns described in the present study would be useful to understand the epidemiology of the infection according to the spatial distribution of the isolates in Brazil.

Acknowledgments

All the colleagues from the Microvet Laboratory.

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Genotypic characterization of strongly hemolytic *Brachyspira* species isolated from pigs in Brazil

AG de Souza Daniel[1], JP Hiroji Sato[1], CE Pereira Real[1], R de Macedo Couto[1], FA Vannucci[2], C Gebhart[3], RM Carvalho Guedes[1]

[1]*Department of Veterinary Clinic and Surgery, Veterinary School of the Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil;* [2]*Microvet, Veterinary Laboratory, Viçosa, MG, Brazil;* [3]*College of Veterinary Medicine, University of Minnesota, Saint Paul, MN, USA,* guedesufmg@gmail.com

Introduction

Swine Dysentery mainly affects pigs in the final stages of the production system. This disease is caused by *B. hyodysenteriae*, a strongly hemolytic species of *Brachyspira*. The disease is characterized by muco-hemorrhagic diarrhea, sometimes associated with fibrin, anorexia and death in untreated animals (2). Although swine dysentery has been largely absent in swine herds in North America in the last 20 years, many outbreaks have been reported in the United States and Canada recently (1). In addition, a new species of strongly hemolytic *Brachyspira*, *"B. hampsonii"*, has recently been reported to cause similar disease in swine herds in North America. In Brazil there were few isolated reports of swine dysentery before 2010. Since then, an increasing number of outbreaks have been reported in different states. Between August and October 2012, due to delivery of contaminated replacement gilts originated from one specific multiplier to different breeding herds with no quarantine facilities, there were at least 18 new outbreaks. In order to confirm the identity of the isolates and understand the real epidemiological link among these outbreaks the characterization of these *Brachyspira* isolates is necessary. The purpose of this study was to characterize the circulating strains strongly hemolytic *Brachyspira* species in Brazil.

Materials and Methods

Samples: Thirty *Brachyspira* species isolates, obtained from 2011 to 2013, in the states of Rio Grande do Sul, Minas Gerais, Santa Catarina, São Paulo and Mato Grosso from pigs with clinical cases of diarrhea and colitis. Isolation: Samples of feces and bowel were plated onto selective medium for *Brachyspira* species [TSA agar with 5% sheep blood, (6.25 mg/µl) rinfampicina, (800 mg/µl) spectinomycin, (25 mg/µl) vancomycin, (25 mg/µl) colistin (3)] and incubated for at least three days at 42 °C in jars with anaerobic atmosphere. Multiple passages were made to obtain pure colonies by the exhaustion technique on plates with selective medium, which were evaluated under phase contrast microscopy to observe the presence of spirochetes without contaminants.

Nox gene sequence: PCR targeting the Nox gene followed by amplicon sequencing was performed in all isolates in order to determine their species of *Brachyspira*. The protocol used was as described by Chander et al. (1). The PCR products were purified using a commercial kit (Invitrogen PureLink PCR Purification) and sequenced in both directions by an outsourced company (BGI Tech Solutions Co., Ltd.) using the Sanger method and automated sequencer. All obtained sequences were aligned and the data shown by a dendrogram, compared to the GenBank database.

Results

Thirty isolates were sequenced. Fifteen of them were from pig herds involved in the outbreak of 2012. The other 15 isolates were obtained from pig herds with no epidemiological association with the 2012 outbreak. Twenty-nine isolates were confirmed as being *B. hyodysenteriae* with 100% similarity to reference strains ATCC 27164, 3140, AN 2420/97, AN 174/92, AN 383:2/00, AN 1409:2/01, and B78 based on the 596 base pairs of the Nox gene. A single isolate was divergent, and identified as *Brachyspira murdochii* with 100% similarity to the *B. murdochii* strain ATCC X2 and C378 and 100% similarity with *Brachyspira* species Canadian strains F65, C47, F62, B60, B70, B64, G81, F66, D71, C52, F68, B31, F87, C61, C35, B58, F56, G70, A62, K07, A63, B7, F52, A22, A58 , A50.

Conclusion and Discussion

All *B. hyodysenteriae* isolates identified in this study were similar to those previously described in Canada, Spain, Sweden, Hungary and the United States, based on the *nox* gene sequence. The *B. murdochii* isolate identified in this study was similar to those characterized and cataloged in Spain and Sweden. None of the isolates were identified as "*B. hampsonii*".

According to the results, there were no differences among the strains identified in different Brazilian states and herds, based on sequencing of *nox*, before or after the outbreaks of 2012. Thus, the *nox* gene sequence, though able to differentiate isolates at the species level, was not sufficiently discriminatory to differentiate the strains within species; therefore, further studies are needed, using for example the multilocus sequence typing technique, in order to better understand the epidemiology of the disease in Brazil.

Acknowledgment

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Use of 23s rDNA PCR for detection of intestinal spirochaetes (*Brachyspira spp.)* from culture positive feces of pigs in México

E Corona-Barrera[1], A Van Kley[2], C Ángel[1], A Gutiérrez[1], M Valencia[1], R Martínez[1], P Pradal-Roa[3], J Thomson[4]
[1]DICIVA-Universidad de Guanajuato, Gto., México; [2]Biology Department SFASU, Texas, USA; [3]FMVZ-UNAM, Ciudad Universitaria. México, D.F.; [4]VSD-SAC, Edinburgh, Scotland, ecorona@ugto.mx

Introduction

Intestinal spirochaetes (IS) of the genus *Brachyspira* have been described as pathogens for pigs in early reports, and other host such as avian (egg layer hens and wild birds), dogs and humans in more recent reports (3). Pathogenic species of importance in veterinary medicine are *Brachyspira hyodysenteriae*-swine dysentery; *Brachyspira pilosicoli*-porcine intestinal spirochaetosis or porcine colonic spirochaetosis; *B. intermedia*-mild colitis in pigs and as a pathogen in egg layer hens, *B. murdochii*-undefined pathogenicity; *B. alvinipulli*-found in chickens and *B. canis*-found in dogs. The isolation of IS from pigs in México was previously reported (1). The use of PCR in this work aimed to detect *Brachyspira* spp DNA at the genus level from IS isolates from México.

Materials and Methods

A total of 73 (12 faecal samples per farm) pig farms were sampled from Northern, Central and Southern pig production areas of México. The samples were cultured on BSM (*Brachyspira* Selective Medium-Columbia Agar no. 2 supplemented with 8.0 % horse blood and an antibiotic compound containing spectinomycin, vancomycin and colistin). Plates were incubated for 7 days at 42 ºC under anaerobic conditions (*AnaeroGen* Oxoid, UK) in jars. The characteristic growth of *Brachyspira* was confirmed by microscopy on Gram stained smears. Such growth was subcultured onto BSM until a pure culture obtained then subjected to DNA extraction. PCR testing was carried out on 20 DNA samples targeting the 23S rDNA for the genus *Brachyspira* was done using the set of primers SF1 5' CAGCTAAGGTCCCAAAATCTATGT 3' and SR1 5' GAACCCGAAAGCCCAGTCAC 3' which generate a characteristic DNA fragment of 555 bp. Amplicons were displayed on a 1.0 % agarose gel added ethidium bromide and visualised on a transiluminator.

Results

A total of 12 out of 20 samples were PCR positive showing the characteristic 555 bp of the genus *Brachyspira*. All the DNA extracts were obtained from *Brachyspira* cultures, for which 8 out of 20 showed strong β-hemolysis, the rest of the cultures were weakly β-hemolytic.

Discussion

The occurrence of IS infections in the pig and other domestic animals in México is not widely documented as compared to other countries such as the (2, 3, 5). The genotyping of *Brachyspira* isolates it is so important as many *Brachyspira* species have been identified in recent times. The epidemiology of *Brachyspira* is becoming more important nowadays as non-domestic animals such as waterfowl (*Anseriformes*) a type of wild duck have been found to be a reservoir for various *Brachyspira* species which could carry these bacteria to animal farms or to water reservoirs (3).

The 23S PCR rDNA used in this study did not detected 100 % of the DNA samples (extracted from culture positive faeces). Discrepancies between diagnostic tests seem to happen along biological studies as bacteriological isolation or molecular methods do not always coincide. A recent study based on PCR method reported a quite low detection level (12.0 %, 10/81) when applied to dried swabs for detection of *Brachyspira* NADH *nox* gene (4). However, running two diagnostic methods gives a better picture of the particularities of the mircoroganism, this makes things more encouraging to keep working on the matter. More studies are needed in México as to assess occurrence of intestinal spirochaetes in the known hosts such as the pig and the unknown hosts such as migratory avian.

Conclusion

The use of bacteriological culture for isolation followed by PCR for detection of Brachyspira spp., was useful in this study to determine the presence of intestinal spirochaetes in the pig in México.

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Update prevalence of *C. perfringens* isolated from diarrheal piglet in Thailand

W Tanomsridachchai[1], S Urairong[2], W Navasakuljinda[2], P Ngamwongsatit[3,4]
[1]*Department of Microbiology, Faculty of Public Health, Mahidol University,Bangkok 10400 Thailand*
[2]*Zoetis (Thailand) Limited, 323 United Center Building, 46th Floor, Silom Road, Bangkok 10500 Thailand*
[3]*Department of Clinical Sciences and Public Health,* [4]*Veterinary Diagnostic Center, Faculty of Veterinary Science, Mahidol University, Phutthamonthon 4 Road, Salaya, Phutthamonthon, Nakhon Pathom, 73170 Thailand,*
puriya.nga@mahidol.ac.th , supanee.urairong@zoetis.com

Introduction

Swine enteric diseases are impact producers, veterinary practitioners and diagnosticians C*lostridium perfringens* is one of the etiologic agents for enteric disease. It is a Gram-positive spore-forming pathogenic anaerobe, which is widely spread in soil and gastrointestinal tract of animals (1). It can be classified into five toxigenic types based on their ability to produce four major toxins, which are alpha, beta, epsilon, and iota (2). The clinical diarrhea in the neonatal piglets is more straightforward to identify, treat, and prevent than weaning and grow-finish diarrhea. Unfortunately, the data of *Clostridium* spp. infection in piglets are extremely limited in Thailand. Detection of *C. perfringens* toxinotypes is therefore critical to better understand the epidemiology and may be helpful in the development of effective preventive measures for *C. perfringens* infection. Therefore, the aim of this current work was to study the prevalence of *C. perfringens* isolated from diarrheal piglets in Thailand.

Materials and Methods

1. Collection of diarrheal piglets' samples. The rectal swab was collected from each one hundred and sixty two neonatal diarrheal piglets (1-14 days) followed the animal protocol No. MUVS-2012-60. Eight local swine farms located in either Western or Eastern parts of Thailand were targeted to compare the prevalence of *C. perfringens*. Rectal swabs were transferred at room temperature in Cary-Blair transport medium for further culture within 24 h.
2. Isolation and identification of *C. perfringens*. *C. perfringens* were isolated by plating on tryptose-sulfite-cycloserine (TSC) agar, incubated at 37°C under anaerobic condition for 24-48 h. The typical three to six black colonies per sample were selected to identify by conventional identification method. All *C. perfringens* isolates were collected and stored in cooked-meat medium at room temperature.
3. Detection of toxin genes by multiplex PCR All *C. perfringens* isolates were extracted DNA and typed by detection of six toxin genes: *cpa* (α), *cpb* (β), *etx* (ε), *itx* (ι), *cpb2* (β2) and *cpe* (enterotoxin) as previously describe (3).
4. Statistical analysis. Significance of difference among Thailand's regions for the prevalence of *C. perfringens* was tested with Chi-Square by SPSS analysis (17.0).

Results

Of the 162 rectal swabs collected from 8 local swine farms isolated the *C. perfringens*, 80 (49.4%) were found the typical black colonies of *C. perfringens* on TSC agar. All of these isolates exhibited the characteristics of Gram-positive, spore formation, rod shape, non-motility, stormy-fermented milk production, ability to utilize lactose, gelatinase production and nitrate reduction. Moreover, the prevalence among the regions found in 39/80 (47.5%) and 41/80 (51.2%) from Western and Eastern, respectively. These were not significantly different each other (P>0.05). All isolates contained *cpa* gene, but not *cpb*, *etx*, *itx*, nor *cpe* genes. Surprisingly, *cpb2* was detected in all isolates. Therefore all of the isolates in our study belonged to *C. perfringens* type A, which exhibited cpa^{+}, $cpb2^{+}$ genotype.

Discussion

The prevalence of *C. perfringens* in specific regions of Thailand was quite similar but lower than other country. The farming management system could be affecting the high prevalence of *C. perfringens*. This study represents an update prevalence of *C. perfringens* type A carrying *cpb2* gene in neonatal diarrheal piglets in two different regions. Toxin typing is important since particular toxin types are associated with specific enteric diseases and this procedure can be adopted for rapid screening of suspected animal samples.

Acknowledgement

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Pathohistology as a diagnostical tool for confirmation of a sudden death syndrome of sows and frequency of distribution of this disease in Ukraine

L Dudar[1], O Ivaschenko[2], O Beh[2]
[1] *Laboratorios Hipra, S.A., Amer (Girona) Spain,* [2]*Bio-Test Laboratory LLC,*
[3]*Vetfactor LLC, Kiev, Ukraine,* liudmyla.dudar@hipra.com

Introduction

The mortality rate of sows is one of the determinants of financial prosperity of the farm [1, 2]. The sudden death of pigs (no matter whether it is a fattening pig, or a lactating sow) with no apparent clinical signs - is one of the most characteristic clinical manifestations of disease caused by *Clostridium novyi.* The death rate from this pathogen usually reaches 4 % of the sow herd in average.

Clostridium novyi is an anaerobic, spore-forming gram-positive bacterium that produces a number of exotoxins (A, B, C, D) [2]. Leading role in the development of the pathology caused by *Clostridium novyi*, is considered toxin A, which induces the formation of massive tissue necrosis [1]. Besides, this toxin increases the possibility of pathogens penetration through the cell membrane and leads to cell-cell contacts disruption. Spores of potentially pathogenic *Clostridium novyi* form normal micro flora of the large intestine. But, it was proved that external factors such as stress, feeding disorders etc. could provoke manifestation of pathogenic form of *Clostridium novyi*. In this case, determined diagnosis must be done. And it couldn't be based on pathological examination only. So, the aim of our study was to analyze the nature of spreading of cases with most typical lesions due to this pathogen, clinical signs and histopathological changes in tissues of pigs affected by *Clostridium novyi* from Ukrainian farms.

Materials and Methods

The analysis of pathological lesions of selected pigs was done to identify and describe the external signs of disease. Besides, the samples of tissues kidney, liver, spleen, lungs, heart, brain, trachea, lymph nodes were collected during necropsy for further histological examination and analysis for signs, specific for *Clostridium novyi*.

Standardized method of histological sections preparation was used with eosin and hematoxylin solutions and microscopic examination under 100x and 400x magnification using Axioskop 2 plus microscope (*CarlZeiss*). Also, the analysis of tissue imprints for detection of *Clostridia novyi*-like bacteria, using Gram staining method, had been done.

Results

During histological examination of liver tissue samples from pigs with specific clinical signs of sudden death, degenerative changes in hepatocytes and multiple large areas of necrosis of liver parenchyma was detected.

In 80% of recorded cases, colored round cavity between hepatocytes (gas bubbles) was observed.

In 20% of cases (in those samples that were taken directly after death) multiple hemorrhages were visualized between necrotic hepatocytes, but no signs of gas bubbles in the liver tissue. Besides, in 90% of recorded cases, classical histopathological signs of necrotic enteritis were found.

In addition, during fresh imprint samples examination from animals with clinical symptoms, large gram-positive rod-shaped bacteria were found in all cases.

With the aim of distribution and frequency determination of sudden death cases, proved as *Clostridiun noviy*, the data from different farms from 5 geographical regions of Ukraine were analyzed. In conclusion, we determined that in 2 of 5 regions, the rate of such cases was above 4% (3,8-4,1 ±0,05%). Also, in 2 of 5 another regions this number was in range from 2,5-3,5% ±0,05%. And in 1 of 5, it reaches no more than 2%±0,05%.

Conclusions and Discussion

Consequently, high efficiency of histological method as diagnostical tool for *Clostridium novyi*-associative pathology was proved. Besides, it was shown that such type of analysis allows not only detecting lesions, specific to *Clostridium novyi,* but also histopathological changes that could determine provoking causes, which leads to development of *Clostridium novyi* infection (such as immunodeficiency, non balanced feeding, allergy, viral hepatitis, etc). The only weak side of this method is significant dependence on the quality of samples.

The established distribution rate of *Clostridium novyi* cases among Ukrainian farms shows the presence of this problem and necessity of its control and specific prophylaxis.

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Antimicrobial resistance and virulence factors in *E. coli* strains isolated from pig in Italy

S Faccini[1], C Rosignoli[1], A Luppi[2], G Franzini[1], AD Nigrelli[1]
[1]*IZSLER, Diagnostic Department of Mantova, Italy,*
[2]*IZSLER, Diagnostic Department of Reggio Emilia, Italy,* silvia.faccini@izsler.it

Introduction

Escherichia coli is part of the commensal flora of the lower intestine of humans and animals. On the other hand pathogenic strains of this species represent a major cause of morbidity and mortality worldwide. Both antimicrobial resistance and virulence genes can be horizontally transferred among *E.coli* strains and other bacterial species through transmissible genetic elements (2). This study evaluated antimicrobial resistances and pathogenic factors among *E.coli* strains, isolated from pig with enteric disorders, in order to describe possible relationships.

Materials and Methods

A total of 549 *E.coli* strains were isolated from pig specimens submitted to the IZSLER diagnostic laboratory of Mantova between 2010 and 2013. All isolates were tested for antibiotic sensitivity by Kirby–Bauer disk diffusion method, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. A panel of 19 different antimicrobial agents, belonging to 10 different classes, was considered: Spectinomycin (Aminocyclitols), Aminosidine, Apramicyn, Gentamicin, Neomicyn (Aminoglycosides), Cefalexin and Ceftiofur (Cephalosporins), Tilmicosin (Macrolides), Amoxicillin, Amoxicillin+Clavulanic acid (Penicillins), Tiamphenicol and Florfenicol (Phenicols), Colistin (Polipeptides), Flumequine, Danofloxacin, Enrofloxacin and Marbofloxacin (Quinolones), Tetracycline (Tetracyclines) and Trimetoprim+Sulfonamides (Sulfonamides). *E. coli* strains, found resistant to more than 3 or different classes of antibiotics, were considered as multi-drug resistant (MDR). A multiplex PCR was performed for detection of 5 different adhesins (F4, F5, F6, F41, and F18) and 3 enterotoxins (LT, STa, and STb) (1). The Chi-square test was used evaluate the difference between the proportions of resistant isolates in strains with pathogenic factors (PFs) and those without (nPFs). A p-value <0.05 was considered as significant.

Results

All the isolates showed resistance against at least 2 antimicrobial agents. The mean number of resistances was 10.04±3.5, in reference to single drugs, and 6.86±1.6 for antimicrobial classes. The most common resistance phenotypes were detected against Thiamphenicol (94.16%), Tetracycline (93.71%), Amoxicillin (93.14%) and Tilmicosin (88.97%). Genes for PFs were detected in 274 strains (49.9%). The most frequent were: STb (37.2%), LT (27.7%), STa (26.6%), F18 (21.7%), F4 (19.7%). The analysis of proportions of resistant strains demonstrated, for some antimicrobial agents, significant differences between the group with genes for PFs and that without (Table1). MDR isolates were 97%. In particular 33% belonged to the group with resistances against 4-6 classes of antimicrobial agents, 50% had resistances to 7-8 antimicrobial classes and 14% to 9-10 classes (Figure 1).

Table 1. Proportions of resistant isolates, among strains without PFs (R nPF) and those with (R PF).

Antimicrobial	R nPF	R PF	p-value
Aminosidine	117/239	146/245	p <0.05
Apramicyn	105/275	154/274	p <0.01
Enrofloxacin	143/275	115/274	p <0.05
Danofloxacin	100/215	73/205	p <0.05
Flumequine	173/275	136/274	p <0.01
Marbofloxacin	114/268	87/265	p <0.05

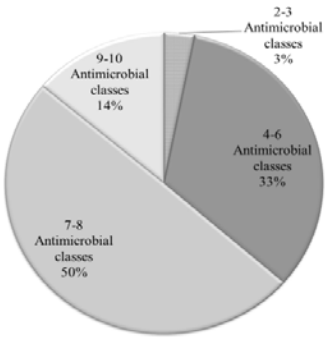

Figure 1. Frequencies of *E.coli* strains belonging to different groups of MDR

Conclusions and Discussion

The study confirms the high prevalence of MDR *E.coli* in swine population. Interestingly resistance against Aminosidine and Apramicyn was more frequent among *E.coli* strains with at least one gene for PFs. On the contrary, in nPFs strains, the proportion of those resistant to Quinolones was significantly higher. No relevant differences were demonstrated for the other antimicrobial agents. The obtained results highlight the importance of deeper investigations on relations between antimicrobial resistance and virulence factors to better understand their mechanism of diffusion.

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Prevalence of F4 hemolytic *E. coli* isolated from pigs with post-weaning diarrhea

A Luppi, P Bonilauri, Y Gherpelli, A Rosamilia, G Biasi, G Maioli, M Dottori
Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), andrea.luppi@izsler.it

Introduction

Post-weaning *Escherichia coli* diarrhea (PWD) is one of the major causes of economic losses for the pig industry, due to either piglet death, or poor weight gain in surviving piglets. PWD is characterized by yellowish or gray diarrhea mainly observed from 5 to 15 days after weaning. PWD is most commonly caused by enterotoxigenic *E. coli* (ETEC), but can also be caused by enteropathogenic *E. coli* that do not posses any virulence factors of classic PWD strains. ETEC strains causing PWD usually have either F4 or F18 as fimbrial adhesins. Because all F4 and F18 *E. coli* causing ETEC PWD are hemolytic, the presence of hemolytic colonies on blood agar are often used as a rapid means for a presumptive diagnosis of these condition (1).
Aim of this study is to asses the prevalence of F4 hemolytic *E. coli* strains isolated from pigs with post-weaning diarrhea from 2002 to 2012.

Materials and Methods

From 2002 to 2012 614 hemolytic *E. coli* were isolated from pathological samples (small intestine) from as many pigs, 28 to 45 days old, with diarrhea. The pigs, sent to the laboratory with a medical history compatible with PWD, were necropsied following standardized methods. The most significant lesions recorded during the necropsies were observed in the gastrointestinal tract. In particular the small intestine was always dilated, hyperemic, with a watery to mucoid yellowish, grayish or slightly reddish content with characteristic smell. During the necropsies samples of small intestine were collected, cultured on blood agar plates and incubated for 24 hours at 37°C. Hemolytic cultures morphologically compatible with *E. coli* were confirmed by Gram staining and standard biochemical methods. Slide agglutination test was used for the identification of F4 positive ETEC.

Results

Following the results of the slide agglutination test 475 strains were classified as F4 positive ETEC (77.3%). The remaining *E. coli* strains (22.3%) were considered F18 ETEC since all strains included in the study were hemolytic. This assumption is supported by the fact that ETEC strains causing PWD usually have either F4 or F18 as fimbrial adhesins and all F4 and F18 *E. coli* causing ETEC PWD are hemolytic (1). On the other hand EPEC strains, ETEC expressing an afimbrial adhesin involved in diffuse adherence (AIDA) and ETEC F5, F6 or F41 (mainly involved in neonatal diarrhea) are not hemolytic. Following the results of slide agglutination the prevalence of F4 *E.coli* strains and the hypothesized prevalence of F18 *E.coli* strains were reported in table 1.

Table 1. Prevalence of F4 and F18 hemolytic *E.coli* isolated from 2002-2012 from pigs with PWD.

Year	% of F4 *E.coli*	% of F18 *E.coli*
2002	89.8	10.2
2003	91.5	8.5
2004	87.5	12.5
2005	86.5	13.5
2006	77.6	22.4
2007	73.5	26.5
2008	78.1	21.9
2009	66.7	33.3
2010	65.9	34.1
2011	69.2	30.8
2012	64.4	35.6

Conclusions and Discussion

The overall prevalence of F4 positive *E.coli* isolated from cases of PWD in the period considered (2002-2012) was 77.3%. However, the proportion of F4 positive *E.coli* showed a progressive decrease over the period considered ($p<0.01$). The variations in prevalence could be influenced by the sampling (passive sampling), because all strains were isolated from pigs with diarrhea sent to the laboratory with diagnostic purpose. In order to confirm the results reported in this study and to asses the actual prevalence of both F4 and F18 ETEC, an active sampling should be performed from cases of post weaning diarrhea. In addition, a genotypic analysis (PCR) of isolates to detect genes encoding for virulence factors such as toxins and adhesins will be done. Future studies, for a better understanding of the epidemiology of the disease, should also include non-hemolytic *E.coli* strains isolated from cases of PWD or from mixed infections (hemolytic and non-hemolytic strains).
Information about the prevalence of ETEC in cases of PWD is of pertinence when measures of control of the disease such as the vaccination must be taken. This is very important considering the need of alternative approaches to the antibiotic therapy, as very high rates of resistance are reported in PWD ETEC (2).

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Characterization of *E. coli* strains associated with urinary infection in sows

MG Spindola[1], VTM Gomes[1], CR Amigo[1], M Moreno[1], KC Silva[1], PHNL Filsner[1], MR Felizardo[1], BLP Costa[1], AM Moreno[1]
[1] *Laboratory of Swine Health - Department of Preventive Veterinary Medicine- FMVZ/USP São Paulo, SP/ Brazil,* morenoam@usp.brr

Introduction

Infections of the most prevalent and important for swine urinary tract are caused by fecal microbiota. In general, among the most commonly found microorganisms in these cases is *Escherichia coli*. The pathotype of *E. coli* commonly related to urinary tract infections is called UPEC. Some factors as hemolysin, aerobactins, fimbriae, capsule, cytotoxic necrotizing factor and serum resistance are associated with this pathotype in humans and animals (1,2). Studies with the international collection of E. coli ECOR strains showed that this species is distributed in four major phylogenetic groups called A , B1 , B2 and D. Groups A and B1 in most studies are identified as part of the intestinal microbiota or causing diarrhea. Strains with extraintestinal features are more frequently belonging to groups B2 and to a lesser extent to group D (2,3). In this study the *E. coli* associated to cystitis in sows were characterized by phylogenetic profile and the presence of virulence genes.

Materials and Methods

A total of 186 strains of *Escherichia coli* strains isolated from sows urine in three swine production systems at São Paulo state were examined. The urine samples showed turbidity, ammonia smell, dark yellow, presence of sediment and nitrite. Isolated strains were evaluated based on the amplification of *Chua, yjaA* and TspE4.C2 genes under the proposed scheme by Clemont et al. (2000) (2) and for the presence of genes *focH*, sfa, afa, hly, cnf and *pap*[1].

Results

Among the 186 strains of *E. coli* evaluated, 3 (1.6 %) were characterized in group A, 65 (34.9 %) in group B1, 55 (29.6 %) in group B2 and 63 (33.9 %) in group D. The *focH* gene was present in 146 strains (78.5 %) and pap gene in 108 (58 %). The distribution of all virulence genes tested are describe in table 1.

Conclusions and Discussion

The characterization of the strains involved in urinary tract infection in pigs by determining the phylogenetic group showed that 118 strains (63.5 %), have the potential to survive in extra- intestinal environments, being gathered in groups B2 and D, and more 50 % have genes encoding adhesion factors which are usually related to this kind of infection in humans and other animal species.

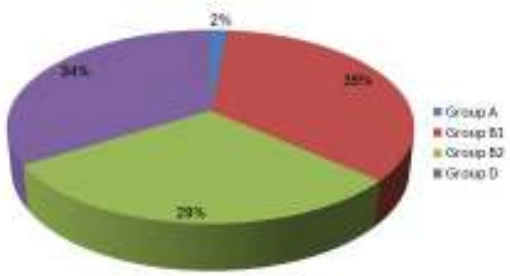

Figure 1. Distribution of strains of *E. coli* isolated from urine of sows according to the phylogenetic group.

Table 1. Occurrence of genes encoding virulence factors in 186 *E. coli* strains.

Phylogenetic group			Virulence genes						
	N	%	focH	pap	sfa	afa	hly	aero	cnf
A	3	1,6	0	1	0	0	0	0	0
B1	65	34,9	46	30	5	3	2	2	8
B2	55	29,6	45	35	8	11	1	5	19
D	63	33,9	55	42	8	11	0	6	16
Total	186	100	146	108	21	25	3	13	43

Acknowledgments

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Phylogenetic classification of strains of *E. coli* associated with edema disease in swine

VTM Gomes[1], M Moreno[1], KC Silva[1], APS Silva[1], MR Felizardo[1], BLP Costa[1], AM Moreno[1]
[1] *Laboratory of Swine Health - Department of Preventive Veterinary Medicine- FMVZ/USP*
São Paulo, SP/ Brazil, morenoam@usp.br

Introduction

Escherichia coli is an important enteric pathogen of intestinal and extra-intestinal diseases in animals and humans worldwide. Edema disease is a systemic disease of weaned piglets caused by host-adapted strains of *Escherichia coli* that produce a variant of Shiga toxin 2 (Stx2e)(1). The disease occurs shortly after weaning and is characterized by swollen eyelids, ataxia, recumbence, convulsions, paralysis, or sudden death. Edema disease has been described in different states of Brazil for many years and has caused economic losses to national swine(2). The objective of the current study was to characterize the strains of *E. coli* positive for Stx2e as phylogenetic classification according to Clermont et al. (2000)(3).

Materials and Methods

In the present study were evaluated 158 *E. coli* strains Stx2e toxin gene positive isolated from 62 animals, from 13 swine herds located at Rio Grande do Sul, Santa Catarina, Mato Grosso, Paraná, São Paulo, Goias and Minas Gerais States. Strains were submitted to phylogenetic group characterization. The phylogenetic group of isolates of *E. coli* stx2e toxin producing *E. coli* was determined based on the amplification of Chua, yjaA and TspE4.C2 genes according to the proposed scheme for Clemont et al. (2000) (3).

Results

Escherichia coli strains can be classified in to four major phylogenetic groups: A, B1, B2 and D. The phylogroups A and B1 are characterized by gut colonizers or diarrhea cause. In contrast, the groups B2 and D frequently cause extra-intestinal infections, with high virulence profile. The characterization of phylogenetic groups permitted the distribution of strains in four groups described as follow: group A 27.2% (43/158), group B1 3.8% (6/158), group B2 39.2% (62/158) and group D 29.8% (47/158).

Conclusions and Discussion

Studies with the international collection of *E. coli* ECOR strains showed that this isolates is distributed in four major phylogenetic groups called A, B1, B2 and D. Groups A and B1 are formed in most studies by strains belonging to intestinal microbiota or causing diarrhea. Strains with extraintestinal features are more frequently belonging to groups B2 and to a lesser extent to group D. The characterization of 158 strains of porcine origin

isolated from fecal swabs, showed that 69% of stx2e producing *E. coli* belonged to B2 and D phylogenetic groups, which is commonly associated with extra-intestinal infection. This data indicates the potential of these isolates to cause extra-intestinal infections in human population(4).

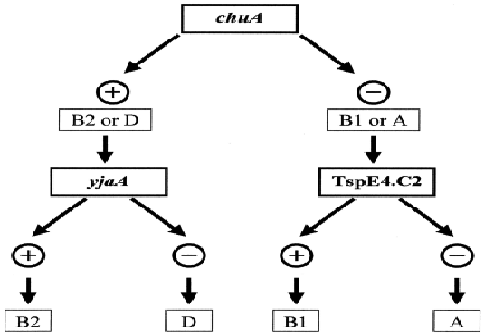

Figure 1. Decision tree to determine the phylogenetic group of an *E. coli* strain by using the results of PCR amplification described by Clermont et al, 2000[3].

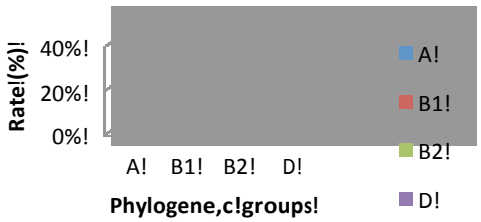

Figure 2. The distribution (%) of phylogenetic groups (A, B1, B2 and D) strains of *Escherichia coli* stx2e +

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Characterization of resistance profile of *H. parasuis* strains from swine in Brazil

GFR Silva[1], TSP Ferreira[1], LZ Moreno[1], BLP Costa[1], CEC Matajira[1], PHNL Filsner[1], VTM Gomes[1], AA Sanches[1], AM.Moreno[1]
[1] *Laboratory of Swine Health - Department of Preventive Veterinary Medicine- FMVZ/USP São Paulo, SP/ Brazil,* morenoam@usp.br

Introduction

Haemophilus parasuis is an important porcine pathogen in Brazil and the etiological agent of Glässer's disease (1). This disease is characterized by fibrinous polyserositis, polyarthritis and meningitis. However, in the acute form it might only be associated with pneumonia and septicemia without polyserositis. Nowadays Glässer's disease has been described as an emergence disease wish causes important economic losses for veterinary industry with mortality, low growth and use of antimicrobials. Although vaccine has been utilized for control, high serovar diversity implies in use of antimicrobial for outbreaks controls (1). Unfortunately, the extended use of antibiotic for growth promotion and treatments has encouraged an increase antimicrobial resistance (2). The aim of this study is understand the resistance profile of *H. parasuis* isolates from Brazilians swine.

Materials and Methods

In order to investigate the antimicrobial susceptibility of *H. parasuis* isolates, a total of 46 strains isolated from swine with pneumonia and polyserositis were evaluated. Samples were plated on sheep blood agar at 5%, supplemented with NAD and then incubated in a jar containing 5% to 10% CO_2 for 24-48 hrs at 37°C ± 1°C. Initial identification was made on the basis of colony morphology. The DNA was extracted from the isolated strains and amplified by Polymerase Chain Reaction (PCR) using specific primers for *H. parasuis* (3). Minimal inhibitory concentrations (MICs) of drugs were determined using BOPO6F MIC Plate - Sensititre ® against the following antimicrobial agents: ampicillin, clindamycin, chlortetracycline, danofloxacin, enrofloxacin, florfenicol, gentamicin, neomycin, oxytetracycline, penicillin, sulfadimethoxine, spectinomycin, trimethoprim/ sulfamethoxazole, tiamulin, tilmicosin, tulathromycin, tylosin, ceftiofur.

Results

It was observed that the most effective antimicrobial agent against *H. parasuis*, or with lower levels of resistance were chlortetracycline (0%), followed by ceftiofur (2.1%), tiamulin (2.1%), gentamycin (2.1%), florfenicol (2.1%), oxytetracycline (4.3%), neomycin (4.3%), ampicillin (6.5%), spectinomycin (6.5%) and tulathromycin (13%). Antimicrobials that showed higher rates of resistance were tylosin and sulfamethoxazole (100% and 98% respectively), followed by danofloxacin (71.7%), trimethoprim/ sulfamethoxazole (60.8%), clindamycin (48%), enrofloxacin (41.3%), penicillin (32.6%) and tilmicosin (21.7%).

Conclusions and Discussion

The data will be of great impact for the control of Glasser disease in pigs in Brazil, with great application in the herds and will contribute to the rational use of antimicrobials in swine production. The information generated can assist also in development of specific MIC breakpoints for these bacterial specie. The strains that were resistant will generate new studies related to the identification of resistance mechanisms involved and the associated genes.

Table 1. *In vitro* sucetibility of *H. parasuis* isolates from swine

Antimicrobial	MIC (µg/mL)			Resistant
	MIC 50	MIC 90	Range	% (n=46)
Ceftiofur	≤0.25	1	≤0.25->8	2.1
Tiamulin	8	16	1 - >32	2.1
Chlortetracycline	≤0.5	1	≤0.5 - 1	0
Oxytetracycline	1	2	≤0.5 - >8	4.3
Penicillin	0.25	2	≤0.12 - >8	32.6
Ampicillin	≤0.25	1	≤0.25 - >16	6.5
Danofloxacin	0.5	>1	≤0.12 - >1	71.7
Trimethoprim/ sulfamethoxazole	>2/38	>2/38	≤2/38->2/38	60.8
Tylosin	16	>32	8->32	100
Tulathromycin	2	64	≤1 - >64	13
Clindamycin	2	>16	0.5 ->16	48
Sulfadimethoxine	>256	>256	≤256 ->2561	98
Gentamicina	≤1	2	≤1 - >16	2.1
Florfenicol	0.5	1	≤0.25 -8	2.1
Neomycin	≤4	8	≤4 - >32	4.3
Spectinomycin	≤8	≤8	≤8->64	6.5
Tilmicosin	≤4	64	≤4->64	30.4
Enrofloxacin	0.25	>2	≤0.12->2	41.3

Acknowledgments

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Typing *M. hyopneumoniae* bacterins by multiple-locus variable-number of tandem-repeat analysis (MLVA)

P Tamiozzo[1,2], R Zamora[1], A Estanguet[1], J Parada[1,2], P Camacho[1], A Carranza[1], JJ Busso[1]
[1]*Deparment of Animal Pathology of the Faculty of Agronomy and Veterinary Sciences, UNRC, Río Cuarto, Córdoba, República Argentina.* [2]*CONICET, República Argentina*. topo.vet@gmail.com

Introduction

Control of *M. hyopneumoniae* (*Mh*) infections can be accomplished in several ways, mainly by optimization of management practices and the use of antimicrobials and vaccines (1). Although previous studies have tested pig immunization using homologous and heterologous or high and low virulence *Mh* strains (2, 3) less is known about their virulence and genetic diversity of the *Mh* strains used to manufacture the bacterins and their relationship with *Mh* field strains. It had been pointed out (3) that most of commercial vaccines are based on J strain, however we have found other *Mh* genetic subtypes from commercial vaccines that can be purchased over the counter in our country in a preliminary *Mh* bacterins genetic characterization (4). The objective of this work was to typing *Mh* from bacterins by MLVA.

Materials and Methods

Six *Mh* bacterins were analyzed. According to the manufacturers and/or web search the *Mh* strains used to make these bacterins are as following: Bacterins A and D: Strain J; Bacterin B: *S*train 11 and bacterins C, E and F: unknown *Mh* strains. Prior to MLVA, *Mh* bacterins were tested by a specie-specific nested PCR (5). All the samples rendered positive results. MLVA scheme from the regions: *p146R1*, *p95*, *H4*, *H5* were performed according to de Castro et al. (7). The region *p146R3* was analyzed by the nPCR developed by Tamiozzo et al. (6) in order to identify a polyserine repeat motif. In order to compare the allelic profiles from the *Mh* bacterins with *Mh* field strains, samples from 28 bronchoalveolar lavages (BAL) from 22 weeks-old pigs at slaughter from three herds were randomly submitted under analysis.

Results

Table 1 shows the allelic profiles obtained. In locus *p146R1*, allele 1 was shared with the field samples as in locus *p95* (data not shown). In locus *p146R3* alleles 1, 2 and 3 showed 17, 18 and 21 serines respectively. Only the allele 1 was found in field samples from one herd (data not shown). Regarding to locus *H4* only bacterin B showed 3 alleles, none of them shared with field samples. In locus *H5* allele 2 was shared in field samples from 3 herd, in which there was additional alleles. Half of the analyzed loci show null alleles.

Table 1. Allelic profiles of the six analyzed *M. hyopneumoniae* bacterins according to the loci studied.

Loci	BACTERINS					
	A	B	C	D	E	F
p146R1	1	2,3,4	1	-	-	-
p146R3	2	3	3	2	1	3
P95	-	1	-	-	1	1
H4	-	3,4,5	-	-	-	-
H5	-	2	-	-	-	2

Conclusions and Discussion

Mh genetic subtypes were identified among bacterins, surely due to the fact of bacterins are manufactured with different strains. In all loci (except *H4*), at least one allele was shared with *Mh* field samples. The high percentage of negative PCR reactions would be due to variability in the primer binding sites or different sensitivities of different PCRs utilized since all the samples had been positive to the screening PCR (5). This is reinforced by the fact that only on the nested PCR used for *p146R3* locus all the samples were positives. According to manufacturer´s information bacterins A and D are based on *Mh* strain J. This was confirmed since *p146R3* region showed an 18 serine repeat motif, agreeing with previous report (7, 8). Unfortunately, the other VNTRs could not be analyzed due to the presence of null alleles. The fact that *Mh* strains used to manufacture bacterins were different to field strains need to be deeper studied.

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Dynamic of *M. hyopneumoniae* infection by clinimetry, PCR and ELISA

P Camacho[1], P Tamiozzo[1,2], J Parada[1,2], A Carranza[1], JJ Busso[1], G Di Cola[1], R Ambrogi[1], A Ambrogi[1].
[1]*Deparment of Animal Pathology of the Faculty of Agronomy and Veterinary Sciences, UNRC, Río Cuarto, Córdoba, República Argentina.* [2]*CONICET, República Argentina,*. pablocamacho1@hotmail.com

Introduction

Mycoplasma hyopneumoniae (Mh) is the causative agent of swine enzootic pneumonia (SEP), which main clinical sign is non-productive coughing (1). However, for accurate diagnosis, complementary laboratory techniques are necessary to Mh detection. The clinimetry (coughing index) has been previously associated to Mh detection by PCR and ELISA in fattening pigs (2). The knowledge of the dynamics is a prerequisite for providing efficient treatment and controlling. The aim of this study was to implement a diagnostic methodology to determine the Mh and SEP dynamics in a herd.

Materials and Methods

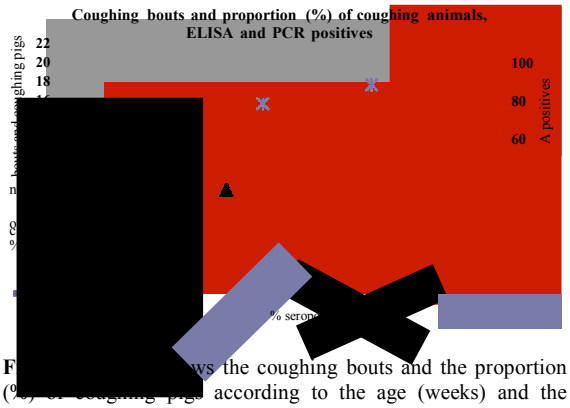
The study was conducted in a 1300-sows, two site herd without any vaccination schedule against Mh. Clinimetric parameters measured were: A) Proportion of pigs with cough, according to Laohasinnarong et al (3) and B) The coughing index, according to Nathues et al (2). These indicators were measured on pigs aged 4, 5, 7, 8, 9, 10, 11, 12, 13, 15, 18 and 22 weeks. To detect Mh by PCR (4), nasal swab samples of 15 pigs at 4, 5, 7, 8, 9, 15 and 22 weeks old were taken. For detection of antibodies against Mh by ELISA (Idexx), blood samples were taken from 15 pigs aged 4, 5, 7, 10, 13, 15, 18 and 22 weeks.!

Results

The dynamic of SEP and Mh was assessed by Clinimetry, PCR and serology (ELISA) is shown in the Figure 1. The coughing index exceeded the 2.5% from 8 to 12 weeks old sampling, reaching peaks at 9 and 11 weeks old. Regarding the percentage of animals with cough, the maximum values (20%, 16% and 15%) were observed at 9, 12 and 22 weeks old, respectively. The seroconversion is observed starting at 7 weeks old. PCR allows to Mh detection early at 4 weeks old until 15 weeks sampling.

Conclusions and Discussion

The coughing index values (greater than 2.5%) coupled with the high proportion of pig with cough observed suggest that in this particular farm the main clinical impact of SEP was taking place in pigs from 8 to 12 weeks old, whereas coughing index remained below the threshold after this period. The use of clinical index combined are consistent and allows a better survillance of the diseases on populations (5).



Fws the coughing bouts and the proportion (%) of coughing pigs according to the age (weeks) and the proportion (%) of PCR and ELISA positives.

Based on PCR findings, Mh was found early as 4 week old precisely a week before the beginning of the coughing, which has been previously reported (1), until 15 weeks old, agreeing with clinimetry. The fact that the seroconversion started at 7 weeks of age is consistent with the highest coughing index values and the rise of coughing pigs proportion and PCR positive percentages. Seroconversion and high proportion of ELISA positives allow Mh indirect detection more delayed than PCR and clinical sign observation, however the ELISA results in latest samplings (100% in 18-22 weeks old pigs), toghether with PCR results suggest that non-productive cough is due to Mh presence in this herd. All this is important taking into account that it has been suggested that when coughing index is ≥2.5% and seroprevalence is up to 50%, SEP is highly likely (2). In conclusion, the combination of clinimetry and PCR allowed an accurate knowing of the dynamic of SEP and Mh in a herd, essential for a successful disease prevention and control.

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Influence of particulate matter (PM10) and NH_3 on production parameters and lung lesions of fattening pigs

A Michiels[1], S Piepers[1], R Del Pozo Sacristán[1], T Ulens[2], N Van Ransbeeck[2], A Sierens[1], F Haesebrouck[1], P Demeyer[2], D Maes[1]

[1]*Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium*

[2]*Institute for Agricultural and Fisheries Research (ILVO), 9820 Merelbeke, Belgium,* annelies.michiels@ugent.be

Introduction

Continuous development of livestock production systems have increased herd size and stocking density, which has caused a rise in concentration of aerial pollutants (1). Particulate matter (PM) and NH_3 are most hazardous for animal health of all indoor aerial pollutants (2). PM10 is defined as particulate matter which passes through a size-selective inlet with a 50% efficiency cut-off at 10 µm diameter (3). To date, no clear agreement is achieved on the impact of PM10 and NH_3 on the production parameters of fattening pigs and specific respiratory diseases, like enzootic pneumonia (EP) caused by *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*). Moreover the impact of particulate matter on performance and respiratory disease is mostly assessed under experimental conditions. The present study investigated the influence of PM10 and NH_3 on EP and performance of fattening pigs in field conditions.

Materials and Methods

A commercial pig herd with clinical problems of *M. hyopneumoniae* infections in grow-finishers (dry non-productive cough) was selected. In total, 1095 fattening pigs of 2 consecutive fattening rounds allocated over 8 compartments (2 replicates) were included. PM10 and NH_3 concentration were measured in all 8 compartments during the entire fattening period (FP) from 11 until 29 and from 19 until 29 weeks of age (½FP). Mortality was recorded during the entire FP and all pigs were weighed at 11 and 28 weeks of age to calculate Average daily gain (ADG). Nasal swabs of 10 pigs per compartment in each replicate were collected one week prior to slaughter to detect *M. hyopneumoniae* with nested PCR (nPCR). The prevalence of *Mycoplasma*-like pneumonia lesions and pleurisy were recorded at slaughter (29 weeks).
Continuous (ADG) and binary outcome variables (yes/no) were analysed using linear regression or logistic regression, respectively. First univariable analyses (UM) were performed. In case of more than one significant variable ($P<0.05$), multivariable models (MM) were used.

Results

The overall values of the different parameters were: ADG 695 (±116)g, mortality rate 3.84%, prevalence of pneumonia and pleurisy 63 and 64%, respectively, and nPCR-positive 28%. First the UM were performed. Significant UM can be seen in table 1. In case of both explanatory variables (PM10 and NH_3) the MM was built (table 2).

Table 1. Univariable models

Outcome variable	Explanatory variable	β1	OR
Prevalence Pleurisy	**avPM10 FP**	3.04	20.9*
Prevalence Pleurisy	**avNH_3 FP**	3.07	21.5*
nPCR	**avPM10 FP**	5.8	328*
nPCR	**avNH_3 FP**	4.3	70*
nPCR	**avPM10 1/2FP**	5.2	185*
nPCR	**avNH_3 1/2FP**	9.0	8275*

Table 2. Multivariable models

Outcome variable	Explanatory variable	β1	OR
Prevalence Pleurisy	**avPM10 FP**	2.18	8.8*
	avNH_3 FP	1.56	4.8
nPCR	**avPM10 FP**	5.0	149
	avNH_3 FP	1.44	4.2
nPCR	**avPM10 1/2FP**	3.18	24
	avNH_3 1/2FP	6.52	681

FP: average value of PM10 or NH_3 calculated over the entire fattening period: 11-29 weeks of age, ½ FP: average value of PM10 or NH_3 calculated over the second half of the fattening period: 19-29 weeks, β_1: regression coefficient of the logistic regression equitation, OR: odds ratio = exp (β_1) per unit increase in the explanatory variable, OR with an asterisk (*) are significant ($P<0.05$)

Conclusions and Discussion

PM10 and NH_3 did not affect performance, extent and prevalence of pneumonia. PM10 and NH_3 only influenced nPCR results numerically. However, PM10 was positively associated with pleurisy lesions. Further research is necessary to assess the influence of PM10 and NH_3 on other important respiratory pathogens in swine.

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Association between diversity of *M. hyopneumoniae* strains in pig herds and lung lesions at slaughter

A Michiels[1], K Vranckx[2], R Del Pozo Sacristán[1], F Haesebrouck[1], D Maes[1]
[1]*Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium*
[2]*Applied Maths, Sint-Martens-Latem, Belgium,* annelies.michiels@ugent.be

Introduction

M. hyopneumoniae(M.h) is the causative agent of enzootic pneumonia. Previous studies have shown that major differences in virulence between field strains of M.h exist and that different strains may be present within a herd and even within one pig (1,2,3). However the clinical importance of infections with different M.h-strains in a herd is not known. The aim of the study was to investigate whether the presence of more M.h. strains was associated with more severe lung lesions.

Materials and Methods

Ten pig herds infected with M. h and that practiced vaccination of the piglets against M. h were selected. Lung lesions of 1 batch of slaughter pigs of these herds were scored according to Morrison et al. (1985) (4). In total 1190 lungs (70, 127, 67, 152, 152, 156, 141, 158, 90, 77 in respectively herd A, B, C, D, E, F, G, H, I, J) were scored. From 20 pigs of each herd, the left part of the lung was collected and flushed with 20 ml phosphate buffered saline (PBS). Next, DNA extraction was performed and M.h -DNA was detected with a nested polymerase chain reaction (nPCR) (5). All positive samples were submitted to a Multiple-Locus Variable number tandem repeat Analysis (MLVA) (2). The serum of 20 blood samples per herd was used to detect antibodies against M.h.

The continuous outcome variable was extent of pneumonia and binary (yes/no) variables were prevalence of pneumonia, fissures, pleurisy and ELISA M.h-results. The farms were divided into 3 categories according to the results of the MLVA (explanatory variable): 1 (1 strain), 2 (2 until 6 strains) and 3 (7 strains and more in the farm). The extent of pneumonia was not normally distributed, therefore non-parametric Kruskal-Wallis tests were used for pair-wise comparisons. Logistic regression was performed on all binary data. Hosmer and Lemeshow test was performed to ascertain the goodness of fit of the logistic regression model.

Results

Of 200 samples tested with nPCR, 149 samples tested positive and were submitted to the MLVA. Descriptive values of the outcome variables can be consulted in table 1. A statistical difference could be seen between category 2 and 3 ($P<0.05$) for the extent of pneumonia. Per unit increase in category of strains present in the farm the odds of finding pneumonia, pleurisy, and ELISA positive animals increased with respectively OR 1.43 ($P<0.05$), OR 2.70, ($P<0.001$), and OR 6.83 ($P<0.001$). The odds of finding fissures per unit increase in category of strains present in the farm was not statistically significant: OR 1.02, ($P>0.05$). The results can be consulted in table 1. Hosmer and Lemeshow test of each model showed that the data fitted the logistic regression model well.

Table 1. Descriptive values of the parameters under study. Continuous variable extent of pneumonia and binary (yes/no) variables pneumonia, fissures, pleurisy prevalence and ELISA-results.

Outcome variable %	Explanatory variable: category			
	1 (1)	2 (2-6)	3 (≥7)	OR
Extent pneumonia	1.14±2.85^{ab}	2.85±8.95^{a}	3.88±9.37^{b}	n.a.
Prevalence pneumonia	16.9	20.2	27.3	1.43*
Prevalence fissures	25.3	35.6	32.4	1.02
Prevalence pleurisy	2.6	13.4	27.6	2.70*
	n=20	n=80	n=100	
ELISA	5	55	86	6.83*

Categories are given by a number 1, 2, 3; OR: odds ratio; n= number of lungs scored in the slaughter house per category/numbers of blood samples per category; n.a.: odds ratio is not applicable for the continuous value extent of pneumonia; values with different superscripts in the row of extent pneumonia are significantly different ($P<0.05$); OR's with an asterisk(*) are statistically significant ($P<0.05$).

Conclusions and Discussion

The number of strains of M.h in the farm was positively associated with the prevalence of pneumonia and pleurisy but was not able to provide an impression of healed infections with M.h (fissures) in the farm. The number of strains in the farm was positively related to the amount of positive animals for M.h- ELISA as well. These results are preliminary and will be extended by further research through investigation of more samples per category and per farm.

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M. hyopneumoniae serological dynamics in vaccinated and unvaccinated pigs from naturally infected intensive swine farms in Argentina

M Biscia[1]; A Perez[2]; L Anthony[1]; M Spadaro[1]; J Sarradell[1,2]
[1]*General and Special Pathology, Veterinary Faculty, National University of Rosario, CP2170, Ov. Lagos y Ruta 33, Casilda, Santa Fe, Argentina;* [2]*College of Veterinary Medicine, University of Minnesota*
jsarrade@fveter.unr.edu.ar

Introduction

Mycoplasma hyopneumoniae (*Mh*) is the causative agent of Swine Enzootic Pneumonia (SEP). SEP, which is typically endemic in swine farms, inflicts severe economic losses to the pig industry. Vaccination is widely used to mitigate the impact of SEP.[2] The objective of the study here was to assess the dynamics of serological response to *Mh* in vaccinated and unvaccinated pigs at *Mh*-naturally infected swine farms in Argentina.

Materials and Methods

The study was conducted in two intensive farrow-to-finish swine farms in Argentina. In one of the farms, referred to as farm "A" (N=230 sows), 2 ml of an inactivated vaccine (SPRINTVAC® MH, Merial Sanofi Company) was applied to pre-service and pre-farrowing (85 days gestation) sows and 28 days old piglets. In the other farm, referred to as farm "B" (N=700 sows), pre-service and pre-farrowing (85 days gestation) sows and 20 and 40 days old piglets were inoculated with 1 ml of a commercial bacterin (M+PAC®, MSD Animal Health). Both vaccines were administered intramuscularly. Ten animals of each age category were kept unvaccinated in each farm. Blood samples were collected by cranial cava vena puncture from 10 (farm A) and 5 (farm B) vaccinated pigs and from the 10 unvaccinated pigs (per farm) at 20, 40, 60, 80, 120, and 160 days of age. Sera were obtained by blood centrifugation and stored at -20°C until processing. Samples were tested using a commercial monoclonal blocking ELISA (Idexx. *Mycoplasma hyopneumoniae* Test Kit, Idexx Laboratories, Inc., Hoofddorp, The Netherlands). The proportion of positive samples (which, for simplicity, was referred to as prevalence) was computed for each age category and group.

Results

Prevalence was high (0.8-1) in 20 days old pigs, which is likely a reflection of maternal immunity. Serological titers were thereafter consistently high for vaccinated pigs, whereas the proportion of positive pigs decreased at 40-80 days and increased again from the 80 days of age in unvaccinated pigs (Figure 1).

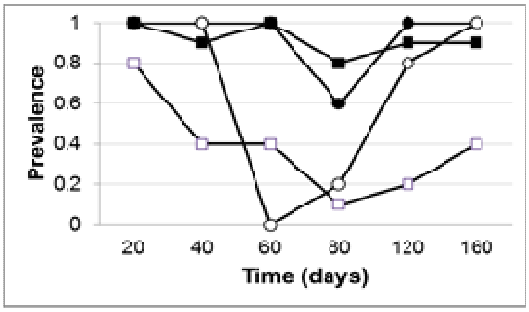

Figure 1: Proportion of *Mycoplasma hyopneumoniae*-positive (prevalence) sera of vaccinated (black marks) and unvaccinated (white marks) pigs in farms A (circles) and B (squares), in Argentina

Conclusions and Discussion

Because commercially available vaccines are produced with *Mh*-inactivated cultures, it is not possible to differentiate between antibodies produced by natural infection from those due to vaccination. Such feature impairs the ability to interpret serological results in vaccinated populations. Additionally, *Mh* vaccination does not protect against infection, but results on an homogeneous immunological sensitization of pigs, which may explain, at least in part, the consistent positive titers obtained in vaccinated animals, extending the antibody levels attributable to maternal protection.[1,2] Alternatively, assessing the serological titers in unvaccinated animals allows for identification of the times and ages at which serological conversion occurs. For those reasons, serological assessment of cohorts of *Mh*-vaccinated and unvaccinated pigs provides information that is useful in the interpretation of the dynamics of *Mh* infection and protection in a farm.

Acknowledgments

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Prevalence of *M. suis* in indoors and outdoors pig farms in Buenos Aires Province by PCR

M Pintos[1], E Bonzo[2], D Posik[3,4], N Fauret[1], C Scodellaro[1], C Perfumo[5], M Arauz[1]
[1] *Central Laboratory of Faculty Veterinary Hospital,* [2] *Epidemiology and Public Health Department,* [3]*Institute Veterinary Genetics (IGEVET), CCT La Plata-CONICET,* [4] *Research Commission of the Buenos Aires Province (CICBA).* [5] *Institute of Pathology, Faculty of Veterinary Sciences, La Plata National University, Argentina,* eugeniapintos@fcv.unlp.edu.ar

Introduction

Mycoplasma suis is a not cultivable epicellular microorganism which parasitize erythrocytes. Both clinical and subclinical *M. suis* infection has a broad distribution in Argentina (4). In preweaning and nursery piglets clinical signs course with jaundice, acute anemia and death. In other categories such growing, fattening pigs and sows reduced feed conversion and reproductive failure has been reported (1,3,4). The presumptive diagnosis is based on signs such anemia, jaundice and reluctance to move and confirmatory diagnosis by PCR procedure (2,3,5). The aim of this study was to determine the prevalence of *M. suis* infection in subclinical pigs from indoors and outdoors herds of Buenos Aires province by means of PCR technique.

Materials and Methods

A cross-sectional study was conducted in twelve herds. Farms and pigs selection criteria in each farm were based on accessibility and advisability. The study comprised seven indoors (IH) and five outdoors (OH) herds. Blood samples from apparently healthy animals were obtained from sows (61), preweaning (67), nursery (47), growing (30) and fattening pigs (37). In total 237 samples were processed by PCR, using a protocol described by Pintos et al. (5). The data obtained were analyzed using Epi-Info (6.04) software package (CDC Atlanta, GA, USA) and 3.1 Epidat.

Results

From a total of 237 samples, the overall positive percentage was 32%. Prevalence percentage in IH was 23.7% (30.7% 16.8 %; $IC_{95\%}$) while in OH 44.7% (34.6%-54.7%, $IC_{95\%}$). (table 1).
Differences between prevalences are significative ($p<0,01$)

Table 1. Prevalence of *M. suis*

	PCR samples		
Herd	Positive	Negative	Total
IH	34	109	143
OH	42	52	94
Total	76	161	237

[1] indoor herds (IH); outdoor herds (OH); positive (samples positive a *M. suis* by PCR); negative (samples negative a *M. suis* by PCR).

Conclusions and Discussion

A previous study carried-out in Argentina on OH by PCR demonstrated a prevalence of *M. suis* 65% (4). However, samples belonged from clinically affected pigs. In the present study a prevalence of 44.7% was found on healthy pigs on the same type of farms and 23.7% on IH. The above results indicate the presence of subclinical infections in which asymptomatic carriers fulfill an important role in the maintenance of *M. suis* infection within the farms. Percentages differences between IH and OH might be due to management, routine health control through antibiotic and parasitic treatments and vaccine schedule particularly PCV-2. It should be noted that clinical signs are generally associated with other viral and immune-mediated diseases (3,4).
The study provides information about prevalence of subclinical infection of *M. suis* as well as the usefulness of the PCR for an active surveillance in order to avoid both clinical and productive disorders.

Acknowledgments

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New variants of R1 and R2 of P97 of *M. hyopneumoniae* field strains from Mexico

L Reyes-Guerra[1], A de la Peña Moctezuma[1], A Ciprián Carrasco[2], A Sahagún-Ruiz[1*]
[1]*Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Ciudad Universitaria, Coyoacán, México, D F, 04510, México.* [2]*Facultad de Estudios Superiores Cuautitlán, Universidad Nacional Autónoma de México, Cuatitlán, Izcalli, Estado de México, México,* asahagun2004@yahoo.com.mx

Introduction

Mycoplasma hyopneumoniae causes enzootic pneumonia, a chronic pulmonary disease of the pig, characterized by low mortality and high morbility. It is one of the most economic important diseases in swine farms. The bacterium attaches to the ciliated epithelial cells lining of the respiratory tract via P97 adhesin that binds to sulphated glycolipids on the host-cell membrane of tracheal cilia. P97 adhesin has two repetitive regions: R1 and R2, that encode for the repeats AKPVA and GAPNQGKKAE, respectively. Both vary in some amino acids and the number of repeats between different strains. The goal of this study was both to characterize R1 and R2 regions of P97 of Mexican isolates as compared to the sequences reported from other countries, and to identify other common bacteria associated to respiratory infection.

Materials and Methods

Injured lung samples (n=384) were obtained from slaughterhouses of the State of Mexico which were processed for bacteriological isolation and DNA extraction with CTAB/phenol/cloroform for PCR. *M. hyopneumoniae* strain J was used as the positive control, and the field strain of *M. hyorhinis* as the negative control. R1-5´ATTAGACGATAATCTTCAGTATTCAT3´ and R2-5´TACCTAAG/TTCAGGAAGGTAATTAG3´ primers based on the sequence of *p97* from *M. hyopneumoniae* were used. *p97* fragment was amplified by PCR and sequenced at the Institute of Biotechnology, UNAM using the ABI System®. Analysis of the sequences was performed with Sequencher 4.1, ClustalW and BLAST from NCBI.

Results

From the 384 samples, 54.7% (210) were negative and 45.3% (174) were positive by bacteriology. From these last ones 2.3% (4) were positive to *Mycoplasma hyorhinis*, 40.8% (71) to *Pasteurella multocida* type A, 6.9% (12) to *Pasteurella* spp, 51.7% (90) to *Trueperella pyogenes*, 17.2% (30) to *Escherichia coli*, 17.2% (30) to *Staphylococcus aureus*, 41.4% (72) to *Staphylococcus* spp, and 17.2% (30) to *Moraxella* spp.

Through PCR *M. hyopneumoniae* was detected in 16.1% (28) of the samples.

A new variant of R1: AK**L**VA was found in two Mexican isolates: 1p97 (EU395827.1) and 79p97 (EU395828.1). Additionally, a new variant of R2: GAPNQGKK**S**E was found in 292P97 isolate (EU395831.1).

Conclusions and Discussion

Staphylococcus spp, *Trueperella pyogenes* and *Pasteurella* spp were detected frequently (40-60%), followed by *E. coli*, *Moraxella* spp and *M. hyopneumoniae* (17%) from injured lung samples.

New variants of R1 and R2 of P97 were found from Mexican *M. hyopneumoniae* isolates. 25 different variants of R1 and 30 of R2 have been reported in the GenBank. R1 variants: AKPVA and AKPEA were more frequently observed. Since it is possible to derive 5 variants of R1 with only one amino acid change from AKPVA, and from AKPEA it is only possible to derive 2 variants. It is suggested that AKPVA is the earliest ancestral variant of R1.

Two Mexican *M. hyopneumonie* isolates present a difference in the amino acid sequence of P97 as in comparison to strains from other parts of the world. Due that the variation occurs in the central proline; this might have important implications with regard to attachment to epithelia, virulence and immune protection induction with vaccines based on P97 adhesin.

Acknowledgments

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Association of *M. hyopneumoniae*, porcine parvovirus and PCV2 in pneumonic lungs from swine detected by multiplex PCR

O Montiel-Velázquez[1],!L Reyes-Guerra[1], H Ramírez Mendoza[1], E Alfonseca-Silva[1], E Lazo-García[1], S Mendoza-Elvira[2], A Sahagún-Ruiz[1]!

[1]*Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Ciudad Universitaria, Coyoacán, México, D F, 04510, México.* [2]*Facultad de Estudios Superiores Cuautitlán, Universidad Nacional Autónoma de México, Cuatitlán, Izcalli, Estado de México, México,* asahagun2004@yahoo.com.mx

Introduction

The Porcine Respiratory Disease Complex (PRDC) is one of the most important problems for the porcine industry at worldwide level. The purpose of this study was to determine pathogen associations in the PRDC using a new designed multiplex PCR for *Mycoplasma hyopneumoniae*, Porcine Circovirus type 2 (PCV2) and Porcine Parvovirus (PPV), a simple PCR for Porcine Pseudorabies Virus, *Actinobacillus pleuropneumoniae* and *Haemophilus parasuis* and bacteriology.

Materials and Methods

Lung samples with- and without pneumonic lesions, from clinically healthy pigs from 2 farms at central Mexico were collected in a slaughterhouse. Samples were taken randomly from cranial and middle lobes, identified with progressive numbers and transported at 4°C. For bacteriology, a 50 mg of lung tissue were inoculated on blood agar with a nurse strain of *S. aureus*, incubated at 37ºC and identified as described. DNA extraction was performed by CTAB/phenol/ Chloroform method and simple and multiplex PCR was performed with primers described in Table 1.

Table 1 PCR primers used in this study

Agent	Gene	Primer	Amplicon
PPV	*NS1*	PPV.*NS1*-F 5' TACCAAGCAACAATGGCTAGC 3'	221 bp
	(AY390557.1)	PPV.*NS1*-R 5'GTTGGCTCGCTCCACGGC 3'	
PCV2	*Replicase*	CVP.*rep*-F 5' CTGATTACCAGCAATCAGACC 3'	427 bp
	(AF027217.1)	CVP.*rep*-R 5' CCACTATTGATTACTTCCAACC 3'	
Mhp	*p97* paralogous	*Mhp*-F 5' GTCTAACTGTCGGACTTAGCA 3'	760 bp
	(AY957500.1)	*Mhp*-R 5' GCCTGTGATTTGCGAAGACTA 3'	
PRV	*glycoprotein E*	PRV.*gE*-F 5' GCGGTGCCTGCTGTACTAC 3'	515 bp
	()	PRV.*gE*-R 5' CGACGACGCACGTCATCAC 3'	
App	*ApxIVA*	App-F 5' TGGCACTGACGGTGATGA 3'	422 bp
	(AF021919)	App-R 5' GGCCATCGACTCAACCAT 3'	
Hps	*16SrRNA*	*Hps*-F 5' GTGATGAGGAAGGGTGGTGT 3'	821 bp
	(M75065)	*Hps*-R 5' GGCTTCGTCACCCTCTGT 3'	

PPV = Porcine Parvovirus, PCV2 = Porcine Circovirus type2, *Mhp* = *Mycoplasma hyopneumoniae*, PRV = Porcine Pseudorabies virus, *App* = *Actinobacillus pleuropneumoniae*, *Hps* = *Haemophilus parasuis*, bp = base pairs

Results

The lungs (n=30) without lesions were positive to PPV (n=16) and PCV2(n=8) by PCR and negative to bacteriology. From the lungs with lesions (N=102), all samples were negative to the porcine pseudorabies virus, *Actinobacillus pleuropneumoniae* and *Haemophilus parasuis* by PCR. Detection of PPV, PRV and *M. hyopneumoniae* by single PCR matched 100% results obtained by multiplex PCR. In contrast, isolation of *M. hyopneumoniae* was not possible even though 21.6% of the samples were positive by PCR. The agent most often detected by PCR was PPV (65.7%), followed by PCV2 (31.4%), *M. hyopneumoniae* (21.6%), and by culture isolation *Pasteurella multocida* (19.6%), and *Trueperella pyogenes* (5.9%) from the lungs with pneumonic lesions. PPV was the most frequently detected both as a single agent (19.6%) and as an associated one (46%). In contrast, *M. hyopneumoniae* was detected only as an associated agent (21.6%), but never as a single one. PCV2 was detected both, as a single agent (10.8%) and as an associated one (20.6%). The most frequent pathogen associations were both PPV/*M. hyopneumoniae* (19.6%) and PPV/PCV2 (19.6%).

Conclusions and Discussion

Infections by associated pathogens were found in 48% of the lung samples, while infections by a single agent were found in 34.3%, indicating that mixed respiratory infections were most frequent than the single ones. The interaction of PCV2 with other pathogens of respiratory and reproductive systems has been reported. Of those pathogens, apparently the PPV could be involved not only in the postweaning multisystemic wasting syndrome but also in the PRDC. In this study, the PPV was found associated with other agents in 46.1% of the lungs with pneumonic lesions. In contrast, the PPV was present in only 5% of PRDC cases during 2000 at the Diagnostic Laboratory, Iowa State University (ISU-VDL). Although, it is well known that PPV is ubiquitous in porcine farms, the high prevalence of PPV suggests a recent infection of the farms. On the other hand, detection of *M. hyopneumoniae* by simple PCR or multiplex PCR was highly efficient as compared to bacteriology.

Acknowledgments

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Use of Baytril® 100 (enrofloxacin) injectable for the treatment of swine respiratory disease associated with *M. hyopneumoniae* in pigs using an experimentally induced infection

D Keil, T Settje, A Holtcamp
Bayer HealthCare – Animal Health Division, andrew.holtcamp@bayer.com

Objectives

The objective of the study was to evaluate the efficacy of a single subcutaneous injection of Baytril® 100 (enrofloxacin) Injectable at 7.5 mg enrofloxacin/kg body weight in pigs experimentally infected with *M. hyo.*

Materials and Methods

This was a placebo-controlled, blinded and completely randomized laboratory study. *M. hyo*-seronegative crossbred barrow and gilt feeder pigs were sourced from a herd with no clinical history of *M. hyo* infection during the preceding six months. Pigs were 9 to 10 weeks of age and weighed between 56-92 lbs on Day 0, the day of treatment. Pigs receive no vaccinations or antimicrobial treatments at the study site. Challenge: A pool of 132 pigs was challenged to place 72 pigs on test (Groups 1 and 2) on Day 0. Pigs were challenged with lung homogenate containing a representative recent *M. hyo* isolate by endotracheal and intranasal inoculation once daily for three consecutive days.

Treatments:

1. Group 1: Saline placebo controls – administered once subcutaneously at the same volume as pigs receiving Baytril® 100. N=36.
2. Group 2: Baytril® 100 – 7.5 mg/kg administered once subcutaneously. N=36.
3. Group 3: Sentinels – No treatment. N=60.

Pigs from all treatment groups were commingled in 12 pens. Each pen ultimately contained three pigs from treatment groups 1 & 2 and, initially, five pigs from group 3. Group 3 pig numbers were reduced as sentinels were necropsied to monitor disease progression and establish when treatment would be initiated

Study Procedures: The total pool of challenged pigs was evaluated daily for coughing until at least 5% had coughing scores of 1 or greater. On that day (Day -2) and each day thereafter until treatment was initiated, five pigs were randomly selected, necropsied and lung lesions scored to monitor disease progression and establish when treatment would be initiated. Treatment was initiated (Day 0) when at least four of five of the necropsied pigs had total lung lesion scores ≥5%. On Day 0, randomly selected pigs were treated with either saline placebo (Group 1) or Baytril® 100 (Group 2). Clinical scores were recorded on Day 0 and daily from Days 3-10. Pigs were weighed, euthanized and necropsied on Day 10. Lungs were harvested, lung lesions scored, and samples collected for *M. hyo* PCR and bacterial culture.

Criteria of Effect and Statistical Evaluation. One individual pig was the experimental unit, with total lung lesion scores at necropsy the primary criterion of effectiveness. In addition, clinical scores for coughing, respiration and depression were evaluated. At necropsy, lungs were cultured for other bacterial pathogens and tested for the presence of *M. hyo* by PCR.

Results

The mean total lung lesion scores, were significantly reduced in the Baytril® 100 (enrofloxacin) group compared to the placebo group with scores of 4% and 27% (P<0.0001), respectively.

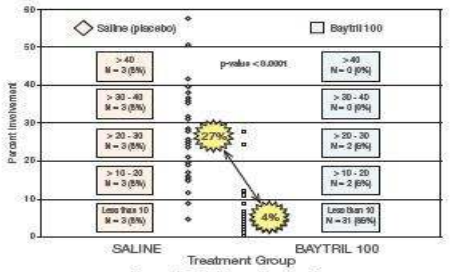

Figure 1: Total Lung Lesion Scores

Significant reductions in % days with abnormal cough score (13.9 vs 53.8 respectively, P<0.0001), % days with abnormal respiration score (19.9 vs 32.6 respectively, P=0.0009), and % days with abnormal depressions scores (7.5 vs 13.2 respectively, P=0.0278) were observed in the Baytril® 100 group compared to the placebo group.

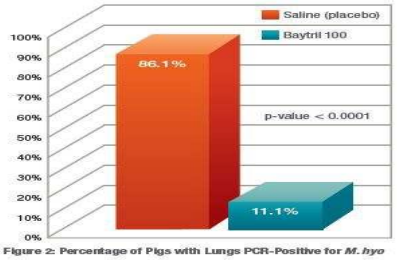

Figure 2: Percentage of Pigs with Lungs PCR-Positive for *M. hyo*

As shown in Figure 2, the percentage of pigs with lungs PCR-positive for *M. hyo* was also reduced in the Baytril ® 100 group compared to placebo controls (11.1 vs 86.1 respectively, P<0.0001).

Conclusions

This pivotal laboratory study demonstrates the effectiveness of a single subcutaneous injection of Baytril® 100 at 7.5 mg enrofloxacin/kg for the treatment of *M. hyo* infection in swine.!

A clinical study measuring the effect of treatment & control of swine respiratory disease over a 28-day period after a single administration of Baytril® 100 (enrofloxacin) in pigs challenged with *M. hyopneumoniae*

DJ Keil, T Settje, A Holtcamp
Bayer HealthCare – Animal Health Division, andrew.holtcamp@bayer.com

Objective

The objective of the study was to measure the outcomes of treatment effect and control of disease for 28 days after a single injection of Baytril® 100 at 7.5 mg enrofloxacin/kg (enrofloxacin) in pigs challenged with *Mycoplasma hyopneumoniae*.

Materials and methods

This was a single location, placebo-controlled, blocked and blinded randomized clinical study. A total of 320, 35-lb healthy pigs that had not received any medication for 14 days, were each challenged for two days with 10 mL of a strain of *M. hyo* recovered from a clinical case of swine respiratory disease (SRD). On Day 0, pigs were scored for SRD and treatments administered. All pigs were randomly assigned to the following five study groups: Group 1 (challenged/untreated, 36, six / pen); Group 2 (36 challenged/treated with Baytril® 100 (enrofloxacin), six/pen); Groups 3— challenged and unchallenged treated with saline (70,10/pen 5 challenged 5 unchallenged); Group 4 challenged and unchallenged treated with Baytril® 100 (enrofloxacin); Group 5 unchallenged and untreated (36/pen). Animals in all groups had scheduled necropsy on days 0, 5, 14, 21 and 28 to determine disease progression.

Enrollment criteria and Data analysis. A total of 147 pigs met the criteria of a temperature ≥ 104˚F and either a respiratory score or a depression score ≥ 1. A total of 99 unchallenged pigs were enrolled as not having SRD (a body temp < 104˚F; a respiratory score ≤ 1; and a depression score ≤ 1). Analysis of the clinical data on Days 5, 14, 21 and 28, An animal was considered a Success if it was alive, had a temp of < 104˚F, and respiratory and depression scores of 0 or 1. If an animal had a temperature ≥ 104˚F and had either a respiratory or depression score of 2 or more, it was deemed a Failure and considered as such for the remainder of the study. The *M. hyo* challenge model was validated by examining both the challenged-untreated and unchallenged-untreated control groups for difference in SRD symptoms. Animals in Group 1 were compared to those in Group 2 and animals in Group 3 to those in Groups 4 to evaluate the treatment and control of Baytril® 100, respectively.

Results

A statistically significant difference in clinical disease & lung pathology was found between the challenged/untreated (Group1) and unchallenged/untreated groups (Group 5).

Significant difference was noted between the number of treated pigs considered a success to untreated pigs on Days 5, 14, and 21. Also, a significant difference in lung lesions was found in treated pigs compared to untreated pigs on Days 14 and 28. PCR identification of *M. hyo* was significantly different in treated compared to untreated on Day 14 and approached significance on Days 21 and28 (Table 1).

Table 1

Study Day	Group 1 (challenged, untreated)		Group 2 (challenged, Baytril 100 treated)	
	% Lung lesions	% PCR positive	% Lung lesions	% PCR positive
5	30	33*	19	0*
14	44[a]	75[c]	3[a]	17[c]
21	32	83**	14	33**
28	29[b]	75**	8[b]	33**

*p = 0.1065Numbers with same superscript (a–c) significantly different (p < 0.05).Rows with ** p < 0.10

A significant difference between the number of treated pigs considered successes compared to untreated pigs on Days 14 and 28 showed the effect of Baytril® 100 in controlling the spread of *M. hyo* infection(Table 2). In addition, a significant difference was noticed in lung lesions and identification of *M. hyo* by PCR in treated pigs compared to the untreated on Day 14 and on Days 5 and 28, respectively(Table 2).

Table 2

Study Days	Group 3 (challenged/unchallenged, saline treated)		Group 4 (challenge/unchallenged, Baytril ® 100 treated)	
	% Lung lesions	% PCR positive	% Lung lesions	% PCR positive
5	25	26[b]	14	0[b]
14	26[a]	29	5[a]	14
21	12	36	6	14
28	16	65[c]	9	20[c]

Numbers with the same superscript are significantly different (p < 0.05)

Conclusion

This study demonstrates the effectiveness of a single subcutaneous injection of Baytril® 100 at 7.5 mg enrofloxacin/kg for the treatment and control of swine respiratory disease infection in pigs.

P.500

Characterization of *E. faecallis* from pigs in Brazil

PHNL Filsner[1], GFR Silva[1], TSP Ferreira[1], KC Silva[1], M Moreno[1], VTM Gomes[1], AM Moreno[1]
Laboratory of Swine Health - Department of Preventive Veterinary Medicine- FMVZ/USP
*São Paulo, SP/ Brazil,*morenoam@usp.br

Introduction

Causative agent of urinary tract infections, endocarditis, meningitis and septicemia in humans and animals, members of the genus *Enterococcus* won epidemiological importance, by the intrinsic and acquired resistance to a wide range of antibiotics (1). Among the 36 species, two are given a greater prominence,*Enterococcusfeacallis* and *Enterococcusfaecium* due to the high frequency of multidrug resistance to antimicrobial agents. Several studies indicate that the use of growth promoters in animal production was an important factor in the increase rate of multi drug resistance in various species of *Enterococcus* (2). Given this, in the present study, *E. faecalis*strains isolated from commercial pigs were evaluated for the antimicrobial resistance profile by determining the minimum inhibitory concentration.

Materials and Methods

In order to investigate the antimicrobial susceptibility of *E. faecallis* isolates, a total of 245 strains isolated from 171 fecal samples collected in 31 swine herds in Brazil. Samples were plated on VRE agar for 24-48 hrs at 37°C ± 1°C. Identification was made on the basis of colony morphology andusing polymerase chain reaction (PCR) with specific primers for *E. faecallis*. Minimal inhibitory concentrations (MICs) of drugs were determined using CMV3AGPF MIC Plate - Sensititre ® against the following antimicrobial agents: tigecycline, ciprofloxacin, daptomycin, vancomycin, tylosin, penicilin, erythromycina, quinupristin./dalfopristin, linezolide, lincomycin, tetracycline, chloranphenicol, nitrofurantoin, gentamicina, kanamycin, streptomycin.

Results

The highest resistance rates were observed against tylosin (98.7%) and lincomycin (98.7%), followed by tetracycline (97.1%), erythromycin (96.7%), streptomycin (96.3%), a combination quinupristin-dalfopristin (95.5%), kanamycin (93.8%), gentamicin (85.3 %), ciprofloxacin (76.7%) and chloramphenicol (71.8%). Strains resistant to vancomycin were not found, and the rate of resistance to daptomycin (0.4%), nitrofurantoin (1.2%) and tigecycline (1.6%) was low.

Conclusions and Discussion

The results of this study indicate that in Brazil, commercial pigs carry a high frequency of *E. faecallis* strains multiresistant to antibiotics and with large genetic variability. Were quite frequent the strains resistant to high levels of aminoglycosides and macrolides among other principles, however, the results indicate that swine offer a low risk to spread Enterococcus strains resistant to vancomycin. Further studies directed to genes involved in resistance patterns observed and the potential virulence of these isolates will be conducted in the future.

Table 1. *In vitro* sucetibility of *E. faecallis* isolates from swine in Brazil.

Antimicrobial	MIC (µg/mL)		Resistant
	MIC 50	MIC 90	% (n=245)
Tigecycline- TGC	0,12	0,25	1,6
Ciprofloxacin – CIP	>4	>4	76,7
Daptomycin- DAP	2	2	0,4
Vancomycin- VAN	1	2	0
Tylosin- TYLT	>32	>32	98,7
Penicilin- PEN	2	4	3,7
Erythromycin - ERY	>8	>8	96,7
Quin./ dalf.-SYN	16	32	95,5
Linezolide- LZD	4	4	6,5
Lincomycin- LIN	>8	>8	98,7
Tetracycline - TET	>32	>32	97,1
Chloranphenicol- CHL	>32	>32	71,8
Nitrofurantoin- NIT	8	16	1,2
Gentamicin - GEN	>1024	>1024	85,3
Kanamycin- KAN	>1024	>1024	93,8
Streptomycin – STR	>2048	>2048	96,3

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P.501

Biochemical characterization of *P. multocida* strains from Brazilian swine

TSP Ferreira[1], LZ Moreno[1], MR Felizardo[1], DDS Gobbi[1], PHNL Filsner[1], GFR Silva[1], M Moreno[1], AM Moreno[1].
Laboratory of Swine Health - Department of Preventive Veterinary Medicine- FMVZ/USP
São Paulo, SP/ Brazil, morenoam@usp.br

Introduction

Infection due Pasteurella. multocida is an important cause of losses in pig farms in Brazil and the world. These losses are related to mortality, growth retardation and weight gain and condemnations of carcasses in slaughterhouses and expenditures on medicines, vaccines and veterinary care. This study proposed the phenotypic characterization of 97 samples of *P. multocida subsp. multocida* isolated from pigs from 67 farms in different epidemiological studies, but associated with molecular methods (Hunt, et al., 2000)[4].

Materials and Methods

The strains were subjected to identification and determination of the capsular type using the polymerase chain reaction described by Townsend et al. (2001)[1]. Production of catalase, oxidase, ornithine decarboxylation (DCO), urease activity and ability to produce indole were performed as described by Cowan (1993)[2]. The ability to ferment L-arabinose, dulcitol, D-glucose, D-lactose, maltose, D-mannitol, D-sorbitol, D-sucrose, D-trehalose and D-xylose were tested using the method described by fermentation microplate Blackall et al. (1997)[3].

Results

All strains tested were positive for catalase, oxidase and indole production, and negative for the presence of urease. The 97 strains were able to metabolize mannitol, sorbitol and glucose, and none was able to ferment arabinose and dulcitol. Compared to other substrates were observed a large variation between strains. Through the use of these sugars and alcohols and ornithine decarboxylase activity (ODC) 12 distinct biotypes were identified. The results concerning the fermentation of sugars, alcohols and activity of ODC are presented in Table 1.!Strains of *P. multocida* ODC negative, positive for lactose and maltose are infrequent according to the literature, therefore samples are considered variants. In the present study, the frequency of samples variants was higher in *P. multocida* isolated lung in relation to isolated in nasal cavity.

Conclusions and Discussion

In the present study we observed a higher frequency of variant strains from lung (49%) and in type capsular A (44%). Variant strains from nasal cavity represented 10% and in from capsular type D only 9%. This finding can not be confronted with the literature since the authors described the occurrence of these biochemical variants only in strains from type capsular A. Since the biochemical typing is subject to greater variations in methodology, reagents used and carbohydrates gene expression is not recommended to use as the only parameter for characterization of P. multocida in

Table 1. Distribution of *P. multocida* strains from swine according biochemical profiles identified.

Nº strains	Biochemical profile [a]											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Test	39	9	2	11	12	14	2	4	1	1	1	1
ODC	+	+	-	-	+	-	+	-	-	-	+	-
Lactose	-	-	-	-	-	-	+	+	-	-	+	+
Maltose	-	-	-	-	-	-	-	+	+	+	+	-
Trealose	-	-	-	-	+	+	-	+	+	-	-	+
Xilose	+	-	-	+	+	+	+	+	+	+	+	+

[a] All the isolates were positive for oxidase, catalase and indole, showed no urease activity nor grew on MacConkey agar. All isolates also fermented glucose, mannitol, sorbitol, and sucrose but not ferment arabinose and dulcitol.
[b] ODC = decarboxylation of ornithine.

Acknowledgments

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Detection of virulence genes from *P. multocida* strains from Brazilian swine

TSP Ferreira[1], SMN Teixeira[1], ATR Costa [2], DDS Gobbi[1], PHNL Filsner[1], GFR Silva[1], M Moreno[1], AM Moreno[1]
Laboratory of Swine Health - Department of Preventive Veterinary Medicine- FMVZ/USP São Paulo, SP/ Brazil,
[2]IPEVE – Instituto de Pesquisas Veterinárias Especializadas - Rua Esmeralda, 786, Prado, CEP 30.410.080, Belo Horizonte - MG, Brasil, morenoam@usp.br.

Introduction

In recent years a considerable number of studies have been conducted in order to determine mechanisms of immunity predilection for hosts, virulence and pathogenicity of *Pasteurella multocida.* In swine is known that this agent is commonly found and its high prevalence makes the complex of respiratory diseases of pigs a major cause of economic loss[1]. In this study strains of *P. multocida* isolated from pigs with rhinitis and pneumonia were characterized by polymerase chain reaction to detect 15 virulence genes.

Materials and Methods

A total of 123 strains of *P. multocida* isolated from lung and nasal cavity of pigs presenting pneumonia or atrophic rhinitis were evaluated. The animals were originated from 80 farms located on different states of Brazil. Strains were submitted to DNA extraction[2] and the species identification and determination of the capsular type was performed using the polymerase chain reaction described by Townsend et al. (2001)[3]. Detection of Virulence genes *toxA, nanB, psl, oma87, nanH, sodA, hghA, ompH, sodC, PtfA, exbBD/ tonB, hgbB, pfhA* and *tbpA* was conducted as described by Ewers et al (2006)[1] and Tang et al, (2009)[4].

Results

Among 123 strains analyzed, 97 strains were characterized as capsular type A (79%) and 26 as capsular type D (21%). All strains were negative for presence of the gene encoding dermonecrotic toxin. The virulence genes were detected in the following frequency: frequencies: 93.5% to *nanB,*92.7% to *psl,* 91.9% to *oma87* and *nanH,* 87.8% to *sodA,* 87% to *hghA,* 83.7% to *omph,* 82.9% to *sodC,* 79.7% to *PtfA* and *exbBD/tonB,* 73.2% to *hgbB,* 14.6% to *pfhA* and 4.9% to *tbpA* (Table 1).

Conclusions and Discussion

The frequencies of different genes was similar to previously described in literature as can be observed in Table 1. The *tbpA* gene was the only exception, since this gene is generally found in ruminant strains (cattle, sheep and buffalo) and was present in 4.9% of the swine strains studied.

Table 1. Frequency distribution of virulence genes detection among *P. multocida* strains in this study and in literature.

Gene	Positive strains %		
	This study	Ewers et al, 2006	Tang et al, 2009
hgbA	87.0	98.1	97.0
hgbB	73.2	86.5	NT
exBD/tonB	79.7	100	97.9
nanH	93.5	100	81.5
psl	92.7	100	NT
ompH	83.7	100	93.1
oma87	91.9	100	94.4
pftA	14.6	21.2	15
sodC	82.9	100	NT
sodA	87.9	100	NT
tbpA	4.9	0	0

Acknowledgments

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Salmonellosis diagnostic serologic test in pigs

J Castillo, E Hernández-Baumgarten[1], MA Mendoza, MC Alvarez, D Trujillo, , S González, A Ciprián, S Mendoza
Facultad de Estudios Superiores Cuautilán-UNAM. juanitacc75@yahoo.com.mx,

Introduction

The pigs subclinically infected with *S. cholerasuis* are considered the most important source of infection in the farm and the clinical pictures can be trigged by immunesuppressing or stressing events such as a viral infection. An infected farm is a source of two types of problems: As disease for pigs which can be caused by *S. cholerasuis, S. typhisuis or S. typhimurium.* Salmonellosis is a zoonotic disease and a public health problem. An infected farm, with or without overt disease can act as a source of infection for humans. The objective of this study was to obtain and antigen from *S. cholerasuis* for the diagnostic of salmonellosis in pigs (1,2, 3).

Materials and Methods

The reference strain of *S. cholerasuis* (ATCC 7001) was grown in an enriched medium for better growth. With the isolated strain, the panels of biochemical tests were conducted (1, 2). For the batch of antigen a 500 ml flash of nutrient broth was inoculated and then passed to a 20 lt fermentor for better biomass yield. The culture was inactivated and centrifuged again to obtain the pure antigen. The purified antigen was conditioned with various buffers, at various pHs and stained with Bengal Rose (BR) or Coomassie Blue (CB). Four hundred ninety five pigs were sampled in the farm. On the other hand, eight weaned pigs, without antibodies against *S. cholerasuis* by ELISA were used. The pigs were challenged with 10 ml of $6x10^6$ PFU/ml culture of *S. cholerasuis* oral via. Serum samples were taken 1, 7, 14, 21 and 28 postinfection. All sera were tested with BR and CB antigens and isolation and identification of *S. cholerasuis* was attempted all animals.

Results

After proper identification, the culture was expanded to 18 lt. in broth and a 20 lt. fermentor. The batch culture yielded 24g of purified antigen. This biomass was divided in two batches, suspended in phenolated saline for inactivation and then one batch was stained with BR , other batch was stained with CB and all three adjusted to an antigen concentration of 11%.

The test of the sera with two antigens gave the following results: BR= 21.81% positive, 78.18 negative, CB= 23.83% positive and 77.18 negative, the correlations between the two antigens using only serum samples and we obtained a 98.79% correlation between them. The results of 21.81 % positive with CB stained antigen is usually prepared to test blood (ring test) samples but the results showed that either antigen can be used with sera. The antigens prepared seems very promising to us for a fast diagnostic test for salmonellosis in pigs.

Conclusions and Discussion

We conclude that *S. cholerasuis* antigen can be used for an agglutination test for fast test in salmonellosis in swine without using many reagents and long tests, the CB stained could be used in the farm with the whole blood and the BR stained antigen in a diagnostic laboratory where sera are sent.

Acknowledgements

Grants: *PAPIIT ITE218711-3 and CONS-23.*

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Evaluation of phage cocktails for control of *Salmonella*

BY Jung[1], K Lee[1], HY Park[1], SC Jung[1], YS Cho[1], JS Son[2], WI Kim[3]
[1]*Bacteriology Division, Animal and Plant Quarantine Agency, Anyang,* [2]*iNtRON Biotechnology Inc., Seongnam,*
[3]*Chonbuk National University, Jeonju, Republic of Korea,* jungby@korea.kr

Introduction

Salmonellae are widely distributed throughout the world, gaining entry to almost all aspects of human food chain. Pork has been reported to be associated with as much as 15% of human cases of salmonellosis (1). It was also estimated that 70% of carcass contamination resulted from infected pigs (2). Despite the multiple treatments to eliminate *Salmonella* spp. in pig farms, it still remains a considerable problem due to limiting effective treatments. The use of specific bacteriophages could be a promising strategy for controlling *Salmonella* colonization in pigs. Therefore, the aim of this study was to evaluate the phage cocktails which were the mixture of *Salmonella* specific lytic phages.

Materials and Methods

Fecal and sewage samples were collected from pig farms for phage isolation. The presence of phages was investigated by a phage enrichment technique using *Salmonella* Typhimurium ATCC 14028 and confirmed by spotting assay. Double layer plaque assay was performed to obtain pure phage isolates. Transmission electron microscopy (TEM) was also performed for investigation of phage morphology (3).

For the estimation of lytic capability, reference strains representing 34 serotypes and 190 *Salmonella* isolates representing 16 serotypes were tested using spotting assay.

Results

Four phage cocktails were prepared with mixture of individual phages: cocktail A (SEP-1, SGP-1, STP-1, SS3P-1, EK9P-1; $\geq 10^9$ pfu/ml), cocktail B (SEP-1, SGP-1, STP-1, SS3P-1, EK9P-1; $\geq 10^{11}$ pfu/ml), cocktail C (SEP-1, SGP-1, STP-1, SS3P-1, STP-2, SCHP-1, SAP-1, SAP-2; $\geq 10^9$ pfu/ml), and cocktail D (STP-2, SCHP-1, SAP-1, SAP-2, EF4P-1, EK8P-1, EK9P-1, CP-3, CP-5; $\geq 10^9$ pfu/ml), respectively. Morphological analysis by TEM revealed that the phages were divided into three types of families; *Siphoviridae*, *Myoviridae* and *Podoviridae*.

All the reference strains were clearly lysed in cocktails B and C. However, only 8 (23.5%) serotypes of reference strains showed complete lysis in cocktails A and D.

In the present study, the numbers of completely lysed isolates were different with the type of individual phage of cocktail and titer of each phage. Among 190 *Salmonella* isolates, 177 (93.2%) and 176 (92.6%) isolates were clearly lysed with cocktails B and C, respectively. However, 96 (50.5%) and 86 (45.3%) isolates were lysed with cocktails A and D, respectively.

All the tested Typhimurium (n = 93), the most prevalent serotype in pig farms in Korea, were completely lysed in cocktails B and C. Serotypes London (n = 11), Schwarzengrund (n = 8) and Derby (n = 6) were also clearly lysed in cocktails B and C. On the other hand, no lysis was observed in serotype Rissen (n = 58) with cocktails A and D, but 47 (81.0%) and 46 (79.3%) isolates were shown with complete lysis patterns in cocktails B and C, respectively.

Conclusions and Discussion

In the present study, phage cocktails B and C showed lytic capability against broad spectrum of *Salmonella* serotypes. Cocktails A and B had the same composition of individual phages, except phage titer. Cocktail B was more efficient compared to cocktail A. This result demonstrated that the phage titer was important to improve the antibacterial effect of phages.

In comparison of cocktails C (mixture of 8 individual phages) and D (mixture of 9 individual phages), cocktail C was much more efficient than D. For this reason, we consider that accurate selection of phages are required for the better efficiency of phage cocktails.

We proposed the usage of phage cocktail as a way to control of *Salmonella* infection in pig farms.

Acknowledgments

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Swine zoonosis risk assessment with new herd seroprofiling assays from QIAGEN

C Schroeder[1], C Sander[1], S Kanitz[1], C Engemann[1], S Hennart[1], N Djuranovic[2]
[1]QIAGEN Leipzig GmbH, Leipzig, Deutscher Platz 5b, D-04103 Leipzig, Germany
[2]QIAGEN Inc., 19300 Germantown Rd, Germantown, MD 20874, nevena.djuranovic@qiagen.com

Introduction

QIAGEN Leipzig developed the pigtype® product line of ELISA tests for screening for swine zoonoses. This product line now includes ELISA for detection of salmonella-, Yersinia-, Trichinella-, and Toxoplasma-antibodies in swine. These pigtype assays are validated for serum and meat juice samples and are officially approved by the German Friedrich-Loeffler-Institut. In order to follow the seroprofiling concept, the pigtype ELISA reagents and assay protocols are standardized. This product concept allows combining serological salmonella monitoring with serological testing for other zoonosis.

Materiasl and Methods

Sets of 8 serum samples per zoonosis parameter were sent to 6 laboratories in Germany and Austria. The samples (each vial 50 µl) were shipped frozen to the participants and storage instructions requested the samples to be stored at –20°C until analysis.

The status of field sera refers to results determined during validation of the respective pigtype ELISA kit. Samples were sourced from field studies, the School of Veterinary Medicine of the Leipzig University, and the! Federal Institute of Risk Assessment in The participant laboratories were requested to test the samples in two runs (e.g., on separate days) according to manufacturer's instructions.

Two data points per sample and laboratory were generated, 12 data points per sample, 96 data points per assay, and 384 data points for the inter-lab trial samples sent. QIAGEN Leipzig pigtype ELISA test kits were sent to each of the participants of the inter-lab trial. All laboratories received ELISA test kits with the same batch number.

Results

Using the pigtype Salmonella Ab, all laboratories scored the correct results for negative and positive samples (Figure 1). The total CV for positive samples of the test panel, using pigtype Salmonella Ab, is 12.7%. Using pigtype Toxoplasma Ab, all laboratories scored the correct results. The total CV for the positive samples of the test panel, using pigtype Toxoplasma Ab is 13.5% (Figure 2). Using pigtype Trichinella Ab, all laboratories scored the correct results. The total CV for the positive samples of the test panel, using pigtype Trichinella Ab is 8.8% (Figure 3). Using pigtype Yersinia Ab, all laboratories scored the correct results. The total coefficient of variation is 8.8% for the positive samples of the test panel in the pigtype Yersinia Ab (Figure 4).

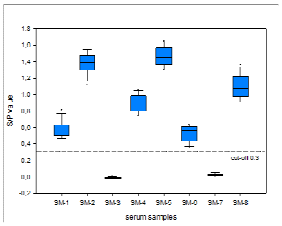

Figure 1. Pigtype Salmonella Ab box plot

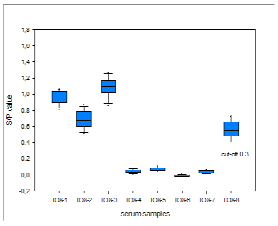

Figure 2. Pigtype Toxoplasma Ab box plot

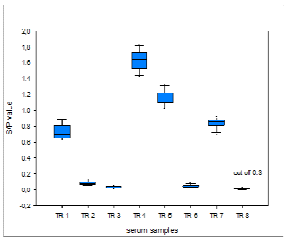

Figure 3. Pigtype Trichinella Ab box plot

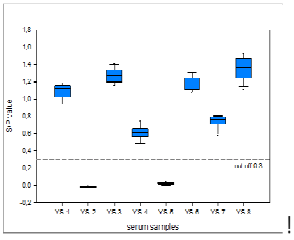

Figure 4. Pigtype Yersinia Ab box plot results

Conclusion

In this study, there was a ≥99% agreement of the test panel results for the participating laboratories. Our data suggest the suitability of the pigtype assays for an easy-to-use and cost-efficient serological monitoring for zoonotic diseases in swine herds. This could be an effective tool to use, under the European Directive on zoonoses, and bring improvements to herd risk assessment and risk oriented meat control.

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Genotypic characterization of *S. hyicus* strains from Brazil

AM Moreno[1], MR Felizardo[1], DE Barcellos[2]; MR Andrade[2], TSP Ferreira[1], FR Silva[1], MC Dutra[1],
[1] *Laboratory of Swine Health - Department of Preventive Veterinary Medicine- FMVZ/USP São Paulo, SP/ Brazil,* [2] Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, RS – Brasil, morenoam@usp.br

Introduction

Staphylococcus hyicus is the causative agent of exudative epidermis in pigs. In Brazil, the occurrence of this disease is widely known, however, studies on the agent dating from the 70s and 80s, with the last 30 years many advances have been made in the characterization of this agent. Epidemiological studies evaluating the genetic diversity of *S. hyicus* are scarce among the techniques described for evaluation of it is the assessment of the diversity of inter 16S - 23S gene and PFGE (1). The present study aimed to characterize isolates of *S. hyicus* and the presence of genes encoding the exfoliative toxins ShetA, ShetB, ExhA, ExhB, ExhC and ExhD and assess the genetic diversity of the strains through the length polymorphism of amplified fragments with a single enzyme (SE- AFLP).

Materials and Methods

A total of 84 strains of *S. hyicus* were characterized for the presence of genes encoding the toxins by PCR as previously described (1,2). Genomic DNA was extracted according to the method described by Boom et al. (1990) (3) and the AFLP protocol (McLauchlin et al., 2000) (4) was processed using *Hind* III endonuclease enzyme.

Results

The following frequencies of these genes were observed: 43 % of ShetA, 0 % of ShetB, 12 % of ExhA, 36 % of ExhB, 52% of ExhC and 8% of ExhD (Figure 1). The strains were subjected to characterization using SE-AFLP and showed 7-12 bands with sizes ranging between 300 and 3000pb (Figure 2). The dendrogram created through this analysis showed a tendency of clustering of the isolates according to the origin and the year of isolation

Conclusions and Discussion

Exudative epidermitis shares many similarities with an infection that occurs in humans, called Staphylococcal scalded skin syndrome, in which *S. aureus* infects the skin of newborns. The exfoliative toxins from S. aureus and *S. hyicus* species have different specificity. The prevalence of positive strains for exfoliative toxins has been studied in several countries. In Brazil, these toxins have not yet been described and the results obtained in the present study Sheta, ExhC and ExhB, toxins are the most frequent.

Genotypic methods such as SE- AFLP have great potential for application in the comparison of *S. hyicus* isolates

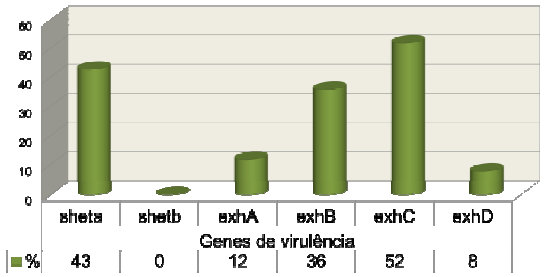

Figure 1. Frequency of virulence genes in 84 *S. hyicus* strains from swine in Brazil.

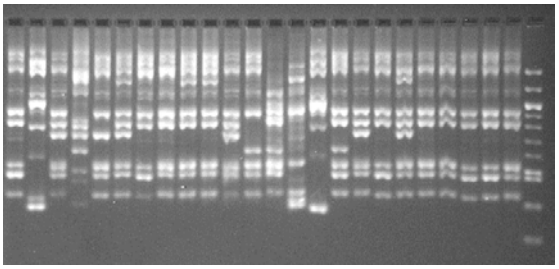

Figure 2. *S. hyicus* profiles obtained using SE-AFLP.

Acknowledgments

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S. dysgalactiae subsp. *equisimilis* resistance profile in strains from swine in Brazil

GFR Silva[1], BLP Costa[1], LZ Moreno[1], CEC Matajira[1], KC Silva[1], TSP Ferreira[1], MC Dutra[1], PHNL Filsner[1], JJ Pereira[1], AM Moreno[1].
[1] *Laboratory of Swine Health - Department of Preventive Veterinary Medicine- FMVZ/USP*
São Paulo, SP/ Brazil, morenoam@usp.br

Introduction

Streptococcus dysgalactiae can be divided in two subspecies based in biochemical properties, *S. dysgalactiae* subsp. *dysgalactiae* and *S. dysgalactiae* subsp. *equisimilis*, which belong to group C and D of Lancefield. They can be isolated from clinical healthy pig, however *S. dysgalactiae* subsp. equisimilis are capable to cause meningitis, arthritis, pneumonia, sepsis, endocarditis and abscesses. These problems cause increase of costs due use of antimicrobial and carcasses losses in slaughterhouse (1). The organism has also been reported to cause a wide variety of human infections such as pharyngitis, cellulitis, sepsis, meningitis and endocarditis (2). The objective of the current study was to describe the antimicrobial susceptibility profile of *S. dysgalactiae* subsp. *equisimilis* isolates form abscesses in swine from Brazil.

Materials and Methods

A total of 14 *S. dysgalactiae* subsp. *equisimilis* strains, from six pigs presenting abscesses were submmited to antimicrobial susceptibility test. Samples were plated on sheep blood agar at 5% and incubated for 24-48 hrs at 37ºC ± 1ºC. Initial identification was made on the basis of colony morphology. The DNA of all strains was extracted and 16S rRNA was amplified by polymerase chain reaction submitted to a sequencing reaction (1). Minimal inhibitory concentrations (MICs) of drugs were determined using BOPO6F MIC Plate - Sensititre ® (3) against the following antimicrobial agents: ampicillin, clindamycin, chlortetracycline, danofloxacin, enrofloxacin, florfenicol, gentamicin, neomycin, oxytetracycline, penicillin, sulfadimethoxine, spectinomycin, trimethoprim/ sulfamethoxazole, tiamulin, tilmicosin, tulathromycin, tylosin, ceftiofur.

Results

All strains of *S. dysgalactiae* subsp. *equisimilis* evaluated were sensible to ceftiofur, penicillin, ampicillin, trimethoprim/ sulfamethoxazole, gentamicin. Antimicrobials that showed higher rates of resistance were danofloxacin and sulfadimethoxine (100%), followed by neomicyn (78.5%), tiamulin, chlortetracycline, oxytetracycline, spectinomycin (71.4%), tylosin, tulathromycin, clindamycin (50%), and tilmicosin (43%). enrofloxacin presented 28.5% of resistance and florfenicol only 14.2%.

Conclusions and Discussion

This is the first report of minimal inhibitory concentrations of drugs against *S. dysgalactiae* subsp. *equisimilis* isolated from swine in Brazil. Probably is also the first description of this organism affecting pigs at our country. Abscesses cases are quite frequent in swine production and cause important losses in animals of different ages, mainly in boars and sows. Treatment is frequently unsuccessfully and the correct antimicrobial choice is essential to improve control chances. The organism also can be a risk to veterinarians and farmers that are in contact with infected animals.

Table 1. *In vitro* sucetibility of *S. dysgalactiae* subsp. *equisimilis* isolates from swine

Antimicrobial	MIC (µg/mL)			Resistant % (n=46)
	MIC 50	MIC 90	Range	
Ceftiofur	≤0.25	≤0.25	≤0.25	0
Tiamulin	>32	>32	≤0,5 - >32	71.4
Chlortetracycline	>8	>8	≤0.5 - >8	71.4
Oxytetracycline	>8	>8	≤0.5 - >8	71.4
Penicillin	≤0.12	≤0.12	≤0.12	0
Ampicillin	≤0.25	≤0.25	≤0.25	0
Danofloxacin	0.5	>1	0.5 - >1	100
Trimethoprim/ sulfamethoxazole	≤2/38	≤2/38	≤2/38	0
Tylosin	1	>32	≤0.5 ->32	50
Tulathromycin	2	>64	≤1 - >64	50
Clindamycin	2	>16	≤0.25 ->16	50
Sulfadimethoxine	>256	>256	>2561	100
Gentamicina	2	4	1 - 4	0
Florfenicol	1	>8	0.5 -8	14.2
Neomycin	16	16	≤4 - >32	78.5
Spectinomycin	>64	>64	≤8->64	71.4
Tilmicosin	8	64	≤4->64	43
Enrofloxacin	0.5	>2	0.25->2	28.5

Acknowledgments

This study was supported by CNPQ and CAPES Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

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The efficiency of supplement egg yolk IgY antibodies of AA-Nutri™ Focus SW6 product in prevention of digestive diseases of pigs from weaning to 70/72 days old

NT Toan[1], DTK Hoang[2], NH Chek[2]
[1]University of Agriculture and Forestry, HCMC, Vietnam, [2]All America Nutrition, Inc.
toan.nguyentat@hcmuaf.edu.vn

Introduction

In swine industry, diarrhea is one of the most common clinical sign and caused by many etiologies. Morbidity and mortality due to diarrhea are key challenges to pig farms, and can cause economic losses to farm owners. Recently, several pig farms in the southern provinces of Vietnam were devastated by many diarrhea causative agents. Especially, the disease occurred quickly with high morbidity in weaning piglets then pig became weakly after weaning period. It is essential to find out a solution to prevent and control the causes of diarrhea.

In fact, there must be more care on the use of antibiotics to prevent diarrhea in livestock. Due to increasing concerns with prophylactic drug use, and high costs and resistance of antibiotic, alternative control methods need to be developed. On the other hand, the demand for safe and healthy foods has always been a consumer concern. Biological and natural products are sought as alternatives to antibiotics prophylaxis applications. AA- Nutri™ Focus SW6 is a product which contain antibodies against specific antigens and neutralize pathogens causing diarrhea in post-weaning pigs. To access AA-Nutri™ Focus SW6 effectiveness on prevention of digestive diseases and improving body weight in pig from weaning to 70/72 days old, the study was carried out.

Materials and Methods

Total 90 piglets of a farm (Farm 1) were randomly allocated into two treatments (control, treatment) and 156 piglets of a second farm (Farm 2) were randomly allocated into two treatments (control, treatment). Both farms were located in Dong Nai province. The supplement levels of AA-Nutri™ Focus SW6 product in the diets of treatment were 500 g per ton of feed in period from 32 to 70/72 days old. The control was not supplemented AA-Nutri™ Focus SW6 in the diet.

Table 1. Trial design

	Period (days old)	Control AANF SW6: *Nil*	Treatment AANF SW6: *500ppm*
Farm 1	32-70	45 pigs	45 pigs
Farm 2	32-72	78 pigs	78 pigs

Parameters including body weight, ADG, FCR, rates of clinical signs, mortality and culling rate in different periods of pig growth from weaning to 70/72 days old were observed and recorded.

Results

The rate of diarrhea and days of diarrhea of the treatment pig groups were lower than the control pig group from 32 to 70 days old as following 48.89% and 2.89%, 53.33% and 5.39% in Farm 1, and 51.28% and 8.71% and 56.41% and 10.59% Farm 2 ($P<0.05$), respectively. The rate of mortality and cull in Farm 1 was higher than in Farm 2. Rates of mortality and cull in treatment groups were lower than control groups, but had no significant differences ($P>0.05$). The weight gain (kg/pig) of the treatment pig groups were higher than the control pig groups for both farms.

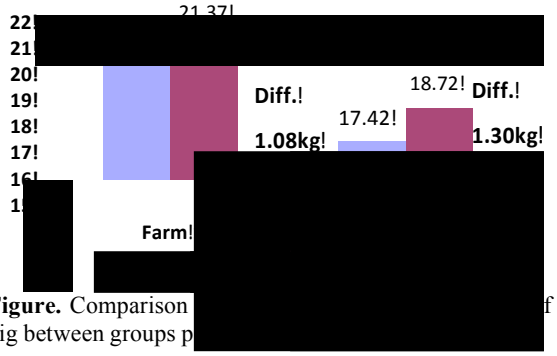

Figure. Comparison f pig between groups p

Daily gain (g/pig/day) of the treatment pig groups were higher than of the control pig groups in Farm 1 and Farm 2 and were 53.2 g/pig/day and 111.2 g/pig/day, respectively. The feed conversion ratio (FCR) of the treatment pig groups were better than the control pig groups (1.50 vs 1.60 for Farm 1 and 1.51 vs 1.70 for Farm 2, respectively).

Conclusions and Discussion

The additions of the egg yolk IgY antibodies in feeds had reduced the rate of diarrhea in pigs, then improved pig body weight. In the current high challenge of diseases in intensive livestock industry, using AA-Nutri™ Focus SW6 has effect on prevention of digestive diseases in pig as follows: reducing morbidity and daily diarrhea rate, increasing weight gain and improving FCR.

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Observations of common breeding farm weaknesses in Canadian swine farms found during application of the RAC, a systematic reproductive audit checklist

B Tully[1,2], S Drapeau[2], A De Grau[2]
[1]*Swine Health Professionals Ltd, Steinbach, MB, Canada,* [2]*Merck Animal Health, Kirkland, QC, Canada,*
btully@shpswine.com

Introduction

Following several years of challenging economic times in the swine industry, those farmers that continue to improve performance and achieve higher reproductive efficiencies have been able to maintain viable farming enterprises. The reproductive efficiency of a sow herd is the result of many separate and interacting factors[1]. Two years ago, we reported the development of the RAC (Reproductive Assessement Checklist), a comprehensive tool used by the herd veterinarian to assess all areas of reproduction on modern sow farms[2]. The RAC is a systematic evaluation of over 100 aspects of the breeding program on swine farms, including; sow/gilt management, farm objectives (including standard operating procedures (SOP) for weaning, breeding, feeding, gilt management, etc), semen management, boar management, insemination, gilt/acclimation management, gestation assessment, farrowing assessment and finally a post-visit summary[2]. This observational study will outline the common findings after application of the RAC on Canadian swine farms.

Materials and Methods

The RAC was used to assess reproductive performance weaknesses on 19 breeding farms across western Canada. Farms were selected for assessment based on having under-achieved breeding performance goals, including farrowing rate, repeat-estrus-rate, total born piglets, etc. Farms involved in this project were not related, had various health statuses, different genetic replacement stock sources, varied in size from 350 sow (using natural service breeding programs) to 3200 sows (using artificial insemination programs), including both farrow-wean and farrow-finish farms. As many farms used different record keeping systems, no attempt to objectively compare breeding performance was made between farms.

Discussion

Many of the under-achieving swine farms in this study had 5 things in common, regardless of the initial reproductive complaint:

- 95% (17/19) farms used poor estrus detection boars (teaser boars) showing low libido, often not exhibiting froth, not vocalizing or showing any interest in females. Most farms did not develop teaser boars well, housing boars together to become familiar, allowing boars to become old and stale and not allowing any natural service to stimulate libido.
- 89% (17/19) farm's sow stimulation and estrus detection post weaning was not performed well and many farms were not starting to observe sows until 4 days post-weaning.
- 84% (16/19) farms had inconsistent execution of SOP's. For example, within a farm, some farm workers started estrus detection on day 2 post-weaning, while others on day 4.
- 89% (17/19) farms spent insufficient time stimulating gilts and detecting estrus within the gilt pool.
- All of the farms' workers (100%) had poor understanding of basic reproductive physiology. Most people doing artificial insemination not understanding what key principles of natural boar breeding need to be applied within AI programs.

Conclusions

The execution of breeding protocols on swine farms are difficult to objectively compare, however the current study identified 5 key areas where more focus would result in improved performance. While no objective or subjective comparisons were made between poor performing breeding farms to those that achieve good reproductive performance, a next step could be to assess performance in herds based on changes to programs outlined in this paper. A benefit to the veterinarian in using the RAC on farms is to outline areas of weakness within breeding programs.

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A survey of infection intensity and various entero-sites invasion due to *B. coli* in weaning piglets at several farms in Southern Provinces, Vietnam

DTMai[1], DT Duy[1], NT Toan[1]
[1]*Faculty of Animal Husbandry and Veterinary Medicine, Nonglam University, HoChiMinh City, Vietnam*
duy.dotien@hcmuaf.edu.vn; toan.nguyentat@hcmuaf.edu.vn

Introduction

Basically, *Balantidium coli* (*B. coli*) is the largest protozoan parasite living in gut contents, colon mucosa and might cause bowel diarrhea in pigs, but rarely causes a severe outbreak[2]. The effects usually were subclinical state and/or cause prolonged mild diarrhea to reduce pig performance [2,3].

In fact, numerous previous studies only focused prevalence survey, without deeper assessment of infection intensity and damaged invasion on different segmental intestines due to *B. coli*[2]. Then, the main purpose of this study was to shortly investigate the intensity of infection, invasion, and damage in different segment of the intestines generated by *B. coli* in piglets collected from several pig-farms bearing with severe diarrhea and respiratory disorders.

Materials and Methods

19 weaning piglets affected complex of diarrhea and respiratory failures were collected from 9 pig-farms (located in four provinces contained high, intensive of pig production of the Southeast region, Vietnam). By routine post-mortem examination, intestinal gross-, microscopic lesions preliminarily assessed. The stool and intestine each pig simultaneously sampled at 3 sites (ileum, ileo-cecal valve, and spiral colon), then evaluated the rate infection, intensity (as described Mc Master method; Coles et al.[1], damage regarding field *B. coli* infection. In addition, samples of pooled intestinal/visceral organs on each surveyed sick pig also collected for testing the co-infection of *B. coli* with PRRSV, PCV2, and CSFV by conventional PCR and RT-PCR [Specific primers, standard protocols generated by Vet. Hospital., Nonglam University, Vietnam: Total RNA/DNA isolation (Promega, Madison, USA); GoTaq Green Master Mix (2X) for DNA amplification and reverse transcriptase (ML-VRT) for RNA amplification].

Results

The results showed that the *B. coli* prevalence through total stool sample was 63.2%. Interesting, the infection rate of *B. coli* found in ileum contain was 16.67% versus 63.2% in spiral colon ($p<0.01$). Infection intensity (table 1.), CPG ≥ 3000 accounted for the highest percentage at 58.3%, 1000≤ CPG <3000 accounted for 25.0%, and the last of CPG <1000 accounted for 16.7%.

In all 3 intensities of infection had the intestinal epithelium damage (necrosis, sloughing), enteritis with leukocyte infiltration and depletion of intestinal lymphoid tissue (image 1.). The level of damage in the intestinal epithelium increased upon the increasing of intensities of infection. In there, the cases of CPG ≥ 3000 appeared the dramatically histo-pathologic changes. There was high correlation between the prevalence of *B. coli* with those of PCV2 and PRRSV, respectively 75(9/12) % and 64.3(9/14) %. Particularly, the set of tri-coinfection of *B. coli*, PRRSV, and PCV2 reached the rate of 83.3(5/6) %.

Table 1. Infection intensity of *B. Coli* from spiral colon contents

No.	Infection intensity (CPG)	Frequency appeared	
		n	%
1	CPG < 1000	2	16,7
2	1000 ≤ CPG < 3000	3	25,0
3	CPG ≥ 3000	7	58,3
Total		12	100

CPG: cysts per gram: number of cyst/trophozoite B. coli insisted in 1gr feces

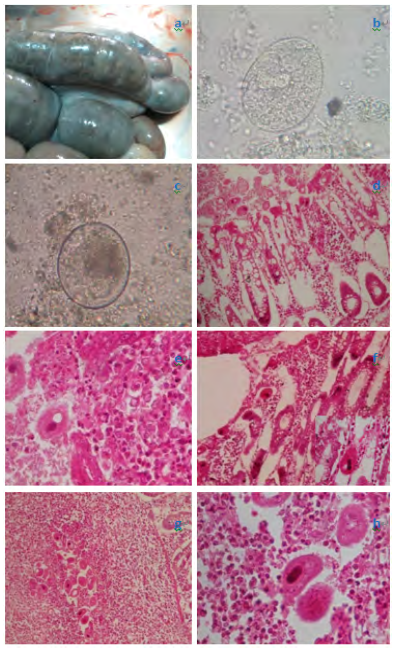

Image 1: Gross- and microscopic lesions in present study. (a) Gross-pathological lesion at spiral colon infected with *B. coli*, necrotic white spots scattered on out-surface. (b, c) morphology of trophozoite and cyst of *B. coli* (10X); (d, e) *B. coli* invaded into epithelium layers of spiral colon, necrosis, sloughing and inflammatory cells infiltration at epithelial layer were showed (10X, 40X); (f) *B. coli* invaded ileo-cecal valve; (g, h) huge *B. coli* cysts deeply invaded into ileum's inner sub-membrane (10X) in case of CPG = 8100, severe damage in mucous membrane with necrotic epithelial cells sloughing and infiltrated inflammatory cells (40X).

Conclusion and discussion

The degrees of damage in the inte nal epithelium w proportionally increased together with the intensities of *B. coli* infection, in that of ≥ 3000 CPG intensity had a biggest histopathology effect. The present study firstly found the invasion of *B. coli* into the small intestinal tissue (ileum; 16.7% prevalent of ileum contain). The result indicated that *B. coli* might be high pathogenic and a big concern in pig health in cases of co-infection with other known pathogens.

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Homogeneity and stability of a flubendazole oral suspension in drinking water

E Bousquet[1], D Leskovar[2], D Uršič[2], K Svetičič Gobec[2], S Combeau[3], J Goutalier[3]
[1]*Virbac Carros France*, [2]*Krka Novo Mesto Slovenia*, [3]*Phatophy Lyon France*, eric.bousquet@virbac.com

Introduction

Flubendazole is a benzimidazole compound active on digestive pig nematodes including an ovicidal effect. Its efficacy in pigs via feed has been reported (1). Nevertheless medication in drinking water would be a valuable alternative (no constraint of medicated feed manufacture, flexibility of treatment implementation), provided that a reliable and easy to use formulation would be available. Thus a new 10% flubendazole oral formulation has been developed, allowing treatment in water over 4 h per 24 h without stirring of medicated water required, followed by an easy cleaning of water equipment (Flimabo®/Flimabend®, Virbac/Krka). Efficacy of this formulation has been reported on *Ascaris sum* (2). Objective of this study was to test homogeneity and stability of the formulation in drinking water from stock suspension to drinkers delivery.

Materials and Methods

In a first step, a stock suspension containing 2.4 g/l of flubendazole was prepared for either the tested suspension (A) or a control 10% flubendazole emulsion (B). Following initial stirring, the stock suspension was either left unstirred (products A and B) or stirred every 30' (product B) for 4 h. Water samples were taken every hour on the top and the bottom of the stock suspension, with or without stirring for product B.

In a second step, a stock suspension was prepared with product A and left unstirred for 4.5 h during which a dosing proportioner incorporated the medicated water through a pipeline to nipples (either pig or poultry model) at the rate of 1.13% (theoretical end flubendazole concentration : 85 mg/l). Water samples were taken every 15' at the nipple level.

In both studies, flubendazole was assayed in water samples by High Performance Liquid Chromatography.

Results

Concentration in stock suspension ranged between 91% and 102% of theoretical one for product A without stirring over 4 h. For product B, when the stock suspension was stirred every 30′, concentrations below 90% of theory were measured at bottom level before each stirring. When the suspension prepared from product B was left unstirred, the phenomenon was enhanced, with concentration decreasing from 97% to 55% over 4 h (Table 1).

Concentrations at nipple level ranged between 94% and 115% of theoretical one for product A without stirring over 4.5 h.

Discussion

As flubendazole is not soluble in water, medication through this vehicle requires a formulation allowing homogeneity and stability of the medicated water suspension during the whole treatment period. The tested formulation (A) fulfilled these criteria from the stock suspension to the end drinkers without requiring any stirring over 4 h. For the control formulation (B), a demixing was observed in the stock suspension, even according to the recommended way of use (stirring every 30′), possibly leading to heterogeneity of medicated water distributed to animals and concern on treatment efficacy.

Conclusion

This study confirms that the tested formulation allows a reliable and practical way of flubendazole medication in water.

Table 1. Flubendazole concentrations in stock suspension (% of theoretical concentration)

		0 h	1 h	2 h	3 h	4 h
A	Top	98.9	94.2	96.4	94.0	95.7
	Bottom	101.4	94.7	90.9	97.8	101.8
B[1]	Top[a]	102.2	101.1	104.6	112.0	98.8
		-	108.6	97.8	108.7	110.1
	Bottom[a]	97.2	76.4	86.7	84.7	88.5
		-	112.3	108.0	101.0	110.2
B[2]	Top	91.8	94.6	98.8	82.7	91.8
	Bottom	97.0	71.3	65.3	58.1	55.2

[1]Stirring every 30′ [2]No stirring
[a]Before stirring (upper row) and after stirring (lower row)

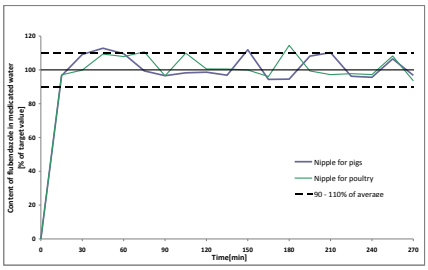

Figure 1. Flubendazole concentrations at nipple level

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Oral fluid collection as a means of diagnostic sampling in loose housed gestating sows

M Pierdon, A Martell, TD Parsons
University of Pennsylvania School of Veterinary Medicine, New Bolton Center Swine Research Group,
mpierdon@vet.upenn.edu

Introduction

Though the use of oral fluids for pathogen detection in swine was first described in the 1970's[3], rope testing to obtain oral fluids from swine is a relatively new diagnostic tool that has been used successfully for pathogen detection in pigs at many stages of production. In 2008 the technique was described in growing swine by Prickett et al.[4]. It has since been described in suckling piglets, gilt developers, and individually housed boars[1,2,5]. There has not been any research published on the use of rope testing for disease monitoring in sows.

As more sows are housed in gestation pens, the need arises for sampling methods that will be effective for this population. Our objective was to determine the optimum oral fluid sampling protocol for sows housed in large dynamic pens.

Materials and Methods

Oral fluid sample collection occurred at 8-10am once a week for 3 weeks at a 750 sow facility with 3 large pre-implantation dynamic pens for gestating sows. Each pen held ~175 sows and sows were fed via an electronic sow feeding system (Compident VI, Schauer Agrotronics). Cotton ropes, 5/8" in diameter, were hung as single or paired ~10 feet from the feeder entrance. Sow activity at the rope was video recorded via handheld cameras and analyzed using the Noldus Observer XT® software package. Number of sows to chew on a rope (NSC), time to first chew on a rope (TFC), and number of aggressive events at the rope (NAE) were scored. Feed rank (FR) was determined by taking the average order in which the sow entered the feed station over the week preceding sample collection. Normal data was analyzed using a two -way ANOVA with sampling day (DAY) and number of ropes (ROPES) as main effects. Shapiro Wilk test was used for TFC as it was not normally distributed. Correlations were performed using Spearmans and point biserial correlations.

Results

Findings are summarized in Table 1. The average number of sows to chew on a rope was ~20. Neither ROPES nor DAY influenced NSC. However, DAY did impact the behavior of sows choosing to chew on the ropes. There was a significant effect of DAY on TFC (H(2)=6.242, P=.0441) with sows approaching the rope ~30 times slower on day 1 compared to day 3 (P=0.0077). There was also a significant effect of DAY on NAE (F(2, 9)=5.25, P=0.0309) as they doubled between day 1 and day 3 (P=0.031). Sows with a lower FR (ate later in the day) were more likely to chew on a rope (R(413)=0.1564, P=.0014) whereas sows with a higher FR (ate earlier in the day) were more likely to initiate aggression at the rope (R(36)=-0.4261, P=.0085).

Table 1. Number of sows to chew, time to first chew and number of aggressive events on different days.

Day	n	Number of sows to chew (NSC)	Time to first chew (TFC)	Number of aggressive events (NAE)
1	3	15.7	644.4[a]	14.3[c]
2	4	20.5	241.9	28.0
3	6	21.7	24.4[b]	38.5[d]
All	13	19.92	234.1	29.7

(a, b) Superscripts indicate statistically significant differences within main effect (p =0.0077)
(c, d) Superscripts indicate statistically significant differences within main effect (p=0.031)

Conclusions and Discussion

Our work demonstrates the feasibility of oral fluid collection for diagnostic sampling in loose housed sows. A valid sampling protocol would include hanging at least 4 ropes per site in order to get a 60 sow or larger sample. The first time sows are sampled it will take longer for sows to approach the rope but the overall number of sows to chew is not affected. Sows that eat later in the day more often chewed on a rope likely because their activity patterns correlated with the time of sampling. However, those sows on the rope with a higher feed rank worked to dominate the rope by initiating more aggressive events. These findings suggest that both time of sampling and social status can impact sows contributing to the oral fluid sample. Further work is needed to investigate how disease presence may alter both sow behavior and social structure and potentially influence which animals interact with the rope.

Acknowledgments

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Comparative stability and performance of doxycyline oral solutions *vs* soluble powder products for administration through drinking water

F Caballero, J Serratosa, X Casas, M Foradada, M Jutglar
R & D Department. DIVASA-FARMAVIC, S.A. Gurb-Vic (Barcelona) Spain, info@divasa-farmavic.com

Introduction

Liquid concentrate oral solutions and soluble powders are two common pharmaceutical forms commercially available for doxycycline (DOX) based products intended to be used for mass treatments administered through drinking water (DW) in porcine and avian production facilities. Although soluble powders are generally accepted as more stable formulations since epimerization phenomena is not significant in this pharmaceutical form and degradation in DW is reduced with the addition of organic acids (2,3,), however, some new concentrated oral solutions are able to avoid epimerization and improve stability in DW due to the solvent system used, resulting in no significant differences in terms of stability between the two pharmaceutical forms. The objective of this work is to evaluate the pros and cons of each formulation based on the measure of different parameters such as solubility, stability and integration in automatic dosage systems.

Materials and Methods

Different commercial DOX products (two soluble powders D500, SP2, and two concentrated solutions D100/10, D200) have been evaluated for the following parameters: 1) stability in the original container (18 months); 2) stability in drinking water at therapeutic recommended dosage (24h); 3) stability in water concentrated solutions (24h); 4) solubility of concentrated water solution; 5) integration in automatic dosage systems (Dosatron®). For the evaluation of stability, DOX concentrations and their degradation products has been determined in the different samples by means of HPLC (4) Determinations related to products dissolved in water have been performed at intervals up to 24 h.

Results

Stability in original containers

All the studied formulations showed stability in their original containers with negligible amounts of epimers. Therefore similar DOX degradation levels were found for powders and oral solutions in their original containers.

Stability and solubility in Drinking Water

Soluble powders containing citric acid (D500) and solutions containing ethanol (D100/10 and D200) showed appropriate stability (degradation <5%) within 24 h study period. The product (SP2) not containing acids in their composition showed significant degradation (>5%) among the same period. Similar stability results were attained when the dosage was done at higher concentrations for use in automatic dosage systems, however for the less stable product (SP2) significant precipitates appeared at the bottom of the tank at 12 h after dilution being more apparent at 24h. Although all the products were dissolved in water at the tested concentrations, for powders, the time of dissolution was higher and agitation was required.

Table 1. Characteristics of tested products.

Product	Dosage form	Doxicicline concentration	Excipients
D100/10	solution	100 mg/ml	PG +EtOH
D200	solution	200 mg/ml	PG +EtOH
D500	powder	500 mg/g	Citric acid
Product 3	powder	500 mg/g	Lactose + Dextrose

PG = Propylenglycol; EtOH = Ethanol; D = DFV- Doxivet®

Table 2. Stability and solubility profiles of the tested products.

Product	Stability in original container	Stability in drinking water at therapeutic dose	Stability in water at high concentration	Solubility
D100/10	+++	+++	+++	+++
D200	+++	+++	+++	+++
D500	+++	+++	+++	++
Product 3	+++	+(+)	+(+)	+

Low = +; Medium = ++; High = +++

Conclusions and Discussion

Concentrated doxycycline solutions containing ethanol in their solvent system show equivalent stability to soluble powders containing citric acid. Although stability in the original container and in DW is equivalent in both dosage forms, more accurate dosage is expected in liquids due to mixing kinetics and lack of solubilization issues (3). Oral solutions have no solubility concerns for optimal performance of automatic dosage systems assuring the right dosage and the efficiency of the preparation even at high concentrations. Reduction of risks associated to tubes and nipples residues and their interaction to subsequent treatments can be also expected (1) with the use of oral solutions.

Table 3. Advantages of the tested products after the administration in drinking water under field conditions.

Product	DILUTION IN DRINKING WATER			SOLUBILITY ISSUES		
	Difficulty Speed		Homogeneity	Drug availability	Risk of tubes and nipples obstruction	Risk of interaction on subsequent treatments
D100/10	+	++	+++	+++	+	+
D200		+				
D500	++	++	++	++	++	++
SP2	+++	+	++	+	+++	+++

Low = +; Medium = ++; High = +++

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P.515

Influence of the solvent system in the stability of concentrated doxycycline oral solutions for veterinary use

M Jutglar, X Casas, J Serratosa, M Foradada, F Caballero
R & D Department. DIVASA-FARMAVIC, S.A. Gurb-Vic (Barcelona) Spain, info@divasa-farmavic.com

Introduction

Epimerization in C4 is a well known phenomena occurring in doxycycline (DOX) when is formulated as solution (1,2,3), being a concern to formulate long lasting concentrate solutions since the inactive degradation product 4-epidoxycycline (4-EDOX) is formed resulting in a significant decrease in the antibiotic activity and subsequent infra-dosage. Several products for veterinary use are formulated under this pharmaceutical form intended to be used for administration through drinking water.

The aim of this work is the evaluation of the influence of different solvent systems used to formulate doxycycline oral solutions for veterinary use on the loss of antibiotic activity due to formation of DOX epimers. The evaluation has been carried out in both, laboratory samples and commercial concentrate DOX oral solutions for administration through drinking water.

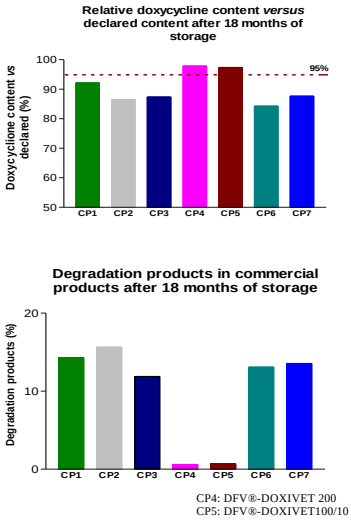
Materials and Methods

Concentrate DOX (10% and 20 % w/v) solutions based on pre-selected solvent systems containing propylene glycol and different co-solvents were prepared in the laboratory. In all prepared samples, the content of DOX and related 4-epimers were analyzed by means of HPLC (3) at different sampling times and after their storage in climatic chambers at 25ºC-60% RH (sampling time: 0,3,6,12 months) and at 40ºC-75% RH (sampling time: 0,3,6 months). Three additional sampling times (18, 24 and 36 months) were added for two of the prepared samples that showed enough stability and low epimer content at 12 months.

Seven different commercial solutions with different expiry dates were evaluated within their 18th month of shelf life. The exactly age of the samples was calculated as the difference of the expiry date and the authorized shelf-life declared in the summary of product characteristics (SPC). The information on the solvent system used was also extracted from the SPC.

Results

Five of the tested solvent systems were unable to stabilize DOX and avoid C4 epimerisation under the experimental conditions. A significant decrease on DOX initial content (degradation > 5%) associated to epimer formation was found in all formulations not containing ethanol as co-solvent. Although increasing amounts of 4-EDOX were found at the different sampling times in all formulations, the 4-epimer kinetics of formation was significantly slower (ANOVA; $p<0,05$) in two of the formulations containing ethanol as co-solvent (CP4 and CP5). Similarly the main degradation product found in different commercial products after 18 months of storage was 4-EDOX and formulations containing ethanol as co-solvent showed low rates of DOX degradation into their 4-epimer.



Conclusions and Discussion

The presence of significant concentrations of inactive degradation product 4-epidoxycycline at the end of the shelf-life is frequent in commercial DOX solutions. The solvent system used in the formulation play an important role in DOX stabilisation and avoidance of 4-epimer formation. Solvent systems based in ethanol avoid high epimerisation rates. An accurate selection of appropriate solvent systems is essential to ensure a proper doxycycline activity in concentrated solution during product shelf-life and avoid their degradation through epimerization.

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P.516

Real time recording for pig farms adopting batch production

J Carr; Y Harco; I Chipenkov, S Samarsky
Globinskiy svinokompleks Ukraine swineunit1@yahoo.com

Pig farm records are often difficult to interpret because standard parameters are summarised and averaged into calendar events, such as months (which have a variable time period) and which have no correlation to the timing of events on the farm. Farms of different sizes and production systems can be difficult to compare. The printouts often do not present the records in a time flow format – for example if the records start on a Monday but the weaning day is a Thursday, the animals bred are not the same animals which farrow. The results do not correlate different parts of farm in the same line. For example this batch's farrowing is 17 weeks behind the same batch which was bred.

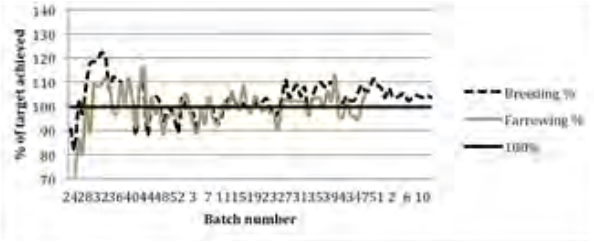
Batch production allows for a farm's records to be synchronised. Batch production starts at weaning and a new batch starts at the next weaning event. If this is accepted it will lead to a revolution in our ability to monitor farms in real time. The same batch of animals can be monitored in real time over the entire pig production cycle from gilt selection to slaughtering the finished pigs. Batch production allows for farms to be explicitly planned and realistic targets to be set. Records then can be used to determine if these targets are in fact achieved.

Having results presented relating to a batch allows for events such as failure to reach breeding targets, the impact of the number of gilts in a batch, the effects of over and under stocking to be easily visualised. Farms of different batch sizes can be easily compared using their ability to reach their set targets. A major cause of poor performance and disease outbreaks are associated with variation between batches.

Having farms with set batch targets has reduced the impact of variation on pig production. It creates more honesty within the system as the records are more transparent. Cost control between batches becomes possible and an area where money is lost becomes clearly apparent. The records can be used to enhance the welfare of the animals within the farm by ensuring that their individual environmental requirements are met. With reduced variation between batches health and medication requirements become easier to monitor.

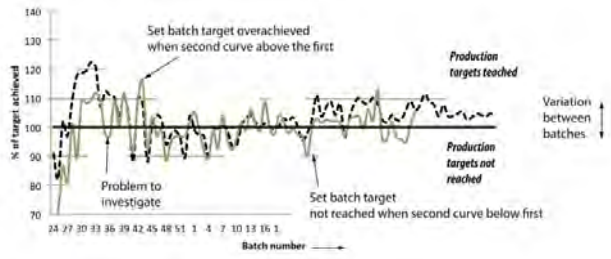
The results can be expressed graphically which can be very informative for the whole farm team.

Example: Breeding and Farrowing



With this example the farm initially had a 40% variation batch on batch (week on week). Once a rational batching programme, with sets production targets, was in place and accepted by the whole farm team, the variation between batches decreased to only 5%.

Comparison of the graphical report allows for instant analysis of production performance over time and in real time.



When the production lines diverge consistently batch targets need to be reassessed. The graphics allow for predictive reasoning of problems and successess. For example it can be seen when weaning numbers will not be achieved because the batch breeding target was not reached.

This method is easily applied to measures of breeding, farrowing, weaning, finishing and slaughter weights. Although finishing may be a compliation of 6 weeks of production (because pigs are sold from multiple batches).

Association of stillborn piglets and blood haemoglobin concentration in sows at farrowing

AK Jensen[1 2], S Bhattarai[1], JP Nielsen[1],
[1] *HERD-centre, Department of Large Animal Science, University of Copenhagen, Denmark*
[2] *God på Gris, Sandnessjøen, Norway,* annakatjensen@gmail.com

Introduction

A high number of stillborn piglets represent both an ethical and economic challenge in the pig production. The number has increased with increasing litter-size in hyper-prolific sows. Herd interventions in order to reduce number of stillbirths are often difficult unless caused by obvious infectious or management factors.

The hemoglobin (Hb) concentration in the sow has previously been studied in relation to stillbirths (1,2,3). Blood Hb is the main blood oxygen binder and transporter, and the level of Hb is influenced by a number of factors including iron-supplementation level.

The objective of this study was to determine the association between number of stillborn piglets and blood haemoglobin concentration in the sows at farrowing.

Materials and Methods

A total of 160 sows from three farrowing batches in a Danish 1700 sow farrow-to-finish herd were studied at the time of farrowing. Jugular blood was sampled within nine days before farrowing and a standard haematological profile was performed. Farrowings were induced with prostaglandin by the herd veterinarian. Dead piglets collected during and after farrowing were autopsied to determine whether they were stillborn. All fully developed piglets with uninflated lungs were considered stillborn. Number of liveborn and parity of sow was recorded after termination of farrowing.

The number of stillbirths was analysed in PROC GENMOD with negative binominal distribution (SAS® 9.2, Cary, NC, USA). Explanatory variables were blood Hb-concentration (g/L), number of liveborn piglets and parity.

Results

The mean Hb concentration of the 160 sows at farrowing was 114.5 (SD: 9) g/l. The mean numbers of liveborn and stillborn piglets per litter were 15.1 (SD:4.2) and 1.2 (SD: 2.1) respectively. The average parity of sows was 2.8 (SD: 1.8).

Factors influencing the number of stillborn piglets per litter was analysed in the model:

Number of stillborn piglets per litter = Hb_{sow} + liveborn + parity + parity*liveborn

Results from the model are shown in table 1.

Table 1. Association of Hb, number of liveborn and parity with number of stillborn piglets per litter.

	μ	Estimate	SE	P-value
Intercept		6.28	1.5	<0.0001
Hb, sow, g/L	115	-0.03	0.01	0.02
Liveborn, n	15.1	-0.2	0.04	<0.0001
Parity, n	2.9	-0.7	0.22	0.002
Liveborn*parity		0.05	0.01	0.0002

Conclusions and Discussion

The number of stillborn piglets per litter was associated with blood Hb-concentration in the sow, as well as number of liveborn piglets and parity.

The association between number of stillborn piglets and Hb-concentration in the sow may be related to oxygen supply during farrowing or related to e.g. the nutritional status of the sow. The sows in this study had a relatively low level of stillbirths (7%), which may be related to good farrowing surveillance. It was not recorded whether the stillborn piglets died before or during farrowing.

Further studies are needed to investigate whether sow Hb values can be increased by e.g. iron supplementation, and thereby serve as a herd intervention to decrease the number of stillborn piglets.

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A method of preventing and treating edema disease of piglets

I Zhirkov[1], G Zozulya[2]
[1]*World Academy for Animal Husbandry*, [2]*Volgograd State Agricultural University, Volgograd, Russia*
zhircov@gmail.com

Introduction

Edema disease of piglets is an acute, often-fatal enterotoxaemia of recently weaned pigs caused by a few serotypes of Escherichia coli. The strains O 8, O 9, O 20, O 137, O 138, O 139, O 141, O 142, O 147 and O 149 very often with hemolytic properties are etiologic agents of the edema disease in Russia. All methods of post weaning diarrhea and edema disease (antibiotics, preventing the use of adhesion, oral administration of specific antibodies, active immunization, passive acidification, etc) treatment(1,2,3), known up to 2005 were not effective enough. Moreover all of these tools are quite costly, and intestinal micro flora is able to adapt to the use of antibiotics. Our method of prevention and treatment of edema disease of piglets is the most economical and environmentally friendly. We proceeded from the hypothesis that the main cause of intestinal colonization by enteropathogenic strains of Escherichia coli is the lack of acid in the stomach barrier.

Materials and Methods

As a means of stimulating gastric acid secretion 2-3% aqueous solution of sodium acetate (JSC Khimprom, Volgograd) was taken. On the first day after weaning piglets were given 10 ml of 2 - 3% aqueous solution of sodium acetate from the cannula of the syringe to the root of the tongue. Then every morning before feeding - 5 ml of the same solution was given. To create additional artificial acidic barrier against pathogenic Escherichia coli (during outbreaks) piglets received an additional 5 ml of 2% aqueous acetic acid. The latter was given in the same way 12 hours after the drinking water solution of sodium acetate.

Experiments were carried out on post weaned piglets of Large White breed at the time of the outbreak. Escherichia coli (strain O 149: K88) was isolated from the feces of the sick animals. Experimental and control groups of animals (58-60 days) were formed from different litters by random sampling. Both groups of pigs (n = 20) were kept in pens with a common wall. Terms of feeding and housing animals were identical. Diseased pigs in the control group were treated with the drug "Pharmasin" (Huvepharma AD) according to the annotation as strains of E. coli isolated from the faeces were most sensitive to tylosin.

Results

In both groups the disease began to appear on the second day after weaning. Live weight of pigs at this point was 8.9 ± 0.7 kg (control) and 9.0 ± 1.2 kg (experience).

As it might be seen from Table 1, in the experimental group 2 piglets died (10%), whereas in the control group - 14 (70%). Moreover, at the end of treatment and preventive measures (on day 11 after weaning) all piglets of the experimental group were healthy and have increased appetite, while in control - the three of the seven animals had symptoms of diarrhea.

Table 1. The incidence of edema disease of piglets (*E. coli* O 149:K88)

Data	Groups of piglets	
	Control	Experimental
Morbidity (days)	115	47
Deaths (piglets)	14	2
Weight gain (kg/10 days)	0.17±0.03	0.8±0.2

Number of days of illness in treated group was 47 days, which is a 144.7% less than in control. Live weight gain of piglets of the experimental group was 0.8 ± 0.2 kg in the control - 0.17 ± 0.03 kg, i.e. 4.7-fold lower ($P < 0.05$).

Conclusions and Discussion

The experimental results showed proposed method for the prevention and treatment of edema disease to be the most effective. During post-weaning stress in the animal body sympatho-adrenal system is activated and leads to blockage of the parietal gland secretory function, which opens the gates for environmental micro flora (including various strains of E. coli)(4). During an outbreak of edema disease in piglets environment is a large number of enteropathogenic strains of Escherichia coli. Microorganisms enter the body through nutritional and colonize the small intestine. Exogenous acetate ion stimulates the synthesis of own gastric acid resulting in closing the entrance for environmental micro flora and stop the very disease. This method is patented in Russia (5).

Acknowledgments

Authors are thankful to Dr. Sergey Volkhonsky for help in research.

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Asian experience with the use of a live genotype-1 PRRS vaccine (productive and economic advantage)

Z Lapus[1], R Masilungan[1],
[1]*Philippine College of Swine Practitioners, Quezon City, Philippines,* renato.bijasa@hipra.com

Introduction

PRRSV has been considered as one of the most economically important swine diseases**. It was reported to cost USD** 67M in breeders and USD 493M in growing pigs. The disease causes high mortality in growing pigs**,** reproductive failures in breeding animals**,** increase in secondary infections **and h**high medication costs.

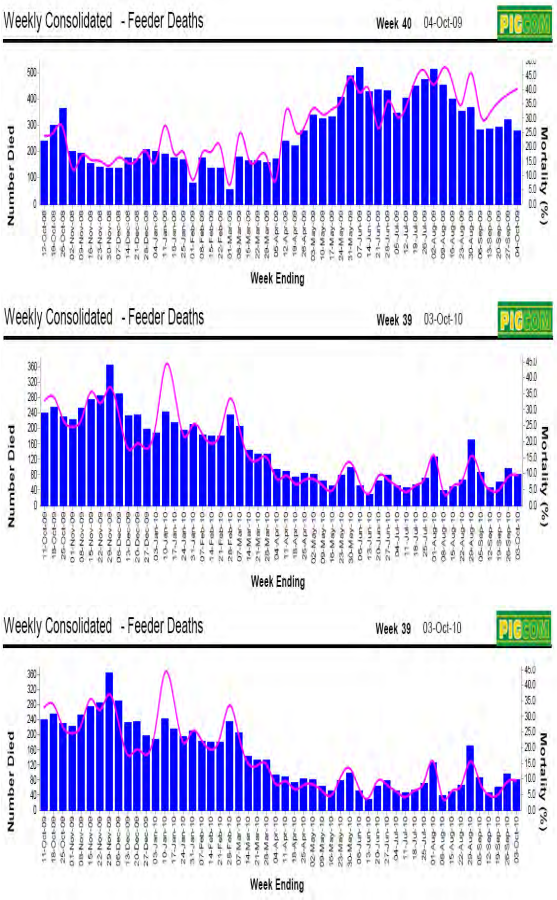
Farm History

This is a case Report of a 3,000 sow level farm located in Northern Luzon, Philippines. The farm was experiencing high mortality in weaning to market (4 to 23 weeks). Diagnosis was done using serology and PCR. The study was done from October 2008 to October 2010. After a pre-vaccination period from October 2008 to October 2009, Amervac PRRS vaccination was implemented from October 2009.Two mass vaccinations in sows with 4 weeks interval between vaccinations; thereafter-mass vaccination every 4 months was done. Further, mass vaccination of piglets aged 18-30 days, thereafter incoming batches of piglets at age 21. PIGCOM® recording system was used in monitoring production data. The vaccination period covered October 2009 to October 2010. A Cost – Benefit analysis was done to compare the two periods

Year	Week Period	Total Herd Mortality
2008- 2009	**12 Oct 2008 – 04 Oct 2009**	**4872**
2009 - 2010	**11 Oct 2009 – 03 Oct 2010**	**998**

Clinical Manifestations

Wean to Market Mortality after vaccination



Cost-Benefit Analysis

Economic Benefits	
Mortality Difference	**3874**
Margin per fattener sold (Php	**2,800.00**
Annual Realized profit (Php)	**10,874,200.00**
Additional Realized Profit/sow 2010 (Php)	**3,615.73**

Conclusions and Discussions

A reduction in mortality could be observed starting from the month of December 2009 and onwards (except for months with PED cases).Additional savings of at least €50/sow/year was realized (one parameter only – wean to market mortality).Vaccination using a MLV Genotype-1 PRRS vaccine is effective in controlling the negative impact of field PRRSV, thus improving farm profit.

Validity of lung scoring at slaughter in comparison with results from gross and microscopical pathology including laboratory diagnostics

M Genzow[1], G Mues[2], D Meemken[4], AP Mesu[3], G Schagemann[1]
[1]*Boehringer Ingelheim Animal Health GmbH, Ingelheim, Germany,* [2]*Veterinary Clinic, Hilter, Germany,* [3]*Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany,* [4]*Institute for Food Quality and Food Safety of the University of Veterinary Medicine Hannover, Foundation, Germany,* marika.genzow@boehringer-ingelheim.com

Introduction

Lung checks at slaughter are widely used to investigate respiratory health of pig farms. In Germany usually the scoring system based on Blaha (1) is used. Due to conditions at the slaughterhouse (line speed, batch integrity, correct identification) and limitations of a gross examination, the validity and interpretation of these scores can be questioned. In the present study the scoring of lungs at slaughter was compared to the scoring obtained in a diagnostic laboratory with both the same criteria and a more detailed scoring method. In addition, a subset of the lungs that presented with consolidation lesions were investigated in more detail by histology, immunohistology, bacteriology and PCR for relevant respiratory pathogens to determine the likely cause.

Materials and Methods

A total of 1433 lungs of fattening pigs from 20 different batches from farms in North West Germany were scored with the Blaha system at the slaughterhouse. Briefly, this method scores lungs from 0 to 3 based on the extent of gross consolidation. Lungs with no visible areas of consolidation are scored as 0. The 9-10 macroscopically most severely affected lungs from each batch were submitted to the diagnostic laboratory and again scored with the Blaha system and additionally with the system of Madec and Kobisch (2). Moreover, the macroscopically most prominently affected 4-5 lungs per batch were submitted for bacteriological, multiplex PCR (*Mycoplasma hyopneumoniae* (M hyo), *Mycoplasma hyorrhinis* (M hyorh), PRRS type 1 and type 2, Swine Influenza virus, PCV2, PRCV and PCMV), histological and immunohistochemical examination. Results were analyzed with SAS version 9.2. In order to determine the degree of association between different scoring systems for lung lesion scores Pearson`s correlation coefficient was computed. Results were considered statistically significant, if $p \leq 0.05$.

Results

The results of the lung scoring at the slaughterhouse according to Blaha revealed that 54% had a score of 0, 32% a score of 1 (lesions <10% of the lung surface), 12% a score of 2 (10% to 30% of the lung surface) and 3% a score of 3 (> 30%). In addition, 41% of the lungs showed apical consolidation (enzootic pneumonia like lesions) and 3% of lungs pleurisy. The scoring at the diagnostic laboratory (191 lungs) showed that 19% had a score of 0, 43% a score of 1, 33% a score of 2 and 5% a score of 3. In 67% of these lungs enzootic pneumonia like lesions and in 23% pleurisy was observed. The comparison of the two scoring systems in 191 lungs revealed a linear correlation between the two methods (Pearson's correlation coefficient of 0,82 ($p \leq 0.05$). Despite further laboratory investigations it was not possible to correlate apical lung lesions to any single specific pathogen. Apical lung lesions were observed in lungs negative for M hyo and without other histological lesions indicative of enzootic pneumonia. However, there was a statistically significant higher frequency of apical lesions in M hyo positive lungs when "suspect" M hyo IHC results were included ($p \leq 0.05$, Fisher`s exact test). In addition, there was a statistically significant higher frequency of BALT (bronchus associated lymphoid tissue) hyperplasia in M hyo positive lungs ($p \leq 0.05$).

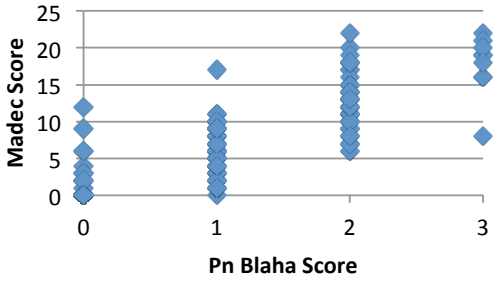


Figure 1. Comparison of 191 lung scores using the Blaha and the Madec & Kobisch system

Conclusions and Discussion

The authors conclude that lung lesion scoring at slaughter is a useful tool to monitor the respiratory health of pigs but is not suitable to identify the cause of lesions. In addition, the Blaha scoring method seems to be a comparable method for slaughter checks particularly with fast slaughterline speeds, but the Madec and Kobisch method may be more suitable in cases where in depth investigations are needed.

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P.521

Case report: PCV2 vaccination of piglets – a must?

C Veldman
DAP Horst, Horst, the Netherlands, dierenarts@daphorst.com

Introduction

PCV2 virus infection is well known for its potential damage in growing pigs (1). PCV2 vaccination is a highly effective tool to prevent growing pigs from production loss due to PCV2 associated disease (2). The objective of this paper is to describe what made up the decision to stop PCV2 vaccination in piglets and finally to re-start PCV2 vaccination.

Materials and methods

In a farrow to finish farm (1300 sows) with high production results the most important diseases were monitored and discussed 4 times a year. Piglets were PCV2 vaccinated (CircoFLEX ®, Boehringer Ingelheim) at the age of 3 weeks (group A, slaughtered January – July 2012). In February 2012 a decision was made to stop PCV2 vaccination of the piglets based upon lack of PCV2 related clinical signs, absence of PCV2 antibodies and reduction of costs (group B, slaughtered August 2012 – March 2013). The effect of stopping vaccination, was evaluated in the finishers every 3 months by serological monitoring (Ingezim PCV2 IgG/IgM ELISA, Ingenasa), clinical signs, production results (source: AgriSyst-PigExpert) and antibiotic use. The antibiotic use was calculated as the averages per month in a period of time, in gram of active matter per pig present at the finishing farm.

Results

After stopping PCV2 vaccination by the end of February 2012 the technical results did not change for 4 months. After 4 months (June 2012) in finishers clinical signs like growth retardation, lack of uniformity and PDNS were first reported. At the same time respiratory disease was an increasing problem and antibiotics were needed to control the situation. No positive serology for PCV2 was found. The decision was made to continue the program not to vaccinate the piglets for PCV2.
8 Months after stopping PCV2 vaccination PCV2 like clinical symptoms and respiratory disease had increased from the age of 15 weeks onwards and PCV2 serology turned out to be positive (table 1), confirming PCV2 infection. PCV2 vaccination was started again in November 2012 at the age of 3 weeks (group C, slaughtered April – December 2013), just about the same time when another kind of genetics was introduced. In time clinical signs disappeared and the amount of antibiotics needed for respiratory disease was reduced by 49% (table 2).

Table 1. Serological results on PCV2 antibodies (Ingezim PCV2 IgG/IgM), presented as positive samples to sample size

	Age (weeks)	IgM	IgG
27.1.2012	16	0/ 8	2/ 8
	23	0/ 8	0/ 8
4.6.2012	16	0/ 6	0/ 6
	23	0/ 6	0/ 6
22.8.2012	16	0/ 8	0/ 8
	23	0/ 8	0/ 8
3.10.2012	16	3/ 6	4/ 6
	23	0/ 6	5/ 6
29.05.13	16	0/ 8	0/ 8
	23	1/ 8	3/ 8

Table 2: Corresponding average monthly results of Average Daily Gain corrected to standard pigs from 25 to 112 kg live weight (ADG) and antibiotic use (AB-use/ pig/ month) of the 3 different groups in time.

Group	A	B	C
CircoFLEX	Yes	No	Yes
ADG (gram/day)	852	820	--
AB-use g/ pig/ month	0,00	0,63	0,32

Discussion

Vaccination is a preventive tool in pig management, but is also an investment. It is hard to predict the consequences of stopping a vaccination program and one has to consider the fact that PCV2 is ubiquitous.
When one decides to stop vaccination it is of importance to monitor the production results and to monitor on important diseases. If done so, one can restart the vaccination protocol in an attempt to prevent loss of production.
At this farm, after stopping PCV2 vaccination, ADG was reduced and antibiotics were needed to control disease. Due to a change of genetics a comparison of the production results between group C and group A or B was not possible. After re-starting PCV2 vaccination the PCV2 like clinical symptoms disappeared and the need for antibiotics in group C compared to group B was reduced.

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Influence of *A. pleuropneumoniae* and swine influenza virus on lung findings at slaughter from pigs vaccinated against *M. hyopneumoniae* and PCV 2

J Seitz[1], R Fux[2], M Ritzmann[1], M Eddicks[1]
[1]*Clinic for Swine, Ludwig-Maximilians-University, Oberschleissheim, Germany,* [2]*Institute for Infectious Diseases and Zoonoses, Ludwig-Maximilians-University, Munich, Germany,* m.eddicks@lmu.de

Introduction

Reasons for respiratory diseases on pig farms are often multifactorial and summarized as the porcine respiratory disease complex (PRDC). Despite vaccination against M hyo and PCV2 economic losses due to pneumonia or pleurisy at slaughter can still be a problem on some farms (1, 2). In the present study farms whose pigs were suffering from pleurisy and/or lung damage at slaughter despite standard vaccination against M hyo and PCV2 were examined for possible responsible co-infections.

Materials and Methods

270 pigs from 9 Bavarian finishing farms were included into this study. Blood samples were taken at the end of the finishing period and examined for antibodies against SIV and APP. 20 % of the samples that were positive in a Swine Influenza Virus (SIV) or Actinobacillus pleuropneumoniae (APP) screening-ELISA were further serotyped (ST). At abattoir a lung score was carried out (3). The vaccination status of each farm is displayed in table 1. To recognize the influence of different APP or SIV serotypes on the lung score the Mann-Whitney U Test was carried out and odds ratios (OR) were calculated to determine the chance develop pleurisy. Only animals from farms not vaccinated against APP (n = 180) or SIV (n=270) were included into the calculations.

Results

The results of the mean lung scores and amount of animals with pleurisy for each farm are shown in table 2. Animals from farms positive for APP serotypes (ST) 12, 1, 9, 11 and SIV H1N1 and H3N2 had significant higher lung scores at slaughter than farms without corresponding findings additionally the chance to get pleurisy was 8.9 and 3.4 times higher for animals from farms positive for APP ST 2 and ST 10 respectively (table 3).

Conclusions and Discussion

In cases of increased lung scores at slaughter despite vaccination against M hyo and PCV2 the involvement of SIV H1N1, H3N2 or APP ST 12, 1, 9, and 11 and in cases of additional pleurisy APP ST 2 and 10 should be clarified to develop additional strategies to improve the lung health on the farm and to avoid financial losses due to lung alteration and pleurisy. These pathogens may then be encountered by optimization of management and environmental conditions and if necessary by adaptions in the vaccination protocol.

Table 1. Vaccination status of each farm.

farm	1	2	3	4	5	6	7	8	9
PCV2	+	+	+	+	+	+	+	+	+
M hyo	+	+	+	+	+	+	+	+	+
APP	-	+	-	-	-	-	+	+	-
PRRSV	-	+	-	-	-	+	-	+	-
SIV	-	-	-	-	-	-	-	-	-

Table 2. Farm status, mean lung score (LS) and percentage of pleurisy positive pigs (PL) at slaughter.

Farm	APP	SIV	LS	PL %
5	c,d*	-	2.0	23.3
1	a,c,d,g*	-	2.2	6.7
8	a,b,c,d*	a°	2.7	13.3
4	a,b,c,f,g*	-	5.2	63.3
6	b,c*	c°	5.3	53.3
3	a,b,c,g*	a°	7.1	70
7	a,c,d*	a°	8.4	23.3
2	b,c,g*	-	11.1	80
9	a,c,d,g*	a,b°	11.2	16.7

Table 3. Results of statistically analysis regarding lung score and pleurisy, APP (n = 180), SIV (n = 270).

	lung score[1]			pleurisy
pathogen on farm	**no**	**yes**	**p-value[2]**	**OR**
APP ST 2	5.1	5.8	p = 0.566	9.6
APP ST 10	5.5	5.2	p = 0.108	3.4
APP ST 12	3.6	6.4	p = 0.039*	-
APP ST 1,9,11	3.6	6.4	p = 0.039*	-
H1N1	4.4	7.4	p < 0.001*	-
H3N2	4.9	11.2	p < 0.001*	-

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Usage of the pig as a surgical model in veterinary teaching

L Aguilar[1], C Romero[1], A García-Contreras[2], J De Loera [3], L Bautista[1]
[1]C.U. UAEM Amecameca, [2]UAM-Xochimilco, [3]FES Cuautitlán-UNAM, cromeron@uaemex.mx

Introduction

Historically, the use of live animals is a common practice in veterinary teaching, in disciplines such as surgery, physiology, biochemistry, anatomy, pharmacology and parasitology (3). However, nowadays many harmless alternatives are available, for example by using simulators, noninvasive auto experimentation and supervised clinical experiences (1). Surgical simulation systems have more advantages than the traditional methods, these are: reduction in costs associated to corpses and live animals, providing experience to the doctor, and allowing the possibility of repetition of the surgical procedures (2). Furthermore, the teachers can serve better to both students and animals and simultaneously save time and avoid hurting the animals (3).

Materials and Methods

The study was conducted in the Centro Universitario UAEM Amecameca (Universidad Autónoma del Estado de México). In this study 95 students participated; 63 students from the veterinary medicine and zootechnics degree (who at the time were studying the surgery module), and 32 Business Administration. Students. Students were divided in 2 groups in order to differentiate the acquired psychomotor skills, in a experimental group (veterinary), we conducted the study based on the use of biologic simulators (skin, intestine, stomach and pig bladder) and the skill acquisition through the surgical practices, likewise we gave the Gibson test (pre and post teaching), and the Gibson test was also given to the control group after 24 hours without surgical practices. The Gibson test was used to evaluate the skill and ability to execute an activity and evaluate the learning method using the simulators, also the relation among the eyes, brain and hand to execute the activities. The variables evaluated were: time in seconds and mistakes made pre and post-surgical practices in the Gibson spiral performance. Additionally, the Tukey and Kruskal-Wallis tests were performed.

Results

We observed that the veterinary students decreased the time to perform the Gibson test after the surgical practice (33.02 ser.) compared with the pre practice evaluation (34.69 ser.), we also significant differences (p=0.035) between both evaluations, on the other hand there were no differences (p=0.359) in the mistakes committed in the psychometric test; but there was a numeric disminution of 10.95% after performing surgical practices. When comparing the psychomotor skills of the students from the control group pre and post teaching, There are differences of 7.73 seconds after the practice, which correspond to 19.32% less of the time.

Conclusion and Discussion

The simulation in medical teaching provides a safe learning atmosphere for the student and patient, Sernal *et al* 2012 argue that the simulation allows the teacher, as well as the student, to define a particular dynamic for a medical activity or for the understanding of a specific concept, and the permanent repetition of the simulation model. In the present study we observed that the simulator training helps achieve learning before the contact with the patients. The pig organs turned out to be a good model for veterinary surgical teaching. Furthermore, they come from slaughterhouses where animals are humanely sacrificed, the size, price and the availability favour their usage, on the other hand the pig as a surgical model replaces other species that cannot be used nowadays such as dogs and cats, given that t the pig is bred to be sacrificed, if appropriate surgical and anesthetics procedures are performed, it can be an excellent surgical model and for human medicine students and biomedical studies.

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Sideropenic anemia in piglets: Comparative study of different iron supplementation forms and products

C Pommellet[1], D Dréau[2], O Merdy[3], F Joisel[3], A Laval[4]
[1]*COOPHAVET, Ancenis, France;* [2]*CECA Vetos, Saint-Allouestre, France;*
[3]*MERIAL SAS, Lyon, France;* [4]*ONIRIS, Nantes, France,* francois.joisel@merial.com

Introduction

In neonatal piglets, iron supplementation may be performed by parenteral administration of dextran iron which is available in complexes of ferric hydroxide and dextran iron or complexes of ferrous glucoheptonate dextran. Previous studies have reported either the superiority (1) of the latter regarding hemoglobin levels and weaning weight or the equivalence between the two forms (2). In addition, iron can also be supplemented *per os* but this route gives also inconsistent results as compared to parenteral route (3,4). The present study aimed to compare on hemoglobin levels and growth of piglets the efficacy of 2 forms of injectable iron and one form of oral iron.

Materials and Methods

The trial was conducted on a 300-sow farrow-to-finish operation located in France. Twenty-seven litters of at least 10 piglets for a total of 298 piglets, born on the same day from multiparous sows of same farrowing date were selected over two consecutive batches. They were allocated to 3 treatment groups :

- Group IM1 (n=9 litters): ferric hydroxide and dextran iron (200 mg/ml), IM, 1 ml at 3 days of age,
- Group IM2 (n=9 litters): glucoheptonate dextran iron (200 mg/ml), IM, 1 ml at 3 days of age,
- Group PO (n=9 litters): oral iron supplement, 10g per administration, at 3, 7 and 10 days of age.

Injectable treatments were administered with 1.3x16-mm needles for the first batch and 0.8x16-mm ones for the second batch. The oral iron supplementation in the PO group was offered directly on the dry & clean floor, near the piglet resting area and out of reach of the sow.

Each piglet was weighted at 3 and 20 days of age. Blood samples were collected at 3 and 20 days of age and assayed for hemoglobin levels by a HEMOCUE® analyzer (Hemocue AB, Ängelhom, Sweden).

Statistical analyses of hemoglobin and bodyweights records were conducted with SYSTAT®12 (Systat Software, San José, CA, USA) by fitting a generalized linear model with batches and group factors as fixed factor, sow factor as nested factor, as well as their interaction. Hemoglobin levels and bodyweights recorded at 3 days of age were respectively included as covariable. Anemia in piglet was defined as a hemoglobin concentration < 9g/100mL. Proportions of anemic piglets were compared using Chi-square tests.

Results and discussion

There was no significant batch effect on average ($p=0.406$); the treatment effect was significant (Figure 1). Hemoglobin levels were statistically lower in PO group as compared to IM1 and IM2 groups ($p<0.01$) for the two batches. This yielded a significantly higher proportion of anemic piglets in PO group (Table 1). Hemoglobin levels were also lower in IM1 group as compared to IM2 group in batch #1 ($p<0.01$) contrary to batch #2 ($p=0.14$). This result was attributed to a leakage of IM1 product during injection due to an inappropriate needle size. The use of smaller size needles in batch #2 corrected this difference: IM1 and IM2 groups had then similar results.

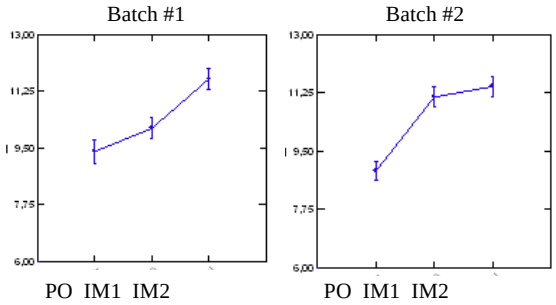

Figure 1. Least-square means of hemoglobin levels at 20 days of age according to batches and groups.

Table 1. Prevalence of anemia according to batch and group.

Group	PO	IM1	IM2
Batch #1	44%	2%	0%
Batch #2	55%	0%	3%

There was no incidence of the treatment group on bodyweights at 20 days of age. However, large litter effect were evidenced, what could have hidden a possible treatment effect.

Conclusion

Under the conditions of the study, there was no difference between injectable forms of iron as far as adapted needles are used. Hemoglobin level in piglets fed oral iron were significantly lower than in the injectable iron groups.

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P.525

Association between pleuritis and esophogastric lesions in Danish finishers

EO Nielsen, S Haugegaard, MBF Nielsen
Pig Research Centre, Danish Agricultural and Food Council, eon@lf.dk

Introduction

Lesions in the oesophageal part of the stomach are found in finishers at slaughter. A Danish study found ulceration or strictures of the oesophageal part of the stomach in 11% out of 1,101 finishers [1]. Fine feed structure is a known cause of oesophagogastric lesions, although other causes such as starvation and illness have also been suggested [2]. This study investigated a possible association between lung lesions and stomach lesions.

Materials and Methods

Lungs and stomachs from a total of 1,518 finishers from 56 pig herds were collected at two abbatoirs. The herds were selected on the basis of prior knowledge of a high frequency of pleurisy lesions. The farmers were contacted for information on feeding. Finishers from each herd were sampled on one day. The macroscopic findings in the stomachs (lesions scored for increasing severity: index 0-10) and lungs were described. A logistic regression analysis was performed using GLIMMIX SAS with stomach index (index 0-7 vs. 8-10) as outcome, explanatory variables were ready-made pelleted feed vs. meal feed, and findings of lung lesions were recorded as yes/no. The herd of origin was included as a random effect.

Results

A total of 19% of the finishers had severe stomach lesions in the form of ulceration or strictures of oesophageus (index 8-10). Lung lesions consistent with pleuritis were found in 59%, lesions consistent with pleuropneumonia were found in 5%, and lesions consistent with Myoplasma-associated pneumonia were found in 19% of the finishers. A total of 33 farmers in the study used meal feed, 18 farmers used ready-made pelleted feed, and the last five farmers provided no information on feeding. Logistic regression analysis gave the following results:

Lungs with pleuritis were significantly associated with severe oesophagogastric lesions (index 8-10) (P=0.032, OR=1.50, 1.03-2.16 95% CI). Other lung lesions were not significantly associated with oesophagogastric lesions.

Ready-made pelleted feed was significantly associated with oesophagogastric lesions (P<0.0001, OR=8.94, 4.59-17.39 95% CI).

Conclusion

A statistically significant association was found between pleuritis and oesophageal lesions in the form of ulceration or strictures in the stomach (OR=1.50). The aetiological background of this association remains to be investigated. Ready-made pelleted feed was strongly associated with the occurrence of oesophageal lesions (OR=8.94).

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Monitoring of important pathogens of swine respiratory disease (PRDC) by serological and PCR-methods in Bavarian sow herds

A Rostalski[1], A Pausenberger[2], M Alex[1], B Janowetz[1], H Niemeyer[1]
[1] *Bavarian Animal Health Service, Poing, Germany,* [2] *ELANCO Animal Health, Bad Homburg, Germany,*
anja.rostalski@tgd-bayern.de

Introduction

For a successful herd health management in modern swine production regularly performed routine diagnostics are an essential part of monitoring the health status in swine herds. The Bavarian animal health service established a serological screening based on testing tissue fluid from testicles collected at castration of piglets for Swine Influenza Virus (SIV), *M. hyopneumoniae(M.hyo*) and Porcine Respiratory and Reproductive Syndrome Virus (PRRSV) (1). Whether the serological status of the sow herd allows any estimation of the colonization prevalence in the nursery tested by PCR on tracheobronchial swabs (TBS) was the objective of this study.

Materials and Methods

32 sow herds from Bavaria with an average size of 158 sows (40 - 430 sows) were included. During castration routine obtained testicles or blood samples of 32 sow herds (425 samples in total, 10-15 samples per herd) underwent standard serology for *M.hyo*, PRRSV and SIV by Herdcheck-ELISA©, Idexx. TBS were collected 5-6 weeks (TBS1) and 11-12 weeks (TBS2) after castration from the nursery and tested by PCR (3) for the same pathogens in the laboratories of Bavarian Animal Health Services, Poing, Germany. Furthermore, data about management and vaccination routines were assessed by questionnaire.

Results

Antibodies against all three pathogens were detected in most of the herds (Tab. 1). None of the farmer vaccinated sows against *M.hyo;* 20 and 18 farmers vaccinated against PRRSV and/or SIV respectively, so serological results are at least in part due to vaccination.

Table1. Number of positive herds (S+) and prevalences within herds (Mean, Standard Deviation)

Pathogen	No. of pos. (n=32) and (%)	Prevalence within herd (Mean / SD)
M.hyo	31 (96,9%)	57,06 (+/- 31,08)
PRRSV	25 (78,13%)	56,87 (+/-39,34)
SIV	31 (96,9%)	90,10 (+/-17,63)

PCR-results from TBS in the nursery did clearly differ from the serology results. Mean intraherd prevalences of antibody and PCR-results are summarized in Fig. 1. In case of SIV the high prevalence of antibodies coincided with a low frequency of PCR-positive results with a decline from TBS1 to TBS2. A similar picture was found for *M.hyo*, but with a slight increase of positive samples from TBS1 to TBS2.

The highest prevalence of PCR-positive results was observed for PRRSV with a clear increase from TBS1 to TBS2. Remarkably, serologically negative herds always delivered negative results from TBS for all three pathogens, whereas within the serologically positive herds all kinds of combinations were found with positive and negative TBS-results no matter whether farms were vaccinating or not.

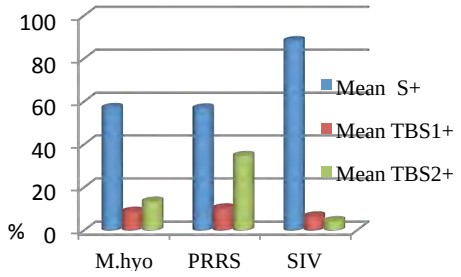

Figure 1. Mean % of seropositive results from sows (S+) compared to PCR-positive results (TBS1+, TBS2+) from nursery

Conclusions and Discussion

Different pathogens are involved in PRDC in swine herds. Herd immunity as well as management has an impact on the degree of severity and frequency of the disease in each production compartment (4). Regular serological examinations of key pathogens in the sow herd deliver a good idea of absence or occurrence and prevalence of pathogens but no forecast estimation of the situation in the nursery.

Acknowledgement

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Ascaris suum and other parasites in intensive farming production in Argentina

L Alarcón[1]; N Streitenberger[1,2]; E Perez[2,], W Galván[1], L Fazzio[1], J Cappuccio[1], E Mateu[3]
[1]Clínica de Grandes Animales y [2] Patología Especial. Facultad de Ciencias Veterinarias, UNLP, 60 y 118 SN. La Plata. Buenos Aires. República Argentina. lalarcon@fcv.unlp.edu.ar, [3]Centre de Recerca en Sanitat Animal (CReSA), UAB-IRTA, Campus UAB, Bellaterra, Spain.

Introduction

Ascaris suum (AS) and *Trichuris suis* (TS) are among the most widespread internal parasites of pigs (1). Monitoring of those parasites is mostly done by the detection of eggs in stool (floatation technique) or at necropsy. However, false negative results may arise with *Trichuris suis* (TS) where egg laying by adults and the emptying of the cecum is sporadic (2). The aim of this study was to determine the presence of parasites in intensive farms in Argentina, by combining classical floatation techniques detection of eggs in stool with necropsy and histopathological studies in order to assess the apparent sensitivity of the different diagnostic approaches.

Materials and Methods

Six farms (500-2,500 sows/farm) designated A to F, were included in the study. Monitoring was initially done by means of a random sampling of faeces of pregnant and lactating sows and pigs of 30, 60, 90, 120 and 150 days of age. Faeces samples were sent to the laboratory where they were examined for the presence of eggs of AS, TS and thin shelled eggs compatible with *Strongyloides* spp according to the modified McMaster egg counting technique. Results were expressed as eggs/gr. In parallel, animals found dead in each of the farms were necropsied and gross lesions were recorded. In farms C to F that presented cases of diarrhea in weaners or fatteners, when intestinal lesions were observed at necropsy, contents were taken for bacteriological culture of Salmonella spp and for PCR for *Lawsonia intracellularis*, *Brachyspira pilosicoli* and *Brachyspira hyodysenteriae* according to previously published methods (4). A section of small and large intestine was taken and fixed in 10% formalin for further histopathological examination.

Results

Ascaris eggs were detected in samples from farms A, B, E and F. Milk spots were seen (3/31) in necropsies of pigs of >60 days of age in farm F. TS eggs were not detected in any of the examined samples. In E and F farms, histopathological examination revelealed larvae of TS in cecum of animals showing catarrhal colitis (1 animal in E and 6 pigs in F). Table 1 shows the results of the McMaster floatation technique for *A. suum*. Thin-shell eggs were seen in samples from all farms but A. In all cases, egg count were always below 100/gr and distributed in all examined ages. None of the faecal contents were positive for *Salmonella*. Farms D and E yielded positive results in the PCR for *L. intracellularis*.

Table 1. Results of the analysis of *A. suum* eggs by floatation of collected faecal samples in six farms.

Farm	A	B	C	D	E	F
G*	5/9 74±42	1/20 160±NA	0/20 NA	0/19 NA	2/20 140±170	16/20 190±180
L**	0/5 NA	1/17 20±NA	0/20 NA	0/20 NA	0/20 NA	17/20 187±168
30***	0/5 NA	0/8 NA	0/10 NA	0/6 NA	0/10 NA	0/10 NA
60	1/9 40±NA	0/1 NA	0/10 NA	0/8 NA	0/10 NA	1/10 20±NA
90	2/8 35±35	0/10 NA	0/9 NA	0/10 NA	1/10 20±NA	0/10 NA
120	3/5 120±40	0/8 NA	0/8 NA	0/10 NA	0/10 NA	0/10 NA
150	4/5 465±640	0/10 NA	0/7 NA	ND	0/10 NA	0/10 NA

*G= gestating sows; **L = Lactating sows, ***age of sampled piglets. NA=does not apply; ND=not done.

Conclusions and Discussion

Most often monitoring of intestinal parasites of swine is done by floatation and/or observation of adult forms in the necropsy. As revealed in the present study negative results in the modified McMaster technique have to be interpreted with caution. Other authors (5) have shown that the sensitivity of this technique is lower than 60% with egg counts/gr $<10^2$. Moreover, the fact that egg laying by adult parasites and the emptying of the cecum is sporadic (1,3) may contribute to false negatives as well. The absence of adult forms in the intestine of necropsied animals did not exclude the presence of larval forms in the gut. The results of the present study emphasize the need for combining techniques in order to have an accurate assessment of the effective control of intestinal parasites.

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Evolution of sow productivity in Colombia during the last 10 years

M Aparicio[1], MA de Andrés[1], J Morales[1], J Naranjo[2], W Silva[2] D Rodriguez[2], C Piñeiro[1§]
[1]*PigCHAMP Pro Europa SL, Segovia, Spain;* [2] *Colombian Swine Producers Association,CENIPORCINO, Bogota, Colombia,* joaquin.morales@pigchamp-pro.com

Introduction

Sow productivity has linearly increased in last 10 years in many countries. In Colombia, the pig sector has made tremendous efforts to increase production and to improve its competitiveness. This effort is reflected in the modernization of pigs farms and the improvement of production parameters, hence products of excellent quality.

A proper collection and analysis of data generated in farm has helped to this improvement, specifically the National Health Improvement Status Program (PNMES Spanish acronym) promoted by the Colombian Swine Producers Association that allowed collecting, analyzing and even monitoring data from different farms distributed across the country.

The objective of this study was to analyse the evolution of sow productivity during the last 10 years the farms attached to the program (PNMES).

Materiasl and Methods

The present study analyses key performance indicators from 20 farms from Colombia. Reproductive data were registered with different software packages and processed and analyzed by PigCHAMP Pro Europa (Segovia, Spain). The current number of reproductive sows in all 20 farms is about 7,600 (average inventory = 380).

In all farms, main variables related to sow productivity were recorded, included total born per sow (TB), born alive (BA), stillborn (expressed as percentage of TB), weaned piglets per litter and farrowing rate. Fertility failures were registered, taking into account data of the event to split into different causes: short return to oestrus (<18 days after artificial insemination; AI), cyclic return (18 to 24 days or 38 to 44 days after AI), acyclic return (25 to 37 days after AI), late return to oestrus (45 to 59 days after AI), sow found not pregnant (in days 60 to 110 after AI), fail to farrow (>110 days after AI), abortion or due to cull or death of the sow.

A descriptive analysis of main variables was conducted to present evolution in last 10 years.

Results

Total born, BA, percentage of stillborn evolutions are presented in figure 1. Prolificacy showed a linear increase since 2004, reaching the maximum value in 2013 (12.7 TB and 11.7 BA). Percentage of stillborn showed a slight decrease since 2004, being currently almost 1 percentage unit lower than 10 years ago. Number of weaned piglets per sow also increased, in a parallel way to BA, reaching the maximum in 2013 (10.7 vs 9.2 in 2004).

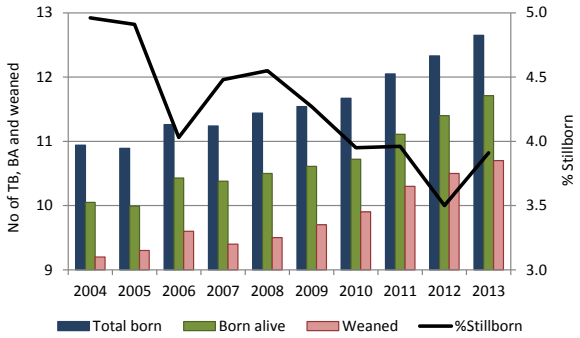

Figure 1. Evolution of total born (TB), born alive (BA), percentage of stillborn and weaned piglets per litter in 2004-2013.

Comparing fertility rate in last two years (2012 vs 2013; figure 2), a reduction of pregnancy losses has been observed. This improvement was mainly associated with a reduction in return to oestrus, both cyclic and acyclic returns, and in not-in-pig sows (sows found not pregnant in days 60 to 110 after insemination and sows fail to farrow).

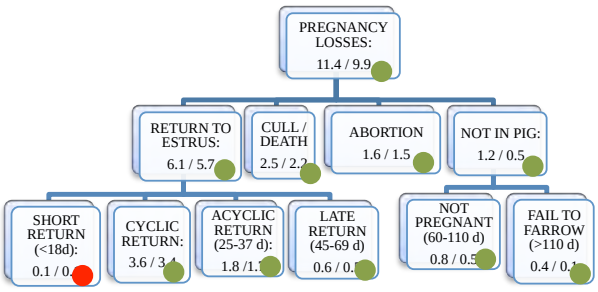

Figure 2. Productivity tree of pregnancy losses in 2012 (left) and in 2013 (right). A traffic light alarm indicates if each variable has improved (green) or not (red) in this 2-year period.

Conclusions

Improvement in TB is the main cause of the increase of BA in these last years, because the ratio between them has been kept almost exact through this time. In addition percentage of stillborn has been also reduced with time. Consequently, we can conclude that the application of the PNMES in Colombian swine farms has a positive effect on the sow productivity. Additionally, the strategies addressed by the PNMES toward the appropriate implementation of biosecurity, management and monitoring of disease status through laboratory diagnosis of main diseases present at farms may be associated with these results.

P.529

Schwannoma in sow's lung: A case report

TP Resende[1], CER Pereira[1], FA Vannucci[2], FS Araujo[4], JL dos Santos[2], GD Cassali[3], KA Damasceno[3], RMC Guedes[1]
[1]*Department of Veterinary Clinic and Surgery, Veterinary School, Universidade Federal de Minas Gerais, Belo Horizonte,* [2]*Laboratório de Microbiologia Veterinária, Microvet, Viçosa,* [3]*Department of General Pathology, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil,*[4] *Veterinarian practitioner, Ponte Nova-MG, Brazil.*
guedesufmg@gmail.com

Introduction

Nodular lung lesions are frequently associated with abscess caused by different bacterial agents (1) or granulomatous pneumonia caused by *Mycobacterium bovis* and *M. tuberculosis*. Anyhow, it is a rare find in modern pig farming (2). However, proliferative lesions are rare in swine, gross finding of nodular lesions need differential diagnosis of granulomatous nodules, abscess or proliferative masses. This is the description of a multiple nodular lesions identified as a Schwannoma in pig lung.

Materials and Methods

A first-parity sow with respiratory signs and clinical suspect of pneumonia was slaughtered. Several whitish firm nodules, not encapsulated, with size ranging from 0.5 to 5 cm in diameter had been found and distributed by pulmonary parenchyma, in all lobe (Fig. 1). Mediastinum lymph nodes were moderately increased. Tumor tissues were fixed in 10% formalin, processed routinely and stained with hematoxilin and eosin (HE). Masson's and Gomori trichromes staining were performed to distinguish cells of the connective tissue, as well as immunohistochemistry (IHC) test with vimentin, desmin, cytokeratin and S-100 protein antibodies

Results

Histologically, the tumors in the nodes were composed by dense nodular cell neoplastic proliferation, with infiltrative appearance. Neoplastic cells arranged in irregular bundles supported by dense connective tissue layer with sarcomatous appearance. Elongated cells with eosinophilic and homogeneous cytoplasm. Marked anisocytosis and moderate anisokaryosis. Central rounded nuclei with loose chromatin. There was moderate mitotic index (Fig. 2). These findings were consistent with malignancy of mesenchymal origin (sarcoma). Masson's and Gomori trichrome staining did not demonstrate staining pattern compatible with connective tissue. At IHC almost all neoplastic cells stained positively for vimentin and S-100 protein and negative for desmin and cytokeratin.

Conclusions and Discussion

Based on the macroscopic findings, the hypothesis of tuberculosis was discarded once the nodules were not encapsulated, without caseous or calcified content and were not filled by purulent or necrotic content as an abscess (1). Neoplasms are rare in swine, especially in lungs. Kubota et al (3) described a myofibroblast sarcoma in lung in a 7-month-old female crossbred pig, but it was characterized by well encapsulated, firm, fibrous masses of varied size and homogeneous grayish white, sometimes with areas of red discoloration, distributed among the diaphragmatic pleura. Microscopically it was composed largely of spindle cells with alpha smooth muscle actin labeling positive and absent staining for desmin. Diagnosis of Schwannoma in dogs is facilitated by distinctive morphological characteristics and IHC labeling of vimentin and S-100 protein (4), which were positive in the present report. In conclusion, gross, microscopic, Masson's and Gomori trichromes and IHC findings were compatible with Schwannoma.

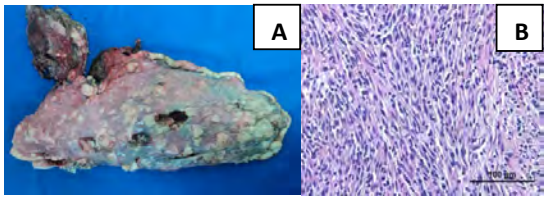

Figure 4. (A) Pig, lung, right lobe. Witish firm nodules, with different size (0.5 to 5 cm), multifocally distribuited. (B) Pig, lung, microscopic cells feature of neoplasma (HE).

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P.530

Producing PRCV serological and virus negative piglets from a PRCV infected farrow to finish herd

B Chappell, T Snider, G van Groenland, E Willems, M Olde Monnikhof
Swine Health Professionals Ltd, Steinbach, Manitoba, Canada, bchappell@shpswine.com

Introduction

Porcine Respiratory Cornovirus (PRCV) negative health status herds can be advantageous in the North America breeding stock industry. PRCV is a coronavirus that is capable of aerosol transmission through long distances. The purpose of the project was to produce PRCV virus and serologically negative piglets from a PRCV positive farrow to finish herd.

Materials and Methods

The goal was to snatch farrow 88 boars from 26 sows over 2 weeks. Immediately after birth piglets were placed into a Camfil L6 filtered incubation chamber where they were hand fed colostrum from a PRCV negative herd. When incubation chambers were full of piglets or the sows were finished farrowing, the incubation chambers were removed to an off site quarantine barn.

The off-site quarantine barn had all incoming air filtered with Camfil L6 filters. After the last piglet entered the quarantine barn they were kept under strict isolation for a 45-day period.

The following PRCV monitoring program was completed.

Day of Isolation	Sample Type	# Piglets	TGE/PRCV Elisa
3	Serum	All Pigs	Yes
24	Serum	All Pigs	Yes

Day of Isolation	Sample Type	# Piglets	PRCV PCR (Pool by 3)
3	Nasal Swab	All pigs	Yes
24	Nasal Swab	All Pigs	Yes

Results

The chart below summarizes the diagnostic:

Day of Isolation	Sample Type	# Piglets	TGE/PRCV Elisa
3	Serum	All Pigs	Negative
24	Serum	All Pigs	Negative

Day of Isolation	Sample Type	# Piglets	TGEPRCV PCR (Pool by 3)
3	Nasal Swab	All Pigs	Negative
24	Nasal Swab	All Pigs	Negative

Conclusions and Discussion

Through strict adherence to protocol and diligent hard working personelle, PRCV virus and antibody negative piglets were produced from a PRCV infected herd using technology like the Camfil L6 filters to prevent aerosolized pathogens.

Acknowledgments

TOPIGS International B.V.
TOPIGS Canada Inc

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P.531

First detection of porcine group H Rotavirus outside the Asian continent

BLD Molinari[1], RAA Otonel[1], E Lorenzetti[1], C Feronato[1], F Possatti[1], R de A Leme, AF Alfieri[1], AA Alfieri[1]
[1] *Laboratory of Animal Virology, Department of Veterinary Preventive Medicine, Universidade Estadual de Londrina, Londrina, Parana, Brazil,* alfieri@uel.br

Introduction

Rotaviruses (RVs), forming a genus of the family *Reoviridae*, are a common cause of viral gastroenteritis in humans and animals. RVs have been classified into 7 groups/species, A-G, based on the antigenicity and genetic characteristics of VP6 (1). Recently, it was proposed the creation of a new RV group H (RVH), based on VP6 analysis that includes the porcine strain SKA-1 isolated from a piglet with diarrhea in Japan (2-5). In this study, we determined the VP6 nucleotide sequence for 3 RVH-positive stool samples obtained from piglets with diarrhea in Brazil, 2012.

Materials and Methods

Three diarrheic stool samples from piglets with 35 days of age from the same farm in Mato Grosso do Sul, Brazil, with similar group B dsRNA pattern on PAGE were selected for molecular analysis. The RT-PCR were carried out using two primer pairs designed based on the complete sequence of the VP6 gene of the porcine RVH strain SKA-1 (3) that amplify products of 590bp and 716bp. The RT-PCR products were sequenced in ABI3500 Genetic Analyzer, analyzed in BLASTn and the phylogenetic tree was realized in MEGA software, version 6.

Results and Discussion

The sequence analysis showed that the 3 fecal samples BR59, BR60, and BR63, shared 100% of identity between themselves and amino acid identity ranging between 75.4% (human strain) and 96.9% (porcine strain) when compared with group H RV and are thus considered to belong to the novel group H RV.

The phylogenetic tree inferred from the VP6 sequences were separated into distinct phylogenetic clusters representative of RV groups. The BR59, BR60, and BR63 samples grouped closest to the cluster containing the novel RVH strains, but were segregated in a different branch (Figure 1).

Conclusions

Our study reports the detection of a novel porcine group H RV from the Americas. With the exception of porcine strain SKA-1 isolated in Japan, there have been no other previous reports of porcine RVH. To date, these viruses have only been detected in China, Bangladesh, Japan, and now in Brazil. The scarcity of information on these viruses may be due to lack of appropriate diagnostic tools. Extensive epidemiologic studies are needed to determine the worldwide dissemination and prevalence of this RV species and its impact on diarrheal diseases.

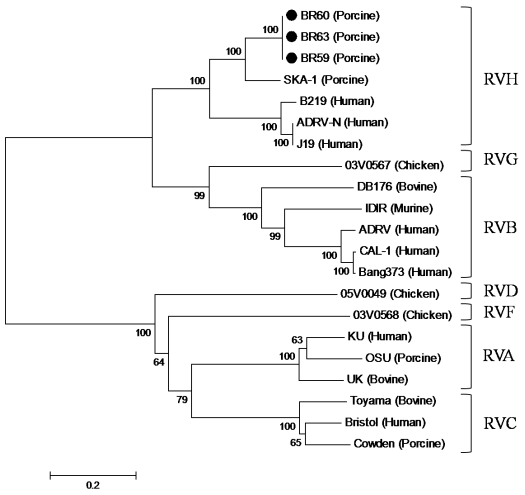

Figure 1. Phylogenetic tree showing the inferred evolutionary relationships among representative rotavirus strains belonging to groups A, B, C, D, F, G, and H, as well as the samples BR59, BR60, and BR63 based on an 1,197bp fragment of the VP6 gene. *The tree was constructed using the neighbor-joining method and the Kimura two-parameter nucleotide substitution model. Bootstrapping was statistically supported with 1,000 replicates. Scale bars indicate nucleotide substitutions per site.*

Acknowledgments

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Abortion in sows in Danish production herds

DV Jensen[1], M Schmidt[2], F Thorup[3], TK Jensen[4]
[1]*Allehelgensgade 28, 4000 Roskilde, Denmark* [2] *Department of Veterinary Reproduction and Obstretric, Faculty of Health and Medical Science, Copenhagen University.* [3]*Danish Agriculture and Food Council, Pig Research Centre.* [4]*Department of Veterinary Diagnostics, National Veterinary Institute (DTU-Vet) Copenhagen,* ft@lf.dk

Introduction

The economical loss from abortion includes the cost of the lost litter and the cost of treatment for the sow. Also, abortions may indicate an outbreak of a newly introduction of a disease in the herd. A study in Denmark in 1990 [1] concluded, that the prevalence of abortions was 1 %. No investigation has since focused on the frequency of abort cases in Denmark. Causes for abortions are either infectious or non-infectious. In cases where an etiology is found, 90 % is infectious [2]. In Denmark the diagnostic detection rate covering 2005 to 2013 in abort cases was 13 % [3], compared to 30-39 % in other countries [4, 5]. This study focuses on the relationship between indicators of inflammatory response at histological examination compared to findings indicating the presence of bacteria in the aborted material.

Materials and Methods

Abort cases from two types of herds were investigated.
Seven cases came from two out of seven herds followed with no history of an increased frequency of abortions while 51 abort cases came from herds with presumed reproduction problems. Necropsy was preformed and Crown-Rump-Length (CRL) was measured for all fetuses to determine the age at death in utero. Three to six fetuses from each case were randomly chosen and tested for *Leptospira ssp*. by immunofluoroscens, Porcine Circovirus (PCV-2) by immunohistochemistry, Porcine Parvovirus (PPV) by Real Time Polymerase Chain Reaction (RT-PCR) and tested for bacterial agents by plating on agar. Presence of Immunoglobulin G was measured in pleural fluid, in fetuses measured to be over 70 days old. Histological examination was performed on lung tissue stained by Hematoxylin and Eosin (H&E.) to reveal neutrophils and bacteria. Fluorescence In Situ Hybridization (FISH) was used to demonstrate bacteria.

Results

A final diagnose was determined in seven cases equal to a detection rate at 12 %. Neutrophils were found in 31 out of 58 cases (53 %), bacteria were found in 16 cases (28 %) when looking at brightfield microscopy and 25 cases (43 %) when looking at FISH. When using FISH, it was possible to differentiate between intra- and extracellular bacteria. In five cases intracellular bacteria were found. In all those five cases with intracellular bacteria, neutrophils were found. In the seven herds without a history of frequent abortions, seven abortions were observed in 3700 pregnancies. The abortion rate in these herds was calculated to 0,2 %. This may be a good estimate for spontaneous abortions in normal herds.

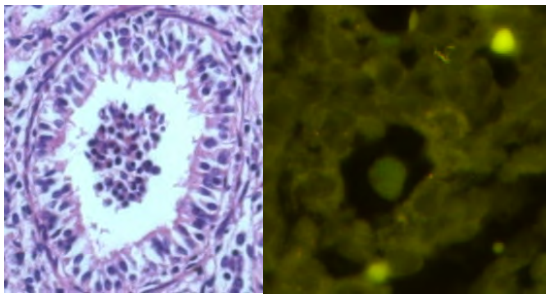

Figure 1. A) Brightfield microscopy - Bronchiole with massive neutrophils (20 x magnification). B) FISH – Intracellular bacteria found in lumen of bronchiole (40 x magnification).

Conclusions and Discussion

The overall abortion rate was calculated on behalf of just seven farms located in a small geographical area. A larger sample size - covering herds all over Denmark would be optimal for an average abortion rate for Danish sow herds, but would not inform about the rate in normal herds. The abortion prevalence was lower than estimated 24 years previously. Diagnosis was made from the clinical findings and test results, but histopathological examination was only carried out on fetal lung tissue It would have been more specific to include histological examinations of more organs per aborted embryo. The overall detection rate of 12 % fit with the general detection rate of 13 % in Denmark over the last eight years.

Acknowledgments

Thanks to the Danish Agriculture and Food Council, Pig Research centre for economic funding of this project.

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P.533

Molecular characterization and clinical aspects of TTSuV infection in swine with low feed conversion efficiency from Rio de Janeiro State, Brazil

ACM Cruz[1], C Baez[1]; RB Varella[1], TX Castro[1], RL Silveira[2]

[1]*PPGMPA, MIP* [2]*PPGMVCR,MMO/MZO. Fluminense Federal University, RJ, BR.* menezescruz@vm.uff.br

Introduction

Torque teno sus virus (TTSuV) is emergent in swine herds and endemic in many swine-producing countries, including Brazil. Until now, two distinct species (TTSuV1 and TTSuV2) and two genotypes (1a and 1b) were reported (1), but the pathogenic role of TTSuV has been still poorly investigated. The aim of this study was to perform the molecular diagnosis and characterization of TTSuV from commercial herd in Rio de Janeiro State, Brazil. In addition, the clinical signs presented by the TTSuV infected animals were reported.

Materials and Methods

A total of 30 pigs from a farrow to finish operation located in Rio de Janeiro State in July 2013 with 5 to 25% low feed conversion efficiency were studied. Pigs were from 40 to 107 days of age, included in two different categories: grower and finisher pigs. A clinical examination and weighing was performed before blood collection. DNA was extracted from serum samples by Wizard Genomic DNA purification Kit (Promega®) and submitted to polymerase chain reaction (PCR) to detect porcine circovirus (PCV1), TTSuV1 and TTSuV2 as previously described (2, 3). All the PCR amplicons were sequenced and nucleotide (nt) similarity with other sequences of TTSuV 1 and 2 deposited in the GenBank database was assessed using the BLAST tool.

Results

TTSuV DNA was detected in 24/30 (80%) serum samples: TTSuV1 in 11/30 (37 %) samples and TTSuV2 was detected in 2/30 (7%) samples. Co-infection of TTSuV1 and TTSuV2 was detected in 11/30 (37%) pigs. All 30 samples tested negative for PCV1. In addition to the weight loss, 19/30 pigs showed some other clinical signs during examination: 10 showed respiratory signs (sneezing and respiratory sounds), 5 showed enteric signs (diarrhea and presence of blood in feaces) and 4 showed systemic signs (sneezing, blood in feaces and arthritis). TTSuV genomes (1 and/or 2) were detected in 14/19 animals (74%). Eleven pigs (11/30) showed no clinical signs after examination and TTSuV genomes (1 and/or 2) were detected in 10/11 animals (91%).

After sequencing, eleven sequences showed enough quality for phylogenetic analysis: Seven TTSuV1 and 4 TTSuV 2. All TTSuV1 strains were characterized as genotype 1a and shared 96.4–98% nt identity with other TTSuV 1a strains from Brazil and Romania. A higher genetic variability was observed in TTSuV2 strains from this study, with a 74,2-88,7% nt identity with others prototypes available in GenBank (Table 1).

Conclusions and Discussion

The variety of clinical signs observed in pigs infected with TTSuV1 and TTSuV2 in this study also suggest that this virus do not possess a particular target organ. Recently, TTSuV1 was associated with clinical porcine respiratory disease complex (PRDC) indicating this virus may play a role in the development of this complex too. As observed in this study, the genetic variability among TTSuV species and multiple infections by distinct genotypes of TTSuV species in a single pig may occur (4). This is a preliminary study and a larger number of samples will be soon evaluated in order to investigate more deeply possible associations between TTSuV and clinical disease in swines. Healthy animals with normal weight gain should also be tested and compared to this group.

Table 1. Clinical signs observed in pigs tested for TTSuV1 and TTSuV2 by PCR

Clinical signs	TTSuV1	TTSuV2	coinfection	negative
Enteric signs	0/5	0/5	3/5	2/5
Respiratory signs	4/10	1/10	3/10	2/10
Sistemic signs	2/4	0/4	1/4	1/4
No clinical signs	5/11	1/11	4/11	1/11
Total	11	2	11	6

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Implementation of degenerate primer nested PCR for detection of TTSuV from PCVAD cases in Mexico

A Vargas-Ruiz[1], H Ramirez-Alvarez[1], J Sanchez-Betancourt[2], J Vazquez-Perez[3], L Garcia-Camacho[1]
[1]*Facultad de Estudios Superiores Cuautitlan,* [2]*Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autonoma de Mexico,* [3]*Centro de Investigacion en Enfermedades Infecciosas, Instituto Nacional de Enfermedades Respiratorias,* luciangie30@hotmail.com

Introduction

Torque teno sus virus (TTSuV) belongs to *Anelloviridae* family within the *Iotatorquevirus* genre with two species, Torque teno sus virus 1 (TTSuV1) and Torque teno sus virus 2 (TTSuV2) (5). Both viruses possess a circular single-stranded negative sense DNA of 2.7 to 2.9 kb composed of 4 open reading frames: ORF1 (encoding for capside and replication proteins), ORF2, ORF 1/1, and ORF 2/2 (former ORF3), and a untranslated region (UTR) which is rich in GC. The UTR is variable in length (20-29% from whole viral genome). In addition, there is a high proportion of variable positions (TTSuV1/46.3% and TTSuV2/23.9%). Few nucleic TTSuV sequences area available in data bases from limited geographic origin. Both genres co-infection is frequent, and they are ubiquitous to domestic and wild pigs (1). Whether TTSuV infection promotes a specific disease as a primary agent or in co-infection with other pathogen, it is unknown (3). However, a high rate of co-infection with PCV2 has been reported (4). The aim of this work was to detect specific TTSuV1 and TTSuV2 sequences by nested PCR based on degenerate primers from well-documented cases of PCVAD in Mexico.

Materials and Methods

Ten TTSuV1 sequences and 15 TTSuV2 sequences available in the GenBank from different countries were used to primer design, and degenerate bases were situated in presence of variable regions in primer binding areas. Forty paraffin-embedded tissues with known-status of PCVAD (20 PMWS cases, and 20 PCV2-RF cases) were selected from case archival (2001-2009) to implement TTSuV1 and TTSuV2 nested PCR. Two amplified products of the expected size from each virus were purified from agarose gel to sequence by high fidelity, processing, and specificity enzyme kits, in a sequencer model 3100 (Applied Biosystems). Then, phylogenetic trees were constructed with the Maximum likelihood method, using the MEGA5 program (Tamura et-al, 2011), with the Tamura-Neg Gamma distance. Statistic trust of the topology was assured with bootstrap values, 1000 repetitions. Bootstraps results over 70 (700) were considered highly similar.

Results

TTSuV1and TTSuV2 nested PCR protocols amplified expected products of 255 bp and 211 bp, respectively from PCVAD cases (Table 1). Sequencing demonstrated that the products were specific of both viruses. The phylogenetic analysis demonstrated a sequence identity of 98% and 94% with German and Chinese TTSuV1 sequences while sequence identity of TTSuV2 was closely related to Chinese sequences. Furthermore, phylogenetic data showed a low homology from each other (different bootstrap).

Table 1. nested PCR results

SYNDROME	TTSuV1+ TTSuV2+	TTSuV1- TTSuV2+	TTSuV1+ TTSuV2-	TTSuV1- TTSuV2-
RF-PCV2	0	0	9	11
PMWS	4	5	4	7

Conclusions and Discussion

TTSuV like PCV2 are ubiquitous in porcine population worldwide. TTSuV has been described in Europe (Germany, Hungry, Italy, France, and Spain), Asia (China, Korea, Japan, and Thailand), and America (Canada, and USA) (2, 3). The present work first documented the presence of both genres of TTSuV in Mexico by using a degenerate primer nested based PCR from PCVAD cases which fulfilled diagnostic criteria (clinical signs, characteristic microscopic lesions, and in situ hybridization positive to PCV2). At present, four full-length genomic sequences have been identified in porcine TTSuV strains which have given different genotypes (TTSuV1a and TTSuV1b) or subtypes (TTSuV2b and TTSuV2c) (3). Scarcity of sequences in data bases has precluded the establishment of phylogenetic relations among TTSuV strains (1). Our preliminary results depict a lower frequency of both viruses than the frequency described in other countries. Given the genomic variability of TTSuV, it is feasible that existing TTSuV strains in Mexico share a lower nucleotide sequence identity than those found in countries with higher prevalence. Currently, phylogenetic comparisons are being performed with an increased number of amplified sequences to further explore such differences.

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P.535

Identification of porcine rotavirus from swine with diarrhea in Brazil

KCP Reis, MR Henriques, LEM Bouillet, FA Vannucci,WV Guimaraes, LF Santos, DL Santos, JL Santos
Microvet – Microbiologia Veterinaria Especial Ltda, Vicosa/MG, Brazil – walter.pdi@microvet.com.br

Introduction

Rotaviruses have been recognized as diarrheic disease agents of swine, other animals and humans. Most rotavirus infections are subclinical in conventionally reared pigs and rotaviruses may be detected in apparently healthy pigs. Nursing and weaning pigs present the greatest morbidity and mortality from rotavirus infections (3). Group A rotaviruses are the leading cause of acute gastroenteritis from young pigs and many other animals species throughout the world (1). Detection of rotaviruses is limited by some of the particular characteristics of these rotavirus groups. Cultivation of rotaviruses is difficult and for this reason sensitive molecular methods are being implemented for direct detection of rotaviruses in faecal samples or other fluids (2). The objective of this study was to confirm the presence of group A rotaviruses, by means of the implemented RT-PCR method, in faecal and intestine samples collected from diarrheic pig in Brazil. The partial sequences obtained have been used for comparison with those available in the Gene Bank.

Materials and Methods

Field samples were usually feces and intestines obtained from diarrheic pigs. Two samples were tested for group A rotavirus by RT-PCR assay with consensus primers (Porva – F: GACGGCAACTCAACCTCTCACAT and Porva – R: TTTACTCTACATAAAGCATCAAT). The RNA extraction and cDNA were performed using the commercial kits lNucleospin RNA virus (MN) and Improm-II Reverse Transcriptase (Promega). The amplicon product is 416 bp. Amplified products were sequenced using the MegaBACE DNA Analysis System (Amersham Biosciences). The expected DNA sequences were based on product size and compared with the sequences available in the Gene Bank using BLAST program.

Results

The results indicated that samples from diarrheic pigs in Brazil were positive for infection with group A rotavirus. It was possible to detect rotaviruses by RT-PCR from faecal or intestine diarrheic animal samples. The amplicons from the RT-PCR assays performed on the intestine and diarrheic faecal samples were specific for their respective group A rotavirus (Fig. 1). The amplicon product showed 100% homology to the sequence rotavirus A strain NSP4 (NSP4) gene, accession number GQ282606.

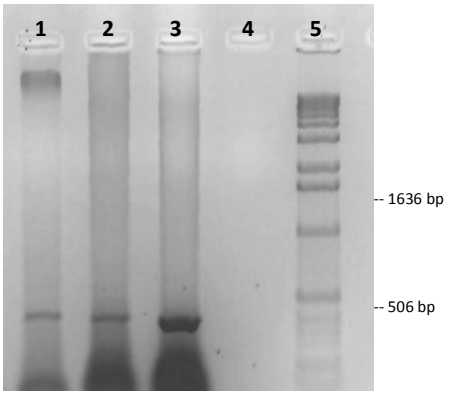

Figure 1. Detection of group A rotavirus in samples from diarrheic swine by RT-PCR. *(1) intestine sample; (2) faecal sample; (3) positive control; (4) negative control; (5) size marker.*

Conclusions and Discussion

The importance of group A rotavirus in the etiology of diarrhea in suckling and recently weaned pigs is well characterized in Brazil. Rotavirus enteritis may cause morbidity and death, as was observed in the cases evaluated in this study. Rotavirus infection in conventionally reared pigs has also been reported to be subclinical or associated with mild diarrhea. Inoculation of piglets with rotavirus has frequently been reported to produce mild disease. The availability of molecular methods to identify rotavirus genotypes, such as the one described here, can facilitate and should intensify studies on the occurrence and distribution of rotaviruses from herds in Brazil. A better understanding of rotavirus infection will contribute to the optimization of vaccine production and prevention programs for rotavirus diarrhea of humans and animals and will help the understanding of the global ecology of rotaviruses.

Acknowledgments

We thank all colleagues in Microvet

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Rapid detection of *Chlamydia*/*Chlamydophila* group in samples collected from swine herds with reproductive disorders

K Rypula[1], A Kumala[1], P Lis[1], K Niemczuk[2], K Płoneczka-Janeczko[1], Z Pejsak[2]
[1]Faculty of Veterinary Medicine, University of Environmental and Life Sciences, Wrocław, Poland, [2]National Veterinary Research Institute, Pulawy, krzysztof.rypula@up.wroc.pl

Introduction

Pathogens from *Chlamydia/Chlamydophila* group in the *Chlamydiaceae* family may be responsible for subclinical intestinal tract infection, pneumonia, polyarthritis, polyserositis, conjunctivitis as well as reproductive disorders such as late-term abortion in sows, an increased rate of perinatal and neonatal mortality, and epididymitis and vesiculitis in boars (5).
The objective of the study was to determine prevalence of *Chlamydia/Chlamydophila* group infection in sows and identify the species of the pathogens infecting sows with reproductive disorders.

Materiasl and Methods

The study was carried out in three (A-C) reproductive herds of pigs. In three of them reproductive disorders like low reproductive efficacy, repeat estrus and purulent, vaginal discharge were observed. The remaining four did not report any such symptoms. Tetracycline had not been used in any of the herds for at least 3 months before the onset of the study. The herds counted 10-500 adult sows. Required sample size was computed in WinEpiscope 2.0 (EPIDECON) assuming expected prevalence of 30% and level of confidence of 95%. From each sow serum sample, conjunctival swab from both eyes and swab from the vaginal vestibule were collected. Serum samples were tested using the complement fixation test (CFT) according to EN ISO/ IEC-17025:2005. Real-time PCR was performed using *Chlamydiaceae* family-specific primers, fluorescent-labeled probe targeting the 23S ribosomal DNA and cycling conditions specified by Everett at al. (4). Then the products obtained in real-time PCR were sequenced (Genomed, Poland) and the species was identified using Basic Local Alignment Search Tool (BLAST;blast.ncbi.nlm.nih. gov) and Molecular Evolutionary Genetics Analysis (6).

Results

All serum samples were negative in CFT. Infected sows were detected in each of the examined herds by real-time PCR. Positivity in regard to examined herds, present or absent reproductive disorders and the place of sampling is provided in the table1. One isolate was proved to be *Chlamydophila pecorum*, whereas all the others were identified as *Chamydia suis* (Fig. 1).

Conclusions and Discussion

Seroprevalence in Europe ranges from 7% to 96% (2). The prevalence of the infection ranged from 23% to 88% in clinically healthy pigs and from 79% to 90% in pigs manifesting clinical symptoms of the infection (1,3). This present study shows that a high number of sows, regardless of the current clinical problems, may be carriers of the pathogen. Development of molecular methods allows quick identification of the isolated pathogenic species of *Chlamydia/Chlamydophila*. Given very low consistency between results of serological and molecular tests, the latter may be proposed a routine diagnostic method.

Table 1. Results of CFT and Rt-PCR the examined herds and clinical manifestation of disorders that may suggest *Chlamydia/Chlamydophila* group infection.

Examined samples	Farms		
	A	**B**	**C**
positive CFT results/total examined			
S[1)]	0/9	0/9	0/6
positive Rt-PCR results/total examined			
CS[2)]	6/9	4/9	4/6
CV[3)]	5/9	6/9	3/6

S[1)] – serum; CS[2)] - conjunctival swabs; SV[3)] - swabs from vestibulum vaginae

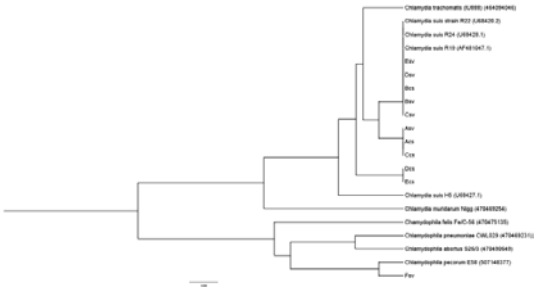

Figure 1. The tree of similarities with comparison to the sequences of the known species of *Chlamydiaceae* family from GenBank.

Acknowledgements

This study was supported by funds from the by grant NN 308 5782 40 from the National Committee for Scientific Research Poland.

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Infection of *C. pecorum* in swine herd – clinical report

A Kumala[1], K Rypula[1], P Lis[1], M Spaliński[2]
[1]*Division of Infectious Diseases and Veterinary Administration, Faculty of Veterinary Medicine, University of Environmental and Life Sciences, Grunwaldzki Square 45, 50-366 Wrocław, Poland*
[2]*Veterinary Practice CHIRION, Brańsk, Poland,* krzysztof.rypula@up.wroc.pl

Introduction

Infections with *Chlamydia* sp. and *Chlamydophila* sp. in swine are associated with various clinical symptoms, e.g., pneumonia, conjunctivitis as well as increased perinatal and neonatal mortality (3).
Chlamydophila pecorum is usually isolated from cattle, sheep and goat-herds, but can be exceptionally found in swine herds. The reported infections were usually related with pneumonia, arthritis, corneitis, conjunctivitis and sporadically encephalitis and myelitis in young cattle. It was also associated with the cases of abortion, metritis and mastitis.

Materiasl and Methods

The research towards identification of *Chlamydia* spp. and *Chlamydophila* spp. infections were conducted in the closed-loop farm with 105 sows in the basic herd. Persistent miscarriages and birth of dead or mummified piglets were earlier reported in this herd. Tetracycline treatment temporarily restrained breeding disorders. The number of serum samples and swabs from the conjunctival sac and vaginal vestibule to diagnose with p_1=10% were calculated using the WinEpiscope® (EPIDECON). All serum samples (n=9) and swabs from both places (n=18) were placed in sterile tubes and transported to the laboratory. DNA was extracted from swabs using Qiagen DNeasy Blood & Tissue Kit (Qiagen, Germany). Samples were analyzed using real-time PCR with the *Chlamydiaceae* family-specific TQF/TQR primers and a fluorescent-labeled probe FAM-CAAAAGGCACGCCGTCAAC-TAMRA (1). Cycling conditions for the real-time PCR were 40 cycles of 15 s at 95°C and 1 min at 60.5°C, with initial denaturation of 3 min at 94°C. Real-time PCR was performed using the Bio-Rad iQ5 (BioRad, USA). DNA from *Chlamydia abortus* (NRVI, Puławy), *Chlamydia trachomatis* (NRVI, Puławy) and *Chlamydia suis* VR-1474 (ATCC, USA) was used as a positive control.
The products obtained using real-time PCR in positive samples were then sequenced (Genomed, Poland) and identified using BLAST (blast.ncbi.nlm.nih.gov).
Antibodies specific to *Chlamydia spp.* were detected using a complement fixation test (CFT) according to EN ISO/ IEC-17025:2005.

Results

A *Chlamydia*-positive sample from vaginal vestibule (4_V) was detected using real-time PCR and later identified by sequencing as *Chlamydophila pecorum* (Fig. 1).
There were no specific antibodies detected in the serum samples.

Conclusions and Discussion

In the swine, *Chlamydia sp.* and *Chlamydophila sp.* infections generally seem to cause subclinical disease and the pathogen may be detect only with use the diagnostic laboratory tests. *Chlamydia suis* is the most commonly isolated, whereas other, including *Chlamydia pecorum*, are rarely found (4). In the described case, infections other than chlamydial were identified in the herd, i.e. PRRSV and PCV-2. These results are consistent with publications where authors presented multi-etiological clinical sings, as PCV-2 and PRRSV are described as the factors causing problems in the reproductive tract (2,5).

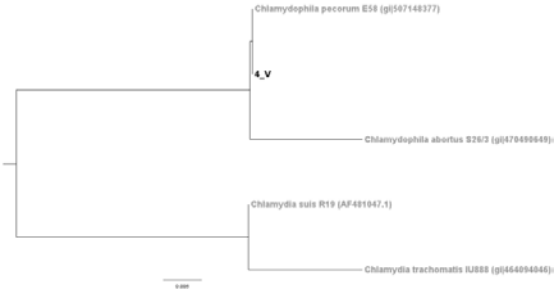

Figure 1. The tree of similarities of the isolated sample (4_V) sequence comparing to the sequences of *Chlamydophila pecorum*, *Chlamydophila abortus*, *Chlamydia suis* and *Chlamydia trachomatis* sequences obtained from GenBank, prepared using MEGA6 (6).

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Plasma concentration of Toltrazuril 5%[c] administered orally in piglets

AF Silva[1], L Catelli[1], L Lima[1], GB Magenis[1], CC Barbosa[1], DMS Cassol[1], MLG Rezende[1], J Cristani[2]
[1]*Departamento Técnico Saúde Animal Ourofino Agronegócio,* [2]*Centro de Ciências Agroveterinárias (CAV/UDESC),*
amilton.silva@ourofino.com

Introduction

Toltrazuril is an antiprotozoal agent with broad anticoccidial and antiprotozoal spectrum action. It is broadly used in the prevention and treatment of coccidiosis in broiler chicken, swine and bovine [2]. It acts on different parasitic forms, mainly schizonts, micro and macro gametocytes [1]. Toltrazuril (TZR) is oxidized and produces toltrazuril-sulfóxido (TZR-SO) and toltrazuril-sulfona (TZR-SO2) metabolites [2]. TZR and TZR-SO2 are effective in controlling oocysts; nonetheless, TZR-SO is the precursor of TZR-SO2. Consequently, the present study aimed to evaluate and quantify the plasma concentrations of toltrazuril 5%[c] (Isocox Pig Doser and Baycox) in piglets subjected to an oral administration of 20 mg/kg live weight, in a unique dose.

Materials and Methods

The experiment was conducted at the Centro de Ciências Agroveterinárias da Universidade do Estado de Santa Catarina (UDESC), under the protocol number 1.31.12 of the Comitê de Ética em Experimentação Animal da UDESC. The analytical methodology followed the criteria established by RDC#27 (ANVISA, 2012). For the pharmacokinetics analysis,theEMEA/CVMP/133/99-FINAL"Guideline for the conduct of pharmacokinetic studies in target animal species" was used as a reference guide. Blood plasma was collected from 53 piglets, 3-days old, with an average weight of 2.0 Kg, from five different litters and randomly distributed into two groups. G1 (treated with 20 mg/Kg live weight of 1[a]-P1 product) and G2 (treated with 20 mg/Kg live weight of 2-P2[b] product). After administering the drugs, blood samples were collected by cranial cava venipuncture at the following times: 0, 6, 12, 18, 24, 36 and 48 hours; and 4, 10 and 20 days for the determination of the pharmacokinetic parameters of TZR and TZR-SO2. The results were analyzed by liquid chromatography in mass detector (LC-MS/MS), in accordance to the Resolution - RDC Nº 27, 2012 ANVISA. The data was analyzed by the Software Phoenix / WinNonlinTM (version 6.3). Considering that the quantity of drug contained in the biological fluid is balanced with the action site, the bioavailability is determined by the concentration of the active ingredient present in the blood, plasma or appropriate biological fluid, with regards to time (CODE OF FEDERAL REGULATIONS, 1998; CONSIGLIERI & STORPIRTIS, 2000).

Results

The Table1 shows the similarity in the TZR and TZR-SO2 concentrations for P1[a] and P2[b]. It took 48 hours to reach the maximum concentration of TZR for P1[a] and 36 hours for P2[b]. The plasma levels of P1* were higher than those of P2[b] for 96 hours. For TZR-SO2, the plasma curves were similar for P1[b] and P2[b] during the entire experiment. According to what was observed after 48 hours of administration, P1[a] presented a higher plasma concentration than P2[b].

Table 1. Mean plasma concentration of TZR and TZR-SO2 collected from 53 piglets during the entire experiment

Moments	**TZR[d]**		**TZR SO2[d]**	
(hours)	P1[a]	P 2[b]	P 1[a]	P 2[b]
0	0,00	0,00	0,00	0,00
6	2,71	5,68	0,00	0,00
12	6,54	4,64	0,01	0,00
18	5,31	6,93	0,06	0,03
24	5,68	7,98	0,07	0,10
36	7,18	8,36	0,18	0,33
48	8,56	5,36	0,75	0,58
96	4,25	1,51	2,39	0,75
240	1,13	1,14	2,86	2,95
480	0,18	0,09	2,11	1,96

[a]Isocox Pig Doser, [b]Baycox, [d]Average - ug/L.

Conclusions and Discussion

The results demonstrated that P1[a] presented plasma levels of TZR and TZR-SO2 higher than P2[b] in 12, 48 and 96 and 480 hours. This leads to infer the better bioavailability of P1[a] when compared to P2[b].

Acknowledgments

Universidade do Estado de Santa Catarina (UDESC), SC/Brazil.

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Characterization of resistance profile of *T. pyogenes* strains from swine pneumonia and abscesses

BLP Costa[1], CEC Matajira[1], LZ Moreno[1], KC Silva[1], GF Silva[1], PHN L Filsner[1], TSP Ferreira[1], MC Dutra[1], VTM Gomes[1], AM Moreno[1].
Laboratory of Swine Health - Department of Preventive Veterinary Medicine- FMVZ/USP
São Paulo, SP/ Brazil, morenoam@usp.br

Introduction

Trueperella pyogenes, previously classified as *Arcanobacterium pyogenes* and *Actinomyces pyogenes*, is a well known opportunistic pathogen causing a variety of suppurative infections in a variety of domestic and wild animals (1,2), and humans (3). In domestic ruminants and pigs, these infections include uterine infections, arthritis, pneumonia, and abscesses (4). Abscesses in pork carcasses generally cause significant losses to the pork industry, requiring trimming or even condemnation that consumes time and compromises investment (5). Antimicrobial treatment is one of the few available short-term alternatives for the treatment of *T. pyogenes* infections. Therefore, the main objective of this study is to determine the antimicrobial resistance pattern of the *T. pyogenes* isolated from lungs and abscesses of swine.

Materials and Methods

In order to investigate the antimicrobial susceptibility of *Trueperella pyogenes* isolates, a total of 19 *T. pyogenes* isolated from suppurative infections involving skin and lungs of pigs (abscesses, pneumonia). Samples were plated on sheep blood agar at 5%, and then incubated in a jar containing 5% to 10% CO_2 for 24-48 hrs at 37°C ± 1°C. Identification was made on the basis of colony morphology, haemolysis on sheep blood agar plates, Gram staining and biochemical characterization (VITEK®). The DNA was extracted from the isolated strains and amplified by Polymerase Chain Reaction (PCR) using specific primers for *T. pyogenes*. Minimal inhibitory concentrations (MICs) of drugs were determined using BOPO6F MIC Plate - Sensititre ® against the following antimicrobial agents: ampicillin, clindamycin, chlortetracycline, danofloxacin, enrofloxacin, florfenicol, gentamicin, neomycin, oxytetracycline, penicillin, sulfadimethoxine, spectinomycin, trimethoprim/ sulfamethoxazole, tiamulin, tilmicosin, tulathromycin, tylosin, ceftiofur.

Results

Among the 19 *T. pyogenes* strains evaluated, all were sensitive to ceftiofur, penicilin, ampicillin, tiamulin, gentamicin, florfenicol and spectinomicyn. All strains were resistant to trimethoprim/sulfamethoxazole and sulfadimethoxine. Resistance to danofloxacin and clindamicyn was 95% followed by 79% to tulathromycin,74% to tilmicosin, 63% to tylosin, 37% to oxytetracycline, 16% to neomicyn and 10,5% to chlortetracycline. Mic 50, MIC 90 and MIC range values are described in Table 1.

Conclusions and Discussion

T. pyogenes are an importan cause of pneumonia and abcess in swine in Brazil. isolation of the agent is held in low frequency due to the demands in culture and difficulties in their identification. The resistance profile of Brazilian isolates were never evaluated or published before and can be of great assistance to veterinarians. it was possible to observe that some antimicrobials largely used in respiratory diseases control as macrolide classes were not very effective against this agent.

Table 1. *In vitro* sucetibility of *T. pyogenes* isolates from swine

Antimicrobial	MIC (µg/mL)			Resistant % (n=19)
	MIC 50	MIC 90	Range	
Ceftiofur	≤ 0,25	≤ 0,25	≤ 0,25	0(0/19)
Tiamulin	≤ 0,5	≤ 0,5	≤ 0,5-1	0(0/19)
Chlortetracycline	1	>8	≤ 0,5->8	10.5(2/19)
Oxytetracycline	2	>8	≤ 0,5->8	37(7/19)
Penicillin	≤ 0,12	≤ 0,12	≤ 0,12	0(0/19)
Ampicillin	≤ 0,25	≤ 0,25	≤ 0,25	0(0/19)
Danofloxacin	>1	>1	0,25->1	95(18/19)
Trimethoprim/ sulfamethoxazole	> 2/38	> 2/38	> 2/38	100(19/19)
Tylosin	>32	>32	≤ 0,5->32	63(12/19)
Tulathromycin	>64	>64	1->64	79 (15/19)
Clindamycin	>16	>16	≤ 0,25->16	95(18/19)
Sulfadimethoxine	>256	>256	>256	100(19/19)
Gentamicina	≤ 1	≤ 1	≤ 1-4	0(0/19)
Florfenicol	0,5	1	≤ 0,25-1	0(0/19)
Neomycin	≤ 4	8	≤ 4-8	16(3/19)
Spectinomycin	≤ 8	≤ 8	≤ 8	0(0/19)
Tilmicosin	>64	>64	≤ 4->64	74 (14/19)
Enrofloxacin	0,5	1	≤ 0,25-1	0(0/19)

Acknowledgments

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Characterization of resistance profile of *Arcobacter* spp. strains from swine

DDS Gobbi[1], PHNL Filsner[1], TSP Ferreira[1], MGX Oliveira [1]; GFR Silva[1], MG Spindola[1],VTM Gomes[1], AM Moreno[1]
Laboratory of Swine Health - Department of Preventive Veterinary Medicine- FMVZ/USP
São Paulo, SP/ Brazil, morenoam@usp.br

Introduction

Among the known species of the genus *Arcobacter*, the species *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* are considered potentially zoonotic and can be transmitted by food of animal origin. The increase in isolation rate of *Arcobacter* spp. from various sources throughout the world, as well as resistance to certain antibiotics reported by several research groups are important aspects from the public health viewpoint (1). Thus, research on the potential of resistance to antimicrobials used in human and veterinary therapeutics is essential to obtain the best results when using antibiotics to treat diseases caused by this micro-organism. The infections caused by this agent are usually self-limiting, but in cases of severe and chronic conditions, adequate medical treatment is needed. This study aimed to isolate and characterize the resistance profile of *Arcobacter* spp strains isolated from swine carcasses and environmental samples from a slaughterhouse located at São Paulo state. The strains were subjected to polymerase chain reaction for species identification and the resistance profile was determined microdilution technique according to the standardized protocol of the M31-A3.

Materials and Methods

The study was carried out on a slaughterhouse of São Paulo State. A total of 30 carcass swabs were submitted to isolation of *Arcobacter* spp as described by Johnson and Murano (1999) (2). Suspect isolates were submitted to DNA extraction and characterized using the primers previously described (3). Strains *A. butzleri* ATCC 49616, *A. cryaerophilus* ATCC 43158 and *A. skirrowii* ATCC 51132 were used as positive control in all reactions. Minimal inhibitory concentrations (MICs) of drugs were determined using Campylobacter MIC Plate - Sensititre ® (TREK Diagnostic Systems Inc., Cleveland, OH). This panel contains the following antibiotics: azithromycin (AZI), ciprofloxacin (CIP), erythromycin (ERY), gentamicin (GEN), tetracycline (TET), florfenicol (FFN), ac. nalidixic (NAL), telithromycin (TEL) and clindamycin (CLI).

Results

Among the 30 carcasses evaluated, 25 were positive for the agent and 70 strains were selected and identified as *Arcobacter* spp. The isolated species were *A. butzleri* (n=61), *A.cryaerophilus* (n=7) and *A.skirowii*(n=2). All strains were susceptible to gentamicin and tetracycline and 77.1% were multirresistant, among these the most common profile of resistance was azithromicin/ florfenicol/ nalidixic acid/ telithromycin/ clindamicycin, followed by florfenicol/ nalidixic acid/ telithromycin/ clindamicycin.

Conclusions and Discussion

The antimicrobial ciprofloxacin, gentamicin and tetracycline are the most suitable for possible infections in humans or animals by *Arcobacter* spp. in our region. Multi-resistance to antimicrobial agents is common in this specie and was present in strains isolated from swine carcasses.

Table 1. Antimicrobial resistance profiles among *Arcobacter* spp. strains from swine.

Profile	Antimicrobials	*A. butzleri*	*A. cryaerophilus*	*A. skirrowii*	Total
P1	FFN/NAL/CLI	3	0	1	4
P2	FFN/ CLI	1	0	0	1
P3	AZI/ERY/FFN/NAL/TEL/CLI	2	0	0	2
P4	AZI/FFN/NAL/TEL/CLI	26	0	0	26
P5	AZI/CIP/FFN/NAL/TEL/CLI	1	0	0	1
P6	AZI/FFN/NAL/CLI	2	0	0	2
P7	FFN/NAL/TEL/CLI	16	0	1	17
P8	AZI/ERY/NAL/TEL/CLI	1	0	0	1
P9	AZI/ERY/TEL/CLI	1	0	0	1
P10	FFN/NAL	1	0	0	1
P11	NAL	0	1	0	1
P12	NAL/CLI	1	0	0	1
P13	CIP/NAL/CLI	0	2	0	2
P14	CIP/NAL	2	3	0	5
P15	CLI	1	0	0	1

Acknowledgments

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PCV2 disease in vaccinated growing pigs in Southern Brazil

R Schaefer[1], D Gava[1], ME Cantão[1], VHB Serrão[2], MC Silva[3], N Mores[1], JRC Zanella[1]
Embrapa Swine and Poultry Research Center, Animal Health Laboratory, Embrapa, Concordia, SC, Brazil[1], Instituto de Física de São Carlos – Universidade de São Paulo-USP, São Carlos, SP, Brazil [2], Centro de Diagnóstico de Sanidade Animal (CEDISA), Concórdia, SC, Brazil, rejane.schaefer@embrapa.br

Introduction

Postweaning multisystemic wasting syndrome, the most common clinical manifestation of porcine circovirus type 2 (PCV2) disease (PCVD), was first described in 1996 in Canada (6). In Brazil, PCV2 was first detected in 2000 in Santa Catarina state where the disease was characterized by wasting and severe lesions in lymphoid tissues (1). PCVD has caused economic losses with high mortality rate in pig farms in several Brazilian states until the introduction of PCV2 vaccine in commercial pig farms in 2008. During December of 2012, a suspect PCVD case was reported in 57-67 days-old pigs in a vaccinated wean-to-finish farm in Southern Brazil. The affected pigs showed coughing, dyspnea, enlargement of inguinal lymph nodes, wasting and diarrhea around 35 to 42 days after housing. A mortality rate up to 5% was registered. The objective of this study was to diagnose and characterize the PCV2 infection in a PCV2 vaccinated pig herd.

Materials and Methods

Lymph node, kidney and lung samples were collected and submitted to laboratory analysis. Tissue samples were processed according to conventional methods for histopathology (HE) and subjected to qPCR (4) and immunohistochemistry (IHC) test (1) to PCV2 detection. DNA sequencing was performed by the Sanger method. The obtained sequences were analyzed and assembled with the Phred/Phrap/Consed software (2). Phylogenetic analyses of the whole genome and the ORF2 gene were performed using the Neighbor-Joining method in the MEGA 5.2 software (7) based on nucleotide and amino acid sequences. Using homology molecular modelling (MODELLER) (5), a structural model of the capsid protein was obtained. This model was validated and the mutated residues were identified.

Results

The diagnostic of PCVD was confirmed by histopathological lesions characterized by multifocal granulomatous lymphadenitis, multifocal lymphohistiocytic interstitial nephritis and multifocal lymphohistiocytic interstitial pneumonia. According to the IHC test results, PCV2 antigen was associated with the lesions. Lymph node samples were positive to PCV2 by qPCR ($5.67x10^{11}$ DNA copies/uL). The complete genome sequence (1.7Kb) of one sample (303/12) was performed. Based on the alignment with other PCV2 strains, the PCV2-303/12 analyzed in this study clustered within the PCV2-b genotype. The comparison among the ORF2 amino acid sequence of the PCV2 described here and other Brazilian PCV2 revealed three amino acids substitutions, in domains 57 (F to I), 178 (N to S) and 190 (A to T). The structural model shows that the N178S mutation probably disrupts the secondary structure of the epitope, which is important to the recognition by antibodies (3).

Conclusions and Discussion

This is the first description of PCVD caused by a PCV2b variant in pigs in a vaccinated herd in Brazil. PCV2 infection was demonstrated by HE, IHC, qPCR and DNA sequencing. In Brazil, both PCV2a and PCV2b genotype have been detected in pigs (1). Nevertheless, after the wide use of PCV2 vaccines, PCV2b became the most prevalent genotype worldwide (6). Moreover, it was possible to build a structural model of the ORF2 gene. The results of the structural modeling indicate a possible disruption in the protein structure around the 178 residue which is an important site for antibodies recognition (3). However, other *in vivo* and *in vitro* studies are needed to confirm this hypothesis.

Acknowledgements

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Evaluation of the effects of CIRCOVAC® in sows´ vaccination, in a Venezuelan farm under field conditions

A Gerardi[1]; J Ochoa[1] ; J Tonassi[2]
[1] NUTRISERVI-MERIAL, Maracay, Venezuela; [2] Grupo La Caridad, Maracay, Venezuela,
adrianagerardi.s@gmail.com

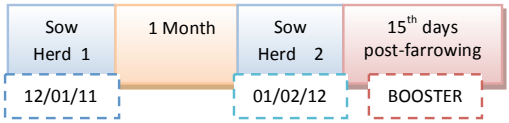
Introduction

PCV2 in Venezuela has caused an impact on reproductive parameters, generating significant economic losses. Vaccination in sows with Circovac® has been producing better reproductive perfomances in different countries around the world (1). The objective of this study was to demonstrate the effects of Circovac® vaccination in sows, in a Venezuelan farm diagnosed with PCV2 under field conditions.

Materials and methods

A Farrow-to-weaning farm with 5000 sows in Venezuela with history of PCV2 and PRRS (-) was studied. PCV2 was detected in 2010 by real-time PCR in fetal tissue (2). Historical reproductive data, since 2010 were collected to this year through the PigCHAMP software in order to evaluate the reproductive parameters: conception rate, births rate and return rate. A comparison was made between unvaccinated sows (UVS) and vaccinated sows (VS) with Circovac®. The vaccination of sows started on December 2011 by the following protocol of vaccination (Table 1).

Table 1. Vaccination Protocol in sows.



In this farm, when the Circovac® was first introduced, all sows were vaccinated in herd. Finally, the sows protocol of vaccination is applied at 15 days postpartum in each cycle.

Statistical analyses were undertaken using the Z-Test with three unequal variances and a one-factor (year) analysis of variance. Tests were carried out using proprietary statistical software.

Results

Table 2. Data of PigCHAMP software. Reproductive parameters of unvaccinated sows (UVS) and vaccinated sows (VS) with Circovac®.

Reproductive parameters	Group/Year		
	UVS/2010	UVS/2011	VS/2012
Total services	13.719	14.043	13.444
Return rate	6,50%	8,30%	5,00%*
Conception rate	82%	84,10%	87%*
Births numbers	11.231	11.702	11.703
Births rate	81,90%	84,10%	86%*

* Statistically significant ($p<0.01$)

Discussions and Conclusions

This study showed that using Circovac® in sow herd, improved reproductive performance (Table 2) optimizing fertility efficiency and productivity significantly ($p<0.01$). A comparison between conception rate, births and return from 2010 to 2011 showed of a greater improvement with a lower rate of return.

Should be mentioned that the system has the same field management conditions in the three years under study.

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P.543

Molecular detection of swine TTSuV 1 and 2, PRRSV and PCV2b, in relationship to PMWS and abortions in pig farms from different regions in Venezuela

V Bermúdez[1], O LaTouche[1], J Moreno[2], A Moscardi[3], A Gerardi[4]
[1]Central University from Venezuela – College of Veterinary Medicine, Dept. of Veterinary Pathology, [2]Laboratorio Carval, [3]Ladivet, [4]Nutriservi-Merial, vbermu2001@yahoo.com; invicto2012@gmail.com

Introduction

Viral diseases interact in pigs farms in Venezuela. Clinical signs and serology give a an idea to pinpoint the most probable agent when several viruses act concomitantly on farm. However, post-weaning multisystemic wasting syndrome (PMWS) has been studied in recent years worldwide, establishing the role of the porcine circovirus type 2 (PCV2) alone or accompanied by Torquetenovirus (TTV1&2) and porcine reproductive and respiratory syndrome (PRRS). Some research has shown TTV 1&2 associated to PMWS by itself (2,3). Eventhough, the virulence of TTV1&2 are not yet fully demonstrated, PCV2b and PRRS alone or synergistically are responsible for great economical losses in our country and worldwide.

Although American, European and Asian farms has studied their status for Bocavirus (SwBoV) and TTV1&2 in PMWS and normal pigs. In our country, the presence of TTV remained unknown until recent years. The main objective of this study was to determine the presence of those TTV1&2 along with known viruses in clinically normal and PMWS affected pigs.

Materials and Methods

One farm (farm 1) wean to finish from Southern Venezuela (3000 sows), 2 farms from Central Region (farm 2, 1000 and Farm 3, 5000 sows respectively) and 3 multisited farms (Farm 4, 2000, farm 5, 3000 and farm 6, 5000 sows) from Central-Western Region were studied the last 4 years. All farms showed some frequent low to moderate percentage of underdeveloped and PMWS pigs from weaning to finish which were serum sampled and necropsied (N=30 pigs each Farm), histopathology and molecular assessment of tissue homogenates set on FTA Cards© (Lymph nodes, Tonsils, Spleen, lung, heart run by Real Time qPCR), for novel viruses (PRRS,PCV2b,SI,TTV1&2,Myh). All farms showed crisis of abortions in which cases sows were bled by parity (N= 20 per farm) and their fetuses tissues (Lungs, Heart, Spleen, Thymus and Lymph nodes) homogenates set on FTA Cards© and evaluated by qPCR to novel viruses and Leptospira (MAT), as well as for histopathology of all viscera and placenta. Data obtained from qPCR from virus genome in pigs versus fetuses histopathology, clinical signs and necropsy findings were control by farm effect, virus detected versus PMWS versus regions studied by using SAS MULTIVARIATE ANOVA, statistical package of the Central University from Venezuela-FCV.

Results

Molecular, clinical and histopathology findings in pigs showed great deal of association ($p<0.05$) between PMWS with PCV2b in farm farm 1 (PRRS -). TTV1&2 was present in 4 out of the 30 pigs studied. The fetuses showed large concentration of PCV2b genome in Heart, Lung, Thymus and spleen while their mother were quite variable on serology but the aborted guilts showed no Ab to PCV2b compared to multiparous sows but guilts showed large amount of PCV2 genome indicating viremic stage prior to abortion. Farm 2 and 3 were PRRS + and PCV2b +. The pigs studied in both farms showed great frequence to TTV1&2 genome. Aborted fetuses showed low presence to TTV but great presence of PCV2 genome. Farms 4,5,6 were PCV2b + and PRRS -. TTV 1&2 genome was detected in 10% average of PMWS pigs and just 5% in aborted fetuses. From our results can be concluded that PCV2b was significantly associated to PMWS ($p<0.05$) and abortions than PRRS or TTV1&2. Farms that showed association of PCV2b and PRRS were more to have circulation of TTV1&2 either in weaned pigs and aborted fetuses. This is the first report of TTV1&2 in swine herds in Venezuela where the molecular detection was first seen in 2010 in a PRRS – farm 1.

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Influence of age on the effectiveness of piglet vaccination against PCV2 in the presence of high maternally derived antibodies

M Haake[1], C Weissenbacher-Lang[3], V Fachinger[4], A Eggen[4], M Ritzmann[1, 2], M Eddicks[2]
[1]*Clinic for Swine, University of Veterinary Medicine Vienna,* [2]*Clinic for Swine, Ludwig-Maximilians-University Oberschleissheim,* [3]*Institute of Pathology and Forensic Veterinary Medicine, University of Veterinary Medicine Vienna,* [4]*MSD Animal Health, Boxmeer* , m.eddicks@lmu.de

Introduction

Vaccination against PCV2 along with good production practice is currently the only way to control PCV2-AD. The use of most PCV2 vaccines is recommended for piglets from 3 weeks of age onwards. However, vaccination of even younger animals is increasingly becoming an area of interest. It is widely believed that the biggest obstacle to the effective vaccination of neonates is the potential for passive interference by maternally derived antibodies (MDA) (1, 2, 3). Antibody responses in neonates have been reported to be diminished and less persistent in the presence of high MDA levels (3, 4).

Materials and Methods

A field trial was carried out in a farm with high levels of MDA in suckling piglets. The animals were allocated to three study groups (**control**: no vaccination against PCV2, n= 200; **1st week**: PCV2 vaccination at one week of age n = 200, (Porcilis PCV®, MSD Animal Health); **3 weeks**: PCV2 vaccination at three weeks of age n = 200 (Porcilis PCV®, MSD Animal Health)). Growth performance, PCV2 antibody titer (ELISA) and amount of PCV2- antigen load (qPCR) in the blood were evaluated. The statistical analyses were performed using SAS software release 8.2 (2001) (SAS, Cary, NC; SAS Institute Inc.). Average daily weight gain was analyzed using analysis of variance with the factors "treatment group", "sex", "farrowing batch". The vaccinated groups were compared with the control group using ANOVA derived Dunnett tests. The Wilcoxon Mann–Whitney test was used in order to assess differences with regard to parameters of viraemia.

Results

At the start of the study, the antibody titers against PCV2 of all treatment groups were high with a mean of 10.5 $\log_2$. Peak levels of viraemia were reached at the end of finishing. Animals vaccinated at 3 weeks of age showed a significantly reduced viral load at the peak of viraemia ($p < 0.0001$) compared to the animals of the control group and animals vaccinated at 1 week of age (figure1). In addition, pigs vaccinated in the 3rd week of life showed a lower percentage of PCV2 DNA positive animals. A significantly higher ADWG in the fattening period and over the entire study period was calculated for the animals vaccinated in the 3rd week of life compared to the animals from the other 2 groups (table 1).

Table 1. Average daily weight gain (g/day) from weaning until end of finishing.

	time of vaccination		
period	3rd week	1st week	control
1.-11. week	332	317*	331*
11.-26. week	807**	792	782**
1.-26. week	606***	592	591***

* p = 0.043; ** p = 0.0061; *** p = 0.0265

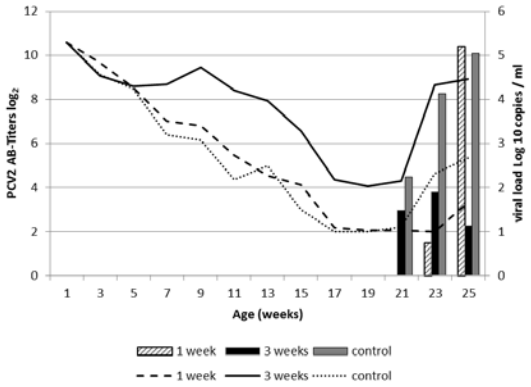

Figure 1. Lines: development of PCV2 Antibodies (AB) Bars: PCV2 viral load (measured on a herd level)

Conclusions and Discussion

Vaccination against PCV2 in the presence of high levels of MDA at three weeks of life leads to long lasting protection as measured by ADWG and reduced viral load following the onset of PCV2 viraemia. Vaccination in the first week of life does not lead to a significant reduction of the amount of viraemic pigs and the level of viraemia compared to the control animals. The matter that MDA titers were comparable high in both, the 1st and 3rd week of life, indicates that MDA levels are not the sole factor in the potential interference with vaccine efficacy. In fact, other factors, such as maternal PCV2-specific lymphocytes and maternally transferred non-specific cytokines, may play an equally important role, and should be taken into account when assessing vaccine efficacy in neonates.

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PCV2b presenting amino acid mutations in the capsid determines enhanced replication in swine testicle cells

TF Cruz[1], AMMG Castro[2], FJ Pedraza-Ordoñez[3], LJ Richtzenhain[2], JP Araújo Jr[1]
[1]*Department of Microbiology and Immunology, Biosciences Institute, Univ. Estadual Paulista (UNESP), Botucatu, SP, Brazil,* [2]*Department of Preventive Veterinary and Animal Health, College of Veterinary Medicine, University of Sao Paulo, SP, Brazil,* [3]*Departamento de Salud Animal, Facultad de Ciencias Agropecuarias, Universidad de Caldas, Manizales, Colombia* tfcruz@yahoo.com.br

Introduction

PCV2 is a small, single-stranded DNA virus that belongs to the family *Circoviridae*. PCV2 is characterized by absence of cytopathic effects (CPE) and lower viral titer in cell culture. Thus, the aim of this study was to describe the increased of viral replication in isolates of PCV2 that have amino acid mutations in the capsid protein associated with the occurrence of CPE.

Materials and Methods

Four isolates of PCV2 (B, C, D and E) with amino acid mutations in the ORF2 were used in this study. The isolates were obtained after viral isolation in swine testicle (ST) cells. The isolated B has one mutation (T200I), isolated C has two mutations (T200I; M72I), isolated D and E has three mutations (T200I; M72I; N77D).

ST cells with semiconfluent monolayer (25-cm^2 flasks) were inoculated with lymph node suspension (original PCV2b; group A), isolated B (group B), C (group C), D (group D) and E (group E). The subcultures were achieved each 48 hours. ST cells (control flask) were inoculated with only minimum essential medium. To evaluate viral replication, aliquots (intracellular viruses) were separated from the infected and control cell suspensions after trypsinization. Extracted DNAs were analyzed by qPCR (SYBR Green I – absolute quantification) for ORF2-PCV2 (1). The total of 17 passages was performed, but for some groups of cells, number of passage was lower due to occurrence of CPE.

Results

The viral loads determined for isolates B, C, D and E were higher when compared to original virus (group A) (Figure 1). All passages from the control group were negative for PCV2 by qPCR. The occurrence of CPE more severe coincided with the peak of viral load (10^{11} DNA copies/ml ST cell suspensions) in groups B, C, D and E. The CPE resulted in cell death. Thus, the number of passages was lower for isolates (D and E) presenting three amino acid mutations in the capsid protein. For isolated B (one mutation), C (two mutations) and original PCV2b, the number of passages performed was higher compared to isolates D and E. After the occurrence of CPE, due to cell death, the viral load was lower in the following passages.

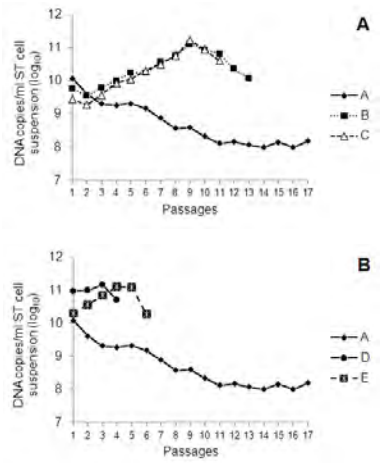

Figure 1. Viral load quantified by qPCR in ST cells infected with isolates of PCV2b. *A: groups A (original PCV2b; CPE: no), B (isolated B; CPE: passage 9) and C (isolated C; CPE: passage 9). B: groups A (original PCV2b; CPE: no), D (isolated D; CPE: passage 3) and E (isolated E; CPE: passage 4).*

Conclusions and Discussion

These results from this study suggested that the amino acid mutations alone or collectively were likely responsible for the enhanced PCV2 replication in ST cells. This is the first report that describes the occurrence of CPE caused by PCV2 in ST cells associated with increased viral load. The CPE observed in group B could be due to the occurrence of three mutations (T200I, M72I and N77D) detected in the thirteenth passage by sequencing (data not shown).

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PCV2b genotype is predominant in captive wild boars in Brazil

JPH Sato[1], D Gava[2], R Schaefer[2], NL Simon[2], ME Cantão[2], JR Ciacci-Zanella[2], DESN Barcellos[1].*Veterinary Medicine School, Federal University of Rio Grande do Sul/UFRGS, Porto Alegre, Brazil[1], Embrapa Swine and Poultry Research Center, Animal Health Laboratory, Concórdia, Brazil[2]*
janice.zanella@embrapa.br

Introduction

Porcine circovirus type 2 (PCV2) is an important infectious agent affecting both domestic and wild pigs (8). Phylogenetically, PCV2 is divided into two major groups, PCV2a (2A, 2B, 2C, 2D, 2E) and PCV2b (1A, 1B, 1C) which differ from each other in some nucleotides of the capsid protein (ORF2). A third genotype (PCV2c) was detected only in Danish pigs (6). Porcine circovirus associated disease (PCVAD) has been characterized in many pork producing countries worldwide (8). In Brazil, PCVAD was identified in 2000 for the first time, although PCV2 was detected in archived material dated from 1988 (2). Since its first detection, different genotypes (PCV2a and PCV2b) have been identified in Brazilian swine population. A single report have identified PCV2a in captive wild boar in 2012 (1). The aim of this study was to investigate the presence of PCV2 in captive wild boars.

Materials and Methods

Bronchial and mesenteric lymph nodes of 129 captive wild boars from two farms were collected at slaughter from January to July, 2012. Captive wild boars were slaughtered with seven months old and 32kg in average. Viral DNA was extracted from pools of lymph nodes of each animal using DNeasy Blood & Tissue Kit (Qiagen). The nested PCR (5) and PCR for sequencing (6) were performed using specific primers for PCV2 (3). The positive samples were tested by quantitative real time PCR (qPCR) (8). The PCR products were gel-purified using BigDye XTerminator Purification Kit (Qiagen) and nucleotide sequences were determined using an ABI3130xI Genetic Analyzer. The obtained sequences were analyzed and assembled with the Phred/Phrap/Consed software (4). Phylogenetic analyses were performed using the Maximun Likelihood and Neighbor Joining methods in the MEGA 6.0 software (10). The nucleotide and amino acid sequences of ORF2 and the complete viral genome were compared with other PCV2 sequences from NCBI database to determine the genotype and cluster classification.

Results

In the total, thirty-eight out of 129 (29.5%) pools of lymph nodes were positive for PCV2 by nested PCR. Seventeen out of eighty samples (21.3%) from farm #1 and twenty-one out of forty-nine samples (42.8%) from farm #2 were positive. Six out of 38 positive DNA samples were selected and qPCR analysed. All six samples were PCV2 qPCR positive, with viral copies ranging from $8.19x10^6$ to $1.19x10^9$ copies/uL.The genome sequence of all six samples were composed of 1,767 nucleotides (nt) which 699 nt codified ORF2 gene. All six samples showed 99% of nucleotide identity with PCV2b genotype. Although there were nucleotide differences on ORF2 sequences, the 233 amino acid analysis showed no difference among those sequences. Phylogenetic tree analyses classified all six sequenced samples as closely related with a PCV2b isolated from pigs in Brazil in 2004 (KC835191) and in 2009 (DQ923523) as well as PCV2b from wild boars detected in Hungary in 2003 (AY874163) and from Romania in 2008 (JN006458), 2010 (JN006464) and 2011 (JN382182). All five samples from farm #1 belonged to cluster 1A and one sample from farm #2 was classified as cluster 1B.

Conclusions and Discussion

This is the first description of PCV2b (cluster 1A and 1B) in captive wild boar. PCV2a infection was reported in PCVAD captive wild boars in Brazil previously (1). In general, PCV2a mainly ocurred before 2003 and PCV2b after 2003 (7). The occurrence of PCV2 in wildlife boars has been reported in different countries (9). The PCV2 is ubiquous and is present in pigs with or without clinical signs (7) but a high viral load was correlated with PCVAD (8). In our study, although the PCV2 load was elevated, which could indicate acute infection or viremia, no clinical condition or gross lesions were found. These findings show that PCV2b clusters 1A and 1B circulate in Brazilian captive wild boars, without clinical signs but with high viral load in lymph nodes. The PCV2 occurrence should be better investigated to determine the viral dynamic in the wild boar population.

Acknowledgement

This work was funded by Embrapa Swine and Poultry, CNPq (578102/2008-0) and UFRGS. JRC Zanella is a fellow of the National Council for Scientific and Technological Development (CNPq).

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Serological Profile of PCV-2 viremia and IgG levels in an unvaccinated herd

H Gauvreau[1], L Whittington[2]
[1]Consulting Veterinarian, Warman Veterinary Services, Saskatoon, SK, Canada, [2] Prairie Swine Centre,
helen.thoday@usask.ca

Introduction

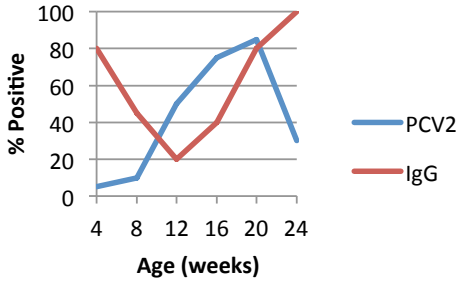
Very few swine herds remain unvaccinated for PCV-2. The Prairie Swine Centre (PSC) is a 300 sow high health farrow-to-finish commercial swine unit located 5 miles south-east of Saskatoon, Saskatchewan, Canada. It is a production research institute affiliated with the University of Saskatchewan and has a significant applied research role for producers in western Canada. The farm has never used sow or piglet vaccination to control PCVAD. The herd is PCV-2 positive and naive to the influence of vaccine in regards to serological profiling and clinical presentation of disease. In this study, PCV-2 viremia and IgG serological profiles were determined prior to further investigations of PCV-2 epidemiology in a naïve herd.

Materials and Methods

Blood samples were collected from groups of 20 pigs at 4, 8, 12, 16, 20, and 24 weeks of age. All the pigs were bled the same day. To generate a quantitative PCV-2 viremia profile samples were submitted to Prairie Diagnostic Services, Saskatoon, Sk. and tested using real-time PCV-2 PCR. A Ct value less than 38 is considered positive. For IgG serum profiling, samples were forwarded to Biovet Laboratories, St. Hyancinth, Quebec and tested using a commercial quantitative PCV2 ab ELISA IgG (Ingenasa) procedure. An S/P ratio of greater than 45 is considered positive. Titer values were calculated and reported for each age group of twenty pigs as the group geometric mean.

Results

PCV-2 Serum Profile



In general, serum IgG levels are elevated in the pigs at weaning (4weeks), decline over several weeks before starting to increase sharply around 12 weeks of age. The level of PCV-2 viremia (% positive pigs) increases slowly from weaning through the nursery period; when transferred to grower rooms there is a rapid increase. Levels of viremia in the oldest pigs declined steeply.

Table 1. Mean Serum Titres for Age Groups

Age Group (weeks)	Geometric Mean
4	720
8	188
12	101
16	320
20	4587
24	8018

Conclusions and Discussion.

At 4 weeks of age the serum IgG levels would be attributed to maternal antibody derived from colostrum intake which declines slowly until the pigs are about 12 weeks old. After 12 weeks IgG produced by their own immune system activity results in the increasing levels of IgG. Thaker et al. reported a similar pattern of serum titres seen in Table 1 although the absolute values were notably different.

A low percentage of piglets were viremic at weaning. The sharp increase in viremia corresponds to approximately two weeks after the pigs are transferred from nursery to grower. At this age they are moved from fully slated flooring to partially slatted concrete floors resulting in a higher level of environmental exposure to manure.

In spite of the high percentage of PCV-2 positive pigs observed in this study the level of clinical PCVAD in the grow-finisher herd remains remarkable low at less than 1%. Other management, environmental, genetic factors influencing this relationship need to be investigated.

Acknowledgements

Funding for this project has been provided by Agriculture and Agri-Food Canada through the Canadian Agricultural Adaptation Program (CAAP).

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Lack of relationship between TTSuV 1 and 2 with PCV2-associated reproductive failure in Mexico

AVargas-Ruiz[1], H Ramirez-Alvarez[1], I Rangel-Rodriguez[1], L Garcia-Camacho[1]
[1]*Facultad de Estudios Superiores Cuautitlan, Universidad Nacional Autonoma de Mexico,* luciangie30@hotmail.com

Introduction

Porcine Circovirus type 2 is a well-acknowledged cause of reproductive failure which promotes increased abortions, stillbirths and neonatal mortality as well as a fetal non-suppurative myocarditis.[1,6,]In Mexico, PCV2-reproductive failure has been described in most Mexican states,[8]displaying a high frequency (up to 39%) as well as a high proportion of transplacental transmission (approximately 50%)[2]. On the other hand, TTSuV1 and TTSuV2 have widely reported in PCV2-associated disease (PCVAD), predominantly in PMWS and PDNS cases.[4,5]Moreover, abortion and transplacental transmission have been described during infection with TTSuV1.[3,4,7]Theaim of the present work was to determine a potential relationship of TTSuV1 and/or TTSuV2 with PCV2-reproductive failure.

Materials and Methods

In order to assess such relationship, 65 paraffin-embedded fetal hearts with known-status of PCV2-reproductive failure (as determined by the presence of clinical signs, non-suppurative myocarditis, and in situ hybridization positive to PCV2) from 2004 to 2009, were selected to perform a nested PCR specific for TTSuV1 and TTSuV2. A contingency table was made based on PCV2-reproductive failure status and both TTSuV1 and TTSuV2 nested PCR results. The data were analyzed by Ji^2test ($\alpha < 0.01$).

Results

The frequency of TTSuV1 and TTSuV2 in the myocardium samples is shown in table 1. All samples were negative to TTSuV2. Overall, 22 out of 65 (33.8 %) were $TTSuV1^+$, and 33.3 % (15/45) of the PCV2-reproductive failure cases were $TTSuV1^+$. Similarly, 35 % (7/20) of the reproductive failure cases not associated with PCV2 were $TTSuV1^+$. However, there is no statistical relation between the presence of PCVAD and the presence of TTSuV1 by Ji^2 test

Table 1. Nested PCR results

SYNDROME	TTSuV1+ TTSuV2+	TTSuV1- TTSuV2+	TTSuV1+ TTSuV2-	TTSuV1- TTSuV2-	Total
RF-PCV2+	0	0	15	30	45
RF-PCV2-	0	0	7	13	20
Total	0	0	22	43	65

Conclusion and Discussion

To our knowledge, the role of both TTSuV species in cases of PCV2-reproductive failure has not been depicted. Considering the high prevalence of PCV2-reproductive failure in Mexico[2], it is of notice the low proportion and the absence of TTSuV1 and TTSuV2, respectively. Furthermore, there is no statistical association between the presence of TTSuV1 and PCV2-reproductive failure. Although high proportion of both TTSuV1 and TTSuV2 have been reported in cases of PMWS, either in single infections or in co-infection,[5] still a similar proportion (36.8%) of PMWS cases were $TTSuV1^+$ in México.[9] Genomic variability of TTSuV might be related with a different pathogenic potential due to a lower nucleotide sequence identity in Mexico strains. Thus, our preliminary results are in agreement with a lack of relationship between TTSuV and PCV2-reproductive failure but this finding must be further validated.

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Investigation of CaCV-1 in swine herds in Brazil

EL Zanella[1]; CH Okino[2]; JPH Sato[3], DESN Barcellos[3], JR Ciacci-Zanella[2].
[1] *College of Agronomy and Veterinary Medicine, University of Passo Fundo, Passo Fundo- RS, Brazil;*
[2]*Embrapa Swine and Poultry Research Center, Animal Health Laboratory, Concórdia, Brazil*[2]
[3]*Veterinary Medicine School, Federal University of Rio Grande do Sul/UFRGS, Porto Alegre, Brazil*[1],
janice.zanella@embrapa.br

Introduction

Circoviridae family comprises of the Circovirus genus whose member species are known to infect only birds and pigs, the Gyrovirus genus, including the Chicken anemia virus (CAV) and the proposed genus Cyclovirus (4, 9). In the past, only porcine circovirus was known to infect mammals and cause economic impact (4). Porcine circovirus type 2 (PCV2) is an important infectious agent affecting both domestic and wild pigs (8). PCV2 disease (PCVD) has been characterized in Brazil as well as in many pork-producing countries worldwide (1, 9). Studies have discovered single stranded DNA viruses co-infections as cause of reproductive problems in pigs in Brazil. Those analyses identified by PCR sequences of single stranded DNA viruses (ssDNA): PCV2, porcine circovirus type 1 (PCV1), torque-teno sus virus (TTSuV1 and TTV2 of the family *Anelloviridae*) and porcine parvovirus (PPV) in fetuses. Recently, a circovirus was isolated from dogs (CaCV-1) and associated with the death or injury in these animals (2, 5). In view of the frequent association and co-infection between PCV2 and other ssDNA viruses, the aim of this study was to investigate the presence of PCV2 and CaCV-1 in domestic and in captive wild boars in Brazil.

Materials and Methods

Samples used in this study included 56 samples of domestic pigs and 129 samples of captive wild boars. For domestic pigs, were analyzed 43 sera samples, pool of organs of 2 aborted fetuses, of 1 mummified piglet and of 1 stillborn piglet. In addition, lungs of 3 nursery pigs and lymph nodes of 6 nursery pigs from PCVD suspect cases were also analyzed. Bronchial and mesenteric lymph nodes of 129 captive wild boars from two farms were collected at slaughter. Captive wild boars were slaughtered with seven months old and 32kg in average. Viral DNA was extracted from pools of lymph nodes using DNeasy Blood & Tissue Kit (Qiagen). The PCV2 nested PCR (3) or PCV2 quantitative real time PCR (qPCR) (6, 7) and CaCV-1 real time PCR (5) were performed using specific primers and probes.

Results

Fifty-five out of 185 (29.7%) total samples (sera, pools of lymph nodes or lungs) were positive for PCV2 by nested and/or qPCR (Table 1). Twelve out of 43 sera samples of domestic pigs and 3 out of 6 lymph nodes of domestic pigs as well were positive for PCV2. In addition, one lung sample and pool of organs of a mummified pig was also positive for PCV2. For captive wild boars, 38 out of 129 lymph node pool of bronchial and mesenteric lymph nodes were positive for PCV2.
All 185 samples used in this study were negative for CaCV-1.

Table 1. qPCR results of 185 porcine samples tested for PCV2 and CaCV-1

Samples	qPCR results	
	PCV2	CaCV-1
Domestic pig		
Total of Samples	56	56
Positive Sera /Total	12/43	0/43
Positive LN / Total	3/6	0/6
Positive Lung / Total	1/3	0/3
Positive AF/ Total	0/2	0/2
Positive MP / Total	1/1	0/1
Positive SB / Total	0/1	0/1
Captive wild pig		
Total of Samples	129	129
Positive LN / Total	38/129	0/129
Total Positive Samples	55/185 (29.7%)	0/185 (0%)

LN: pool of bronchial and mesenteric lymph nodes
AF: pool of organs of aborted fetuses
MP: pool of organs of mummified piglet
SB: pool of organs of stillborn piglet

Conclusions and Discussion

Although all analyzed samples were negative for CaCV-1, this is the first description of molecular diagnostic of CaCV-1 in swine tissues, fetuses or sera in Brazil for both domestic and captive wild boars. This diagnostic tool is important to be accessible for studies of the pathogenesis and epidemiology of ssDNA viruses co-infections in economically important species such as swine.

Acknowledgement

The authors thank Neide Simon for technical support. This work was funded by Embrapa Swine and Poultry, CNPq (578102/2008-0) and UFRGS. JRC Zanella is a fellow of the National Council for Scientific and Technological Development (CNPq).

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Genetic characterization of ORF2 gene of the PCV2 from Sonora, Mexico farms

M Reséndiz-Sandoval, A Burgara-Estrella, A Arvayo-Zatarain, J Hernández*,
Laboratorio de Inmunología, *Centro de Investigación en Alimentación y Desarrollo A.C (CIAD), Hermosillo, Sonora, México*. *jhdez@ciad.mx

Introduction

Porcine circovirus type 2 (PCV2) affects swine production industry causing significant economic losses (1). The PCV2 genome contains three open reading frames (ORF1, ORF2, ORF3). ORF2 codes for the major capsid protein and is the most variable among strains. ORF2 is used for phylogenetic analysis and allows the classification of PCV2 into five genotypes (PCV2a, PCV2b, PCV2c, PCV2d, and PCV2e). The aim of this work was to characterize the ORF2 gene from viruses circulating in farms from Sonora, Mexico.

Materials and Methods

Serum samples were collected from 11 swine farms located in Sonora, Mexico. The DNA was isolated using a commercial system column-based following the manufacture's recommendations (Qiagen® CA, USA). PCR was performed using a recombinant Taq polymerase (Invitrogen® CA, USA) and primers previously reported (2). The amplification conditions were as follow: 94°C for 3 min, 35 cycles of 94°C for 1 min, 55°C for 30 seg, 72°C for 1 min and 72°C for 10 min. The products of 800 bp (n=9) were sequence in the GATC sequence service from the University of Arizona. The sequence alignment was carried out with BioEdit and phylogenetic tree was constructed with MEGA4 software.

Results

The analysis showed that the percentages of identity among the sequences from Sonora were 97-100% at nucleotide level and 96-100% at amino acid level. When compared the ORF2 sequences from viruses circulating in other countries, the identity was 85-100% at nucleotide level.

The phylogenetic tree revealed that the viruses circulating in Sonora were classified as PCV2a genotype (Fig. 1).

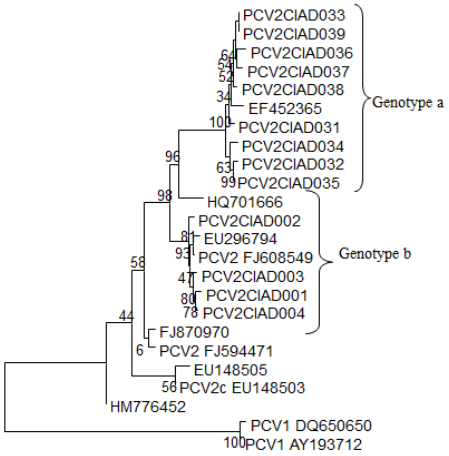

Figure 1. Phylogenetic relationship between ORF2 sequences of PCV2. Sequences from Sonora (n=9) were compared with 49 sequences from the GenBank.

Conclusions and Discussion

The high percentage of identity observed between sequences from different countries is in agreement with other authors (3, 4, 5). Previous studies conducted in our laboratory with samples from Sonora farms, reported viruses that belonged to the PCV2b genotype; all these samples were obtained from pigs with PMWS (5). In this work all PCV2 strains come from farms without clinical signs of PMWS and were grouped into the PCV2a genotype

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Mutation in the capsid protein of PCV2b determine cytopathogenicity in swine testicle cells

TF Cruz[1], FJ Pedraza-Ordoñez[2], AMMG Castro[3], LJ Richtzenhain[3], JP Araújo Jr[1]
[1]*Department of Microbiology and Immunology, Biosciences Institute, Univ. Estadual Paulista (UNESP), Botucatu, SP, Brazil,* [2]*Departamento de Salud Animal, Universidad de Caldas, Manizales, Colombia,* [3]*Department of Preventive Veterinary and Animal Health, College of Veterinary Medicine, University of Sao Paulo, SP, Brazil,*
tfcruz@yahoo.com.br

Introduction

Porcine circovirus type 2 (PCV2) is a DNA virus characterized by absence of cytopathic effects (CPE) in cell culture. Thus, the objective of the present study was to describe the occurrence of CPE in swine testicle (ST) cells infected with PCV2 presenting mutations in the viral capsid.

Materials and Methods

Four isolates of PCV2 (B, C, D and E) with amino acid mutations in the ORF2 were used in this study. The isolates were obtained after viral isolation in ST cells. The isolated B has one mutation (T200I), isolated C has two mutations (T200I; M72I), isolated D and E has three mutations (T200I; M72I; N77D).

To compare the CPE, ST cells with semiconfluent monolayer (25-cm^2 flasks) were inoculated with lymph node suspension (original PCV2b; group A), isolate B (group B), C (group C), D (group D) and E (group E). The subcultures were achieved each 48 hours. ST cells (control flask) were inoculated with only minimum essential medium. The total of 17 passages was performed, but for some groups of cells, number of passages was lower due to viral action. The PCV2 viruses from last passage of groups A, B, C, D and E were genetically characterized by determining the complete sequences (1).

Each flask was stained with Giemsa to assess cell morphology, except flasks of the first passage. The cell counting was performed on ten different fields randomly selected, and the magnification of 400X was used for evaluation. The results of cell counting were analyzed by the Fischer test ($\alpha = 0.05$), followed by Duncan test ($\alpha = 0.05$).

Results

One mutation (T200I) was observed in the sequence of virus from 17th passage of group A. Three mutations (T200I, M72I and N77D) were found in the last passages of groups B (13 passage), C (11 passage), D (4 passage) and E (6 passage). The cell counting data are shown in Figure 1. The cells are fusiform to rounded, becoming necrotic with eosinophilic cytoplasm, condensed chromatin, karyorrhexis and karyolysis. Finally, a decrease is observed in the number of cells that are more elongated (by the space they have to grow) and the morphologic characteristics are maintained (Figure 2). The CPE was more severe in the 9th passage of group C and B, 3rd passage of group D and 4th passage of group E. The control group and A did not show CPE.

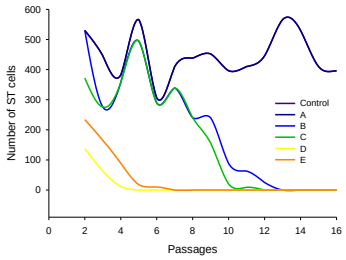

Figure 1. The evaluation of cell survival by the cell counting infected with five isolates of PCV2.

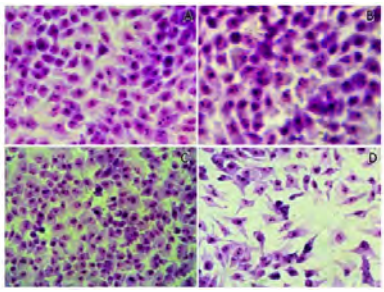

Figure 2. CPE, ninth passage. A: Control group (no virus). B: Group A (original PCV2b). C: Group B (isolated B). D: Group C (isolated C).

Conclusions and Discussion

This is the first report that describes the occurrence of CPE caused by PCV2 in ST cells. The two (T200I, M72I) and three (T200I, M72I, N77D) amino acid mutations in the viral capsid were observed in groups that shown CPE. The occurrence of one mutation (T200I) did not cause CPE in ST cell. The CPE observed in group B could be due to the occurrence of three mutations (T200I, M72I and N77D) detected in the 13th passage by sequencing. The presence of mutations in the viral capsid likely favoreced the viral replication, wich caused lower cell survival.

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Development of a single chain variable fragments antibody against of the capsid of PCV-2

MR Almeida[1], MR Santos[1], MB Heinemann[2], C Ueira-Vieira[3],GC Bressan[1], JL R Fietto[1], A Silva-Júnior[1]
[1]*Laboratory of Molecular Animal Infectology, BIOAGRO, Universidade Federal de Viçosa, Viçosa, Brazil,* [2] *Federal Universirty of Minas Gerais, Belo Horizonte, Brazil.*[3]*Federal Universirty of Uberlandia, Uberlandia, Brazil,* abelardo.junior@ufv.br.

Introduction

Porcine circovirus-2 (PCV2) is an emerging virus associated with a number of different syndromes in pigs known as Porcine Circovirus Associated Diseases (PCVAD) (1). The genome of PCV2 encodes the viral capsid protein (cap). The cap protein is an important structural protein that is the main target for diagnostic tests (2). Single chain fragment variable (scFv) is a molecule that consist of the variable region of the light chain (VL) and heavy (VH) joined by a flexible linker polypeptide. The scFv molecule can be select by recombinant phages and ELISA assays (3). In this work, scFv were selected against recombinant cap protein (rcap-PCV2) by phage display technique.

Materials and Methods

The selection of scFv used an immune phage display library of antibody fragments fused to PIII protein of M13 phage (4). Were performed two cycles of selection against rcap-PCV2. Positive clones for expression of the scFv fragments were tested for their affinity with rcap-PCV2 by ELISA. To confirm the affinity of the positive clones with rcap-PCV2 was performed western blotting. Heavy and light chains from positive clones were sequenced. Bioinformatics analyzes were performed.

Results

The presence of scFv in solution was verified. Eighteen clones were selected based on the best readings of optical density. In figure 1, it was possible to identify 14 clones that have significantly higher affinities with rcap-PCV2 compared to the control. Among these clones, F1 and F5 presented higher reactivity with rcap-PCV2 (Fig 1). In Western blotting assay scFv clones F1 and F5 showed reactivity with rcap-PCV2 (Fig 2). Sequence analysis of selected F1 and F5 clones showed differences between the hyper-variable (CDR) regions, especially in the light chain (Table 1).

Table 1. Sequence of amino acid of CDR of light chain of the F1 and F5 clones. The DNA sequences were analyzed using the Ig-BLAST alignment program.

Clone	CDR1	CDR2	CDR3
F1	RASQSISSSL	AASSLQS	TELQNP
F5	PGQSEYLVGE	RQSRITK	STELQN

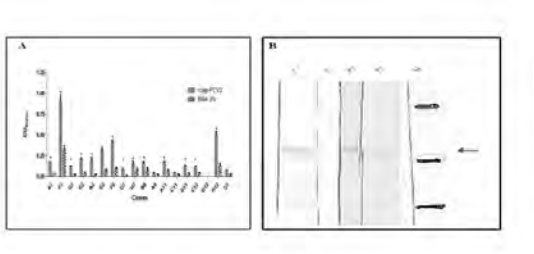

Figure 1. A: ELISA analysis of scFvs showed affinity with rcap- PCV2 and 3% BSA (negative control). B: Western blotting to evaluate the reaction between scFv clones generated by F1 and F5 samples. C+: Commercial serum anti-PCV2. C-: negative serum. M: marker. The arrow indicates the molecular weight (30 kDa).

Conclusions and Discussion

Monoclonal antibodies as scFv have been produced by the technique of phage display against several types of molecules. All these antibody fragments have good potential for use in therapy or diagnosis (5). A definitive diagnosis of the PCVAD is based in detection of the antigen associated with the clinical signs and lesions caused by the disease (6). Recombinant monoclonal antibodies developed in this study could be used as candidates for diagnostic of PCV2.

Acknowledgments

CAPES, CNPq and FAPEMIG for finnancial support.

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Antibodies response and identification of immunoreactive regions of the capsid of PCV-2

JLR Fietto[1], MR Santos[1], MB Heinemann[2], C Ueira-Vieira[3],GC Bressan[1], MR Almeida[1], A Silva-Júnior[1]

[1]Laboratory of Molecular Animal Infectology, BIOAGRO, Universidade Federal de Viçosa, Viçosa, Brazil, [2] Federal Universirty of Minas Gerais, Belo Horizonte, Brazil

.[3]Federal Universirty of Uberlandia, Uberlandia, Brazil, abelardo.junior@ufv.br.

Introduction

Porcine circovirus-2 (PCV2) is the causative agent of postweaning multisystemic wasting syndrome (PMWS), one of the most important swine diseases worldwide (1). The genome of PCV2 encodes the viral capsid protein (Cap). The cap protein is an important structural protein that is immunogenic to PCV2 and is the main target in the fabrication of vaccines against PCV2 (2). In this work, mimetic peptides of the recombinant cap protein were identify by Phage Display technique and antibody response in mice immunized with the peptides were evaluated.

Materials and Methods

The selection of mimetic peptides used phage libraries that exhibited the required molecules merged to the PIII protein of the viral capsid (Ph.D.™ 12 and Ph.D. ™ - 7C7, New England BioLabs®Inc). Phages expressing peptides were selected according to their affinity to anti-PCV2 swine serum, anti-rcap-PCV2 rabbit serum and monoclonal antibodies. Female Balb/c mice aged 5 weeks old were randomly assigned to eigth groups of 5 animals. The animals were vaccinated with phages that contained peptide mimetics to cap protein. In C+ group, the animals were vaccinated with recombinant Cap protein. In C- group, the animals were inoculated with PBS. In wt group, the animals were vaccinated with wild type phage. In C12-S14 groups, the animals were vaccinated with five phages per group. The animals were vaccinated three times in interval of 15 days. Blood samples were collected on day 45 for serological testing by ELISA.

Results

The characterization of selected peptides using bioinformatics analysis and validation by phage display technique revealed antigenicity in five regions of the cap-PCV2 (Table 1). To reinforce these predictions checking the induction of antibodies against PCV2 cap in animals immunized with phages clones were performed (Figure 1).

Table 1. Sequence of peptides selected after alignment and phage-ELISA assays.

Clone	Sequence	Cap region	Alignment[1]	O.D. ratio[2] value
S14	TMGYTQRGLVTV	52-56	**T**M**GYT**QRGLVTV	1.94
S34	SVIQKATAEPYL	130-142	S**VI**Q**KATA**E**PYL**	2.77
C12	DSTIDYT	165-169	**DSTIDY**T	2.63
F1	GGDSNPDPTSMP	83-88	**GGDSNP**DPTSMP	1.35
F7	ASPHLEHTTQQP	145-153	A**SP**HLE**HT**TQ**QP**	1.94

[1]To perform the alignment BLASTp (6) was used.
[2]Value of optical density of mimetic peptide phage relative to wild phage.

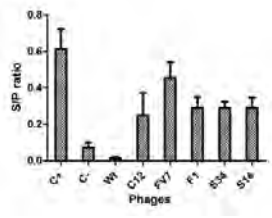

Figure 1. Antibodies response by ELISA in mouse immunized with phage clones.

Conclusions and Discussion

Some studies that use bioinformatics analysis or monoclonal antibodies have investigated the regions of epitopes in the capsid protein of PCV-2 (3, 4, 5). Use of Phage Display proved its efficiency for selection of peptides with similarity to regions of the Cap protein of PCV2. The immunoreactivity was detected in clones S14, S34, C12, F1 and FV7, which indicate that regions 52-56, 83-88, 130-142, 148-153 and 168-173 of the cap protein are immunogenic.

Acknowledgments

CAPES, CNPq and FAPEMIG for finnancial support.

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PCV2 isolated during an outbreak in vaccinated pigs in Brazil

A Silva-Júnior[1], RL Salgado[1], PM Vidigal[1], LFL Souza[1], TS Onofre[1], NF Gonzaga[1], MR Eller[1], GC Bressan[1], JLR Fietto[1], MR Almeida[1]

[1]*Laboratory of Molecular Animal Infectology, BIOAGRO, Universidade Federal de Viçosa, Viçosa, Brazil,* abelardo.junior@ufv.br

Introduction

Porcine circovirus-2 (PCV2) belongs to family *Circoviridae*, genus *Circovirus*, and was initially identified as the causative agent of Post-weaning Multisystemic Wasting Syndrome (PMWS) (1). Since its identification, PMWS became an endemic syndrome in most pig-producing countries and PCV2 has reached a worldwide distribution (2). The PCV2 isolates currently are classified into three major genotypes: PCV2a, PCV2b, and PCV2c (3). Vaccination against PCV2 has only been introduced in Brazil after 2007 as a measure to control PMWS. Here, we presented the complete genomic sequences of a new PCV2 strain (PCV2-UFV1) isolated from pigs in a farm located in Brazilian Southeast during a PMWS outbreak in September 2013. All animals were vaccinated against PCV2.

Materials and Methods

Tissue samples of nine vaccinated animals with clinical symptoms of PMWS were collected, and PCV2 infection was confirmed by PCR using two pairs of primers. The PCR products were purified and sequenced. For comparison purposes, 1,126 genomic sequences of PCV2 isolates were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/Genbank), including the reference isolates of genotypes PCV2a (AF055392), PCV2b (AF055394), and PCV2c (EU148503).

Results

The genotyping of the PCV2-UFV1 was performed as described by Vidigal et al. (3), being classified in PCV2d genotype (Figure 01). In pairwise genomic comparisons, its genome showed high identities (>99%) with other 154 viral isolates from China, Serbia, South Korea, and USA. Among the isolates, the Chinese BDH strain (HM038017) is associated to antigenic changes in PCV2 (4), and the North-American US22625-33 (JX535296) and US22664-35 (JX535297) strains are associated to vaccine failures (5). All these strains, including PCV2-UFV1 have an additional lysine residue at position 234 in the capsid protein (4, 5).

Conclusions and Discussion

We have reported a new Brazilian strain (PCV2-UFV1) isolated from vaccinated pigs during a PMWS outbreak, which are similar to isolates associated to outbreaks and vaccination failures in China and USA. These data support the recent theory on the occurrence of an antigenic drift of PCV2 that might be the cause of vaccine failures throughout the world.

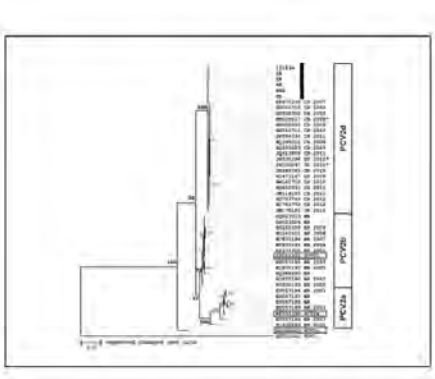

Figure 1. Genetic grouping based on nucleotide sequences of PCV2. *The length of the horizontal bar between the groups is proportional to the genetic distance among the isolates. The positions of the PCV2 isolates detected in this study are indicated by black column.*

Acknowledgments

CAPES, CNPq and FAPEMIG for finnancial support.

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Effect of PCV 2 vaccine application on production of a commercial 6000-sow farm in China

Z Xin, [1] C Min, D En Qiu, [1] S Laizhu [2]
Yan-tai long-da breeding co., LTD Shandong Lai yang 265200
[2] *Zoetis International Trading (Shanghai) Co., Ltd, Shanghai China*
zhangxin@longdameat.cn

Introduction

PCV2 is one of the main pathogens of respiratory disease syndrome in pigs. Since 1997, it has brought huge economic losses to the pig industry. In China, from 2000 to 2007, 58.75% of the farms were positive to PCV2 and in those farms, 38.47% of the pigs were positive [1]. The direct economic losses of PCV2 in affected farms were mainly due to high mortalities in the nursery and growing pigs, and indirectly on the cost of production due to the increase in use of antibiotics used to treat secondary infections.

Materials and Methods

This trial started from September 2012 to February 2013 in a 6000 sows in one-site farm in Shandong province, China. Two commercial vaccines were used in this study; piglets were vaccinated in 14-old days with 0.5 dose of A or 1dose with B vaccine.

A total of 15 batches were enrolled in this study (each batch has approximately 650 piglets). Piglets in each batch have been randomly assigned to each of the vaccine groups. In total, there were 9635 vaccinated piglets in this study, including 5176 vaccinated with A and 4459 vaccinated with B. These piglets were fed by the same formula, and reared in the same environment from the start until the end of the study.

The parameters measured to compare the 2 PCV vaccines in this study include: mortality rate, average daily gain, feed conversion rate and cost of weight gain. SPSS13.0 software was used for statistical analysis.

Results

The results are summarized in the table below.

Table 1. Summary of Results on Production Parameters

Production Parameters	Vaccine Group	
	A	B
Mortality Rate	4.29±0.013	7.80±0.035
ADG	555.34±13.60	537.59±28.92
FCR	2.56±0.08	2.62±0.12
Cost of Gain	CNY 8.53±0.28	CNY 8.78±2.12

Note: different lowercase letters peer shoulder showed significant difference ($P < 0.05$), and different capital letters showed extremely significant difference ($P < 0.01$), the same letter indicates no significant difference ($P > 0.05$), the same below.

Discussion

The health status of piglets usually becomes unstable after weaning. It is usually due to both seroconversion to PRRS and disappearance of the maternal antibodies against PCV2. However, with the use of commercial vaccines, the clinical impact of PCV2 is significantly reduced. In a study done by Jacela et al., they showed that pigs had superior growth performance and lower mortality after vaccination with commercial PCV2 vaccines[2]. There are quite a number of studies about the impact of PCV vaccines on production performance, however, the sample size are relatively small so it was decided to do this large scale study.

Differences in production performance between vaccine A and Vaccine B has been observed in this study in terms of mortality rate (4.21% vs.7.80%, difference -3.79%; p=0.1507), average daily weight gain (555g/day vs.537g/day; difference +18g/day; p<0.0001), feed conversion rate (2.56 vs. 2.62, difference - 0.06) and cost of gain (CNY8.53 vs CNY8.78, difference – CNY0.25). Moreover, the author observed that there are more pigs that had clinical signs of PCVAD, such as inguinal lymph node enlargement and typical PMWS symptoms in Vaccine B group.

The results on production performance and the other clinical observations, we conclude that vaccine A is more effective than vaccine B in the conditions in this study.

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The features of PCV2 as an emerging in Ukraine

L Dudar[1], V Polishchuk[1], I Sobko[2]
[1-] *Virology department at the NNC "Institute of Biology" Taras Shevchenko' Kyiv National University, 64 Volodymyrska str., Kyiv 01033, Ukraine;* [2]*Bio-Test Laboratory LLC, Kiev, Ukraine.* liudmyla.dudar@hipra.com

Introduction

Ukraine is a high-risk country for new infectious diseases of pigs to transmit. Porcine circovirus 2 (PCV2) is causal agent of porcine multisystemic wasting syndrome (PMWS) and other PCV2-assocciated diseases. PCV2 has some genetic variation and has been found in both domestic and wild pigs (2). This virus is highly stable even under high temperatures, under many different pH-values, and resistant against many common disinfectants (iodine, alcohol, phenol, and formaldehyde) (3).

The clinical signs of PCV2-assocciated infection of pigs in Ukraine were described from beginning of 2000s. However, the information about the detection of PCV2 isolates, their characterization and distribution in different regions, have been not published yet.

The aims of this study were to investigate the disease transmission dynamics in the livestock interface in Ukraine with PCV2 as a model, and to estimate the prevalence of PCV2 in domestic pigs. Therefore, we have conducted a study to identify the causative agent of the mentioned disease.

Materials and Methods

The samples of pathological material of 352 animals from 109 farms all over Ukraine regions were carried out during 6 years. The samples (lungs, mesenteric and lymph nodes, small intestine etc.) were collected during pathology examination after observation of clinical signs and frozen -20 °C.

DNA extraction from samples from 2007 to 2009 year has been proceeding by sorbent approach and from 2010 to 2012 has been done with ready-to-use filtrating columns kit «Tissue DNA» (*Machery Nagel*).

The specific primers, recommended by OIE, were used for PCV2 genome detection and optimized. The PCR amplification using different commercial Taq-polymerases and Master-Mixes was carried out on DNA extracted from mentioned samples by ordinary amplification protocol.

Results

During examination of the tissue samples from animals, the external and internal pathological changes, specific for PCV-associated pig syndromes, were described as followed:

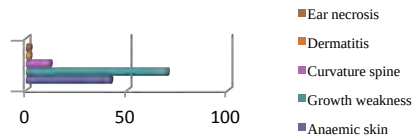

Figure 1. Index of external pathological features found among studied animals.

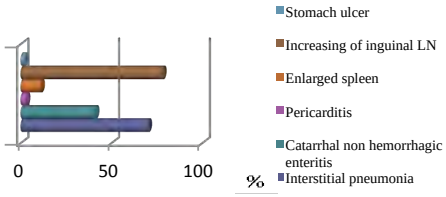

Figure 2. Index of internal pathological features found in the studied material.

The detection of PCV-2 in dependence of the localization showed some relationship. In samples of intestine and lymph nodes the virus was detected in 100% samples with external signs of PCV2-associative disease; in blood samples - 60%; in the combined samples of intestines and lungs - 80%; and in intestines and lymph nodes - 66.6%; lungs, intestines and lymph nodes - 50%.

In conclusion, it was postulated that prevalence of PCV-2 on the territory of Ukraine is about 41%, because the viral DNA were detected in 144 of 352 samples.

Conclusions and Discussion

Consequently, the research results indicate the possibility of detecting of PCV-2 by PCR in all of the mentioned tissues, but with varying frequency.

Summarizing the data of the described long-term and large-scale studies, we can state that PCV-2 is extremely common virus throughout the territory of Ukraine.

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Farrowing parameters enhancement in a Brazilian farm using PARVOSUIN MR®
(Swine erysipelas and porcine parvovirus inactivated vaccine)

I Rodríguez-Ballarà[1], W Grieder[2], L Crestani[3]
[1]*Technical services, HIPRA, Spain,* [2]*HIPRA SAUDE ANIMAL Brazil,* [3] *Granja Folhados, Minas Gerais, Brazil.*
isaac.rodriguez@hipra.com

Inroduction
Vaccines against Swine Erysipela (SE) and Porcine Parvovirus (PPV) are widely used in intensive swine farms. In Europe most of the swine farms use a bivalent vaccine, besides in America Erysipela and Parvovirus vaccines include Leptospira sp (Le). PPV infection after 6 days of conception until 35 days of gestation results in embryonic death and maternal resorption of fetal tissues. At or about day 70 of gestation, the fetus is able to mount effective immune response and eliminate the virus (1). Besides, subacute and chronical SE infection can cause infertility, litters with increased number of mummies or small litters. In some cases these signs may be so mild as to remain unnoticed (2).
SE and mainly PPV are widespread among swine so can cause reproductive losses in unvaccinated herds or when the vaccine is not working correctly.
The objective of this study is to compare the historical reproductive sow performance in a Brazilian farm before and after the change from a trivalent reproductive vaccine (SE, PPV, Lepto) to a bivalent reproductive vaccine (SE, PPV).

Materials and Method
The study was carried out in a 1800 sow's farm in Minas Gerais State, Brazil. The vaccinal program included a trivalent (SE, PPV, Le) vaccine (Vaccine A). In November 2012 the reproductive vaccine was changed to a bivalent (PPV, SE) vaccine (Parvosuin MR®). The vaccinal program implemented with the bivalent vaccine was the same used with trivalent vaccine. Parvosuin MR® was administrated at 14 days after farrowing in sows, and 2 doses before mating to the gilts.
In order to assess the field efficacy of Parvosuin MR® reproductive data from lactation units was recorded and compared between 5 months before to start the vaccination with Parvosuin MR® and 5 months after the vaccination change. Three main farrowing parameters were selected to evaluate the impact change vaccination in reproductive parameters: total piglets born per litter, total born alive piglets and percentage mummified piglets. The total sow's data (multiparous sows and primiparous sows) and particularly primiparous sow's data were evaluated.

Results
Total piglets born per litter and total born alive piglets increased significantly (*P<0.05, t-test for independent samples*) after Parvosuin MR® implementation of (Figure 1), the increase was also significant in primiparous sows (Figure 2). The percentage of mummified piglets after Parvosuin MR® vaccination was reduced by 0,3 %.

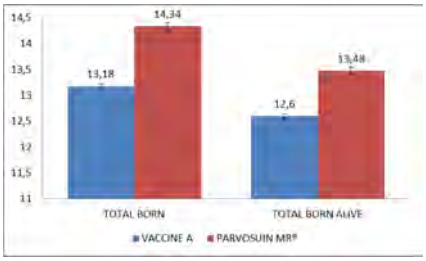

Figure 1. Mean Total Piglets Born / Litter and Total Born Alive/Litter from the entire reproductive herd (± SEM).

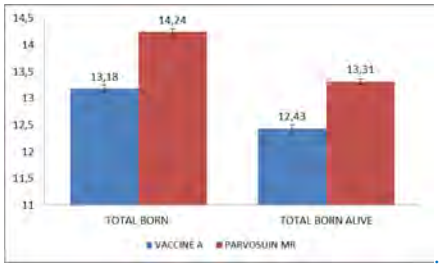

Figure 2. Mean Total Piglets Born/Litter and Total Born Alive/Litter from first parity sows (± SEM).

Conclusions and Discussion
After the implementation of Parvosuin MR® vaccination farrowing parameters improved significantly. The significant increase of total piglets born was improving directly the total piglets weaned and definitely the productive parameters in farrowing units. Besides, this case is showing the importance of SE and PPV infections in the sow reproductive failure (3). This historical comparative field study is showing again the efficacy differences in field between trivalent and bivalent reproductive vaccines as was demonstrated in previous studies (4).

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Experimental infection of a highly pathogenic PRRSV-QY1 strain at different passage levels

W Lu[a], B Sun[a], Q Xie[a], Y Bee[a], J Ma[a]*
[a]*College of Animal Science, South China Agricultural University, Tianhe District, Wushan Road, Guangzhou 510642, Guangdong, P.R. China* mjy000713@gmail.com

Introduction

PRRSV has been widespread in most swine-producing countries worldwide and causes substantial economic losses to the swine industry. The highly pathogenic PRRSV (HP-PRRSV) emerged in mainland China in 2006, and was the most significant variant due to its outcome of clinical severity (1). In the present study, a virulent strain of HP- PRRSV QY1 was serially passaged in Marc145 cells for up to 100 passages and the final and intermediate passage levels were used to perform an animal infection experiment.

Materiasl and Methods

A total of fifty 35-day-old PRRSV-free piglets were randomly divided into five groups with 10 animals in each group. At 6 weeks of age, piglets in group 1, 2, 3 and 4 were inoculated intramuscularly with 2×10^5 tissue culture infective does (TCID50) QY1 virus of P5, P60, P80 and P100, respectively. Group 5 was intramuscularly injected with Dulbecco's Modified Eagle's Medium (DMEM) and used as the negative control. During the course of the study, viraemia, serology, clinical signs, rectal temperature, and weight gain were monitored. Animal were euthanized on 14 DPI (n=5 per group), followed by lungs examination for gross and microscopic changes.

Results

It was found that QY1 was gradually attenuated during the in vitro passage. The earlier passage virus P5 developed typical clinical symptoms of HP-PRRSV including high fever, anorexia, depression, lethargy, dyspnea, skin cyanosis and 2/10 piglets in this group died on 9 dpi and 11 dpi, respectively. QY1 P60 exhibited mild to moderate clinical symptoms, and the P80 showed very mild clinical. Animals inoculated with QY1 P100 did not show any significant clinical symptoms throughout the experiment. Gross lung lesions above 10% in P60 and P80.

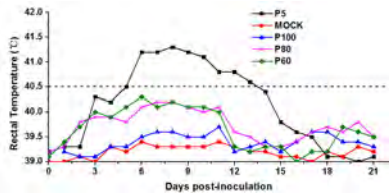

Figure 1. Mean rectal temperature following inoculation with different QY1 passage virus.

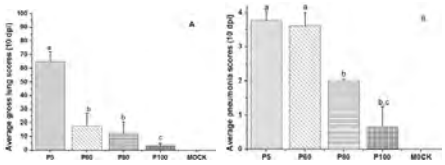

Figure 2. Average gross lung scores (A) and pneumonia scores (B) were recorded at 10 dpi. The data were expressed as the mean±S.D. from 5 pigs in each group.

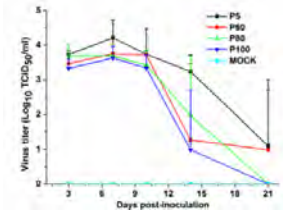

Figure 3. Level of viral titers on Marc-145 cells in the serum. The data were expressed as the mean±S.D. from 5 pigs in each group.

Conclusions and Discussion

The present study had clearly displayed that HP-PRRSV QY1 P100 had been attenuated in vitro and virus attenuation was further supported by pathological examination. The virulent phenotype grew to significantly higher levels in vivo than cell-culture adapted isolates.

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Virulence comparison of four type 2 PRRSV strains

WH Lu[a], HM Tun[b], BL Sun[a], YZ Bi[a], FC-C Leung[b], JY Ma [a]
[a]*College of Animal Science, South China Agricultural University, Tianhe District, Wushan Road, Guangzhou 510642, Guangdong, P.R. China,* [b]*School of Biological Sciences, University of Hong Kong, Pokfulam Road, Hong Kong SAR, P.R. China,* mjy000713@gmail.com

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) was first reported in China since late 1995 and several variants were further reported in subsequence years, causing huge economic losses to Chinese swine industry. To date, three lineages (lineage 3, 5.1 and 8.7) of PRRV were reported in China based on our global genotyping (1). In the present study, we compared the pathogenicity and clinical presentations among currently isolated viruses. One pathogenic isolate that belonged to the HP-PRRSVs cluster which emergence in China in 2007, two pathogenic isolates were associated with the re-emergence of lineage 3 PRRSV variants.

Materiasl and Methods

A total of thirty 5-weeks-old piglets from a herd negative for both PRRSV antibody and antigen were divided into 5 groups (group1 to 5) of 6 pigs each. Pigs in group 1-3 were intramuscularly inoculated with 2ml of either PRRSV strain (QY1, QYYZ, and GM2) containing 10^4 median cell culture infectious does (CCID50), group4 was intramuscularly vaccinated with two doses of Ingelvac-PRRS MLV (10^5 CCID50/ml) and group 5 was challenged with non-infected cell culture medium as negative control. Clinical signs and body temperature were monitored daily. Serums were collected for virus load and PRRSV specific antibody detection. All pigs were euthanized and necropsied on 21DPI.

Results

In vivo experiment showed that only HP-PRRS variant QY1 caused 2 piglets death on 7dpi and 10 dpi. However, other PRRSV viruses caused 0% mortality rate until 21dpi.

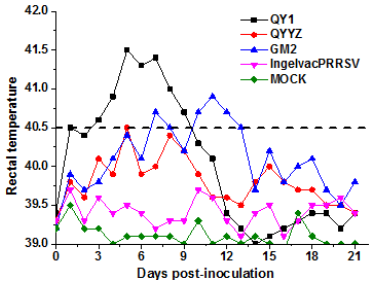

Figure 1. Mean rectal temperature for each experimental group from 0 days of inoculation to the end of the experiment

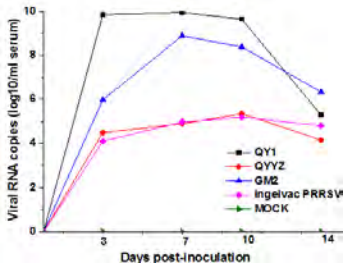

Figure 2. Viral copies in the serum at each collection time point. The data were expressed as the mean from 3 pigs in each group. No PRRSV were detected from any of the samples collected pigs in the Mock infected group.

Conclusions

The results obtains indicated that type 2 PRRSV exhibit variability in genome and pathogenicity and HP-PRRSV showed more virulent than the re-emergence of lineage 3 PRRSV variants.

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P.560

Phylogenetic analysis and molecular characteristics of five variant Chinese field isolates of PRRSV

Z Pei, R Chen, D He, X Zhang, H Liu
Guangdong Dahuanong Animal Health Products Co.,Ltd, Xinxing, China peizhangfu2003@aliyun.com

Introduction

Porcine reproductive and respiratory syndrome (PRRS) has now been widely recognized as an economically important disease. The objective of this study was to compare the molecular and biological characteristics of porcine reproductive and respiratory syndrome virus (PRRSV) field isolates in China to those of the modified live virus (MLV Resp PRRS/Repro) PRRS vaccine and its parent strain (ATCC VR2332).

Results

The two genes (GP5 and NSP2) of five isolates of PRRSV from China, designated AH4, GX5, ZJ1, ZJ2 and ZJ3, were sequenced and analyzed. Phylogenetic analyses based on the nucleotide sequence of the ORF5 showed that the five Chinese isolates belonged to the same genetic subgroup and were related to the North American PRRS genotype. Comparative analysis with the relevant sequences of another Chinese isolate (NVDC-JXA1) and North American (VR2332 and MLV Resp PRRS/Repro) viruses revealed that these isolates have 87.2-89.4% homology with VR2332, and 87.2-89.1% identity with MLV Resp PRRS/Repro vaccine and 89.9-99.2% with NVDC-JXA1. All NSP2 nonstructural protein of these five isolates exhibited variations (a 29 amino acids deletion) in comparison with other North American PRRSV isolates. Therefore, these isolates were novel strain with unique amino acid composition. However, they all share more than 96.8% identity with other highly pathogenic Chinese PRRS strains. Additionally, there are extensive amino acid (AA) mutations in the GP5 protein and the NSP2 protein when compared with the previous isolates.

Conclusions and Discussion

These results might be useful to study the genetic diversity of PRRSV in China and to track the infection sources as well as for vaccines development.

Acknowledgments

Funds for this research were provided by *Guangdong Wens Foodstuff Group Co.Ltd*.

Dr Ruiai Chen is a professor in the Department of Veterinary, South China Agricultural University. Her research interests are PRRSV and porcine epidemic diarrhea.

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Phylogenetic analysis of PRRSV in Colombian intensive pig farms

MA Rincon[1], JN Castro[1], CP Calderón[1], AV Castillo[1], LM Perez[1], DC Gomez[1], Y Chimbi[1], E Mendoza[1]
Laboratorio Nacional de Diagnóstico Veterinario, Instituto Colombiano Agropecuario-ICA, maria.rincon@ica.gov.co

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is characterized by its genetic variability (1). Open reading frame five (ORF5), encodes the structural glycoprotein GP5, this fragment is the most variable in viral genome and for this reason is ideal for understand the genetic relationship between different PRRS isolates and is used for the phylogenetic tree construction (2).Therefore, the aims of this research was to analyze ORF5 sequences of PRRSV isolated in field samples of swine farms in the highest productivity departments in Colombia, from 2012 to 2013.

Materials and Methods

The field samples were collected from intensive swine farms from Antioquia and Valle del Cauca in Colombia, (n=32). RNA was extracted from tissue (lung, linfonode or tonsil), serum or oral fluid by the use of RNeasy kit, Qiagen. Then, we realized a nested RT-PCR using a specific set of primers designed on ORF5 fragment (3). PCR products were sequenced in both directions, using the intern amplification primers by a 3130 Applied Biosystems genetic analyzer.

Results

Phylogenetic tree is shown in Figure 1. All the isolates were ranked in genotype 2. Thirty of them were included with the **VR2332** reference strain group and two isolates were included with the high patogenicity MN184 virus. Nucleotide variation between isolates of the same farm and geographic region, were noted.

Conclusions and Discussion

This study found that American genotype predominated in Colombian pigs. It is well known that Colombia keeps a wide commercial exchange with North American countries, which includes the import of live animals and semen, so it is not a surprise that the strains analyzed in this work were found to be closely related to Canadian and American strains. These studies contribute to the understanding of PRRSV molecular epidemiology in these geographic regions.

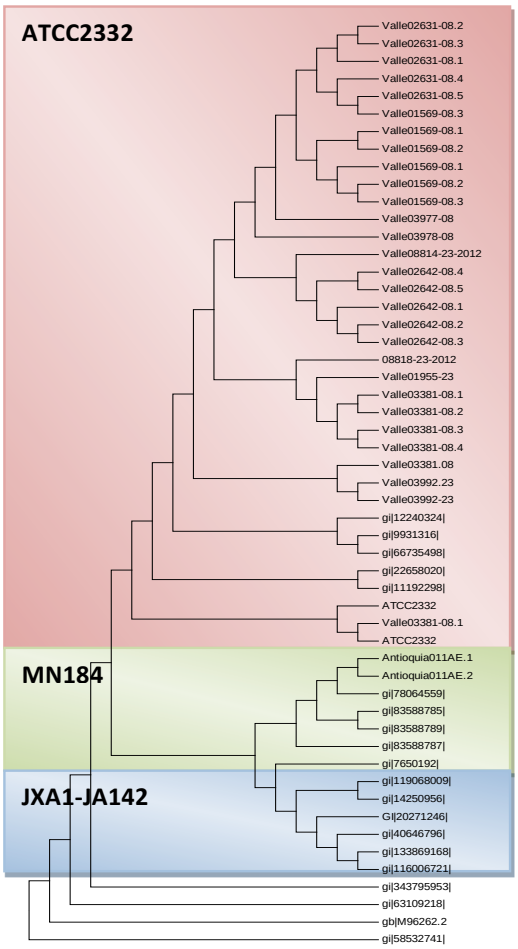

Figure 1. Phylogenetic tree constructed by the neighbor-joining method based on **ORF5 PRRSV fragment.**

Acknowledgments

This work was supported by the Colombian Swine Producers.

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The initiative of PRRS area regional control/elimination in Japan (P-JET: PRRS-Japan Elimination Team)

S Otake[1,13], S Arai[2], Y Hayakawa[11], K Ikeda[10], H Iseki[4], S Ishizeki[3], R Kano[9], F Koike[5], T Furuichi[7], M Furukawa[7], M Miyashita[9], Y Mizukami[8], S Nakatake[12], M Notsute[6], T Shibuya[9], H Ishikawa[3], H Tsunemitsu[4]

[1]Swine Extension & Consulting, Inc., [2]Azabu University, [3]Summit Veterinary Services, [4]Natinal Institute of Animal Health, [5]SMC, [6]Notsute Swine Clinic, [7]Toyoura Veterinary Clinic, [8]Akabane Animal Clinic, [9]Boehringer Ingelheim Vetmedica Japan, [10]IDEXX Laboratories K.K., [11]PIGLETS, [12]Miyazaki Prefectural Economic Federation of Agricultural Co-operatives, [13] Swine Disease Eradication Center, University of Minnesota

satoshiotake@hotmail.co.jp

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically significant diseases in the Japanese swine industry. The annual economical loss due to PRRS in Japan was reported as ￥28 billion ($373 million)[1]. To initiate PRRS area regional control/elimination in Japan, P-JET (PRRS-Japan Elimination Team) has been founded since July 2011

Objectives

To organize a working group consisting of swine practitioners, researchers, and industrial partners who focus on PRRS area regional control/elimination in Japan.

To establish and provide a network, technical know-how, and educational support for pig producers and veterinarians who are active in their PRRS area regional control/elimination projects in Japan.

To create and publish a hands-on manual of PRRS control/elimination, which will be tailored to some of the specifics of the Japanese pig industry. The manual will be available for pig producers and veterinarians in Japan.

Materials and Methods

Demographics (updated by January 2014)

A total of 10 regions in 8 prefectures (see Figure 1)

Approximately 270 sites, 45,700 sows

Type of production system: farrow-to-grow (20%), farrow-to-finish (80%)

Main strategies

Routine P-JET member meeting (periodically)

Workshop for each project region and case

Presentation at industrial and academic seminars

Publication for industrial and academic journals

Producing and providing technical/educational materials for pig producers and veterinarians

Preliminary results (achievements)

To date (January 2014), a total of 14 P-JET member meetings and 4 P-JET workshops have been completed.

A number of seminars and publications have been made.

P-JET herd classification has been established and is being widely used among pig producers and veterinarians in Japan.

P-JET biosecurity educational brochure has been established and is being widely used among pig producers and veterinarians in Japan.

Figure 1. Optional map of regions

Discussion (next steps)

This is the first initiative of PRRS area regional control/elimination in the history of the Japanese swine industry. In order to maintain this initiative and make it more effective/productive, we will focus our next steps as follows:

To hold workshops that are more technical-oriented, adapted specifically for each project region or case

To establish P-JET biosecurity risk assessment tool

To establish P-JET management recommendation manual

To establish P-JET sampling/testing manual

References

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Influence of Ingelvac® PRRS MLV vaccination on variability of S/P values serology in a breeding herd

Dedicated in the memory of Yang Xianjin

D Xu[1], P Sun[2], L Zhu[1], T Tao[1]

[1]*Boehringer Ingelheim Int'l Trading (Shanghai) Co. Ltd., Beijing 100004, China*

[2]*Anhui Agricultural University, Hefei 230061, China*

Tao.tan@boehringer-ingelheim.com

Introduction

PRRS modified live vaccine plays an important role in controlling Porcine Reproductive and Respiratory disease. Serology is a useful monitoring tool to assess PRRS exposure dynamic herds under a PRRS stabilization program. This study describes SP values variation during 4 years in a farrow to finish farm applying Ingelvac® PRRS MLV as a primary tool for achieving stabilization.

Materials and Methods

The study was conducted in a 12-year old farm with 1200 sows single-site with continuous flow system located in east China. In 2006, this farm suffered an outbreak with highly-pathogenic porcine reproductive and respiratory syndrome (HP-PRRS) and the main symptoms were abortions and high mortality in lactating and nursery pigs. Weaned piglets per sow per year in 2008 was only 16 and nursery phase was clinically unstable. In February 2009, Ingelvac® PRRS MLV was implemented in a whole herd approach to control the situation. Breeding herd was mass vaccinated every 3 months while the piglets were vaccinated at 14 days of age. 100 blood samples annually distributed in 20 gilts, 20 sows from Parity 1-2, P 3-4, P 5 and P 6+ respectively were taken and tested for IDEXX PRRS ELISA at the Veterinary Research Institute of Jiangsu Agricultural Science Academy. The S/P values were analyzed at the beginning of MLV implementation in 2009 and during the control program (2010, 2011, 2012 and 2013) at summer season each year. The analysis was done using MINITAB 16.2.3 (State College PA USA), SP values were analyzed in BoxPlot chart and Kruskal-Wallis analysis. Also a description of weaned pigs per sow and growing mortality at each year was analyzed.

Results

Figure 1 describes a clear reduction of variability of SP values at each sampling point. S/P values median was statistically significant reduced along the line. The variation of S/P values was also reduced (Inter Quartile Range from 1.141 to 0.600) through the vaccination period. This reduction of variability on S/P values matches with productivity improvement where there were 2.6 more pigs weaned per sow and the growing mortality was reduced from 4.3% to 1.8% (table 1).

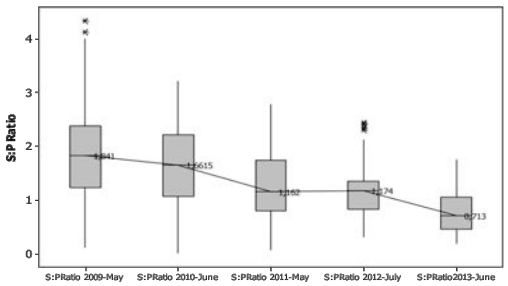

Figure 1. Mean value and variation of S/P value of the herd from 2009 to 2013

Table 1. Values of S/P ratio amd Performance.

	2009	2010	2011	2012	2013
%Positive	99%	94%	92%	97%	78%
SP value Median	1,841a	1,661a	1,162b	1,174b	0.713c
Standard Deviation	0.919	0.727	0.628	0.436	0.377
InterQuartile Range	1.141	1.133	0.946	0.518	0.600
Weaned pigs per sow	22.5	23.7	24.6	24.3	25.1
Growing Mortality	4.3%	3.1%	2.2%	2.4%	1.8%

Conclusions and Discussion

Breeding herd stability can be defined as a reduction of PRRS resident virus circulation within the population. This is an important milestone in any PRRS control program. Considering that IDEXX PRRS ELISA measures exposure, the reduction of variability in S/P ratios along the line, can be interpreted as a reduction of circulation-exposure of resident virus that reflects a stronger stabilization process in the breeding herd (no resident virus circulation). This farm has been using Ingelvac® PRRS MLV since 2009. This is an innovative and practical way to analyze serology as stabilization measurement tool in vaccinated breeding herds[1]. In addition, during the period of 2011-2012, the farm suffered less losses compared to other farms co-infected with PED and Aujeszky's disease.

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Seroprevalence of PRRSV in Colombian breeding herds

C Corzo[1], J Naranjo[2]
[1]PIC, Hendersonville, TN, USA, [2]Colombian Swine Producers Association, Bogota, Colombia,
cesar.corzo@genusplc.com

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is an important swine disease from an economic standpoint (1). The virus is present in most swine producing countries worldwide and the Colombian swine industry is not an exception (2). The virus has been detected locally through different means (3,4); however, data on the seropevalence of this virus at the breeding herd level is scarce. Therefore, the objective of the present study was to estimate the seroprevalence of PRRSV in Colombian breeding herds.

Materials and Methods

A Colombian swine producer association database was obtained for farm selection. The database included all major breeding stock companies. For this study, it was decided to consider pig farms that had at least 50 sows. Farms were both randomly and conveniently selected. Producers that owned a commercial farm were invited to participate. In case a producer declined the invitation to participate on the study the farm located immediately below on the list would be contacted with the same purpose.

Because there were no estimates of breeding farm PRRSV seroprevalence in Colombia, it was decided to estimate a 50% (±5% precision) farm prevalence with a 95% confidence. A total of 25 blood samples were collected per farm during the last quarter of 2012. Depending on the farm, (i.e. farrow-to-finish, farrow-to-feeder or farrow-to-wean) finishing, feeder pigs or gilts were used for sample collection. Samples were tested for PRRSV specific antibodies by ELISA (IDEXX Laboratories, Inc., Westbrook, ME, USA). Singleton reactors were further tested with an indirect immunofluorescent antibody test (IFA) to rule out false positive results. A general questionnaire was developed to gather farm specific data.

Results and Discussion

The number of farms required to estimate the desired seroprevalence was 171 farms. Unfortunately some producers were not interested in participating in the study; therefore 154 farms participated in the study including all but one major breeding stock supplier.

Out of the participating breeding pig farms, 21 (13.6%) were classified as positive for PRRSV. There were 9 additional farms that had singleton reactors but tested negative by IFA. Mean number of PRRSV ELISA positive samples in positive breeding herds was 19.3, ranging between 6 and 25. Mean within herd seroprevalence was 78%.

Positive breeding pig farms were distributed among the three different breeding farm types being farrow-to-finish the one with the most positive farms followed by farrow-to-feeder breeding farms (Table 1). There was no significant difference between breeding farm types with regards to PRRSV status (p=0.6).

Table 1. Number of PRRSV positive and negative breeding farms in Colombia.

FARM TYPE	PRRS +	PRRS -	TOTAL
Farrow-to-Finish	15	83	98
Farrow-to-Feeder	4	24	28
Farrow-to-Wean	2	26	28
TOTAL	21	133	154

With regards to gilt introduction, 64 breeding herds do have a designated off-site quarantine area for isolation and acclimation; however, 70 breeding herds bring in gilts directly without quarantine which poses an important risk for virus dissemination.

Even though the required number of farms needed to estimate a 50% prevalence were not tested, the results of this study ilustrate that PRRSV is present in the country. Furthermore, the virus continues to be a threat to the industry especially when there continues to exist an important proportion of PRRSV free breeding farms.

The Colombian swine industry has an interesting opportunity to control and perhaps in some regions eliminate the virus since the number of positive breeding farms is considered to be low.

Acknowledgments

Colombian swine producers for participating on the study and the Colombian swine producer association (Asoporcicultures-FNP) for funding.

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Improvement of reproduction parameters in a German sow herds after vaccination with Unistrain

S Baier[1], O Niemann[2]
[1]Swine Health Service Agricultural Chamber of Lower Saxony, [2]Hipra Deutschland GmbH, olaf.niemann@hipra.com

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) infection is the leading cause of economic casualty in swine industry worldwide[1,2]. The virus can cause reproductive failure, respiratory disease, and growth retardation in pigs. Vaccination with modified live vaccines (MLV) is still the principal means used to control PRRSV infection. This report presents observations made with a new genotype 1 based MLV against PRRS (UNISTRAIN®) in Germany. The main aim was the evaluation of reproductive parameters after a change in the vaccine.

Materials and Methods

The observation was carried out on a farm located in the Northwest of Germany with a well-documented history of PRRS.

The farm was a one-site piglet producer site with 470 sows and nursery with a 2-week-production rhythm. In May 2013 the PRRS vaccination protocol in sows (piglets were not vaccinated) was changed from a 6/60 vaccination with a genotype 1-based MLV vaccine, to mass vaccination every four month with UNISTRAIN®.

Farm was visited on a regular basis for clinical examination and blood sampling for PRRS monitoring. Aborted material, when available, was sent to the laboratory for routine examination, as well as for screening of PRRSV, PCV2 and SIV. Reproductive data was collected and checked during visits by the monitor.

Analysis of the collected data was done in January 2014, after 9 months of vaccination. The data was compared to the data of the last 12 months before vaccine change.

Results

After 9 months of continuous vaccination with UNISTRAIN®, the reproductive data of the farm improved. In comparison to the 12-month period before vaccine change the return-to-estrus decreased in sows and also in gilts (Fig 1.). Number of weaned piglets/sow/year increased significantly from 26.3 to 29.1 (Fig. 2.). Also all other recorded data showed an improvement: Farrowing rate +1.7%, piglet mortality -4.3%; farrowings/sow/year +0.06; no. piglets born/sow/year +1.3 and no. piglets born alive /sow/year +1.3.

During 2013 a total of 13 abortions have been recorded, and aborted material has been tested. No PRRSV has been found so far.

Serology showed a stabilization of the entire herd. No virus was transmitted from sows to piglets. Virus circulation in the nursery could be found during the whole observation.

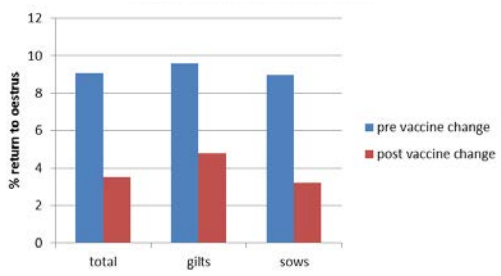

Figure 1. Return-to-oestrus rate

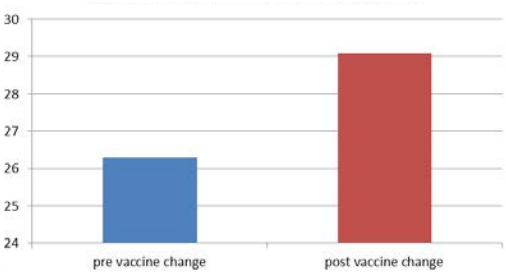

Figure 2. No piglets weaned/sow and year

Conclusions and Discussion

After the change of vaccine the reproduction data of the farm improved. No side effects occurred and the production was successfully stabilized.

Nevertheless the transmission of PRRSV from sows to piglets couldn`t be found the nursery remained PRRSV positive and showed clinical signs of PRDC. A full clinical stabilisation would require a piglet vaccination.

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Impact of sow vaccination with UNISTRAIN® on the prevalence of PRRSV antibodies at nursery age in a pig farm in Germany

P Veltmann[1]; G Klossok [1],O Niemann[2]
[1]*Veterinary practice Thesings Kreuz, Vechta, Germany ;* [2]*HIPRA Deutschland; Düsseldorf, Germany*
olaf.niemann@hipra.com

Introduction

The Porcine Reproductive a nd Respiratory Syndrome (PRRS) is one of the most economically important diseases in the swine industry. Vaccination with modified live vaccines (MLV) is the preferred strategy used to control PRRS, in combination with some pig husbandry practices. The causal agent of PRRS is the PRRSV (PRRSV), which is represented by two main genotypes (type 1 and 2), and diverse genotypes and variants, occurring all over the world. Evidence of vaccine-induced protective immunity against heterologous challenge has been published[1]. Concerning this matter, the PRRSV MLV UNISTRAIN® that was recently introduced in the European market, has demonstrated clinical protection against heterologous infection in gilts and sows [2, 3]. Furthermore, the vaccination using UNISTRAIN® enabled pregnant gilts to clear the virus and reduced its vertical and horizontal transmission to fetuses, thereby reducing the infection pressure at the herd level [4]. The main focus of this study was to evaluate the prevalence of specific serum antibodies in piglets against PRRSV, after vaccination with UNISTRAIN®, and the evolution of seroprevalence over time, as an indirect measure of the infection pressure dynamics in the studied population under field conditions.

Material & Methods

The case was recorded in a 1000-sow herd with a 2-site production system. Site 1 is the farrow-to-nursery farm. Piglets were being weaned at 24 days of age and moved to the nursery barn. The sow herd was being vaccinated with a genotype 1 MLV PRRS vaccine other than UNISTRAIN® vaccine (mass vaccination on a 4 month interval). ELISA serology and PCR on serum samples from nursery piglets showed the presence of antibodies against PRRSV and PRRSV viremia (DV-vaccine strain and PRRSV field strain). In addition, PRRSV was identified as the cause of abortion, in a vaccinated sow, and was linked to the increase in the titres of antibodies against PRRSV in the nursery period. As a result of these findings, PRRS vaccination was changed to UNISTRAIN®. No changes were made in other vaccination regimens. The impact of vaccine change was documented by testing serum samples by ELISA (IDEXX HerdCheck PRRS X3) at different time points during nursery.

Results

3 months after the vaccine change the percentage of seropositive animals, and also the average of ELISA antibody titres in nursery piglets dropped significantly (Fig 1, Fig 2). This was accompanied by an overall improvement of the clinical performance in regards to PRRS-related respiratory disease in the nursery. The use of antibiotics decreased in the last quarter significantly compared to the same quarter in the previous year.

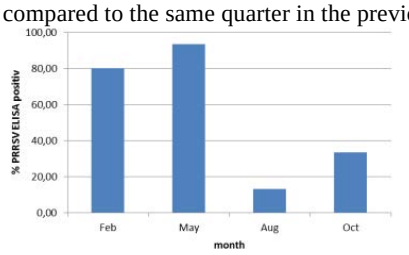

Figure1. % PRRS positive ELISA

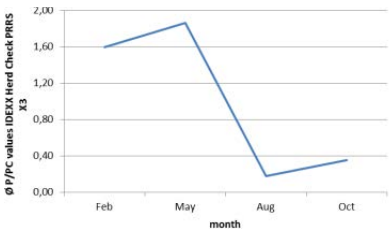

Figure 2. Average P/PC value IDEXX ELISA

Conclusion and Discussion

The results may be indicative of a decrease in the infection pressure exerted by PRRSV in the nursery as a consequence of the active vaccination of the sows. Nevertheless, it was not possible to eradicate the infection from the herd, but the productive parameters show clinical and virological stabilization of the breeding herd and the nursery.

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Antiviral activity of *A. pleuropneumoniae* against PRRSV

C Provost[1], C Lévesque[1], J Labrie[1], Y Hernandez Reyes[1], JA Burciaga Nava[2], M Jacques[1], CA Gagnon[1]
[1]*Centre de recherche en infectiologie porcine et avicole (CRIPA) et Groupe de recherche sur les maladies infectieuses du porc (GREMIP), Faculté de médecine vétérinaire, Université de Montréal, St-Hyacinthe, Québec, Canada,*
[2]*Departmento de Bioquímica, Facultad de Medicina, Universidad Juárez del Estado de Durango, México,*
chantale.provost@umontreal.ca

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is the most economically devastating viral disease affecting the swine industry worldwide (4). The etiological agent, PRRSV, induces a disease that has many clinical manifestations but the two most prevalent are severe reproductive failure in sows and gilts (1,3) and respiratory problems in pigs of all ages (1,3,6). App is the causative agent of porcine pleuropneumonia, a severe and highly contagious respiratory disease responsible for major economic losses in the swine industry worldwide (2). In the present study, the *in vitro* interactions between PRRSV and *App* were investigated. Thus, MARC-145, SJPL and pulmonary alveolar macrophages (PAM) cells were the cell models used since they are permissive to PRRSV infection and replication (4,5). Results indicate that App possesses a strong antiviral activity against PRRSV *in vitro*.

Materials and Methods

Cells were infected with 0.5 MOI of PRRSV and incubated in DMEM for 4 hours, then wash using PBS. Thereafter, fresh medium was added. *AppΔapxIΔapxIIC* (a genetically modified App that those not produce active ApxI and II toxins) kindly provided by Ruud P.A.M. Segers (MSD Animal Health, Boxmeer, The Netherlands), from an overnight culture grown at an OD_{600nm} of 0.6 were resuspended at 10 MOI in complete cell culture medium. To obtain *AppΔapxIΔapxIIC* supernatant, resuspended *AppΔapxIΔapxIIC* at an MOI of 10:1 were centrifuged at 4000 rpm for 15 minutes and harvested supernatants were passed through a 0.22 µm filter to remove all residual bacteria. Bacteria or 1 ml of the suspensions was added after 4 hours PRRSV infection, and incubated for 48 hours. The presence of PRRSV N antigen was determined by IFA and by the Kärber method (5). Fractionated, heated (56°C) and UV inactivated *AppΔapxIΔapxIIC* supernatant, as LPS from *App* were also used as treatment. Other viruses were also tested for App antiviral activity: BHV-4, PCV2b, BAV3, BHV-1, CPV, EHV-1, BVDV1, swine H1N1 and H3N2.

Results

AppΔapxIΔapxIIC and its supernatant (sup) inhibit PRRSV replication in SJPL (Figure 1) and PAM cells. However, UV inactiveated *AppΔapxIΔapxIIC* and LPS did not block PRRSV replication (Figure 1). Fractionation of *AppΔapxIΔapxIIC* supernatant demonstrated that the inhibitory effect on PRRSV infection is mediated by App metabolite(s) weighting < 1 kDa. To a lesser extent, *AppΔapxIΔapxIIC* supernatant also inhibited swine influenza H1N1 and H3N2.

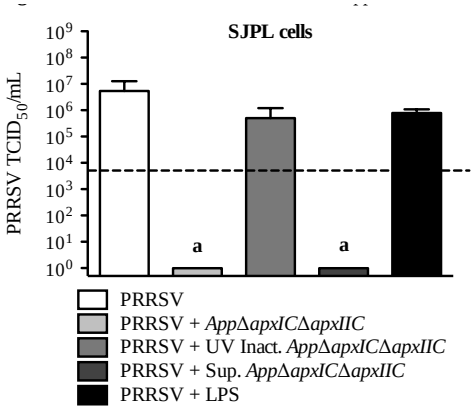

Figure 1. PRRSV titers after treatment with *App*

Conclusions and Discussion

App possesses a strong antiviral activity against PRRSV *in vitro*. This activity is mediated via a small molecule, smaller than < 1 kDa that is secreted by the bacteria. App antiviral effect is not mediated by App's LPS. An App antiviral activity was also detected against swine influenza. Those results suggest that this research might lead to the discovery and future identification of a new antiviral molecule that is secreted by App.

Acknowledgments

Natural Sciences and Engineering Research Council of Canada (NSERC), Fonds de recherche du Québec Nature et technologies (FRQNT). CP was a recipient of a Canadian Swine Health Board (CSHB) postdoctoral fellowships.

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Influence of immune response to Japanese isolate of PRRSV on subsequent manifestation of highly pathogenic PRRS

H Iseki[1], M Takagi[1], K Kawashima[2], T Shibahara[2], O Mikami[2], M Ikezawa[2], N Tung[3], K Inui[3], M Yamakawa[1]
[1]*Viral Disease and Epidemiology Research Division,* [2]*Pathology and pathophysiology Research Division, National Institute of Animal Health, Tsukuba, Japan,* [3]*National Center for Veterinary Diagnosis, Hanoi, Vietnam,* hiseki@affrc.go.jp

Introduction

The emerging porcine reproductive and respiratory syndrome (PRRS) outbreaks in 2006 swept over nearly half of the People's Republic of China and involved >2,000,000 pigs, which posed great concern to the global swine industry and public health (1). A similar PRRS outbreak was also observed in Vietnam in 2007, and further spread of the disease has been noted in other countries in the region (2). Although this highly pathogenic PRRSV (HP-PRRSV) has not yet been detected in Japan, we must operate under the assumption that the virus could eventually spread there. Here, to evaluate the cross-protective immune response between HP-PRRSV and the Japanese isolate, we examined PRRS pathogenicity using HP-PRRSV from the 2010 outbreaks in Vietnam in pigs which had been previously infected by the Japanese isolate under experimental conditions.

Materials and Methods

Twenty-one pigs free from PRRSV and other specific pathogens at four weeks of age were used in the present study. Five (group 1) and another five (group 2) pigs were intranasally inoculated with 1 ml inoculum containing 10^5 $TCID_{50}$ of Japanese isolate. Group 2 and another 6 pigs (group 3) were additionally intranasally inoculated with 10^5 $TCID_{50}$ HP-PRRSV/pig at 28 days post-infection (DPI) following Japanese isolate challenge. Five pigs (group 4) used as uninfected controls were housed in a separate animal facility during the experiment. All animals were monitored daily for body temperature, body weight, and clinical signs. Serum was collected at 0, 1, 5, 8, 11 and 14 DPI following HP-PRRSV challenge. The amounts of PRRSV in the collected serum were measured by quantitative real time RT-PCR. All pigs were necropsied at 14 DPI following HP-PRRSV challenge, and pathological findings were evaluated.

Results

Regarding findings on infection with the Japanese isolate, although tachypnea and depression were shown slightly in group 1, no significant differences in body weight, body temperature, or clinical score were noted between group 1 and control animals. In contrast, animals infected with HP-PRRSV alone (group 3) exhibited high fever and depression with anorexia, edema, and dyspnea, and one pig in this group died at 12 dpi; postmortem examination of that animal revealed consolidated pneumonia, thymus atrophy, and lymphadenopathy. While some animals in group 3 (HP-PRRSV alone) exhibited renal petechiae and swelling of knee joints. However, no significant differences in body weight, body temperature, or clinical score were noted between group 2 (Japanese isolate and HP-PRRSV) and control animals. Of note, the viral load in group 2 was significant lower than in group 3. No clinical signs, lesions, or viral antigens were observed in control animals.

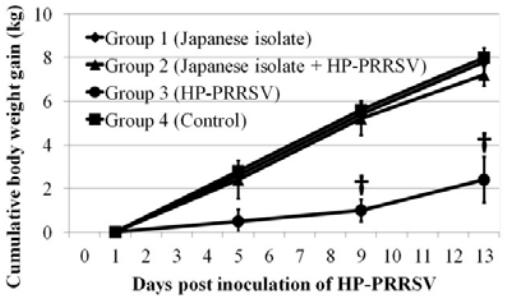

Figure 1. Body weights are expressed in kg as the mean ± standard error of the number of pigs alive at measurement. *Superscripts indicate statistically significant differences from control group ($p < 0.05$).*

Conclusions and Discussion

Viral replication and clinical signs were significantly reduced, and average daily weight gain was improved in pigs inoculated in advance with the Japanese isolate compared with non-pre-inoculated animals. These findings indicate that the immune response to the Japanese isolate was able to strongly alleviate subsequent manifestation of HP-PRRS. However, this also means that recognition of HP-PRRSV invasion in Japan may be difficult, as symptoms will not be obvious.

Acknowledgments

This work was supported by JSPS KAKENHI Grant Number 24780301.

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Real time RT-PCR; detection of PRRSV

R Sina[1], B Lewis[1], V Lazar[1], A Musarra[1], R Pogranichniy[1].
[1]*Department of Comparative Pathobiology, Animal Disease Diagnostic Laboratory, Virology, Purdue University, West Lafayette, IN,* rmp@purdue.edu

Introduction

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) has presented a challenge to the swine industry for over twenty five years. The disease causes weight loss and death in pig populations costing the industry economic losses[2]. Early detection of PRRSV is crucial, and cost effective means of accurate detection are needed.

Four commercially available PRRSV Taqman based real time RT-PCR detection methods, including a previously developed in-house SYBR green assay, were analyzed. Their relative analytical sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) were compared.

Materiasl and Methods

Four commercially available PRRSV Taqman based Real time RT-PCR including a previously developed in-house SYBR green assay were analyzed for their relative sensitivity (ability to identify positive samples) and specificity (ability to identify negative samples)[1]. In this study a diverse sample set was selected to compare the multiple kits' performance relative to the sample type. A total of 287 samples were tested including serum, oral fluid, and semen as well as lung, lymph node, spleen, and tonsil tissue homogenates. All samples were submitted to Indiana Animal Disease Diagnostic Lab (ADDL) for testing between 2009-2013.

Results

Overall the findings of this study show that kits designated A, B, C, D, E had average sensitivities of 90.2%, 95.9%, 96.8%, 78.2% and 95% with average specificities of 95.0%, 95.7%, 85.2%, 96.8% and 80% respectively across all sample types table 1. Furthermore kit B demonstrated the highest positive predictive value (PPV), and kit C displayed the highest negative predictive value (NPV) among all the sample types.

Table1. Data from all samples analysis. Percentages of Se, Sp, PPV, NPV corresponding to each kit were calculated.

Total	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Kit A	91.4	94.6	94.1	92.1
Kit B	95.7	95.3	95.0	96.0
Kit C	96.4	83.8	84.8	96.1
Kit D	76.6	96.3	95.5	80.0
Kit E	95.0	80.8	82.7	94.4

Conclusions and Discussion

The results from this study indicated that specific PRRSV diagnostic kits' performance (sensitivity, specificity) was dependent on sample type. Kit A demonstrated the best performance in tissue homogenate samples and had consistent composite ratings between 91.4% and 94.6% (Table 1). The overall positive and negative predictive values (PPV, NPV) for kit A were 94.1% and 92.1%, respectively.

In conclusion, this study determined that the best performance of kit A was in tissue homogenates, kit B in oral fluid and semen, kit C and E in serum samples.

Acknowledgments

NIFA/PRRSV-CAP2 funding for support and ADDL staff.

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Investigations on the detection of PRRSV in straw

H Nienhoff[1], J Boehmer[2], K Strutzberg-Minder[2]
[1]Swine Health Service, Chamber of Agriculture Lower Saxony, Germany, [2]IVD GmbH Innovative Veterinary Diagnostic Laboratory, Hannover, Germany, hendrik.nienhoff@lwk-niedersachsen.de

Introduction

Straw is still a common litter in stables. Especially in boar studs it used to intersperse the pens because of its good influence on hoof quality. Straw is also important in the debate about animal welfare (2). As straw is used in stables and also in stables of high health herds this raises up questions on biosecurity. Is straw a potential source of entry of PRRS-virus in high health herds? The following study emblazes the methods of detection of PRRS-virus in straw and the length of detection.

Materials and Methods

A bale of straw (0.9 x 0.5 x 0.4 m) was incubated with 5 x 4.0-6.3 $\log_{10}$ $GKID_{50}$ of a European attenuated PRRSV life strain (DV-strain) via an aerosol can. The bale was stored in an open barn so that the conditions of storage were the same like in commercial pig farms. Swiffer® samples (1) and straw samples of approximately 5 cm^3 were taken at six dates over four weeks. 15 ml of PBS were added tot he samples, washed by shaking and decanted. On both, Swiffer®-samples and straw samples PCR was performed with a commercially available and officially registered kit (*virotype*® PRRSV RT-PCR Kit, QIAGEN, Germany) and results documented as respective ct-values.

Results

PRRSV ct-values for the different methods and dates of sampling are shown in Table 1. The temperature in the barn was between -2°C and +13°C, humidity between 68 and 100%. No rain could reach the bale.

Conclusions and Discussion

The study shows, that it is possible to detect PRRSV in straw sampled by two different methods by RT-PCR . The straw samples were positive four times, the Swiffer®-samples three times. Virus could be detected by combining the methods until the end of the study. Only on sampling day 19 no virus could be detected. Although a high amount of virus was taken for incubation, only small amounts of virus with high ct-values over 30 could be detected. This indicates that straw is not the best medium for detection of PRRSV. On the other hand the study shows, that under conditions similar to the field, PRRSV can be found in straw for at least 26 days.
In conlusion high health herds have to be very careful to bring straw into their barns. The straw can be tested for PRRSV by Swiffer®-sampling of straw samples. Because of the low recovery rate a combination of both methods is suggested.

Table 1. ct-values of different (environmental) samples tested by PRRSV RT-PCR

Days p.i.	Method	PRRSV Type 1 (EU)	EU ct	PRRSV Type 2 (NA)
1	straw	pos	32	neg
	swiffer®	pos	30	neg
3	straw	neg		neg
	swiffer®	pos	32	neg
5	straw	pos	33	neg
	swiffer®	pos	35	neg
12	straw	pos	32	neg
	swiffer®	neg		neg
19	straw	neg		neg
	swiffer®	neg		neg
26	straw	pos	33	neg
	swiffer®	neg		neg

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Lesion development and viral distribution in pigs following infection with virus of highly pathogenic PRRS

K Kawashima[1], M Takagi[2], H Iseki[2], T Shibahara[1], N Tung[3], K Inui[3], M Yamakawa[2]
[1]*Pathology and pathophysiology Research Division,* [2]*Viral Disease and Epidemiology Research Division, National Institute of Animal Health, Tsukuba, Japan,*[3]*National Center for Veterinary Diagnosis, Hanoi, Vietnam*
kawaken@affrc.go.jp

Introduction

Porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV) is the causative agent for the disease characterized by reproductive failure in sows and respiratory symptoms in piglets and growing pigs (1). Atypical PRRS, characterized by high fever and high mortality in animals of all ages, emerged in 2006 in China and was named "highly pathogenic PRRS" (HP-PRRS) (2). This pandemic disease has spread rapidly among Southeast Asian countries, inflicting severe economical losses, and countries free from the disease are now at high risk of HP-PRRS invasion. While a variant of the PRRSV (HP-PRRSV) has been isolated in affected pigs, its pathogenicity has not yet been fully determined. Here, we aimed to clarify the sequential development of lesions and viral distribution due to HP-PRRSV infection under an experimental condition.

Materials and Methods

Seventeen 4-week-old specific-pathogen-free pigs were used. Thirteen pigs were intranasally inoculated with $10^{5.5}$ $TCID_{50}$ HP-PRRSV, while the remaining 4 animals were kept as uninfected controls. The HP-PRRSV used was isolated from an infected pig in Vietnam in 2010. All pigs were monitored daily for clinical signs. Three or four infected pigs each were necropsied on 3, 7, 14, and 21 days post-inoculation (dpi), and any pathological **alterations** were assessed in the collected tissues. Immunohistochemical detection for PRRSV was performed using an anti-PRRSV antibody (SR30; Rural Technologies, Inc., Brookings, SD, USA) and a commercial kit (Nichirei Biosciences Inc. Tokyo, Japan). Apoptosis was identified with a commercial kit (ApopTag®; CHEMICON® International, Inc, Temecula, CA, USA) in selected tissue sections. This experiment was conducted in compliance with the animal experimentation code of the National Institute of Animal Health.

Results

All infected pigs exhibited high fever and depression with anorexia, edema, and dyspnea. One pig died at 10 dpi. Consolidated pneumonia, thymus atrophy, and lymphadenopathy were prominent gross lesions. The distribution and intensity of the lesions and the viral antigens are summarized in Table 1. Severe interstitial pneumonia, characterized by marked alveolar exudates due to apoptotic or necrotic cells with positive reaction for apoptosis, was observed from 3 to 21 dpi. In lymphoid tissues, focal necrosis or abundant single cell necrosis at 3 dpi and follicular hyperplasia at 14 and 21 dpi were noted. Other organs, including the kidneys, developed lymphohistiocytic infiltration around vessels. Perivascular cuffing and glial nodules were found in the brain. PRRSV antigen was found in necrotic cell debris and monocyte/macrophage lineage cells in all organs examined. Of note, copious amounts of viral antigens were detected in the lung and lymphoid tissues as early as 3 dpi, and antigen findings remained positive in the lungs and tonsils at 21 dpi. No clinical signs, lesions, or viral antigens were observed in control animals.

Table 1. Distribution and intensity of the lesions and PRRSV antigens in selected tissues.

	3dpi	7dpi	14dpi	21dpi
Lung	+++/+++[a]	+++/++	++++/+++	+++/+
Tonsil	+++/++++	+/++	++/++	+/++
Lymph node	+++/+++	+/+	++/+	+/-
Kidney	+/+	+/+	+++/+	++/-
Brain	-/-	+++/+	+++/+	++/-

dpi, days post infection; [a] Lesion intensity (- = no lesions, + = mild, ++ = moderate focal, +++ = moderate diffuse, ++++ = severe)/immunohistochemical intensity (- = no signal, + =1-10 cells in the section are positive, ++ = 11-30 positive cells seen, +++ = 31-100 positive cells seen, ++++ = >100 positive cells seen)

Conclusions and Discussion

Striking necrotic or apoptotic lesions in lung and lymphoid tissues with copious amounts of viral antigens were characteristic in the early phase of HP-PRRSV infection in the present study. While tissue distribution and target cells for viral replication observed in the present study were similar to those of conventional PRRSV infections (3,4), both quantitative and qualitative exacerbation of lesions in the early phase of the infection were noted.

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Successful elimination of PRRS from small one-site pig farm in Slovenia

M Štukelj[1], Š Malovrh[2], I Golinar Oven[1]
[1]*Veterinary Faculty, Institute for health care of pigs, Gerbičeva 60, 1000 Ljubljana, Slovenia,* [2]*Biotechnical Faculty, Department for Animal Science, Groblje 3, 1230 Domžale, Slovenia,* marina.stukelj@vf.uni-lj.si

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) infection is difficult to control due to large heterogeneity among the isolates. Elimination of a disease is defined as disappearance of all clinical cases of a specific disease (1) which is the consequence of desistance of virus replication and circulation in the population of pigs. PRRS elimination is a long term goal and first step is stabilization of the breeding herd. Stabilization can be achieved with immunization. Exposure to a homologous strain provides a high level of protection against the same or nearly the same virus strain (2). Herd closure is also required to achieve herd stability. In the period of herd closure new pigs cannot be introduced to the farm. This applies also to internal replacements of gilts to the breeding herd (3). The success of PRRS elimination depends also on the biosecurity practices and cooperative work (4).

The objective of this study was elimination of PRRS from a small farrow-to-finish pig farm with herd closure and improved biosecurity.

Materials and Methods

Farm

The study was conducted between January 2012 and November 2013 on one-site pig farm with 80 sows and 1 boar, free of classical swine fever and Aujeszky disease. Farm was surrounded with other PRRS positive farms. After confirmation of PRRS, farm was closed in February 2012 for six months.

Samples

In total 354 serum samples were taken in 4 samplings for antibody detection (Table 1) and 278 serum samples in three samplings for PRRSV antigen detection.

Methods

354 serum samples were tested with IDEXX PRRS ELISA (HerdChek X3, IDEXX Laboratories Westbrook, Maine, USA). Results were expressed in S/P (sample: positive) ratios **(N –** Negative S/P ratio less than 0.4, **L** – Low positive S/P ratio between 4.4 and 1, **P** – positive S/P ratio between 1 and 2**, H** – High positive S/P ratio more than 2).

278 samples were screened with one step RT-PCR (Qiagen, Germany) and specific primers for detection of EU/NA PRRSV in highly conserved region of ORF 7 (5) and two PCR positive samples were subjected to direct sequencing.

The owner was acquainted with the obligatory measures of the strict biosecurity protocols.

Results

Table 1. Results of ELISA

Sampling		No. of tested samples			No. of samples according to S/P			
		BP	W	F	N	LP	P	HP
I.	11.1.2012	66	/	10	6	28	27	15
II.	21.5.2012	68	10	10	32	28	23	5
III.	14.5.2013	81	10	9	55	21	21	3
IV.	5.11.2013	79	5	6	59	14	16	1

BP – breeding pigs, W – weaners age of 8 weeks, F – fatteners

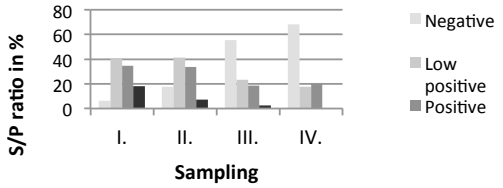

Figure 1. S/P ratios in breeding pigs.

By RT-PCR, PRRSV was detected only in II. sampling at weaners and fatteners. In each consecutive sampling S/P ratio decreased (Figure 1). The sequencing results of 258 nucleotides in ORF7. The detected PRRSVes shared an 93% nucleotide identity with the Lelystad virus and 64.3% identity with VR2332.

Conclusions and Discussion

The positive effect of herd closure and following strict biosecurity protocol was evident already after three months by serological testing. In following sampling S/P ratio was constantly decreasing. Therefore we were expecting an eradication of PRRS in a next few months.

Elimination of PRRS is possible even in situations when neighboring farms are PRRS-positive and also on one-site farms.

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Antigenic characterization and genetic diversity of PRRSV in Mexican strains

A Toiber[1], A Gayosso[1], R Tellez[1], A Morilla[2], R Alonso[1]
[1]*Departmento genética y estadística, Facultad de medicina veterinaria y zootecnia, Universidad Nacional Autónoma de México* [2]*Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias,* liz.toiber@gmail.com

Introduction

Porcine respiratory and reproductive syndrome (PRRS) is one of the most devastating swine diseases worldwide.

PRRSV are distinguish by high genetic variability, ORF-5 gen is the most variable and is commonly employed for phylogenetic studies (4,5); in contrast the ORF-7 gen is one of the most conserved and its product is highly immunogenic, because of that it is use as antigen in ELISA test for diagnosis of this disease (3). Based in nucleotide sequences of ORF-5 (2) and ORF-7 (1) in Mexico had been reported unique strains of PRRSV. Our laboratory has clone and expressed the ORF-7 gen from a local variant in baculovirus system. A characteristic feature of the variant is the presence of an additional amino acid. This recombinant antigen was incorporated in an ELISA system for the detection of antibodies. The aim of this work was to analyze the antigenic and genetic variability of PRRSV strains circulating in México.

Materials and Methods

We study 622 serums from different farms at the center of Mexico. The serum samples were evaluated with two commercial ELISA kits (C1 and C2); and with the ELISA system developed by us, called ELISA-UNAM (EU). The study of genetic variability was performed by amplifying the ORF-5 and the ORF-7 genes from purified RNA of these same samples by nested RT-PCR and nucleotide sequence analyses.

Results

ELISA analysis of 622 serum samples defined 8 antigenic groups (A-H), by their positive (+) or negative (-) reactivity with the different ELISA systems. In the Group A (+EU, -C1, -C2), contained 38% of the samples; in group B (+EU, -C1, +C2) identified 14%; the Group C (+EU, +C1, -C2) enclose 6%; in Group D (+EU, +C1, +C2) we detected 27%; for the Group E (-EU, -C1, -C2) resulted 6%; in group F (-EU,-C1,+C2) were 4% of the samples; in group G (-EU, +C1, -C2) we find 5% and in group H (-EU, +C1, -C2) 1%. We processed for PCR 300 samples been positive 80 samples. Interestingly, some of the negative samples for the commercial ELISA render positive PCR amplification. Only few ORF-5 and ORF-7 PCR amplicons were sequenced. The resulting sequences were BLAST queried with other sequences available in NCBI. We find that most of the sequences were very similar to the vacunal and the VR2332 strains.

Conclusions and Discussion

The immunoreactivity groups found with the different ELISA systems, suggests the presence of viruses with different antigenic determinants, implaying that each ELISA system detects only an antigenic subset.

We observed in the same farm, samples with different antigenic groups. Notably, negative samples to commercial ELISA systems were found positive to our recombinant antigen system. By other hand some samples were positive for all ELISA systems.

All the ORF 5 y ORF7 sequences were identical or very similar to the vacunal virus, giving evidence that circulating virus may be revertants of the vacunal strain. Because same sequences are divergent make believe that they have been circulating long ago. Revertant vaccine virus had been previously reported (.). In a previous study it was found a great extent of genetic diversity, finding revertant strains as well, but also mexican local strains and world-wide circulating ones (1).

We can conclude that there are an extensive antigenic and genetic diversity of the PRRSV in Mexico. This scenario calls for more extensive and deeper research of the viral and antigenic diversity, in order to understand its origins and biological properties. Likewise, for the control and eradication of PRRSV in the country, it is required more efficient diagnostic tools covering all the antigenic diversity and the development of more effective and safe vaccines.

Acknowledgments

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Northwest Indiana PRRS ARC project: What is success?

T Gillespie[1], M Inskeep[1], M Ash[2]
[1]*Rensselaer Swine Services, Rensselaer, IN,* [2]*Indiana Board of Animal Health, Indianapolis, IN,* tom.gillespie@rssvet.com

Introduction

The Northwest Indiana (NW IN) Porcine Reproductive and Respiratory Syndrome (PRRS) Area Regional Control (ARC) project began in 2009 with a goal to develop a network of producers that voluntarily collaborate to control PRRSV in a specific region.[1] Growing confidence of participating producers has led to continual growth toward achieving the original goal. Individual producers are realizing how cooperation can ultimately improve the productivity of their farms.

Materials and Methods

Current boundaries of the NW project are from US 421 west to the Indiana/Illinois line and from SR 24 north to Lake Michigan. Each year the project has grown in number of producers, number of pigs represented, and geographical area. In 2010, 38 sites were tested, representing 19,850 sows and 144,705 grow-finish pigs. In 2013, 55 sites participated, representing 35,485 sows and 188,375 finishing pigs. There are currently 8 sites not in the project, representing about 5% of finisher pigs and < 3% of sows and several are small exhibition operations.

Figure 1. Project expansion beyond early counties.

Results

Early implementation of PRRS control at individual sites has minimized outbreaks of PRRS in this area. There has been only one break in a negative sow farm since the project began. Testing in the summer of 2013 revealed 21 of the 55 sites were ELISA positive and 11 of the 55 were PCR positive. Currently 21 of 55 sites utilize modified live virus vaccine to help control PRRS on their farms. With ORF5 sequencing, 6 of 11 positive PCRs were determined to be vaccine virus at that time.

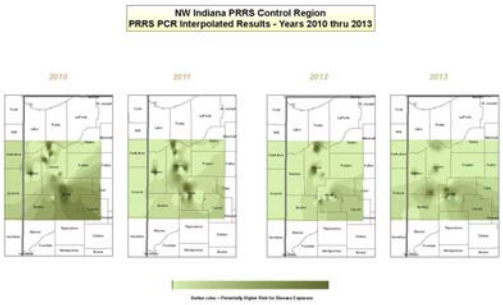

Figure 2. Interpolated maps show PRRS results tracked annually. Darker areas represent where PRRS positive PCR samples were collected.

Results are reported monthly to one representative from each of the farms/companies that have signed participation agreements. If severe breaks occur between updates, neighbors in close proximity to the new positive farm are notified by phone.

Conclusion and Discussion

Joint meetings have occurred with the West Central Indiana PRRS ARC project in an attempt to find synergies that would strengthen both projects. Also, there are farms in the NW IN PRRS ARC project receiving pigs from out-of-state sow farms that participate in other PRRS ARC projects. The efforts of other projects can only strengthen the efforts in the NW IN PRRS ARC project. Perhaps more important than eradicating PRRS, the NW PRRS ARC project is building trust among neighbors, allowing the sharing of information not only for PRRS, but for other diseases as well. Several times in the past year, health status information has been shared between the participants when PEDV and TEGV outbreaks occurred. This helped neighboring farms develop updated trucking routes and biosecurity plans to keep disease from spreading. It is this crucial communication that will ultimately lead to area regional control not only in NW Indiana, but in area regional control projects across the country and world.

Acknowledgments

Indiana Board of Animal Health, Indianapolis, IN

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Analysis of spray dried porcine plasma indicates absence of PRRSV infection in Brazilian pigs

J Crenshaw[1], J Pujols[2,3], J Polo[1,4], J Campbell[1], C Rodríguez[4], N Navarro[2], E Pileri[2], JR Ciacci-Zanella[5], L Rangel[6]
[1]APC Inc., Ankeny, IA, [2] CReSA, Fundación UAB-IRTA, Cerdanyola del Vallès, Spain, [3] Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Barcelona, Spain, [4]APC Europe S.A., Granollers, Spain, [5] Embrapa Swine and Poultry Research Center, Animal Health Laboratory, Concórdia, Brazil, [6] APC Inc-Brasil,
joe.crenshaw@functionalproteins.com

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most economically devastating diseases of the global pig industry. The PRRSV is endemic in most major swine producing areas of the world with some exceptions. A recent study in Brazil reported absence of PRRSV genome and antibodies against PRRSV in the analysis of 4020 samples of either serum, plasma, or oral fluid collected from quarantined imported boars, feral pigs, or from domestic pigs at 113 commercial farms located in eight states during 2008 to 2012 (2).

To indirectly confirm these results, the following strategy was planned. Pathogens infecting pigs during their productive life cause the immune system to produce antibodies, which are still detectable in blood even at slaughter age. Spray dried porcine plasma (SDPP), a standard high quality protein used globally in diets for nursery pigs, has been proven as a good source material to monitor evolution of antibodies in pig populations, especially in regards to enzootic agents (5). The objective of this study was to provide the results of analysis, of different lots (batches) of SDPP collected from a Brazilian spray-dried plasma producer plant, for the occurrence of antibodies against PRRSV as a way to demonstrate the presence or absence of this virus in the Brazilian pig population.

Materials and Methods

During November 2012 samples from 8 different manufacturing lots of SDPP were obtained from a commercial spray-dried plasma producer located in the state of Santa Catarina, Brazil. This Brazilian producer collects blood from abattoirs that slaughter pigs from farms located within about a 500 km circumference of the manufacturing plant. The Brazilian SDPP powder samples were analyzed at CReSA for antibodies against PRRSV. Prior to analyses, the SDPP powder was reconstituted in sterile distilled water at a concentration of 9% w/v to obtain a similar concentration to that of liquid porcine plasma. The presence of antibodies against PRRSV was determined by a commercially available ELISA kit (HerdChek PRRS 2XR, IDEXX Laboratories). According to the manufacturer, sample to positive control ratios (S/P) > 0.4 were considered positive. A qRT-PCR was also performed on these samples as an attempt to detect PRRSV genome (4).

Results

None of the SDPP lots analyzed from the Brazilian commercial plasma producer contained antibodies against PRRSV and no PRRSV genome were detected. Each of the 8 manufacturing lots of SDPP were produced from the blood collected from approximately 35,000 to 40,000 pigs; therefore the study involved the blood from about 300,000 pigs which represented approximately 1% of the total Brazilian pig population and approximately 3.1% of the total Santa Catarina state pig population.

Conclusions and Discussion

The use of SDPP in nursery pig diets has been a common practice by the Brazilian swine industry during the past 10 years. During this period the use of SDPP has expanded significantly and presently it is estimated that around 80% of Brazilian pigs consume diets containing SDPP during the post-weaning period. The nutrition provided by the inclusion of SDPP in diets and duration of feeding diets with SDPP to pigs in Brazil has been reported to reduce clinical symptoms associated with Porcine Circovirus Associated Disease (PCVAD) and improve nursery pig performance compared to diets without SDPP (3).

Under the conditions of this study results indicated that PRRSV is not present in the Brazilian pig population as previously reported (1) and confirmed by recent studies of Brazilian swine herds (2).

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PRRSV and SIV detection in individual blood samples, nasal swabs and pen oral fluids in a field longitudinal study in post weaning piglets

E Giacomini[1], MB Boniotti[1], N.Ferrari [1], C Salogni [1], P Pasquali[2], GL Alborali[1]
[1]*Istituto Zooprofilattico Sperimentale Lombardia Emilia Romagna, Brescia, Italy,*
[2] *Istituto Superiore Sanità , Roma, Italy,* giovanni.alborali@izsler.it

Introduction

Oral fluids have recently been used as a surveillance tool for Porcine reproductive and respiratory syndrome virus (PRRSV) and SIV using reverse transcription polymerase chain reaction (RT-PCR). The sampling of oral fluids (OF) through cotton rope, where animals chew on the material and deposit oral fluid, represents an easy and welfare-friendly alternative to serum surveillance in pig pen. Surveillance of PRRSV and SIV in post-weaning pigs by OF has allowed to know and monitor the health status of different batches of pigs before moving to other fattening herds.
The aim of this study was to estimate and compare the detection of PRRSV and SIV with different sampling approaches: individual blood, nasal swab and OF.

Materials and Methods

The study was carried out in one farrow to growing herd in Northern Italy with endemic PRRSV and SIV infection in the post-weaning site. Farm consists of 10 post-weaning building, each of them organized in 24 pens with 60 animals each. Pigs are not vaccinated against PRRSV and animals were moved in post weaning unit at 25 days of age until 85 days of age old. Then, they are moved in another fattening herd. The study unit consists of 15 pigs coming from 3 different pens of the same building. The animals were marked with ear tags and weighted at the beginning and the end of the post-weaning period. Sex and dam's parity were also recorded. The sampling protocol included individual blood and nasal swabs and OF from 5 animals. Three sampling were performed: T1, 25 days of life when weaning, T2 at 55 days and T3 at 85 days of life. PRRSV detection was performed by using the NucleoMagVet kit (Macherey-Nagel), for RNA extraction, and TaqMan NA and EU PRRSV kit (Life Technologies). Antibody detection was done by the IDEXX X3 ELISA kit. SIV detection was done using the primers described by Spackman et al 2002 (2).

Results

The results of one study unit is shown as an example of the 12 groups collected over one year.

Table 1. Example of PRRSV results of study unit 1

					SERUM						NASAL SWAB			COTTON ROPE		
					PRRS ELISA			PRRSV RT-PCR			PRRSV RT-PCR			PRRSV RT-PCR		
ANIMAL	Kg T0	Kg T2	SEX	Sow parity	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2
1	6	29	F	1	0,6	2,2	1,8	1	1	1	1	0	0			
2	5	32	M	1	0,6	1,6	1,1	1	1	1	0	0	0			
3	5	31,5	M	1	0,4	1,7	1,9	1	1	1	0	0	0	1	1	1
4	7	40,5	M	2	1,5	1,3	1,4	1	1	1	0	0	0			
5	6,5	38	F	2	1,1	2,1	2,1	1	1	1	1	1	0			
6	6,5	26	F	3	0	0	1,7	1	1	1	0	1	0			
7	7,5	21	M	3	0	0	0,9	1	1	1	0	0	0			
8	8	33	F	4	1,4	1,1	1,8	1	1	1	0	0	0	0	1	1
9	6	30	F	4	1,4	0	1,9	1	1	1	0	0	0			
10	6,5	34	M	5	1,1	0	1,1	1	1	1	0	0	0			
11	7	24	F	5	1,1	1,6	1,4	1	1	1	1	0	0			
12	5,5	28	M	7	0	3	2,3	1	1	1	0	0	0			
13	7	26	M	7	1,2	1,6	2,5	1	1	1	0	0	0	1	0	0
14	7	32	F	8	0	0,8	1,8	1	1	1	0	0	0			
15	7	34,5	M	8	0	0,7	0,6	1	1	1	0	0	0			
					SERUM						NASAL SWAB			COTTON ROPE		

Table 2. Example of SIV results of study unit 1

					NASAL SWAB			COTTON ROPE		
					SIV-A			SIV-A		
ANIMAL	Kg T0	Kg T2	SEX	Sow parity	T0	T1	T2	T0	T1	T2
1	6	29	F	1	0	0	1			
2	5	32	M	1	0	0	1			
3	5	31,5	M	1	0	0	0	1	1	1
4	7	40,5	M	2	0	0	0			
5	6,5	38	F	2	0	0	0			
6	6,5	26	F	3	0	0	0			
7	7,5	21	M	3	0	0	0			
8	8	33	F	4	0	0	0	0	0	1
9	6	30	F	4	0	0	0			
10	6,5	34	M	5	0	0	0			
11	7	24	F	5	0	0	1			
12	5,5	28	M	7	0	0	0			
13	7	26	M	7	0	0	0	0	1	1
14	7	32	F	8	0	0	1			
15	7	34,5	M	8	0	0	1			
					NASAL SWAB			COTTON ROPE		

In this study unit PRRSV was detectable in blood samples from T0 to T2 while OF was more sensible than nasal swabs for PRRSV and SIV detection.

Conclusions and Discussion

OF sampling is a promising approach for increasing the efficiency and cost effectiveness of virus surveillance in swine herds. Although OF performance is not always comparable to blood sampling, OF is easy and rapid to perform, it doesn't stress the animals and shows reliable diagnostic performance. The data collection of 12 study units will allow a more reliable evaluation of OF performances and to take into account the biological and seasonal variability.

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Stability of PRRSV type 1 in oral fluid samples

R Graage[1], M Viehmann[1], H Soellner[1], I Hennig-Pauka[1], A Ladinig[1], M Ritzmann[1,2]
[1]*University Clinic for Swine, University of Veterinary Medicine, Vienna, Austria,* [2] *Clinic for Swine, Ludwig-Maximilians-University, Munich, Germany,* robert.grrage@vetmeduni.ac.at

Introduction

PRRSV (porcine reproductive and respiratory syndrome virus) is one of the most common pathogens in swine medicine. Therefore the surveillance of PRRSV is a major tool to control this pathogen. In routine diagnostics, invasive sampling methods are used, i.e. serum and tissue samples. The detection of PRRSV in oral fluid was first reported in 19974. Since 2008, oral fluid samples are optimized for the detection of PRRSV in the USA1,2. Oral fluid has been shown to be a convenient diagnostic specimen for the detection of several infectious agents and antibodies3. In Europe, oral fluid samples are rarely used for the detection of PRRSV.

The aim of the present study was to analyze the stability of PRRSV Type 1 in oral fluid samples.

Materiasl and Methods

Two methods of collecting oral fluid samples were compared: 1) the TEGO Oral Fluids Kit ([TEGO] ITL Animal Healthcare, Melbourne, Australia) and 2) cotton ropes ([CR] Sankt Josef Werkstatt, Dorfen, Germany). Oral fluid samples were collected from PRRSV negative pigs. To ensure uniformity an equal dose of 0.96 mL PRRSV (2.5 x 107 per mL RNA copies, Porcilis® PRRS, MSD, Boxmeer, The Netherlands) was added to both pools of 8.6 mL. Samples were stored for 0, 2, 12, 24, 48, or 72 hours at different temperatures (10 °C, 4 °C and -20 °C). Aliquots of both collection methods were stored for the different time periods at each temperature, before being frozen at -80°C until further testing. Each aliquot was tested in duplicate. Viral RNA was extracted from oral fluid specimens using a commercially available viral RNA isolation kit (QIAamp® Viral RNA Mini Kit, QIAGEN GmbH, Hilden, Germany). A commercial qRT-PCR kit (Applied Biosystems, Forster City, CA) was conducted for genome quantification. PCR diagnostic was based upon orf 7 amplification.

Results

Type 1 PRRSV RNA could be detected in oral fluid collected with both, the TEGO Oral Fluids Kit and cotton ropes. PRRSV genome was detectable within 72 hours at all assessed temperatures (figure 1). With an increasing sample storage time, the detected virus load (copies per mL) decreased. After 48 hours, a distinct reduction of the initial virus concentration of 107 mean copies per mL to 106.8 - 105.5 had occurred. After 72 hours, the lowest reduction of the mean copies per mL was found at 4 °C (TEGO) and 10 °C (CR). The highest reduction was found at -20°C in both oral fluid samples (TEGO and CR).

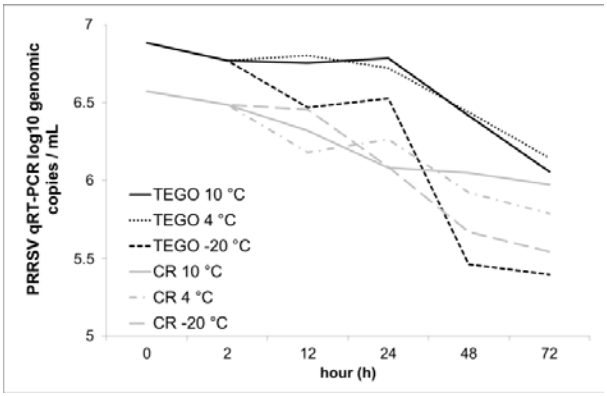

Figure 1. Stability of PRRSV RNA (log10 genomic copies/mL) over time influenced by storage temperature. TEGO: TEGO Oral Fluids Kit; CR: cotton rope

Conclusion

Results obtained by this study will help to formulate recommendations for the collection and handling of oral fluid samples in order to optimize PRRSV detection. PRRSV RNA in oral fluid is stable for 72 hours. The highest quantity of copies was preserved at 4-10 °C. Results are comparable to those of other studies5. Data of this study implicate, that the PRRSV genome is detectable in oral fluids within 72 hours at storage temperatures between -20 and 10 °C.

An appropriate specimen-handling protocol would be a fast cooling-down of oral fluid samples and storage at a temperature between 4 and 10 °C.

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Experimental evaluation of individual and collective oral fluid sampling for the early detection of PRRSV- infected piglets

E Gibert[1], E Pileri[1,2], E Cano[1], RM López[1], S López-Soria[1], GE Martín-Valls[1], Mateu E[1,4].
[1]*Centre de Recerca en Sanitat Animal (CReSA),* [2]*Dept. Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain*
enric.mateu@uab.cat

Introduction

Oral fluids (OF) are becoming increasingly popular as a sample for PRRSV testing. The main advantages of OF testing are the easiness of collection, the possibility of testing large numbers of pigs by analysing just a few pen samples and the decrease in the need of restraining pigs. Additionally, OF are though to be more sensitive than serum for the detection of late infections, when viremia has usually disappeared (1). The objective of the present study was to evaluate, in an experimental model, the sensitivity and the specificity of individual OF for testing viral shedding, particularly in early infections.

Materials and Methods

Samples were collected from 45 4-week-old piglets distributed randomly in 7 pens. In 4 pens (n=22) pigs were inoculated intranasally with 2 ml (1 ml/nostril) containing $10^{5,5}$ $TCID_{50}$/ml of the genotype 1, subtype 1 PRRSV isolate 3267. The remaining 3 pens were kept as negative controls. Collective (pen) OF samples were collected with a non-treated cotton rope as described by (2) while individual OF samples were collected using a non-treated cotton device (Salivette®, Sarstedt AG & Co.). Sera were also collected. Sampling was done at 0, 1, 2, 3, 7 and 14 days post-inoculation (dpi). Viral RNA was extracted using the Nucleospin® RNA Virus (Macherey-Nagel) kit. The qRT-PCR reaction was performed with One-Step RT-PCR Master Mix (Applied Biosystems) and primers designed to bind specifically ORF7 of strain 3267. A series of decimal dilutions (10^0-10^7 genomic copies) of a standard (ORF7 amplicon) were included. Statistical analyses were done with StatsDirect v.3 considering sera as the "golden standard". Kappa value was calculated with WinEpiscope 2.0.

Results

Most inoculated pigs (22/27; 81.5%; $CI_{95\%}$: 61.3-93.0%) become viremic at 1 dpi and from 2 dpi until day 14 all remained so. For individual OF samples, first positive results were obtained at 2 dpi (8/27; 29.6%; $CI_{95\%}$: 14.5-50.3%). Average viral loads per ml of sample are shown in table 1. Compared to serum, the sensitivity of the real-time qRT-PCR using individual OF always below 36.4% but the specificity was 100% (Table 2). The highest Kappa value for the comparison of blood sampling and individual OF was 0.30. Using ropes, at 1 dpi ¼ inoculated pens was detected as positive being all pen positive onwards. In all cases, uninoculated pigs remained negative all throughout the study.

Table 1. Average viral load qRT-PCR in serum and oral fluids (OF) of experimentally inoculated pigs.

	AVERAGE VIRAL LOAD (genomic copies/ml)*	
Dpi	Serum	Oral fluids
0	NA	NA
1	5.75±0.92	NA
2	5.65±0.93	4.74±0.62
3	7.08±0.72	4.51±0.56
7	6.07±0.72	4.63±0.52
14	5.51±0.65	NA

*positive samples; NA = not applicable.

Table 2. Sensitivity and specificity (relative to serum) of PRRSV detection by qRT-PCR using individual OF.

Dpi	Sensitivity	Specificity
0	N.A.	100% (45/45)
1	0% (0/22)	100% (20/20)
2	36.4% (8/22)	100% (13/13)
3	35.3% (6/17)	100% (11/11)
7	23.0% (3/13)	100% (9/9)
14	0% (0/7)	100% (8/8)

Conclusions and Discussion

Analysis of individual OF apparently resulted in low sensitivity compared to blood for the detection of PRRSV shedding. At this point it is difficult to elucidate the causes of this lack of sensitivity but it is worth to note, that the amount of OF collected per pig was highly variable (0.19-0.37 g of OF/sample) and with relatively low concentrations of virus compared to blood. Handling and restraining is highly stressing for small pigs. At present we are working on new methods of individual OF collection without restraining of pigs based on the natural curious behaviour of pigs.

Acknowledgments

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P.580

Inhibition replication of PRRSV on MARC 145 cell culture using glycyrrhizinic acid aqueous solutions and a potential nanoparticulated formulation

Z Urbán[1], S Mendoza[1], A Jiménez[1], H Ramírez[2], S González[1], H Lara[3], F Quezada[3], A Ciprián[1], D Quintanar
[1]*Facultad de Estudios Superiores Cuautilán-UNAM;* [2]*Facultad de Medicina Veterinaria y Zootecnia-UNAM,*
[3]*Laboratorios Avi-Mex, SA de CV. México.* Contact: mzum_1212@hotmail.com

Introduction

Glycyrrhizinic acid (GA), a saponin from licorice root has been used as anti-inflamatory, anti-ulcer, anti-tumor, anti-viral, etc.[1,2], for many years[1]. It has also antiviral activity against several viruses[3,4]. Since the late 80´s, the porcine reproductive and respiratory syndrome (PRRS) has been the cause of important economic lost all over the world due to the lack of an efficient treatment against the virus. Nanoparticles are structures that carry substances or drugs so that they can reach organs or even cells of interest. The aim of the research was to study the effect of GA solutions on uninfected and PRRSV-infected cells in culture. An attempt of testing loaded nanoparticles with GA on cells was explored.

Materials and Methods

MARC cells were maintained in RPMI medium at 37 ºC and 5% CO_2. PRRS strain VR 2332 was a gift from Laboratorios Avi-Mex, S.A. de C.V. For cytotoxicity determination, when confluence was reached 100 µl of the sterile solutions of GA (1-30 mg/ml and 0.1-0.9 mg/ml) were added. Daily observation of the plate was done (144 h). At the end, trypan blue staining (TB) and MTT assay were performed. Inhibition of the cytophatic effect was evaluated as follows: 100 µl of virus (10^6 $TCID_{50}$/ml) in RPMI were added to confluent cells. The plate was kept at 37 ºC for 1 h. After this time, the cells were treated with GA solutions at concentrations 0.1-0.9 mg/ml and maintained for 144 h. Viability and selectivity index were calculated. The simultaneous assay of virus-infected cells treated with GA was performed on cells that previously were in contact with virus (10^1-10^8 $TCID_{50}$/ml); GA solutions (0.1-0.9 mg/ml) were added to the cells. Virus titer was calculated daily using the Reed & Muench method. Nanoparticles were obtained by the microemulsion method[5]. Once sterilized, they were tested on cells previously infected with virus 10^1-10^8 $TCID_{50}$/ml, at a GA concentration of 0.54 mg/ml. Observation of the culture was performed daily during 72 h and TB staining was applied at the end of the assay.

Results

The viral titer decreased two logarithms compared to that obtained in the first viral titration without GA treatment. EC_{50} was determined at 0.5 mg/ml. Although the selectivity index (CC_{50}/EC_{50}) was relatively low (1.73), GA showed reduction of PRRS replication *in vitro*. After testing GA loaded nanoparticles, TB dye exclusion results indicated an inhibition of virus replication (viability of 98-99%) compared to control infected cells (20% viability) after 72 h.

Conclusions and Discussion

Although GA exhibited a low selectivity index (1.73), it showed inhibition of the replication of PRRS. Crance et al., 2003, reported selectivity index values of 6-13 for viruses of the *Flaviviridae* family[4]. GA showed a low selectivity index but it was a potent inhibitor of all flaviviruses. It is important to point out that selectivity indices of a compound could be moderately influenced by the strain of virus tested.

The higher viability observed in cells treated with GA loaded nanoparticles; suggest that the drug exerted its effect as the viral replication was inhibited and that these carriers reached the inner space of the cell. Formation of needle like structures was observed at 24 h of the assay, they could be the result of precipitation of the drug. GA showed reduction of PRRS replication *in vitro.* It can be concluded that GA is active, though not selective against PRRSV. Nanoparticles can be used as potential carriers of GA with the probably advantage of reaching anatomic organs as lungs. We are conducting further assays to have more evidence at this respect. To our knowledge it is the first time that GA is proved against PRSS in cell culture.

Acknowledgements

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Peptides from non-structural proteins of PRRSV inducing IL-10 responses can suppress recall responses to genotype 1 virus

AJ Burgara-Estrella[1], I Diaz[2], IM Rodríguez-Gómez[3], J Hernández[1], E Mateu[2,4]
[1] *Centro de Investigación en Alimentación y Desarrollo A.C (CIAD), Hermosillo, Sonora, México.*
[2] *Centre de Recerca en Sanitat Animal (CReSA), UAB-IRTA, Campus UAB, Bellaterra, Barcelona, Spain.*
[3] *Departamento de Anatomía y Anatomía Patológica Comparadas, Facultad de Veterinaria, Universidad de Córdoba, Córdoba, Spain.*
[4] *Departament de Sanitat i d'Anatomia Animals, UAB, Bellaterra, Barcelona, Spain.* enric.mateu@uab.es

Introduction

Non-structural proteins (nsp) of PRRSV (PRRSV) are involved in the down-regulation of type I interferons (1). Previously, we reported that peptides in nsp of PRRSV may induce IL-10 responses both as natural or recall responses. Moreover, we showed that some of those peptides have the ability to inhibit IFN-γ response in peripheral blood mononuclear cells (PBMCs) stimulated with PHA (2). In the present work, we explore the potential nsp peptides as inhibitors of recall responses to PRRSV.

Materials and Methods

Fourteen 3-week old piglets were used for the experiments. Three of them were vaccinated with a live attenuated genotype-1 vaccine and three were kept as controls. PBMCs from pigs were collected at 56 days post immunization. Peptides previously reported as specific IL-10-inducers (2) were tested for their potential for inhibiting PRRSV-specific IFN-γ responses by ELISPOT ($5x10^5$ cells/well). For this purpose, PBMCs were stimulated with vaccine virus (m.o.i. 0.01) for 20 h in the presence of the selected peptides at 10, 1 and 0.1 µg/mL. Cell culture supernatants were examined for IL-10 by ELISA using an appropriate antibody pair (R&D Systems). Kruskal-Wallis test was used to compare groups.

Results

Three peptides, one from nsp2, one from nsp9 and one from nsp11 showed some potential for the inhibition of PRRSV-specific IFN-γ responses in PRRSV vaccinated pigs (Table 1). The strongest inhibitory effect was observed for peptide GTPGVVSYY located in nsp11. The addition of such peptide at 10 µg/ml reduced significantly the frequencies of INF-γ secreting cells induced by the virus in the three vaccinated pigs. The specific IFN-γ response of the unvaccinated pigs was negligible (<5 spots/$5x10^5$ PBMC). The analysis of cell culture supernatants resulted in an average of 58 pg/ml of IL-10 in PBMCs of vaccinated pigs while were negative for that cytokine for unvaccinated animals.

Table 1. Effect of the addition of selected IL-10 inducing peptides to PRRSV-stimulated cultures of vaccinated pigs on the frequency of virus-specific IFN-γ secreting cells as determined in ELISPOT.

	Frequency of IFN-γ secreting cells/$5x10^5$ PBMC†		
	Pig number		
Stimulus	**1**	**2**	**3**
Whole PRRSV	55±9	66±14	13±2
nsp2-GRFEFLPKM	24±7**	75±16	16±0
nsp9-VLPGVLRLV	36±10¶	69±4	19±7
nsp11-GTPGVVSYY	28±5**	47±5¶	7±2*

† Arithmetic mean and standard deviation of triplicate assays

*p<0.05; **p<0.01; ¶p<0.07

Conclusions and Discussion

In the present work we identified peptides in the nsp of PRRSV with capability to inhibit the virus-specific IFN-γ responses of vaccinated pigs. These results suggest that nsp may contribute to reduce the strength of the adaptive response in the case of a situation of partial immunity in which the vaccinated pig could be infected by a field strain. Since the peptides studied here induced IL-10 responses only in vaccinated pigs, the present results indicate that adaptive immunity against PRRS may also induce regulatory or anti-inflammatory components.

Aknowledgements

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P.582

Tylvalosin tartrate inhibits the replication of highly pathogenic PRRSV *in vitro*

M Takagi[1], H Iseki[1], N Hattori[1], K Kawashima[2], T Shibahara[2], M Yamakawa[1]
1, Viral Disease and Epidemiology Research Division, 2, Pathology and Pathophysiology Research Division, National Institute of Animal Health, Tsukuba, Japan, m7takagi@affrc.go.jp

Introduction

Porcine reproductive and respiratory syndrome (PRRS), an infectious disease of pigs, causes reproductive failure in sows and respiratory disorder in young pigs, and is an economically important disease worldwide. Recently, an atypical and highly pathogenic PRRS variant (HP-PRRS) has occurred in China and Southeast Asia. This disease was characterized by a high fever of above 41˚C, anorexia, red discoloration of the ears (blue ear) and body, and high mortality in pigs of all ages.

Currently vaccines have provided limited protection against heterologous PRRSV or HP-PRRSV strains. Recently, it has reported that macrolide antibiotics may have an anti-viral effect on PRRSV[1, 2]. The combination of vaccine and drugs may be more effective for the prevention of PRRS. In this study, the macrolide antibiotic, tylvalosin tartrate, was tested for anti-viral activity against HP-PRRSV replication *in vitro* and for mitochondrial superoxide production in cells.

Materials and Methods

MARC145 cells were treated with tylvalosin tartrate (API of Aivlosin®, Ecopharma Inc.) at various concentrations (0.1, 1, 10 and 100 µg/ml) for 4 hours at 37˚C. After the treatment, the cells were washed with PBS 5 times. In indirect immunofluorescence assay (IFA), cells were inoculated with HP-PRRSV Vietnamese isolate 100186-614 at 10^2 and 10^3 $TCID_{50}$/ml, and were incubated for 24 and 48 hours. The cells were fixed with 80% acetone, and IFA was conducted with serum obtained from a pig infected with isolate 100186-614 and FITC-conjugated anti-porcine IgG, The fluorescence was visualized under fluorescence microscope.

In flow cytometry (FCM), after the treatment with tylvalosin tartrate, the cells were inoculated with isolate 100186-614 at 5 x 10^5 $TCID_{50}$/ml, and were incubated for 48 hours. The cells were fixed with 4% paraformaldehyde, and were permeabilized with 0.5% saponin. The cells were stained by a method like IFA. The total number of HP-PRRSV antigen-positive cells was determined by counting the positive cells present in a total of 10,000 cells by EPICS XL ADC and EXPO32 software (Beckman-Coulter).

For mitochondrial superoxide production in cells, after the treatment with 100 µg/ml tylvalosin tartrate, the cells were incubated with 5 µM MitoSOX (Molecular Probes, Invitrogen, Eugene, OR, USA) at 37˚C for 30 min, washed two times with Hank's buffered salt solution containing calcium and magnesium and fixed with 4% paraformaldehyde. For the determination of the mitochondrial superoxide generation in cells, MitoSOX was excited by laser at 488 nm of FCM and the data was collected at 575 nm (FL2) channel of FCM. The data presented by histogram of mean intensity of MitoSOX fluorescence.

Results

It was clearly shown that pre-treatment of the cells with tylvalosin tartrate at a concentration of 100 µg/ml reduced the number of cells expressing HP-PRRSV antigen in IFA. However, the reduction in virus positive cells was not observed at other concentration in comparison with control. In FCM, when cells were treated at 100 µg/ml, the replication of HP-PRRSV was reduced by approximately 20% (Table 1). The virus positive ratio in cells treated with tylvalosin tartrate at other concentration was not different from it in control. In mitochondrial superoxide production in cells, histogram of FCM showed 2.1 ± 0.3 fold increase in MitoSOX fluorescence intensity with 100 µg/ml tylvalosin tartrate following 4 h treatment (Figure 1).

Table 1. Ratio of positive cells to HP-PRRSV detected by FCM

	Treatment of tylvalosin tartrate		
	No treatment	100 µg/ml	10 µg/ml
1st test	51.64%	39.68%	43.62%
2nd test	38.82%	28.08%	43.02%

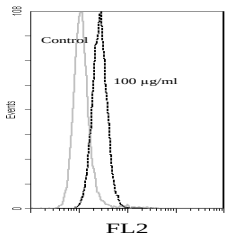

Figure 1. Tylvalosin tartrate increases some mitochondrial superoxide in MARC145 cells. Control: no treatment. Three times of tests were carried out.

Conclusions and Discussion

In this study, treatment with tylvalosin tartrate reduced the number of cells expressing HP-PRRSV antigen. These data demonstrated that tylvalosin tartrate had an anti-viral activity against HP-PRRSV replication *in vitro*. This anti-viral activity could be related to increasing production of mitochondrial superoxide in cells.

It will be necessary to elucidate the anti-viral mechanism of tylvalosin tartrate and to inspect the effect of it *in vivo* in future.

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P.583

Phylogenetic and amino acid analysis of some PRRSV strains from Romania

V Petrovan[1], L Buburuzan[1], M Zaulet[1]
[1]*University of Bucharest, Department of Biochemistry and Molecular Biology, 91-95 Splaiul Independentei, 5th district Bucharest, Romania,* petrovan.vlad@gmail.com

Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) is the most devastating and economically challenging disease to the swine industry worldwide due to reproductive failure including late abortions, high mortality in weaned pigs and respiratory disorders associated with secondary bacterial infections (2, 3). This study was conducted on national scale, because PRRSV is one of the most rapidly evolving RNA viruses (1). To establish the genetic diversity in the swine farms, 605 samples were collected from different individuals (3 months – 4 years). the purpose of the study was to make a population screening, strain identification and phylogenetic analysis on Romanian farms. The aim of this study was to evaluate the genetic diversity and amino acid analysis of PRRSV isolates from different areas in Romania by comparing the nucleotide sequences obtained for ORF5 gene and ORF7 gene with reference sequences from GenBank.

Materials and Methods

The detection of the virus was made by Real Time PCR using specific kits and protocols, followed up by Sanger sequencing and data interpretation.

The sequences obtained were proofread manually, truncated to the real dimensions of the genes (606 bp for ORF5 gene and 387 bp for ORF7 gene) using MEGA 5.05.

The phylogenetic trees for ORF5 and ORF7 were generated from the aligned sequences in MEGA 5.05 program using a Maximum Likelihood method. The amino acid sequences of both genes were aligned using BioEdit program, resulting in a 201 amino acids alignment corresponding to ORF5 gene and a 128 amino acid alignment corresponding to ORF7 gene.

The analysis of amino acid sequences evidenced for both GP5 and N-nucleocapsid proteins the belonging Romanian virus to type 1.

Results

We identified for ORF5 two hypervariable regions, one in the signal pepdide and one in the beginning of the mature chain. Interestingly, for ORF7, the amino acid sequence for the isolate Rom22 has an asparagine inserted at position 12 of the sequence and one substitution in position 42, for the isolate Rom26, three amino acid substitutions in positions 4, 8 and 16 and for the isolate Rom30 one amino acid substitution in position 124.

The analysis of amino acid sequences evidenced for both GP5 and N-nucleocapsid proteins the belonging Romanian virus to type 1.

Secondly, phylogenetic analysis was performed for both genes and the sequences revealed that the Romanian PRRSV nucleotide sequences clustered in three groups within the subtype 1.

Conclusions and Discussion

In conclusion, the results obtained from the amino acid sequences and from phylogenetic trees confirm the affiliation of all Romanian isolates to the subtype 1 of PRRSV.

Acknowledgments

The study was conducted in the laboratoires of the Department of Biochemistry and Molecular Biology, Faculty of Biology, Bucharest, Romania

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P.584

ORF5 diversity of PRRSV Mexican strains isolated from 2009 to 2013 compared with reference strain VR-2332

A Massa[1], A Flores[1], S Ramírez[2], M Macías[1], R Raya[1], V Orozco[1], A Franco[1], C Armenta[1]
[1]*Lapisa S.A. de C.V.; Carretera La Piedad-Guadalajara Km. 5.5, Col. Camelinas, C.P. 59375, La Piedad, Michoacán; México.* www.lapisa.com, [2]*Private practice.*

Introduction

Two genotypes of porcine reproductive and respiratory syndrome virus (PRRSV) have been reported: North American (Type II) and European (Type I). In Mexico, only strains of North American genotype have been identified. The glycoprotein 5 (GP5) is the most variable external protein[1] and the main responsible for inducing the production of neutralizing antibodies[2]. The GP5 contains an open reading frame named ORF5. The genetic comparison of the ORF5, is a common procedure for the identification of heterologous strains. This study describes genetic variation, at nucleotide and amino acid levels of the ORF5 of Mexican isolates compared with reference strain VR-2332.

Materials and Methods

Field samples were obtained from animals raised in commercial swine production companies from nine states of Mexico. For PRRSV detection, a commercial real time RT-PCR was performed. The samples were processed and isolation was carried out following a modified technique[3]. The ORF5 regions of the isolates were amplified and sequenced.

For the comparison, 107 ORF5 sequences were selected randomly and proportionally, from the total number of isolates obtained from 2009 to 2013. The sequence of reference strain VR-2332 was obtained from GenBank® with access number U87392 . The nucleotide sequences were translated into amino acids for comparison. GP5 encodes for 200 amino acids and a stop codon (603 base pairs). The pairwise alignment and identity matrixes were created and analyzed using the BioEdit sequence alignment editor 7.1.3.0 software.

Results

The homology of Mexican strains compared with VR-2332 for nucleotide and amino acid composition ranged from 83.4 to 99.6% and 83.5 to 99.0% respectively. The complete data (ranges) for the years analyzed are presented in Table 1.

Conclusions and Discussion

Different percentages of ORF5 nucleotide homology comparing with VR-2332 have been reported, 85-99% in Canada[1], 87.8-89.0% in Korea[4], 86.9-99.0% in China[5], and 88% for a Mexican isolate[3]. In the Mexican strains analyzed, the 83.4% lowest similarity detected in the lower limit of nucleotide comparison indicates a wide range of variation as reported for isolates from different countries. The 83.5% similarity of Mexican strains observed in the lower limit of amino acid comparison demonstrates continued changes, higher than the 15% difference (85-92% similarity) reported for Canadian isolates[1]. Previous reports of North American strains indicated 88-97% similarity[5]. These results demonstrate, that the nucleotide changes have also evolved into amino acid composition variations in the Mexican isolates.

Table 1. Similarity of sequences of the ORF5 gene from 107 Mexican isolates compared with strain VR-2332.

		Percentage of similarity (interval endpoints)	
Year	Strains	Nucleotides	Amino acids
2009	6	86.7 – 98.8	87.5 – 97.0
2010	7	86.7 – 91.8	85.5 – 92.5
2011	13	85.7 – 98.1	85.0 – 95.5
2012	24	85.7 – 98.3	85.0 – 97.0
2013	57	83.4 – 99.6	83.5 – 99.0
Total strains 2009-2013	107	83.4 – 99.6	83.5 – 99.0

A continued decline in the percentage of similarity demonstrating gradual changes in the virus from 2009 to 2013, was identified. In contrast, the highest similarity for nucleotides, 99.6%, and amino acids, 99%, were found in 2013. The results demonstrate the circulation of strains closely related to the prototype VR-2332, probably as the result of vaccination with similar strains, and at the same time, with others that have evolved into gradual changes in the ORF5 gene, possibly causing variations that favor virus immune response evasion. These variations should be considered for the election of control strategies aimed to produce effective immunization against PRRSV in the field.

Acknowledgments

Lapisa® Diagnostic Laboratory; Michoacán, México

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P.585

Stabilization of PRRSV circulation in a farm using a vaccination program with PROGRESSIS® at the end of gestation

P Defoort[1], T Meyns[2], S Van Poucke[2], V Dekens[2], F Joisel[3]
[1]*Provet DAP, Torhout, Belgium;* [2]*MERIAL NV, Diegem, Belgium;* [3]*MERIAL S.A.S., Lyon, France;*
pascal.defoort@provet.be

Introduction

PRRS is considered as the most important economic viral disease of intensive swine production. It is characterized by reproductive failure in sows and respiratory disease and poor production in nursery piglets. PROGRESSIS®, an inactivated EU type PRRSV vaccine, was shown to reduce the number of viraemic piglets, born from vaccinated sows (1). Additionally, it has been shown that maternal immunity can protect piglets during their nursery period by vaccination of sows at day 60 (2) or at day 90 of pregnancy with PROGRESSIS (3). The present case report describes the stabilization of a conventional swine farm infected with PRRSV by vaccinating the sows and gilts during almost 2 years at day 60 with an EU type MLV vaccine and at day 90 of gestation with PROGRESSIS.

Materials and Methods

This case involved a 1200-sow farrow-to-finish farm, with 5 production lines of a recent date. Genetics are Danbred sows x Piétrain. At the time of the problems, the sows and gilts were vaccinated in block with a EU type MLV PRRSV vaccine 3 times a year. The nursery piglets were housed in 2 large buildings.

Results

In January 2012, severe problems associated with PRRSV were diagnosed. The problems included early births, weak and death born piglets and problems in the nursery room, bad production results (piglet mortality around 4.5%) and increased use of antibiotics. At that time, serological data (IDEXX ELISA) confirmed circulation of PRRSV in sows and gilts (Table 1) and in piglets (Table 2). PRRSV was also detected in blood by PCR in piglets of 3 and 7 weeks of age.

Table 1. PRRSV S/P ratios in sera collected from sows and gilts between Jan 2012 and Oct 2013

	01/2012	11/2012	10/2013
Gilts	1.78	3.18	1.63
	2.26	1.52	1.44
	2.15	1.12	0.96
	0.87	2.14	1.69
	2.51	3.84	1.92
Sows	0.9	4.23	2.29
	0.53	1.58	2.22
	1.6	1.87	2.02
	3.16	3.25	2.14
	1.26	2.49	2.4

To solve these problems, the vaccination program in the sows was adapted to vaccination with an EU type MLV vaccine at day 60 combined with PROGRESSIS at day 90 of gestation.

In November 2012, around 11 months after the start of the adapted vaccination program, a clinical and serological stabilization of PRRSV circulation in the nursery piglets was observed (Table 2). At that time, the serological profile in sows and gilts was still indicative for recent circulation of PRRSV (titers > 3). In October 2013, after 22 months of vaccinating all sows and gilts repeatedly at day 90 of gestation with PROGRESSIS, also the titers of the sows and the gilts became uniform (Table 1), while S/P ratios in piglets at the end of nursery remained low. At the end of 2013, the mortality in the nursery was reduced to ≈ 2%.

Table 2. PRRSV S/P ratios in sera collected from piglets of 6 and 10 weeks of age between Jan 2012 and Oct 2013

	01/2012	11/2012	10/2013
Piglets of 6 weeks of age	2.14	2.96	1.04
	1.11	1.54	1.60
	2.35	1.15	1.88
	2.34	0.69	2.57
	2.19	2.8	2.30
Piglets of 10 weeks of age	2.55	0.28	0.67
	2.46	0.78	0.17
	3.06	0.08	0.08
	2.25	0.34	0.55
	2.87	0.58	0.56

Conclusion and Discussion

After the start of the vaccination with PROGRESSIS at day 90 of pregnancy, there was a relative quick recovery of the production results in the nursery due to a lower infection pressure. After 22 months, there was in addition a remarkable stabilization of the PRRSV circulation in the sows and gilts. These results indicate that the use of PROGRESSIS just before farrowing can be an efficient tool to induce an increased maternally derived immunity in piglets and to control virus circulation at herd level in order to stabilize a herd.

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Introduction of PRSSV vaccination with PROGRESSIS® in an Italian herd vaccinating sows with CIRCOVAC®: A case report

F Salvini[1], G Leotti[2], O Merdy[3], F Joisel[3]
[1]DVM, Brescia, Italy; [2]MERIAL SpA Italia, Milano, Italy; [3]MERIAL S.A.S., Lyon, France; giorgio.leotti@merial.com

Introduction

This case report illustrates the benefits of modification in herd management practices and particularly the progressive introduction of PRSSv vaccination with PROGRESSIS® in addition to PCV2 vaccination (CIRCOVAC®) on reproductive performance in a well-managed operation located in Italy.

Case Description

The farm was a 1200-sow farrow-to-finish operation managed under a continuous flow management system. The gilts came from a self-replacement stock housed separately from the sows until their first farrowing. The herd was Aujeszky's disease free and PRRSV positive as well as PCV2 positive. Key evolutions of herd management practices are reported in the table below (Table 1):

Table 1. Schedule of events.

Period	Operation
2010-2012	Progressive improvement of feed characteristics
2011/2012	Weaning age delayed from 3 to 4 weeks of age
End 2012	Synchronization procedure abandoned
Mid-2013	Modification of the genetic background
February 2010	CIRCOVAC vaccination implementation in gilts and sows according to the manufacturer recommendations
2011	PRRSV vaccination with PROGRESSIS introduced in gilts only (2 ml, IM, primo-immunization in quarantine + one booster injection at 70-80 days of gestation
2012	Sow mass vaccination with PROGRESSIS + one booster injection at 70-80 days of gestation
2nd quarter 2012	PROGRESSIS vaccination at 7days of lactation and 70 days of gestation for each sow
Mid-2013	Systematic sow medication at farrowing

Average reproductive performances of the herd were extracted over four consecutive years from the farm monitoring software.

Results and Discussion

Fertility and prolificacy data are described in Table 2. The modifications introduced in reproductive cycle schedule led to an increase of the inter-parity interval. A clear lengthening of gestation duration was observed over the monitoring period as well as a definite increase of the farrowing rate . Following the implementation of PRSSv vaccination in gilts, the farrowing rate in gilts increased from 82.8% to 91.7% in 2012. The hot 2013 summer and a behavior change are supposed to have negatively impacted gilt gestation/farrowing rate in 2013. Following sow vaccination implementation, the gestation rate ranged between 91.5% in 2012 and 92.1% in 2013 for the whole herd. Consequently, almost a 10% improvement of the farrowing rate of the whole herd was obtained between 2010 and 2012-2013.

Table 2. Fertility and prolificacy data over 4 consecutive years.

	2010	2011	2012	2013*
Total Nb of gilts or sows	1116	1044	1123	1118
Nb AI	3007	2845	2914	2284
Nb Farrowing	2507	2540	2695	2105
% Farrowing	83.4%	89.3%	92.5%	92.1%
Nb Abortion	58	40	38	23
% Abortion	2.3%	1.6%	1.4%	1.1%
Nb AI (Gilts)	711	609	638	467
Nb Farrowing (Gilts)	589	560	585	397
% Farrowing (Gilts)	82.8%	92.0%	91.7%	85.0%
Weaning-to-estrus interval	6.77	7.32	6.24	5.75
Days of gestation	114.56	115.41	115.76	116.05
Days of lactation	24.64	27.74	27.81	27.91
Inter-parity interval	145.97	150.47	149.81	149.71
Total born alive / litter	11.87	12.84	13.81	13.70
Total weaned / litter	9.94	10.47	11.46	11.74
Total weaned / sow / year	24.87	25.40	27.92	28.61

*2013 data were collected in the dams inseminated up to 15/10/2013.

A clear and steadily improvement of prolificacy indexes was observed despite the lengthening of the reproductive cycle. Particularly, litter size increased by 1.94 born alive piglets per litter between 2010 and 2012. Consequently the number of piglets weaned per dam increased by 3.05 in this period. At the time of writing, the 2013 indexes are still impacted by seasonal variation. Nevertheless, the number of weaned piglets per dam and per year increased by 3.74 between 2010 and 2013.

Conclusions

This case report showed PRSSv vaccination implementation with PROGRESSIS in a herd vaccinating sows with CIRCOVAC as an effective tool to help improving fertility and prolificacy parameters in a herd with good performances and management practices under Italian conditions.

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Genetic variability of PRRSV identified by RT-PCR in pig herds from six states of Mexican Republic

G Socci[1], E Carrera[2], F Diosdado[1], A Martínez[1], J Santiago[4], A Coba[1], L Zapata[1], E Corona[3]
[1]CENID-Microbiología, INIFAP [2]Independiente, [3]Universidad de Guanajuato, [4]INER
socci.guadalupe@inifap.gob.mx

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most important infectious diseases of pigs throughout the world (1). PRRS is caused by an arterivirus. The genome is composed of nine open reading frames (ORFs). ORF5 and 6 encode for membrane proteins and ORF7 for nucleocapsid protein. Sequencing studies about ORF5 show high genetic and antigenic variability (2). This makes PRRS diagnostics being complicated and vaccination ineffective (3). So the purpose of this study was to identify PRRSV by RT-PCR in pig herds with suggestive signs of PRRS in six states of the Mexican Republic and analyze its genetic variability.

Materiasl and Methods

Thirty four pig herds with suspected clinical signs for virus occurrence were conveniently sampled. Farms sampled were as follows: 7 in Estado de Mexico, from Otumba, Temascaltepec, Teotihuacan, Texcoco, Toluca, Xonacatlan, and Zumpango; 8 in Guanajuato from Abasolo, Aldama, Iramuco, Irapuato, Jaral del Progreso, Juventino Rosas and Silao; 3 in Puebla, from Atlixco and Izucar de Matamoros; 8 in Queretaro from Colon, Corregidora, El Marques, Ezequiel Montes, Huimilpan, Queretaro, San Juan del Rio and Tequisquiapan; 4 in Michoacan from La Piedad; and 4 in Veracruz from Jalapa. In total 167 samples were collected: 106 bloods, 8 sera and 53 tissue pools (tonsils, lung and lymph nodes). In order to detect PRRSV a first RT-PCR was assay using primers that amplify about 300 bp from ORF7 (sense 5'-CCAGCCAGTCAATCARCTGTG-3' and antisense 5'-GCGAATCAGGCGCACWGTATG-3') (4). Positive samples were amplified for phylogenetic analysis using primers that amplify 803 bp from ORF5-ORF6 adjacent region (sense 5'-TTGACGCTATGTGAGCTGAATG-3', and antisense 5'-ACTTTCRACGTGGTGGGC-3') (5). Amplicons were purified and sequenced. The obtained sequences were used to construct the respective phylogenetic tree by Mega 5.05 software with the Maximun Likelihood method with 500 bootstrap replicates.

Results

PRRSV was detected in pig farms as indicated: 7 in Guanajuato (Silao, Aldama 1, Aldama 2, Juventino, Irapuato, Iramuco, and Abasolo); 1 in Estado de Mexico (Xonacatlan); 1 in Michoacan (La Piedad), 1 in Puebla (Atlixco); and 1 in Veracruz (Jalapa); for a total of 11 pig farms. PRRSV was identified in 27 (16%) out of 167 studied samples: 3 bloods, 3 sera, and 21 tissue pools. Phylogenetic tree inferred for identified PRRSV is shown in Fig. 1.

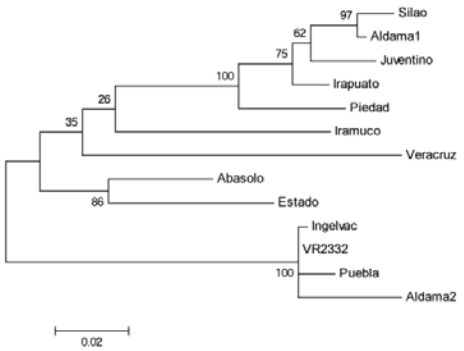

Figure 1. Phylogenetic tree based on the analysis of ORF5-ORF6 adjacent region from 11 PRRSV identified in pig herds from different states of Mexican Republic. *Sequences from Ingelvac vaccine and VR2332 reference strain were included. The scale indicates the number of substitutions per site.*

Conclusions and Discussion

Our results show that PRRSV is circulating in pig herds in several states of the Mexican Republic, and that there is a significant genetic variability among viruses even isolated from the same region (Aldama1 and Aldama2 in Fig. 1). On the other hand, some isolates, Puebla and Aldama2, showed high similarity with the Ingelvac vaccine strain. In both corresponding farms young pigs were vaccinated after weaning; however a number of fattening pigs showed PRRS signs. Our results confirm PRRS variability reported elsewhere (2), and support the usefulness of genetic analysis for epidemiological prospective studies.

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Assessment of infection degree of PRRS in pigs from farrow to finish farms in Mexico

F Diosdado[1], A Martínez[1], L Zapata[1], MA Coba[1], G Socci[1], E Carrera[1], E Corona[2].
[1]CENID-.Microbiología, INIFAP. Km. 15.5 carretera México-Toluca, 05110, México DF. [2]DICIVA, Irapuato-Salamanca Universidad de Guanajuato. diosdado.fernando@inifap.gob.mx

Introduction

The porcine reproductive and respiratory syndrome (PRRS) is one of the most important diseases of pigs at international level, since causes reproductive disorders in sows and respiratory problems in pigs of any age (1). By ELISAs laboratories can serological and indirectly determinate the infection stage in pig herds by evaluating sample-to-positive (S/P) ratio (2). Therefore the objective of this work was to assess the infection degree in sows and fattener pigs, in pig farms in five states of the Mexican Republic.

Materials and Methods

Non-vaccinated pig farms in Veracruz, Puebla, Estado de México, Michoacán and Jalisco states were serologically and opportunely sampled. For this, 30 blood samples from fattener pigs 4-6 months old, and 30 blood samples from sows with 1-6 parturitions were collected from each farm. Sera were obtained and stored at -20°C until evaluation. Serum specific PRRSV (PRRSV) antibodies and ratios S/P were determined by ELISA (HerdCheck, IDEXX, Laboratories, Maine, USA) (3). Mean S/P values were finally analyzed.

Results

Table 1 shows mean S/P values calculated.

Table 1. Sample to PRRS positive (S/P) ratios in Mexican pig herds.

State	Farms	Total of samples	Ratios in sows	Ratios in fattener pigs
Veracruz	5	165	0.92	1.92
Estado de México	10	526	0.88	2.00
Puebla	3	95	0.98	1.68
Michoacán	16	152	1.22	1.93
Jalisco	11	107	1.09	1.82

Conclusions and Discussion

Accordingly to the literature, S/P values lesser tan 1.5 indicates that farms have acquired the PRRSV infection, but there is not any possibility for transmission of viral infection; intermediate values from 1.6 to 2.5 suggest a recent infection; and values greater than 2.6 indicate active infections (2). Our results show that sows have S/P ratios lesser than 1.5, and fattener pigs, ratios from 1.68 to 2.00. We conclude that in these farms piglets should be better sanitarily managed, and it is fundamental to assess the effect of a vaccine given to post-weaning pigs to reduce PRRSV infections, reducing also other associated infections.

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Identification of PRRSV, PCV2 and BEDV of pigs with respiratory signs

AMA Coba[1], VF Diosdado[1], LA Martínez[1], SL Zapata[1], EG Socci[1], SE Carrera[2], H Ramirez[3], F Rivera[3]
[1]CENID-MA, INIFAP, Km 15.5 carretera México-Toluca, CP 05110, México, D.F. [2]Práctica privada, [3]FMVZ, UNAM.
coba.maria@inifap.gob.mx

Introduction

Porcine respiratory reproductive syndrome virus (PRRSV), blue eye disease virus (BEDV), and porcine circovirus type 2 (PCV2) are important pathogens associated to the porcine respiratory disease complex (PRDC), although many other viruses and bacteria are common (1,3). PRRSV, BEDV, and PCV2 have been reported to replicate in different cell lines under very specific conditions (3). The objective of this study was to identify these three virus in tissue samples from pigs with respiratory signs in five states of México.

Materiasl and Methods

We studied lymphatic node, lung and tonsil samples from pigs in five states of Mexico (Table 1).

Table 1. States of Mexico sampled to detect PRRSV, BEDV, and PCV2 in pig farms.

Farms	States	Samples
7	Guanajuato	42
10	Querétaro	63
1	Michoacán	6
1	Veracruz	3
3	Puebla	10

For virus isolation (VI), tissue samples were macerated, centrifuged, and filtrated through 0.45 µm sterile membranes. MARC-145 cells at 60% confluence in 24 well plates were inoculated in duplicate using 200 µl of inoculum. Positive and negative controls for each virus were also prepared. Cell cultures were incubated at 37°C, 5% CO_2, for five days. All specimens received at least three consecutive passages. All third passages were analyzed by end point PCR using primers specific for ORF7 (PRRS), ORF V1 (PCV2) and N (BEDV) sequences.

Results

Under our conditions, only some inoculated cell cultures showed cytophatic viral effect, which consisted of clusters of rounding cells, mainly observed between the fifth and the seventh day postinoculation. However, PCR was sensitive enough to identify all three virus studied. We observed coinfection cases as shown in Table 2; all three combinations in 4/7 farms in Guanajuato; a triple combination in Michoacán; only simple infection in Veracruz (PCV2) and Querétaro (7/10 farms, PCV2 or BEDV); and the Puebla state resulted negative to any isolation.

Table 2. Viral coinfections detected by PCR in pigs with respiratory signs.

State	Farm number	PCR DIAGNOSTIC [1]PRRSV	[2]PCV2	[3]BEDV
Gto (4/7)*	1	+	+	-
	2	-	+	+
	4	+	+	-
	5	+	-	+
Mich. (1/1)	1	+	+	+

Abreviations: Gto, Guanajuato; Mich, Michoacán; *, positive farms/total farms. [1]ORF7, [2]ORF V1, [3]GEN N

Conclusions and Discussion

Our combined system allowed to detect all three viruses studied. Interestingly all three virus replicated onto MARC-145 cells, in which only PRRSV had been previously grown (1,3). Dual and triple coinfections were clearly involved in respiratory disorders observed in the pig farms studied. Before this study only PRRS-PCV2 association had been reported (2). In this work we found all three dual and triple combinations for viral coinfection of pigs, now including BEDV. Our results support the idea that PRRSV, BEDV and PCV2 coinfections have important roles in pathogenesis of PRDC.

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Heterologous cell-mediated immune responses against PRRSV in gilts vaccinated with UNISTRAIN® PRRS

J Miranda[1], I Rodriguez-Ballarà[1], M Fenech[1], E Perozo[1], D Llopart[1], D Torrents[1], E Mateu[2], I Díaz[2]
[1]*HIPRA, Amer (Girona), Spain,* [2]*CReSA (Centre de Recerca en Sanitat Animal), Barcelona, Spain*
joel.miranda@hipra.com

Introduction

Vaccination is one of the main tools to minimize porcine reproductive and respiratory syndrome (PRRS) impact in endemic areas. Current knowledge of PRRSV (PRRSV) immunology is still limited but it seems clear that modified live vaccines (MLV) are a reasonable choice for the immunization of pigs (1). Induction of neutralizing antibodies (NA) after one single dose of a MLV is limited (2). In this scenario, cell-mediated responses after MLV vaccination could be responsible for limiting the duration of viremia, and consequently the spread of the virus (1,2). The aim of the present study was to assess the cell-mediated response against several heterologous PRRSV isolates in vaccinated gilts with a commercial genotype 1 live vaccine (UNISTRAIN® PRRS, HIPRA).

Materials and Methods

Eight Landrace x Pietrain six-month-old gilts were selected from a PRRSV-free farm. Negative PRRSV status was individually confirmed by quantitative RT-PCR (qRT-PCR) and ELISA (CIVTEST® SUIS PRRS, HIPRA). Six gilts were IM vaccinated with 2 ml of UNISTRAIN® PRRS ($10^{5,2}$ $TCID_{50}$/dose; HIPRA) and two gilts were IM injected with 2 ml of PBS (controls). Heparinized blood samples were collected at the day of vaccination (D0) and at days 14, 28, 42 and 56 post-vaccination (pv) to obtain peripheral blood mononuclear cells (PBMC). Frequencies of PRRSV-specific IFN-γ-secreting cells (IFN-γ-SC) were measured as reported before (3) to assess the heterologous responses against five genotype 1 isolates retrieved from clinical outbreaks in 4 to 10 week-old piglets (Table 1).

Table 1. Field PRRSV isolates used in the present study with detail of the ORF5 similarity to the vaccine strain.

Country of isolation	Year of isolation	Similarity to the vaccine strain (ORF5)
Slovakia	2005	90%
UK	2011	88%
Hungary	2011	98%
Spain	2005	92%
Italy	1992	89%

Comparison of frequencies of IFN-γ-SC for each isolate was done by means of the Kruskal-Wallis test using Statsdirect 2.8.0.

Results

For all strains, IFN-γ-SC were already detected at day 14 pv (Figure 1). The peak of secreting cell frequencies was observed at day 14 pv for two isolates (Spain and UK), and at day 28 pv for three isolates (Italy, Hungary and Slovakia). As it was expected, IFN-γ-SC mean values for all strains were null or <5 in control groups.

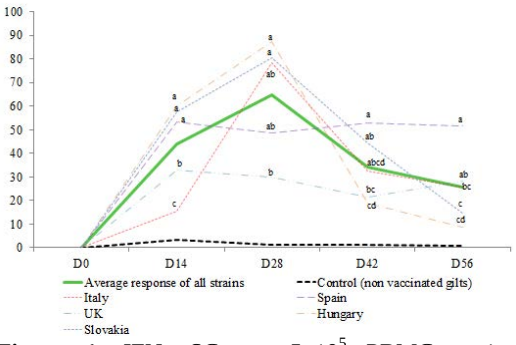

Figure 1. IFN-γ-SC per $5x10^5$ PBMC against 5 genotype-1 field isolates in vaccinated and control gilts. *Different superscript letters indicated statistically significant differences ($p<0.05$) among the five isolates.*

Conclusions and Discussion

Genetic/antigenic diversity and variability in the immunobiological properties of the PRRSV (3,4) may compromise the heterologous protection generated by vaccination in PRRS control strategies. Although PRRS immunity is not yet fully understood, the significance not only of the NA but the cell-mediated immunity is important for a better understanding of vaccine performance (3). The present study showed that primo-immunization of naïve gilts with UNISTRAIN® PRRS induced a significant specific cell-mediated immunity against 5 heterologous PRRSV strains. The mean IFN-γ-SC response was significantly higher than non-vaccinated group regardless the virus strain origin, year of isolation and genetic homology. Briefly, the results demonstrate that a single immunization with UNISTRAIN® PRRS of replacement gilts can generate an optimal cell-mediated response against pathogenic field PRRSV.

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Seroprevalence of PRRSV on PRRS suspected farms in Belgium and the Netherlands

HG Prüst[1], FX Tribó[2], D Llopart[2]
[1] *HIPRA BENELUX, Brusselsesteenweg, Belgium,* [2]*HIPRA HQ, Amer, Spain,* herman.prust@hipra.com

Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) has become one of the most economically important diseases in the swine industry. The economic damage has been calculated at €126 per sow per outbreak (1). Classification of PRRS status of a herd is a basic step to control PRRSV-infection on a farm. The aim of this study is summarizes the results of PRRS serology on Belgium and Dutch farms in 2013.

Materials and Methods

A total of 1307 blood samples belonging to 25 different farms which were suffering reproductive problems were analyzed for PRRS serology. Breeders were grouped into 5 groups according to sow parity (group 1: Gilts; group 2: Sows of 1-2 parities; group 3: sows of 3-4 parities; group 4: sows of 5-6 parities; and group 5: sows of >6 parities). Pig samples were grouped in 3 groups according to the age (4, 7 and 10 weeks of age). If fatteners were present (above 10 weeks of age) also these were sampled with approximately 4 week age-interval. From these blood samples serum was collected, frozen and sent by cool transport to DIAGNOS, HIPRA in Spain. Samples were analyzed for antibodies with ELISA (CIVTEST® SUIS PRRS E/S; HIPRA)

Results

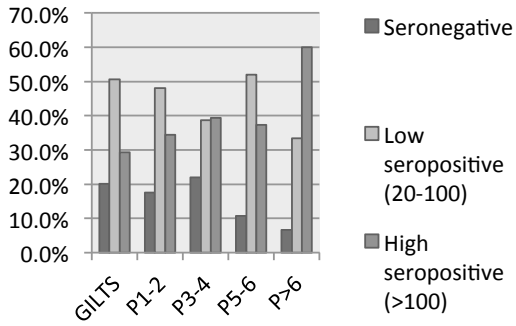
Serology results show 100% of the farms were seropositive. A total of 63.7% of the samples were found seropositive against PRRSV. Moreover, 82.9%, 38.7% and 57.2% of sows, nursery pigs and fatteners respectively, were seropositive for PRRS.

Table 1. Number of positive, negative PRRS samples on 4, 7 and 10 week-old (W) pigs.

	4W	7W	10W
N samples	105	116	114
Seronegative	55	90	53
Seropositive	50	26	61
% SEROPOSITIVE	47.6%	22.4%	53.5%

Table 2. Number of positive, negative PRRS samples on fatteners

	early	mid	end
N samples	96	110	98
Seronegative	52	61	17
Seropositive	44	69	61
% SEROPOSITIVE	46%	63%	62%



Graph 1. Percentage of PRRS seropositive sows grouped by parities. N=621

Conclusions and Discussion

The high percentage of seropostive piglets of 4 weeks can be attributed to maternally derived antibodies. At 7 weeks of age the percentage is a combination of maternally derived antibodies and active infections. We see that the highest percentage of seropositive piglets are seen at the end of the nursery (10 weeks), indicating an infection 2-4 weeks earlier. The trend in fatteners is also increasing as pig age.

The present study only show the prevalence of seropositive animals. However it is very important to classify the PRRS status of the farms in order to carry out a good assestment.

The blood sampling will continue the next year.

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In vitro inhibition o12345 PRRSV replication by specific DNA aptamers

C Savard[1], C Provost[1], C A Gagnon[1]

[1] *Swine and Poultry Infectious Diseases Research Center (CRIPA), Faculté de médecine vétérinaire, Université de Montréal , Saint-Hyacinthe, QC, Canada,* christian.savard@umontreal.ca

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is the viral disease with the highest economic impact on swine production in North America. PRRS is caused by an enveloped, single-stranded RNA virus (PRRSV) belonging to *Arteriviridae* viral family. PRRSV cause reproductive failure and increased mortality in young pigs as a result of severe respiratory disease and poor growth performance[1]. Currently, vaccination is the principal available measure to control and prevent the disease. However, the success obtained with vaccines is rather mitigated. Therefore, finding new, safe, effective and inexpensive ways to control PRRSV infection is imperative. Aptamers are a new class of therapeutic molecules composed of synthetic nucleic acid capable of binding to a broad range of targets with high affinity and specificity[2]. Aptamers has the potential to inhibit viral infection at any stage in the viral cycle, including viral entry. The objective of this study was to select PRRSV-specific DNA aptamers and evaluate their antiviral capacity *in vitro* in MARC-145 and porcine alveolar macrophages (PAM) infected cells.

Materials and Methods

Synthetic ssDNA library[3] was used to select PRRSV-specific aptamers using systematic evolution of ligands by exponential enrichment technique. PRRSV strain FMV09-1155278 (FMV09) used as target was previously isolated by our Diagnosis Lab. Virus was propagated in MARC-145 cells and purified on a discontinuous sucrose gradient. After 4 rounds of selection, candidate's aptamers were cloned and sequenced. 6 candidates aptamers (S4,15,16,17,18 and 19) were selected based on their binding capacity against FMV09. Thereafter, a viral inhibition assay with selected candidates and control aptamers (LB32 selected against VR-2332 PRRSV strain[4] and A22 selected against influenza A (H1N1)[5] was conducted. Aptamers were incubated with homologous (FMV09) and heterologous genotype II (IAF-Klop) and genotype I (LV) virus at 37˚C for 1h and then adsorbed on MARC-145 or porcine alveolar macrophages (PAM) cells for 3h. Then, cells were washed and incubated for 72 h. For MARC-145 cells, viral cythopathic effect was evaluated using the CytoTox96® NonRadioactive cytotoxicity assay (promega). For PAM cells, viral titers were evaluated in MARC-145 cells.

Results

Two aptamers (S18 and S19) had a significantly higher binding capacity to FMV09, at a similar level of the PRRSV-specfic aptamer (LB32). Only S18 aptamer significantly decreased the mortality caused by the homologous virus in MARC-145 cells, suggesting an antiviral effect of this aptamer. Aptamers S15, S16 and S17 have also demonstrated a partial inhibitory effect on cell death induced by the homologous virus but was not statistically significant. In addition, S18 and S15 aptamers, also demonstrated a significant antiviral effect against a heterologous genotype II strain (IAF-Klop). All tested candidate aptamers had no effect on a heterologous genotype I strain (LV), showing genotype specificity. All aptamers except aptamer S19 significantly decreased the viral titers of homologous virus (FMV09) in PAM cells (Figure 1). In addition, the aptamer S15 decreased significantly the titer of heterologous virus IAF-Klop (Figure 1). As in MARC-145 cells, any aptamers had significant effect on genotype I LV strain.

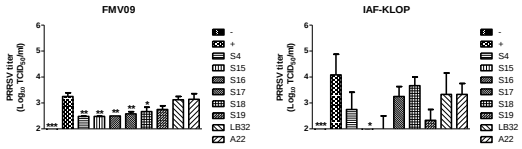

Figure 1. Inhibitory effect of candidate aptamers on PRRSV infection in porcine alveolar macrophages.

Conclusions and Discussion

In conclusion, S15 and S18 aptamers showed some *in vitro* inhibitory effect on homologous and heterologous PRRSV genotype II strains, in MARC- 145 and PAM cells. Both aptamers demonstrate specificity for PRRSV genotype II strains. Antiviral effect of these two aptamers was greater than the LB32 PRRSV aptamer previously reported in the literature[4]. Both aptamers showed similar structure, which could explain their comparable antiviral effect.

Acknowledgments

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Utilization of laboratory testing for monitoring PCV2 vaccination programs

J Seate[1], B Thacker[2]
[1]Murphy-Brown LLC – South Division, Rose Hill, NC, [2]Merck Animal Health, DeSoto, KS brad.thacker@merck.com

Introduction

Vaccination for porcine circovirus type 2 along with My*coplasma hyopneumoniae* (Mhp) is performed in nearly all swine operations. That said, vaccination compliance along with logistic considerations related to other procedures, pig health at the time of vaccination and the potential for vaccine interference by maternally-derived antibody (MDA) need to be considered when developing and implementing vaccination programs. Serological monitoring is commonly done to assess the potential for MDA interference, to evaluate post-vaccination immune responses which can serve as an indicator for vaccination compliance and to determine the onset of infection.[1] The objective of the study reported here was to evaluate different serological assays for Circumvent® PCV M compliance monitoring.

Materials and Methods

Pigs were vaccinated with Circumvent® PCV M at processing (3-4 days of age) and weaning (3 weeks of age) as directed by the herd veterinarian. Immediately after weaning, selected pigs were ear tagged and blood sampled at the nursery site. The initial plan was to bleed the pigs again at 6 weeks of age (WOA). Due to the poor antibody responses at 6 WOA, additional samples were collected at 10, 15 and 21 WOA. A second group (B) was started 8 weeks after the first group (A) using the same altered protocol. All laboratory testing was performed at the Iowa State University Veterinary Diagnostic Laboratory. PCV2 serum antibodies were measured by 4-Dilution PCV2 IFA, Ingezim Circovirus IgG (INGEL) (Ingenasa, Madrid, Spain) and PCV2 ELISA (PCVEL). Mhp serum antibodies were measured by ELISA (MHEL) (IDEXX, Westbrook, ME). Titer values are reported as the group geometric mean. For the IFA test, a titer $\geq$640 was considered positive. S/P ratios and PCR cycle times (CT) are reported as group means. Selected time points were tested for PCV2 by PCR.

Results

Table 1 presents the Group A data. Overall, post-vaccination responses at 6 and 10 WOA were lower than expected. MDA interference may have been responsible for lower Mhp titers but were not a consideration for the low PCV2 IFA titers. The INGEL and PCVEL were not done at 3 and 6 WOA due to misplacement of the samples. Subsequent testing indicated exposure to PCV2 and Mhp by all assays. PCV2 PCR and IFA indicated onset of PCV2 infection between 15 and 21 WOA.

Table 2 presents the results from Group B. PCV2 PCRs were negative up through 20 WOA. The post-vaccination antibody response is consistent with proper vaccination. PCV2 MDAs were low and Mhp MDAs were lower than observed with Group A.

Table 1. Group A Results

Age	No. positive/No. tested				
(wks)	IFA	INGEL	PCVEL	MHEL	PCR
3	8/20	ND	ND	17/20	ND
6	4/20	ND	ND	6/20	ND
10	4/19	15/19	17/19	3/19	ND
15	2/19	8/19	8/19	0/19	0/19
21	17/19	19/19	19/19	13/19	16/19
	Titers		S/P Ratios		CT
3	134.5	ND	ND	0.985	ND
6	109.3	ND	ND	0.462	ND
10	119.5	333.2	0.662	0.242	ND
15	92.6	122.5	0.416	0.100	>37
21	856.9	1097.6	1.073	0.738	30.1

Table 2: Group B Results

Age	No. positive/No. tested				
(wks)	IFA	INGEL	PCVEL	MHEL	PCR
3	0/25	25/25	25/25	7/25	0/25
6	22/23	23/23	23/23	23/23	0/23
10	19/24	24/24	24/24	23/24	0/24
16	17/22	ND	9/22	22/22	0/22
20	0/22	ND	13/22	24/24	0/24
	Titers		S/P Ratios		CT
3	80	653	0.748	0.303	>37
6	1037	8299	0.987	1.737	>37
10	640	1427	0.816	1.692	>37
16	273	ND	.299	1.542	>37
20	128	ND	.413	1.300	>37

Conclusions and Discussion

It appears that Group A was not properly vaccinated as all assays showed poor responses to PCV2 and Mhp. Group B appears to be properly vaccinated with nearly all pigs showing positive responses in all tests With regard to the different assays, the IFA, INGEL and MHEL appear to be more discriminating than the PCVEL based on the titers or S/P ratios at 3 vs. 6 WOA in Group B.

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Comparison of three commercially available ELISA kits for detection of anti-influenza A antibodies in Argentine swine farms

J Sarradell,[1,3]; M Biscia,[1]; R Di Masso,[2]; L Anthony,[1]; F Garófolo,[1]; A Perez, [3]

[1]*General and Special Pathology, Veterinary Faculty, National University of Rosario. Argentina* [2] *Genetic, Veterinary Faculty, National University of Rosario. CP 2170. Ov.Lagos y Ruta 33, Casilda, Santa Fe, Argentina.* [3] *College of Veterinary Medicine, University of Minnesota. e-mail. jsarrade@unr.edu.ar*

Introduction

Swine influenza virus (SIV) causes respiratory disease in pigs worldwide.[1,2] A number of ELISA kits are commercially available for SIV-diagnosis. The aim of this study was to quantify the pairwise concordance in the detection of anti-type A-SIV antibodies (positive, negative) for, three commercial ELISA kits available in Argentina.

Materials and Methods

Commercial hibrid lines from 3 intensive Argentine swine farms, with unknown SIV health status, were assessed. Serum samples were collected from 88 pigs from different categories. Sera were obtained by puncture of cranial cava vena and blood centrifugation and stored at -20 °C until processing.

Each sample was analyzed using three commercial ELISA kits for anti-type A SIV antibodies, namely, 1) Influenza A test kit from IDEXX Laboratory, The Netherlands; 2) CIVTEST Suis Influenza from HIPRA Laboratory, Spain, and 3) ELISA ID Screen Influenza A Antibody Competition Kit from IDVet Montpellier, France. Samples were processed following the protocols provided by each laboratory.

The pairwise degree of concordance was estimated by calculating Cohen's Kappa coefficient (k) corrected by the agreements due to random atributes [3]. Results were interpreted using a qualitative scale (excellent, good, moderate, fair, poor) proposed elsewhere.[2]

Results

The proportion of positive results was 43-56%, depending on the kit (Table 1).

Table 1. Results of the 88 serum samples from pigs in Argentina assessed using three commercially available ELISA kits for detection of Swine Influenza Virus antibodies.

	ELISA kit		
Results	*HIPRA*	*IDEXX*	*IDVET*
Positive	38	49	42
Negative	50	39	46

Pairwise agreement between the tests was moderate (Table 2).

Table 2. Pairwise agreement (95% confidence interval), as indicated by the kappa index (k) between ELISA kits for detection of Swine Influenza Virus antibodies commercially available in Argentina.

	IDEXX	IDVET
HIPRA	0.48 (0.35-0.61)	0.59 (0.47-0.71)
IDEXX	NA	0.52 (0.39-0.65)

Conclusions and Discussion

Pairwise agreement between the serological kits commercially available in Argentina was found to be moderate. The proportion of positive results was higher for the IDEXX kit, which may be due to higher sensitivity, lower specificity, or a combination of both, compared to the other tests assessed here.

The differences in test results detected here may be related, at least in part, to the antigens included and the technology used in the manufacturing of the kits and/or the SIV strains in the farms. Consequently, interpretation of ELISA results are likely appropriate and robust if performed at the herd level, whereas individual interpretation of tests results seems to be substantially influenced by the diagnostic test used.

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Surveillance of PRRS-negative swine farms in Costa Rica using the IDEXX PRRS of antibody kit for oral fluids

S Lizano[1], F Soto[2], L Salazar[2], F Chacón[3]
[1]*IDEXX Laboratories Inc., Westbrook, ME,* [2]*Genetak Análisis, San José, Costa Rica,*
[3]*Merck Sharp & Dohme I.A. Corp., San José, Costa Rica,* sergio-lizano@idexx.com

Introduction

PRRS is one of the costliest diseases in the swine industry worldwide and is endemic to most swine producing regions, particularly those with large animal populations. The frequent monitoring of PRRS infection in animal populations is therefore a key factor in the control of the disease and in the improvement of herd health and productivity. Recently, diagnostic screening of PRRS infection by antibody detection using swine oral fluids has provided an easy, cost-effective, and non-invasive way to conduct frequent monitoring and surveillance of pig populations (1). In this study, we demonstrate the utility of this approach to monitor PRRS exposure status in commercial farms in Costa Rica.

Materials and Methods

The study was conducted on different commercial farms housing pigs at different age groups up to 21 weeks. The number of pigs per pen ranged from 14-20 in barns ranging from 672-1200 pigs per barn (approx. 48-60 pens per barn). Cotton ropes were hung on 5-10 pens per barn and oral fluids collected as previously described (1). Oral fluid collections conducted every two weeks or whenever possible according to the site. Oral fluid samples were tested using a commercial PRRS oral fluid antibody ELISA (IDEXX Laboratories, Inc., Westbrook, ME, USA). Samples with S/P $\geq$ 0.4 were considered positive.

Results

Oral fluids collected from 6 different sites were monitored at different times (Figure 1). All samples tested negative for PCR. Most sites tested antibody-negative throughout the testing period except for PTJ-1, which had a PRRS antibody-positive sample on week period 6-7 (Figure 2). Other sites (PFS-1 and PTP-1) had one sample each that tested negative but at an S/P close to cutoff between weeks 4-6.

Table 1. Number of positive oral fluids (S/P $\geq$ 0.4) in each farm site, at different weeks of age

Site	Age in weeks								
	4-5	6-7	8-9	10-11	12-13	14-15	16-17	18-19	20-21
PSM-1				0		0			
PTJ-1		1	0	0	0	0	0	0	
PFS-1	0*	0	0	0	0	0	0	0	0
PGL-1			0	0	0	0	0	0	0
PRN-1	0	0	0				0		
PTP-1		0*	0		0				

*all samples were negative, with at least one sample exhibiting S/Ps close to cutoff (0.374 for site PFS-1 and 0.324 for site PTP-1).

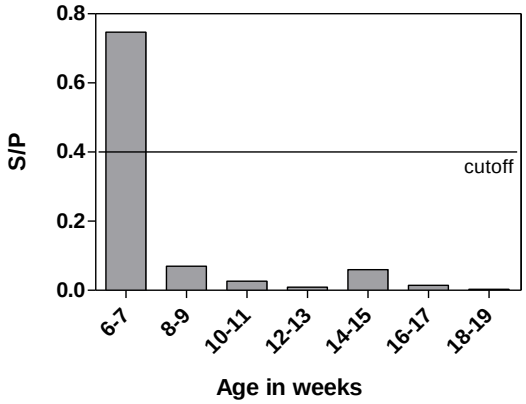

Figure 1. S/Ps for site PTJ-1 suring the testing period.

Conclusions and Discussion

In this study, the monitoring of pig populations for PRRS antibodies in oral fluids using the IDEXX PRRS OF ELISA proved useful in ascertaining the negative status of herds over time.

While all samples were antibody-negative, some "dirty" negatives and a positive sample were detected in the early post-weaning period. This could be attributed to maternal antibodies or contamination from porcine plasma-based feed supplements that are usually administered in the first weeks post-weaning (2,3). After this period, oral fluids across all sites tested consistently negative for PRRS antibodies up to week 21, indicating that the populations were not exposed during this period. This highlights the specificity of the IDEXX PRRS OF ELISA.

Oral fluids sampling is easy to implement. Rope collection in a pen allows ample coverage of the testing population by increasing the number of animals represented in the barn. This approach affords the possibility to conduct constant monitoring over time in an efficient and cost-effective manner

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Monitoring for antibodies to PRRSV in oral fluids in growing pigs in Costa Rica

S Lizano[1], F Soto[2], L Salazar[2], F Chacón[3]
[1]*IDEXX Laboratories Inc., Westbrook, ME,* [2]*Genetak Análisis, San José, Costa Rica,* [3]*Merck Sharp & Dohme I.A. Corp., San José, Costa Rica,* sergio-lizano@idexx.com

Introduction

The frequent monitoring of swine herds to test for exposure to the Porcine Reproductive and Respiratory Syndrome virus (PRRSV) in animal populations is a key factor in disease control and eradication to improve herd health and productivity, as well as reduce production costs. Sampling for oral fluids using cotton rope facilitates the easy, cost-effective, and non-invasive monitoring and surveillance of pig populations (1). In this report, we present a case study validating the usefulness of frequent oral fluids sampling in a barn to monitor disease exposure by detection of antibodies to PRRSV using the IDEXX PRRS OF Antibody ELISA.

Materials and Methods

The study was conducted by hanging cotton ropes to collect pen oral fluids from 8 pens in a continuous flow barn housing non-vaccinated post-weaning pigs ranging from 4 to 22 weeks of age. Sampling included the pens housing gestation sows. Oral fluids collected from the ropes were processed as previously described (2) and tested for antibodies to PRRSV using the IDEXX PRRS OF Ab ELISA Test. Samples with an S/P value ≥0.40 were considered positive. The samples were also tested by RT-PCR for the presence of PRRSV.

Results

In barn 1, oral fluids collected from the gestation pens (G1 and G2) as well as the pens containing post-weaning pigs from weeks 4 to 6 were positive for antibodies to PRRSV at moderate S/P levels (S/Ps from 1.11-3.58), while S/P levels were negative in pigs aged weeks 7-9 (Figure 1). In contrast, samples from barn 2 were strongly positive (S/Ps from 4.72-10.46) in pigs aged 11 weeks onwards. All samples tested negative by RT-PCR.

Conclusions and Discussion

The moderately positive S/Ps from barn 1 in gestation pens as well as in pens housing pigs in the early stages post-weaning likely indicate the presence of maternal antibodies transferred onto weaning pigs by passive immunization instead of an active infection. This is supported by the observation that older pigs aged weeks 7-9 and housed in the same barn have negative S/P values. Conversely, older pigs housed in barn 2 tested strongly positive for anti-PRRSV antibodies, suggesting exposure to PRRSV during the growing period. In both pens, the negative PCR results indicate the absence of an active infection in both pens at the time of sampling.

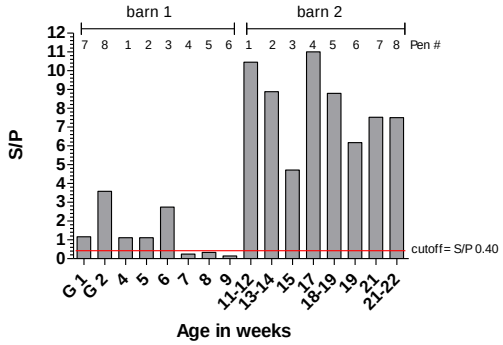

Figure 1. Antibody levels in oral fluid samples from pens housing pigs at different weeks of age and in gestation sows (G1 and G2). *G1/G2 (gestation pens)*

This study underscores the importance of using a combination of diagnostic tools (e.g. PCR to determine active viral shedding; antibody-testing to determine exposure) when assessing the exposure status of a herd in a growing pig population. A sampling program consisting of periodic oral fluids collections of 8-10 pens per barn (e.g. bi-weekly, same pens sampled each time) would provide a powerful surveillance tool to monitor the exposure status of herds over time. In addition, the inclusion boars as well as gestation sows and replacement gilts in the monitoring program would provide a more complete picture of the PRRS status of the farm. Once the exposure status of each barn is established, the goals are to implement control programs aimed at stabilizing the PRRS status of the herd and eventually produce PRRS-negative piglets.

Oral fluids sampling is easy to implement, thus providing the opportunity to sample often. Rope collection in a pen allows ample coverage of the testing population by increasing the number of animals represented in the barn. The introduction of diagnostic tools for antibody detection designed for oral fluids such as the IDEXX PRRS OF ELISA will facilitate the implementation of disease surveillance in swine herds.

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Development of the IDEXX PRRS of ELISA for the detection of PRRS antibodies in swine oral fluids

S Lizano[1], S Koller[1], C Goodell[1]
[1]*IDEXX Laboratories Inc., Westbrook, ME, USA,* sergio-lizano@idexx.com

Introduction

Testing of swine oral provides a convenient and cost-effective tool for disease surveillance in commercial pig herds. Recently, detection of antibodies to Porcine Reproductive and Respiratory Syndrome virus (PRRSV) in oral fluids has been described using an adapted overnight sample incubation format adaptation of the HerdChek® PRRS X3 ELISA, (IDEXX Laboratories, Inc.).[1] In this study, we describe a new PRRS oral fluids ELISA (IDEXX PRRS OF Ab Test) for same-day detection of PRRS antibodies in swine oral fluids. An S/P ≥ 0.40 is considered a positive result.

Materials and Methods

Sample set 1 consisted of a temporal series of pooled oral fluids from a barn of 1200 PRRSV vaccinated pigs (PRRS MLV, Boehringer Ingelheim Vetmedica Inc.).
Sample set 2 consisted of a temporal series of paired oral fluids and serum collected from individual boars 0-7 days before (negative exposures status) and 21 days after (positive exposure status) vaccination or experimental infection with type I or type II PRRSV.[2]
Sample set 3 consisted of pen-based oral fluid samples collected at various levels of prevalence (0%, 4%, 12%, 20%, and 36%) of antibody-positive vaccinated pigs introduced at 14 days post-vaccination into pens of PRRS-negative pigs.[3]
Sample Set 4 consisted of paired oral fluids and serum comprised of 147 samples from a PRRS-positive farm and 151 samples from a PRRS-negative sow farm, for a total of 298 sets of paired samples.

Results

A comparison between the new protocol and the standard overnight protocol (SOP) using sample set 1 indicated 100% agreement between the two tests, with average S/P values 1.4 to 1.5-fold higher than SOP. Analysis of Sample sets 2 and 4 using the serum test (PRRS X3) as a reference standard indicated a specificity of 98.7% and a sensitivity of 100% for the IDEXX PRRS OF Ab Test. Figure 1 summarizes the results for Sample set 4. A temporal analysis of oral fluids from the pigs inoculated with either type 1 (EU) or type 2 (US) strains of PRRSV revealed equivalent times to detection of anti-PRRSV in oral fluids with PRRS OF compared to the performance of PRRS X3 on the paired serum samples from the same temporal series. Finally, an evaluation of sample set 3 indicated that the estimated probability of detecting antibody to PRRSV using the PRRS OF Ab Test Kit is 91% in pens with a prevalence of 20% and increased to over 99% in pens with at least 32% sero-positive pigs. These results were obtained using a statistical model with pen considered as a random effect.

		PRRS X3 (serum)		
		Pos	Neg	Totals
PRRS OF (oral fluid)	Pos	147	2	149
	Neg	0	149	149
	Totals	147	151	298

		95% Confidence Limits	
	%	Low CL	High CL
Sensitivity	100.0	97.52	100.00
Specificity	98.7	95.30	99.84

Figure 1. Performance of the IDEXX PRRS OF ELISA compared to results obtained with PRRS X3 on field populations.

Conclusions and Discussion

- These results describe a new sensitive, same-day test for anti- PRRS antibody detection aimed to support the emerging use of oral fluids for frequent surveillance of pig herds. The IDEXX PRRS Oral Fluids Ab Test is now commercially available from IDEXX Laboratories.

Acknowledgements

The authors would like to thank Drs. Jeff Zimmerman, Apisit Kittawornrat, and Chris Olsen at Iowa State University for their research and for supplying the sample sets of oral fluids and serum that made this study possible.

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Design and construction of a nebulization chamber for pigs

F Sotres, S Mendoza, E Hernández-Baumgarten, D Trujillo, E Martínez, A Ciprián
Facultad de Estudios Superiores Cuautilán-UNAM; [2]Facultad de Medicina Veterinaria y Zootecnia-UNAM,
pila_rh_dz@hotmail.com

Introduction

The respiratory pig diseases have been important in diminishing profits for several decades for the world pig producing industry. In most cases, the reproduction of respiratory diseases under controlled conditions has been made by inoculating these agents by not natural routes or using very aggressive vehicles. For these reasons the need to have a system that can fatefully reproduce what happens under natural conditions is very important. For these reasons in Mexico we have worked for or over 20 years in designing and constructing a nebulization chamber that allows us to control the most important variables in order to obtain reproducibility in the challenges . There are references of nebulization chambers in mice, such as the one written by López Dávalos (1983). In pigs, the first of such chambers in Mexico was described by Caballero (1985), afterwards the one made by Ciprián et al. (1988) and Sotres et al (2007). In this cas the chamber will be made by modifying Sebunya *et al* (1983) original model

Materiasl and Methods

The chamber's Shell was constructed from 1 ½ inch PTR the walls and roof were covers with fiberglass sheetand the floor was former with plastic resin slats cappable of supporting 120 Kg/Cm^2. The ramp-door 1.00X1.00 M has a built in drainage system. On the side walls three 1 ½ round holes were cut to install one disposable nebulizer in each one (Devilbiss), and then connected to three heavy duty nebulizer equipments (Devilbiss Pulmo Aid). The chamber is hermetic and was also equipped with filter system connected to an air inactivation by air washing, in order to be able to work with pathogens that are very hazardous to disseminate.

Results

The size of the constructing allows accommodating 10 week old piglets in a temporal manner. The entire chamber is light weight and the nebuilizers work at a pressure of 12 to 18 $psig^2$ and produce a 9 liters per minute of nebulizad fluid each. With the operating conditions specified, the particles produced are between 0.5 a 5 µm, adequate to reach the alveoli in the lungs and thus guaranteeing the deposition of the nebulized material.

Conclusions and Discussion

It is of utmost importance the capacity to fatefully reproduce the natural conditions of naturally occurring diseases of the pathogenic agents and to have available a trustworthy equipment that can fatefully and reproducibly imitate the conditions of exposure to a given pathogen. It is vital to ensure that the clinical pictures and lesions observed in the field are caused only by the pathogens used for the challenge and not to factors out of our control.

Acknowledgements

PAPIIT ITE218711-3 and CONS-23

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Field comparison of PCV2 vaccines: A retrospective production data analysis

B Thacker[1], R Blomme[2], D Holtkamp[3], J Creel[1]
[1]Merck Animal Health, DeSoto, KS; [2]AMVC Veterinary Services, Audubon, IA; [3]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, brad.thacker@merck.com

Introduction

In this case study, we present a retrospective production record data analysis that found significant differences in the performance of PCV2 vaccines and estimates the economic impacts of these differences. The operation purchases weaned pigs for two separate flows. One flow consists of terminal line animals where all progeny are sold for slaughter. The other flow consists of maternal line animals where barrows and some gilts are sold for slaughter while most gilts are sold as replacements. Both flows are considered to be of high health status and are managed the same. Originally, a one dose PCV2 vaccine, CircoFLEX® (CFLEX) (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) was used in both flows. The maternal flow was switched to Circumvent® PCV (CVENT) (Merck Animal Health, Summit, NJ) in response to concerns about viremia in replacement gilts. The producer judged that the maternal flow was outperforming the terminal flow and decided to switch the terminal flow to Fostera™ PCV (FOST) (Zoetis, Florham, NJ). The performance of the terminal flow still lagged behind the maternal flow so the producer requested assistance with analyzing the operation's production records along with an economic assessment.

Materials and Methods

Production data in this operation was collected by site as each group was closed-out and the site was emptied. Only finisher data was evaluated; evaluation of nursery performance revealed no differences between the two flows. For statistical comparison, the data was organized into two time periods. In Time Period 1, CFLEX was compared to CVENT. In Time Period 2, FOST was compared to CVENT. The overall average values for average daily gain (ADG), feed conversion ratio (FCR), mortality rate and cull rate were weighted by the number of pigs placed in each group. The genetic supplier was consulted to determine if there were expected differences in the performance of the two lines. They indicated that ADG and FCR would be impacted such that the maternal line would have lower ADG (0.064 lb per day) and increased FCR (0.100 less efficient). Accordingly, the data was analyzed with (genetic line adjusted-ADJ) or without (actual-ACT) group adjustments. The data was analyzed on a group basis by ANOVA or an individual pig basis by Chi square. A swine enterprise budgeting model was used to determine the economic differences between the vaccines within each time period. The outcome is reported as the difference in profit per pig.

Results

A summary of the group close-out performance is in Table 1. For both periods, the mortality and cull rates were significantly ($P<0.05$) lower in CVENT vaccinated groups compared to CFLEX or FOST. For ADG, no differences were found using the actual or adjusted values. For FCR, no differences were found between vaccines within time period using the actual values. However, FCR was significantly better in CVENT groups using the adjusted values.

For the individual pig based analysis, for CFLEX and FOST pigs, the odds of dying were 1.87 and 1.98 times greater compared to CVENT pigs and the odds of being culled were 1.90 and 1.76 times greater, respectively.

The economic analysis revealed that the increase in profit per pig provided by CVENT compared to CFLEX ranged from \$0.99 to \$6.27 and compared to FOST ranged from \$0.70 to \$6.16, depending on the parameter values used in the model. The largest differences were calculated using the ADG and FCR adjusted values.

Table 1. Summary of group close-out performance.

Parameter	Results			
Flow	TERMINAL		MATERNAL	
Period	1	2	1	2
Vaccine	CFLEX	FOST	CVENT	
Groups	29	30	25	20
No. pigs	56,830	60,847	66,611	54,202
% Died	2.67%	3.01%	1.45%	1.55%
% Culls	1.61%	1.31%	0.87%	0.76%
ADG-ACT	1.91	1.97	1.89	1.91
ADG-ADJ			1.95	1.97
FCR-ACT	2.74	2.63	2.74	2.63
FCR-ADJ			2.64	2.53

Conclusions and Discussion

This situation provided a unique opportunity to compare the performance of PCV2 vaccines under field conditions. The operation has switched the terminal flow to Circumvent PCV and close-out data from several initial groups reveals reduced mortality and cull rates similar to the CVENT vaccinated maternal flow.

Aknowledgments

Thank you to the farm and office staff that made this data available to us and their assistance in the analysis.

Improvement of farrowing parameters in a Mexican farm using a bivalent reproductive vaccine (Parvosuin MR®)

I Rodríguez-Ballarà[1], H Delgado[2], JA Mares[3]
[1]*Technical services, HIPRA, Spain,* [2]*HIPRA MEXICO, Mexico.* [3] *Porcina Ernesto Aceves, Mexico.*
isaac.rodriguez@hipra.com

Inroduction

Vaccines against Swine Erysipela (SE) and Porcine Parvovirus (PPV) are widely used in intensive swine farms. In Europe most of the swine farms use a bivalent vaccine (PPV, SE), besides in America Erysipela and Parvovirus vaccines include Leptospira sp (Le). PPV infection after 6 days of conception until 35 days of gestation results in embryonic death and maternal resorption of fetal tissues. At or about day 70 of gestation, the fetus is able to mount effective immune response and eliminate the virus (1). Besides, subacute and chronical SE infection can cause infertility, litters with increased number of mummies or small litters, and pre or post parturient vulvar discharges. In some cases this signs may be so mild as to remain unnoticed (2).

SE and mainly PPV are widespread among swine so can cause reproductive losses in unvaccinated herds or when the vaccine is not working correctly.

The objective of this study is to compare the historical reproductive sow performance in a Mexican farm, after the change of the reproductive vaccine from a trivalent reproductive vaccine (SE, PPV, Lepto) to a bivalent reproductive vaccine (SE, PPV).

Materials and Method

The study was carried out in a 1380 sow's farm in Michoacán State, Mexico. The vaccinal program included a trivalent (SE, PPV,Le) vaccine (Vaccine A). In May 2011 the reproductive vaccine was changed to a bivalent (PPV, SE) vaccine (Parvosuin MR®). The vaccinal program followed by the bivalent vaccine was the same as used with trivalent vaccine, Parvosuin MR® was implemented 14 days after farrowing in sows, and 2 doses before mating were applied to the gilts.

In order to assess the field efficacy of Parvosuin MR® reproductive data from lactation units was recorded and compared between 33 months before to implement Parvosuin MR® vaccination and 33 months after the vaccination change. Two farrowing parameters were selected to evaluate the impact of bivalent vaccination: total piglets born per litter and percentage of mummified piglets.

Results

A total piglet born per litter mean from data recorded before Parvosuin MR® vaccination and after was calculated. Total piglet born per litter increased significantly (*P<0.05, t-test for independent samples*) after the implementation of Parvosuin MR® (Figure 1). Besides, the evolution of percentage of mummified piglets showed a clear trend to decrease after Parvosuin MR® vaccination as it is shown in Figure 2.

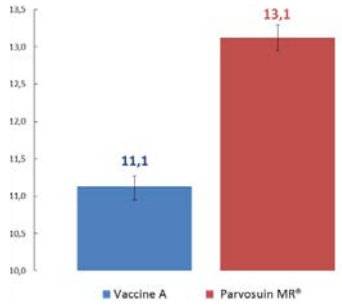

Figure 1. Mean TOTAL PIGLETS BORN / LITTER (± SEM).

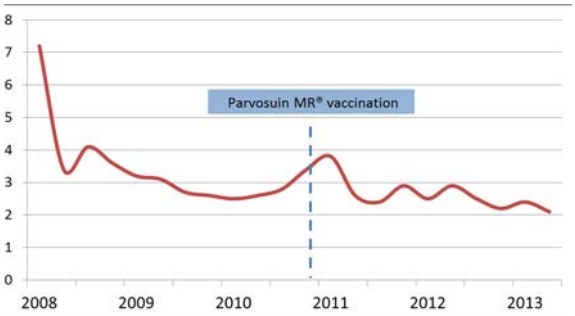

Figure 2. Evolution of the percentage of MUMMIFIED PIGLETS from 3rd trimester 2008 to 4th trimester 2013.

Conclusions and Discussion

After the implementation of Parvosuin MR Vaccination, farrowing parameters improved significantly. The significant increase of total piglets born enhance directly the total piglets weaned and definitely the productive parameters in farrowing units. This historical comparative field study is showing again the efficacy differences between trivalent and bivalent vaccines as it has been demonstrated in previous studies(3).

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PRRSV antibody monitoring in sows – observations using oral fluid antibody detection

C Goodell[1], C Gomez[1], C Díaz Rayo[2], A Bedoy[2], M Serrano[3], S Zimmerman[1]
[1]*IDEXX., Westbrook,ME;* [2] *Diagnosticos Integrales en Patologia Animal-ITSON, Mexico,* [3]*Investigacion Aplicada S.A. de C.V, México* Christa-goodell@idexx.com

Introduction

As farms grow and consolidate, sow farms and their progeny are often combined into one production flow. However, when sows are housed in distinct buildings, PRRSV status can differ between sow barns and PRRSV can actively circulate as populations of pigs are comingled. This study evaluated such a situation in a continuous flow, farrow-to-finish farm.

Materials and Methods

This study was conducted in a 950 sow farrow-to-finish farm in Sonora, México. The farm experienced a PRRS outbreak 9 months earlier, had instituted PRRS vaccination (Ingelvac PRRS® MLV, Boehringer Ingelheim) to control the outbreak, and continues to vaccinate the sow herd quarterly. Replacement gilts were raised on farm and moved into the gilt developer unit (GDU) at 10weeks of age. In the GDU, they were vaccinated with PRRS MLV at approximately 20 weeks of age.

Sows were housed in 2 gestation barns located approximately 100 meters apart. In this study, the mean and variation in the PRRS oral fluid antibody ELISA S/P ratios were evaluated in the two gestation barns, one farrowing barn housing weanling piglets, and the GDU. Oral fluids were collected by suspending a cotton rope (20 minutes) at the front of individual sow stalls in each gestation barn (n=12 and n=8), in farrowing crates with ~18 day old litters (n=8), and in pens of growing gilts in the gilt developer unit (n=11). Oral fluid samples were tested using a commercial PRRSV oral fluid antibody ELISA (IDEXX., Westbrook, ME). Samples with S/P ≥ 0.4 were classified as positive.

Results

PRRS oral fluid antibody ELISA S/P ratios by sow barn and by animal age in the GDU are given below.

Table 1. Mean sow S/P and standard deviation by barn

Sow site (no. of samples)	Mean S/P	SD
Gestation 1 (n=12)	4.20	2.90
Gestation 2 (n=8)	1.00	0.84
Farrowing (n=8)	2.22	2.62

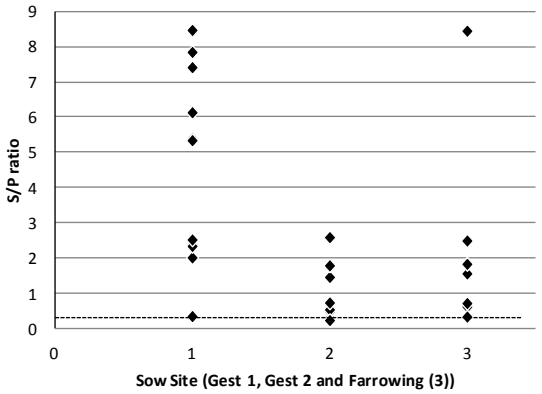

Figure 1. Scatterplot of sow oral fluid sample S/P ratios by sow housing location (positive cut-off ≥0.4)

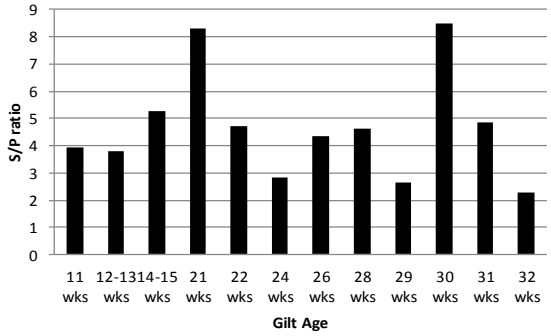

Figure 2. Pen-based PRRS S/P ratio by age in GDU

Conclusions and Discussion

This study provided a cross sectional illustration of the antibody status of a subset of the females within this farm. Oral fluid ELISA results were positive as expected, however a large difference was observed in the mean S/P ratios between sow sites and high variation was detected among the sow samples between barns. In the GDU, animals entered previously exposed to PRRSV, indicating current PRRSV circulation in the on-site nursery. A booster response was seen after vaccination, however, there was also evidence of more PRRSV circulation in the older animals. These results suggest continued PRRS circulation within this well vaccinated farm site, and emphasize the importance of PRRS antibody monitoring in site 1 an GDU locations if PRRS stabilization is desired. Biweekly oral fluids PRRS antibody monitoring is a valuable tool for PRRSV transmission control.

The neutrophil infiltration in the PRRSV infected porcine lung

J Liu, M Hou, X Wu, G Liu
Department of Basic Veterinary Medicine, College of Veterinary Medicine,
Huazhong Agricultural University, Wuhan, Hubei, China, liuguoquan@mail.hzau.edu.cn

Introduction

Neutrophils are innate immune cells and play a crucial role in the first line of host defense (1). However, neutrophil lung recruitment and infiltration may also cause lung injury(2). Porcine reproductive and respiratory syndrome (PRRS) is the most economically important infectious viral disease caused by porcine reproductive and respiratory syndrome virus (PRRSV). Diffuse interstitial pneumonia and a large number of bleeding spots in the lungs have been reported in the infected pigs (3,4). There is little known about the pathological mechanisms how PRRSV infection causes pig lung injury. Recent genome-wide transcriptome studies on the PRRSV infected porcine lung tissue, suggested the upregulation of the molecules involving in neutrophils adhesion and migration (5). Therefore, we examined the neutrohpil infiltration in the PRRSV infected swine lungs.

Materials and Methods

A number of normal swine lungs were purchased from slaughterhouse and PRRSV infectious swine lungs were collected from clinic. All of the samples were examined by PCR method to examine infection of PRRSV or PCV2 (porcine circovirus type 2), and then divided into three groups: the control group (n=8) without PRRSV and/or PCV2 infection, the PRRSV alone infected group (n=4) and the one with both infection as PRRSV+PCV2 (n=4). All the lung samples were processed routinely for haematoxylin and eosin (HE) staining. Then the quantitative real-time PCR (qPCR) method was used to determine the expression of ICAM-1, myeloperoxidase (MPO), IL-8, MCP-1. To determine neutrophil infiltration, the MPO activity of porcine lung lysates was measured.

Results

H&E staining results showed the infiltration of immune cells in the infected lungs of both PRRSV and PRRSV+PCV2 groups. QPCR showed that the mRNA expression of ICAM-1 and IL-8 was strongly upregulated in the infection groups, while the expression of MCP-1 was markedly downregulated (Figure 1) in both groups in comparison of the control group. QPCR aslo showed that the mRNA expression of MPO was highly upregulated in both infection groups. Neutrophil lung infiltration determined by MPO activity was increased 4-fold in PRRSV group and 6-fold in PRRSV+PCV2 group (Figure 2).

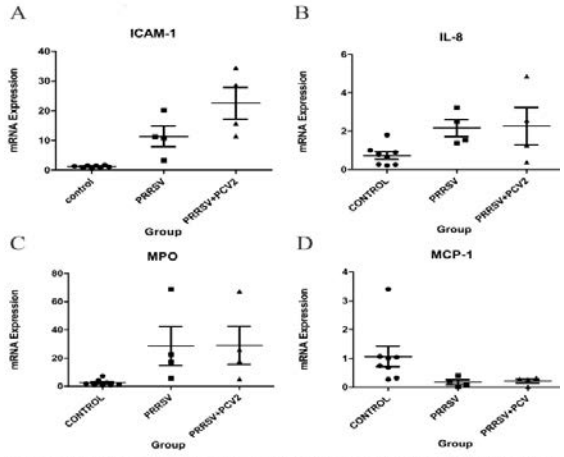

Figure1.mRNA expression of ICAM-1,IL-8,MPO,MCP-1 according to different groups (A) ICAM-1;(B)IL-8;(C)MPO;(D)MCP-1.The mRNA expression of ICAM-1,IL-8,MPO was strongly upregulated in PRRSV alone group and PRRSV+PCV2 mixed group,while the expression of MCP-1 was markedly downregulated.

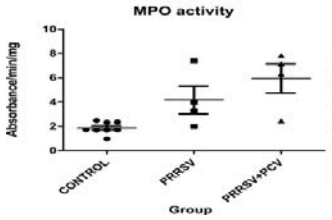

Figure 2.MPO activity assay. Neutrophil lung infiltration determined by MPO activity was increased in PRRSV alone group and PRRSV+ PCV2 mixed group.

Conclusions and Discussion

Histological studies indicated the recuitment and infiltration of immune cells in the infected lung tissues. Upregulations of ICAM-1 and IL-8 expression suggested more neutrophils recuitment and infiltration. Down-regulation of MCP-1 suggested that less monocytes were recuited. MPO QPCR and MPO activity assay indicated the recrutiment of a large number of neutrophils in the infected swine lungs, which may contribue to the lung injury in infected animals.

Acknowledgments

This work was supported by the grant from Natural Scienc Foundation of China (37312428) and Fundamental Research Funds for the Central Universities (2013PY054).

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A comparative study of two *E. coli*/*C. perfringens* combination vaccines

M Collell[1], M Murmans[2], M Witvliet[2]
[1]*Global Marketing, MSD Animal Health, Summit, NJ, USA,* [2]*Microbiological R&D, MSD Animal Health, Boxmeer, The Netherlands,* miquel.collell@merck.com

Introduction

Sows and gilts are commonly vaccinated to protect their offspring against neonatal diarrhea caused by Enterotoxigenic *Escherichia coli* (ETEC). Because *Clostridium perfringens* can also be involved in neonatal disease, combination vaccines have been developed against ETEC and *Clostridium spp.* over the years, but it is crucial that the vaccine-induced antibodies against both organisms reach the progeny via the colostrum in sufficient levels. In the present study, the safety and efficacy characteristics of two of these combination vaccines were compared. Clinical observation, palpation of local reactions and rectal temperature were used to assess safety of the vaccines and colostral antibody levels were determined as a measure for efficacy.

Materials and Methods

In the study, three groups of 8 pregnant sows housed on a conventional farm without neonatal diarrhea problems were used. Group 1 was vaccinated with Porcilis ColiClos (2 ml) at 6 and 2 weeks before the expected farrowing date. Group 2 was vaccinated with Suiseng (2 ml) at 6 and 3 weeks before farrowing and group 3 was left unvaccinated. After vaccination, the animals were observed daily for clinical abnormalities and local reactions. Rectal temperatures were measured just before vaccination and 6 hours and one day post vaccination. Colostrum samples were taken shortly after farrowing and tested for antibodies against the ETEC F4ab, F4ac, F5 and F6 fimbriae and heat-labile toxin (LT) and the *C. perfringens* beta-toxin by ELISA with purified antigens. Antibody levels were expressed as $\log_2$ titers and the means of the three groups were compared per antigen using Duncan's multiple range test (SAS® Enterprise Guide 4.3, Cary, NC, USA). Titers below the detection limit (5.6 $\log_2$) were set at 4.6.

Results and Discussion

Vaccination did not result in any acute systemic reactions and the average temperature increases at 6 hours post vaccination were low. After the first vaccination, the sows in group 1 did not show an increase at all and the mean increase after the second vaccination was 0.5°C. In group 2, the mean increase was 0.4°C and 0.3° after the first and second vaccination, respectively. On the day after vaccination, the rectal temperatures had returned to normal. Other than one sow in group 2 that had a lack of appetite on the day after vaccination, became severely ill and aborted one day later, no clinical abnormalities were observed. Vaccination did not induce local reactions in either group.

The colostrum antibody titers are summarized in the Table below. In the unvaccinated control group, the colostrum titers were low for all antigens except the *E. coli* F6 fimbrial antigen

Table 1 Mean antibody titers in colostrum ($\log_2$)

Antigen	Group 1 Coliclos	Group 2 Suiseng	Group 3 Control
F4ab	$13.1^{a} \pm 2.6$	$9.0^{b} \pm 2.0$	$4.6^{c} \pm 0.0$
F4ac	$12.5^{a} \pm 2.3$	$10.3^{b} \pm 1.7$	$4.8^{c} \pm 0.6$
F5	$10.7^{a} \pm 2.3$	$7.9^{b} \pm 1.5$	$4.9^{c} \pm 0.8$
F6	$11.2^{a} \pm 1.6$	$9.1^{b} \pm 1.5$	$8.7^{b} \pm 0.8$
LT	$10.5^{a} \pm 1.5$	$7.0^{b} \pm 2.7$	$5.9^{b} \pm 1.7$
beta-toxin	$7.6^{a} \pm 2.0$	$6.4^{a,b} \pm 1.5$	$4.8^{b} \pm 1.1$

Groups with different superscripts within a row are significantly different.

The mean antibody titers against all five *E. coli* virulence factors were significantly higher in group 1 that had been vaccinated with Porcilis ColiClos than in group 2 that had received Suiseng or in the unvaccinated control group 3. The difference between groups 1 and 2 in antibody titer against *C. perfringens* type C beta-toxin was not statistically significant. However, the difference between group 1 and group 3 was significant and between group 2 and 3 was not.

Early detection of PRRSV infection in an "expected negative" herd using pen-based oral fluid sampling

S Zimmerman[1], C Gomez[1], C Goodell[1], L Barron[1], A Garcia-Rendón[2], H Jiménez[2], E Lucio[3], W Gonzales[3], P Avalos[3]; J Chapa[3], G Aguila[3], G Gutierrez[3], B Bautista[3], M Serrano[3]

[1]*IDEXX Laboratories Inc., Westbrook,* [2]*Granja Rancho Covadonga;* [3]*IASA Investigacion Aplilcada,Mexico,*

Silvia-zimmerman@idexx.com

Introduction

PRRS is one of the most costly diseases swine producers confront. PRRSV is endemic in Mexico and producers and government are working together to reduce the economic impact of the disease. There are several effective approaches for PRRSV elimination, but re-infection is not rare. Therefore, surveillance of negative farms for the purpose of early detection/response is vital. The long-term aim of this study (currently in progress) was to evaluate antibody-based PRRSV monitoring in an "expected negative" farm using oral fluid samples.

Materials and Methods

This study was conducted in a 2500-sow 3-site farm. The farm was presumed negative to PRRSV because of the absence of history of clinical signs suggestive of PRRSV infection. Neither PRRSV immunoprophylaxis or PRRSV vaccine had been used in the herd.

The number of oral fluid samples collected is given in Table 1. Samples were collected three times at two week intervals. Note that Site 1 quarantine and acclimation housed 10-20 gilts per pen. Sites 2 and 3 housed 20-30 pigs per pen. In Sites 2 and 3, oral fluids were collected by suspending a cotton rope between pens for ~30 minutes. In this way, each rope was chewed by pigs in 2 adjacent pens.

Samples were tested using a commercial PRRSV oral fluid antibody ELISA (IDEXX Laboratories, Inc., Westbrook, ME, USA). Samples with S/P $\geq$ 0.4 were classified as positive.

Table 1. Number and location of oral fluid specimens collected at each of 3 samplings

		# Pens or crates per barn	# oral fluids (3 samplings)			Total
			1	2	3	
Site 1	Gestation (2 Barns)	600 - 900 crates	17*	6**	5***	28
	Aclimation (1 Barn)	21 pens	5	5	na	10
	Quarantine (1 Barn)	10 pens	6	6	6	18
Site 2	Nursery (1 Barn)	7 rooms w/ 20-28 pens	35	35	35	105
Site 3	Finishing (6 Barns)	24-50 pens/barn	35	35	35	105
Number of oral fluid samples per week =			98	87	81	266

* individual sows (13) and boars (4)
** individual sows (2) and boars (4)
*** individual sow (1) and boars (4)

Results

SITE 1: At the first sampling, all samples from quarantine and acclimation were negative, but one animal in one of the two gestation barns tested positive (S/P = 0.77). Two weeks later - the second sampling - two oral fluid samples collected from the same gestation barn were positive (S/Ps = 6.33 and 5.48).

SITE 2 (nursery): At the first sampling, all 25 samples from rooms 1 to 5 tested negative, whereas all 10 samples from rooms 6 and 7 tested positive (mean S/P 3.15; min S/P 1.36; max S/P 4.65).

All 35 samples tested negative on the second sampling.

At the 3rd sampling, the 10 samples from rooms 1 and 2 were positive (mean S/P 5.31; min S/P 2.92; max S/P 6.80), but all other samples were negative.

SITE 3 (growing/finishing): Among 105 samples collected over the 3 samplings, just 5 negative samples were identified.

Conclusions and Discussion

The intent of this study was to explore PRRSV surveillance using oral fluid sampling and PRRSV oral fluid antibody detection. The initial identification of PRRSV-positive sows in an "expected negative" herd was unexpected. However, these results were confirmed by the detection of PRRSV antibody in samples from Sites 2 (nursery) and 3 (grow/finish).

The absence of clinical signs and the low number of antibody-positive samples from Site 1 suggested that PRRSV had been introduced recently and that a small number of sows were infected. In Site 2, the restriction of antibody positives to just 2 of 7 rooms supported this interpretation. Of course, PRRSV transmission and massive infection occurred as pigs were mixed at Site 3.

This surveillance approach was done at low cost, but most importantly, PRRSV infection was detected early enough for veterinarians to act to control the disease in the sow herd -- before the appearance of clinical signs and production losses.

Acknowledgments

Granja Rancho Covadonga

PRRSV surveillance in an "expected negative" herd in Mexico

S Zimmerman[1], J Gomez[2], C Gomez[1], M Serrano[3], W Gonzalez[3], E Lucio [3] P Avalos[3], G Gutierrez[3], B Bautista[3], G Aguila[3], L Barron[1], J Chapa[3], C Goodell[1]

[1]*IDEXX Laboratories Inc., Westbrook, ME USA,* [2]*Alcer y Rocer Farm, México,*

[3]*Investigacion Aplicada S.A. de C.V, México* Silvia-zimmerman@idexx.com

Introduction

Eliminating PRRSV and then protecting the herd's PRRSV-free status is one of the greatest challenges veterinarians and producers face. This study was conducted in a PRRSV-free farm (since 2007) located in a herd-dense region and surrounded by PRRSV-positive farms. An efficient, effective, and affordable surveillance program was needed to protect this herd. The aim of this study was to evaluate PRRSV surveillance based on antibody testing of oral fluid samples. Herein we present the results from the first three samplings.

Materials and Methods

The study was conducted in a PRRSV-negative, great-grandparent sow farm (n = 100) in central Mexico. As part of the farm's PRRSV prevention strategy, InmunoPRRS® (Investigacion Aplicada SA de CV) was given to sows (85 and 100 days of gestation); pigs (1, 77, 92 days of age and 90 kg) and on a herd basis every four months. PRRSV vaccine was not used on the farm.

Oral fluids were collected by hanging a cotton rope in each pen or gestation crate for ~30 minutes. Samples were collected from all barns and from all ages and stages (nursery, growing/finish, replacement gilts, gestation). A total of 66 samples were collected and tested, i.e., 16, 25, and 25 samples in the 1st, 2nd, and 3rd sampling, respectively. Samples were tested individually (not pooled) using a commercial PRRSV oral fluid antibody ELISA (IDEXX Laboratories, Inc.). Samples with S/P ratios ≥ 0.4 were classified as positive.

Results

All oral fluid specimens (n = 66) tested negative (S/P < 0.40) on the PRRSV oral fluid antibody ELISA.

- mean S/P ratio = 0.15
- S/P ratio 99% confidence interval (0.13, 0.18)
- min S/P ratio = 0, max S/P ratio = 0.38

Conclusions and Discussion

Tests used in PRRSV surveillance must meet strict requirements: (a) diagnostic sensitivity, (b) diagnostic specificity, and (c) consistency, i.e., agreement in testing results within (repeatability) and between (reproducibility) laboratories.

PCRs are usually assumed to meet these criteria, but the assumption is rarely tested. In one study on PRRSV RT-PCR performance (1) ***only 1 of 19*** participating laboratories correctly identified the PRRSV status of the 30 samples tested (19 positive, 11 negative). In the remaining 18 laboratories, diagnostic sensitivity ranged from 32-95% and diagnostic specificity from 37-100%.

In contrast, a study (2) based on 492 positive and 367 negative oral fluid samples estimated the diagnostic sensitivity and specificity of PRRSV oral fluid antibody ELISA at 94.7% (95% CI 92.4, 96.5) and 100% (95% CI: 99.0, 100.0). A separate study (3) in which 12 laboratories each tested the same 263 oral fluid samples found that the test was highly repeatable (consist results within a laboratory) and highly reproducible (consist results between laboratories).

PRRSV-free farms have 3 primary concerns regarding surveillance: (a) false positive results, (b) false negatives results, e.g., rapid detection and (c) cost.

(a) FALSE POSITIVES: Consistent with prior reports (2), no false positives were observed in this study and the use of InmunoPRRS® in the herd did not interfere with the test.

(b) FALSE NEGATIVES: Detection of PRRSV viremia by RT-PCR is fast, but the presence of PRRSV RNA in serum or oral fluid is transient and false negative RT-PCRs occur. PRRSV antibody is detectable in oral fluids ≥9 days after infection and remains detectable for months. Thus, early detection can be achieved by collecting samples at 2 week intervals.

(c) COST: Testing an oral fluid sample for PRRSV antibody costs far less testing for PRRSV nucleic acid by RT-PCR. Excellent surveillance can be implemented at a lower cost.

Acknowledgments

Granja Alcer and Rocer

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Effects of immunological castration on weight variation at marketing

B Cowles, MA Mellencamp, MK Senn
Zoetis Inc. Florham Park, NJ, USA, bobby.cowles@zoetis.com

Introduction

Physical castration (PC) early in life is effective in controlling boar taint. However, production losses associated with its use are substantial and include increased preweaning mortality (1.6%), reduced feed efficiency (6-10%), and loss of carcass lean (4-8%).[1] Immunological castration (IC) offers producers an alternative to PC that uses the animal's immune system to reduce boar taint close to the time of slaughter. The difference in timing allows IC pigs to grow as intact males for most of their life, benefitting from the natural improvements in feed conversion and carcass composition.

Improvest® (*gonadotropin releasing factor analog - diphtheria toxoid conjugate*, Zoetis) is an FDA-approved veterinary prescription product that is a safe and effective alternative for PC. The 1st dose is given after 9 wk of age to prime the immune system. The 2nd dose is given at least 4 wk after the first and 3-10 wk before harvest. This approach allows the IC barrow to express its naturally efficient growth potential resulting in substantial feed savings per pound of gain compared to PC barrows. Twelve US Improvest® trials showed that IC improves average daily gain by 4.2%. Increased growth rate is associated with reduced variation in market weights because of the improved performance of the slowest growing pigs.[2] The objective of this study was to determine the impact of Improvest® on reducing wean-to-finish body weight variation.

Materials and Methods

Three different commercial wean-to-finish farms in the Midwest were enrolled in the study. Male pigs were randomly allocated to one of two treatments: PC or IC at 3-7 days of age. Pigs in the PC group were physically castrated at 3-7 d of age. Improvest® was administered at 64 and 122 d of age. Pigs were individually weighed at weaning and at marketing. Each farm marketed pigs according to their usual protocol and targeted a final weight of 285 lb for IC barrows.

Results and Discussion

These commercial farms had different genetics, housing systems, diets, and marketing strategies so statistical comparisons were not possible. Farm A housed PC and IC in the same barn and marketed the animals over a 5 week period starting May 20, 2013, with animals being 4-8 wk post Improvest® dose #2. Average market wt was 277±12.07 lb for PC and 286±10.80 lb for ICs. Variation in market weights was reduced by 10% for IC's on this farm compared to PC, as shown by reduced live weight standard deviation: 10.80 vs. 12.08 lb, respectively. Farm B housed PC and IC barrows at 2 different sites and in different barns at each site. They marketed over a 3 wk period starting July 15, 2013. Animals were 3-7 wk post 2nd dose. The average market weight was 264±17.54 lb for PC and 271±18.58 lb for ICs. Farm C housed PC and IC on the same site but in different barns and marketed the animals over a 3 wk period starting Aug 8, 2013 with animals being 7-9 wk post dose #2 of Improvest®. The average weight to market was 265±22.75 lb for PC and 268.76 ±24.10 lb for IC. Farm B and C did not demonstrate reduced market weight variation. On these farms, IC weights were 14 and 16 lb less than the target marketing weight, respectively.

This study showed that Improvest® delivered heavier live weights for the same number of days on feed on all farms (Fig. 1). This gain was seen despite two farms experiencing severe summer heat during the finishing phase. IC barrows had reduced variation in live and hot carcass weights on one farm. Reduced market weight variation is important because it leads to decreased sort loss by increasing the proportion of pigs that qualify for packer premiums. This improves farm profitability. However, the ability to capture the value of reduced variation depends on several factors, including marketing strategy and sorting accuracy. The inability of two farms to capture these benefits was likely due to their marketing and sorting protocols. Farms may need to review and recalibrate their marketing protocols to capture the full value of Improvest® in their system.

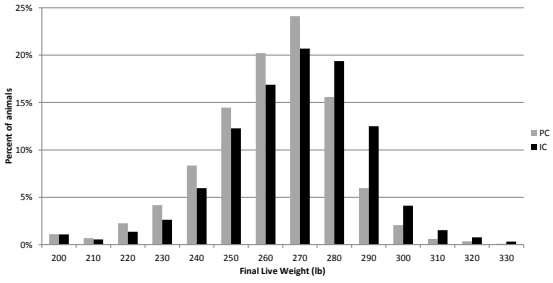

Fig. 1. Combined distribution of marketing live weights for the three study farms.

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Effects of physical castration on mortality from processing to weaning

B Cowles, MA Mellencamp, MK Senn
Zoetis Inc. Florham Park, NJ 07940, bobby.cowles@zoetis.com

Introduction

In the US, as in most countries, male pigs are physically castrated (PC) during the first week of life, primarily to reduce boar taint. Improvest® (*gonadotrophin releasing factor analog-diphtheria toxoid conjugate*, Zoetis) is a PC alternative which allows male pigs to remain intact for most of their life. This takes advantage of the natural production efficiencies and carcass advantages of intact males. Pigs are immunologically castrated (IC) later in life by an immunization process. The 2nd immunization (given 3-10 wk before slaughter) results in a temporary IC by suppressing testicular function and, consequently, reducing boar taint. Anecdotal reports indicate that castration causes a small but significant level of preweaning mortality post processing (PWMPP). A meta-analysis of 15 Improvac trials in Europe showed that PWM was 1.6% higher in physical castrates (PC) than intact males (1). In 24 trials in China, the PWM difference was 2.8% (2).

The goal of this study was to determine the death loss of PC and intact males on 4 US farms with different types of management from the time of processing to weaning.

Materials and Methods

Four different commercial sow farms in the Midwest were enrolled in the study. The study began on the day of processing (age 4-7 days). Male piglets needed to weigh at least 2.2 lb and be in good health. Study pigs were randomized to treatment by selecting every other pig for castration. Castration was performed by the farm's usual method. Records were kept of all animals that died and those receiving treatment.

Results and Discussion

Records were available for 4,099 PC and 4,105 intact males (Table 1) (3). PWMPP on all farms ranged from 0.9% to 4.98%, which is less than the US industry average (12.9%) (4). However, this is most probably due to starting the study on the day of processing, weight requirement, and health status of the animals at the time of enrollment into the study. Three sites showed a reduction in PWMPP for intact males compared with PC. Only Farm C's reduction was statistical significant (P=0.0001). This reduction ranged from 1.24% to 2.57%. Farm B showed 0.61% less mortality in PC than intact males. Overall, there was a 1.22% difference in PWMPP between IM and PC. Statistically as a group there was no difference between PC and intact male groups at the 0.05 level. The main reason for mortality in both groups during this period was being laid on, followed by ruptures. Rupture deaths and repairs during this time varied from farm to farm.

This study was designed to determine the effects of castration on PWMPP under different management systems. This study confirms that, depending on the management system, most farms will have a reduction in PWMPP when castration is discontinued. On average for all farms in the study, PC increased PWMPP. Implementation of Improvest and eliminating castration can increase the number of animals that survive to weaning, which in the long run will impact the pounds of marketable product to the producer and the profitability of that operation. Economic modeling indicates this savings will be about $1.61 per pig (5).

Table 1. Preweaning mortality from processing to weaning of physical castrates and intact males.

Trait	Farm	Treatment	
		Castrate	Intact
No. pigs	A	996	1003
	B	994	995
	C	1110	1110
	D	999	997
Processing wt, lb	A	5.41±1.37	5.37±1.33
	B	4.10±0.73	4.10±0.82
	C	5.84±1.42	5.90±1.52
	D	3.26±0.61	3.29±0.62
Weaning wt, lb	A	13.84±2.99	13.88±2.91
	B	13.52±2.24	13.49±2.45
	C	13.79±2.87	13.99±3.01
	D	11.93±2.48	12.23±2.27
Mortality[a], %	A	4.62	3.19
	B	1.01	1.61
	C	3.51	0.9
	D	4.9	3.61

[a]Statistical analysis for mortality: Farm 1: P=0.0835; Farm B: P=0.2351; Farm C: P=0.0001; Farm D: P=0.1679.

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The use of cross-sectional serological profile for appropriate implementation of *A. pleuropneumoniae* vaccination program in farrow-to-finish farms

E Czyżewska, A Dors, M Pomorska-Mól, K Kwit, P Kwieciński, P Matyba, J Wojciechowski, Z Pejsak
Department of Swine Diseases, National Veterinary Research Institute, Pulawy, Poland, z.pejsak@piwet.pulawy.pl

Introduction

Porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae* (App) may cause serious economic losses, especially in fattening units (1). Usually, control of pleuropneumonia in endemically infected herds requires combinations of vaccination, medications and improved husbandry. Commercially available vaccines can reduce clinical symptoms and improve performance. Generally, vaccination is recommended in pigs at 6 week of age or older. However, vaccination at early age may be ineffective due to interference with maternally derived antibody (MDA) which with regard to App may persist for 12 weeks (1, 2).

Materials and Methods

Three farrow-to-finish herds (A–C) with histories of App infections were selected. Vaccination against App was not carried out in these herds. In all herds piglets were weaned at 28 day of age. Serum samples were taken from 10 pigs of 3, 6, 9, 12, 15 and 18 weeks of age. For detection of specific antibodies against App commercial ELISA test were used: The APP-ApxIV ELISA test kit (IDEXX).

Results

In herd A, antibodies to App were detected in at least 60% of pigs till 9 weeks of age. At 12 weeks of age none of examined animals have detectable antibodies. The prevalence of antibody was increased thereafter, and reached 90% of pigs at age 18 weeks (Figure 1.). The same trend (decrease followed by increase) was also observed with regard to the S/P value. In herd B, antibodies to App were detected in at least 50% of animals till 9 weeks of age. Seroprevalence of App-specific antibodies increased slightly at the 12 week of age (60% of animals had App-specific antibodies) and continued to rise at the 15 and 18 week of age, reaching 80% and 100% respectively (Figure 2.). In herd C, MDA were detected in 100% of piglets at 3 weeks of age, 80% of pigs at 6 weeks of age and in 60% of 9 weeks old pigs. At age of 15 weeks none of the examined pigs have detectable antibodies to App. At the next sampling period (18 weeks of age) the seroprevalence increase to 80%, however S/P value were lower at that time compared to values observed in 3-6 week-old piglets (Figure 3.).

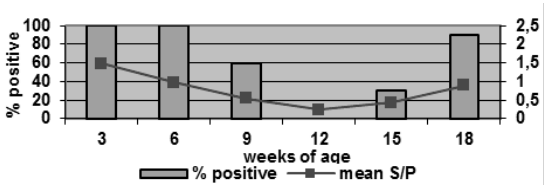

Figure 1. Serum profile of App in herd A

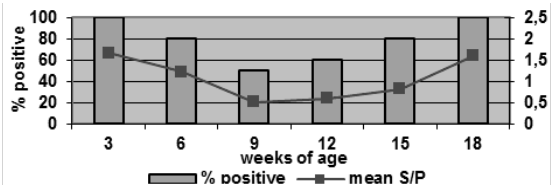

Figure 2. Serum profile of App in herd B

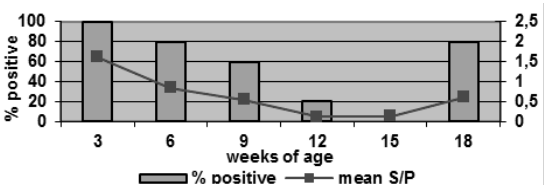

Figure 3. Serum profile of App in herd C

Discussion

The results of present study showed that there were significant differences between the patterns of antibody circulation on various swine herd. In herds A and C, the MDA decayed to undetectable level at age of 12 or 15 weeks (herd A and C, respectively). In herd B there were no significant drop in the percentage of pigs with App-specific antibodies, and no clear border between MDA and post-infective antibodies could be identified. In all examined herds the significant increase of seropositive pigs was observed in fattening units. Information on the persistence of App-specific MDA and age at which pigs become infected with *App* are important for implementation of proper vaccination program. Because, antibody response after natural infection can be detected approximately 10-14 days post infection (1), the best age for the first vaccination of pigs in herd A is 8 weeks, and for the second 11 weeks. In herd C the best age for the vaccination is 10 and 13 weeks. In herd B onset of seroconversion was observed at around 12 weeks of age, thus the first vaccination should be done at 6 weeks of age, but at this age the high level of MDA was observed. Thus, to overcome the MDA interference, beside second vaccination at about 9 weeks of age, the third vaccination around 12-14 weeks should be considered (2). Summarizing, the serological profile could be a useful tool in planning a farm specific vaccination program.

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Probiotic strains modulateimmune response to *S. choleraesuis* on swine mesenteric lymph nodes dendritic cells

M Arenas-Padilla[1], A González-Rascón[1], L Félix-Valenzuela[1], J Hernández[2], V Mata-Haro[1]
[1] *Laboratorio de Inmunología y microbiología, Ciencia de los Alimentos,*
[2]*Laboratorio de Inmunología, Nutrición, Centro de Investigación en Alimentación y Desarrollo, Hermosillo, Son, Mex.*
vmata@ciad.mx

Introduction

Probiotic microorganisms have been used widely as they confer health benefits to the host (3). Probiotics are able to influence the maturation of dendritic cells (DC), this includes the expression of different membrane molecules and cytokine production (2,5). On the other hand, in the swine industry, *Salmonella choleraesuis* is one of the main etiological agents of infectious diarrhea in piglets (1), for which antibiotics have traditionally been used for control and treatment, therefore probiotics are proposed as a preventive measure to reduce the use of antibiotics (4). The objective of the present study was to analyze the modulation of the immune response of swine mesenteric lymph node dendritic cells stimulated with probiotic bacteria and *Salmonella choleraesuis*.

Materials and methods

DC were obtained from swine mesenteric lymph nodes (MLN-DC) and cultured (DMEM, Invitrogen Co., Carlsbad, CA, USA) for 12 h with: *Bifidobacterium animalis* subsp. *lactis* Bb12, *Bifidobacterium thermophilum* 108, *Lactobacillus reuteri* 703, *Lactobacillus reuteri* 1447 or *Lactobacillus reuteri* ATCC 53608. The cultures were washed to remove probiotics and MLN-DC were incubated with *Salmonella choleraesuis* for additional 12 h. The surface membrane molecules expression of MHC-II, CD1 and CD163 (VMRD, Seattle, WA, USA) on MLN-DC stimulated with different probiotics and infected with *S. choleraesuis* was analyzed by flow citometry. Finally, IL-10 and IFN-γ production were determined on supernatants from these cultures by ELISA. Data were analyzed using one-way ANOVA and Fisher-LCD test for comparison of means (NCSS, 2007).

Results

All strains significantly increased ($p<0.05$) the number of MHC-II positive cells (not shown). *Lactobacillus reuteri* ATCC 53608 significantly increased ($p<0.05$) the mean expression of MHC-II+ on MLN-DC in *Salmonella choleraesuis* presence (Fig. 1A). There was no significant difference ($p<0.05$) among treatments on CD1 molecule neither CD163 (not shown). *Bifidobacterium animalis* subsp. *lactis* Bb12 ($p<0.05$) was the only probiotic bacteria that produce IL-10 after stimulation, but this production was diminished after inoculation with *S. choleraesuis* (Fig. 1B).

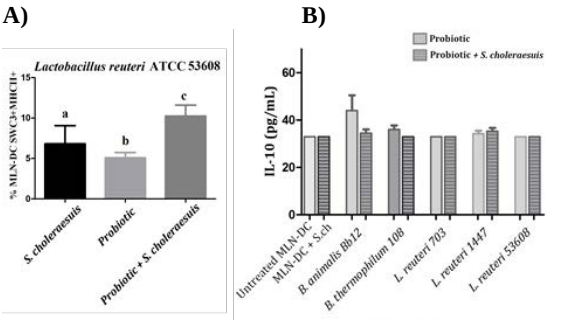

Figure 1. Difference on percentage of MLN-DC SWC3+MHCII+ with *Lactobacillus reuteri* 53608 stimulation and *Salmonella choleraesuis* challenge (A). IL-10 production, treatments with probiotics and *S. choleraesuis* challenge (B).

Conclusions and Discussion

The results show that some probiotics have stimulating and modulating capacities. *Lactobacillus reuteri* ATCC 53608, which was able tomodulate the response to *Salmonella choleraesuis* infection as shown by the increase of the expression of molecules associated with antigen presentation. *Bifidobacterium animalis* subsp. *lactis* Bb12 could induce an anti-inflammatory response, which could help in the regulation of inflammation as a result of an infection by *Salmonella*.

Acknowledgments

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Lack of regulatory T cell induction by porcine dendritic cells exposed to probiotics

A González[1], M Arenas[1], L Felix[1], J Hernandez[2], V Mata[1],
[1]Department of Food Technology, [2]Department of Nutrition, Food and Development Research Center, Hermosillo, Sonora, México, vmata@ciad.mx

Introduction

In the swine industry, probiotics have been proposed as a prophylactic mean to prevent enteric problems, one of the main causes of economic losses (1). The immunomodulatory properties of certain probiotic bacteria can guide maturation of dendritic cells (DCs) towards a cellular response (2). This is an adaptive response mediated by T lymphocytes, in which regulatory T cells (Treg) play an important role suppressing immune responses. Due the important role of Tregs in leading the immune response, probiotics could exert influence in these cells. The aim of this investigation was to analyze the regulatory phenotype of T lymphocytes stimulated with dendritic cells exposed to probiotics

Materials and Methods

DCs from mesenteric lymph nodes (DCGM) were obtained by an immunomagnetic column and lymphocytes were separated from blood. The strains used in this study where *Lactobacillus reuteri* ATCC 53608, *Bifidobacterium sub lactis* Bb12, *Lactobacillus reuteri* 703, *Lactobacillus reuteri* 1447, and *Bifidobacterium thermophilus* 108; the last three strains were previously isolated and characterized at CIAD. The DCs where stimulated with different probiotic in a 1:100 (DCs:probiotics) ratio. Twenty four hours after stimulation, a co-culture with lymphocytes was setup in a 1:10 ratio (DCs:lymphocytes). Samples were taken at 24 h for RT-qPCR using primers for IL-10, TGF-β and Foxp3. Flow cytometry assays where made to measure intracellular TGF-β at 48 h, and the percentages of cells with phenotypes CD4+CD25+Foxp3+ and CD8+CD25+Foxp3+. IL-10 and TGF-β were also measured in supernatants of cultures by ELISA.

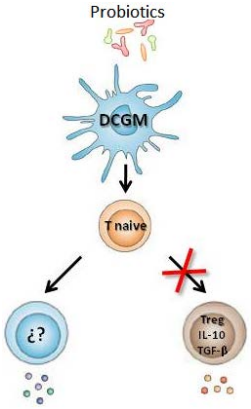
Results

We found no significant difference in the induction of Treg CD4+ and CD8+ phenotypes. Intracellular TGF-β analysis showed that there was not a regulatory environment established at any moment between 48-72 h, which is in agreement with the ELISA assays where TGF-β was inhibited and IL-10 was not detected. The RT-qPCR results show what only *L. reuteri* 53608 induced Foxp3 and TGF-β transcripts in T cells, nonetheless this was not reflected in the in vitro assay.

Conclusions and Discussion

The addition of probiotics to swine diets promote health and grow benefits due their capability of immunomodulate within the gut and induce homeostasis. It is important to maintain a balance between the pro and anti-inflammatory signals so the immune system can respond in a fast and efficient way. An excess of anti-inflammatory (regulatory) signals in the gut could lead to poor immunological response to pathogens, and therefore the pigs would be prone to a variety of sicknesses. The administration of probiotics is a practice that should be determined according to the age and health state of the pigs to assure the benefits.

The probiotics used in this study did not promote regulatory response, indicating they could modulate the response towards a non regulatory state. However, this doesn't mean that they can't be helpful. Arenas 2013 (3) proved that some of these strains helped the immune system to detect and react better at pathogen challenges. Nowadays we continue this project to determine the immune response.



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The study was funded by CONACyT project 105575.
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Clinical, serologic and performance evaluation between two PCV2/Mh vaccination schemes in piglets from a complete cycle farm in Mexico

J Palacios[1], R Huerta[2],
[1]Private Consultant [2]Universidad Autónoma de Puebla
juanmanuelpalacios7@gmail.com

Introduction

PCV2 and *Mycoplasma hyopneumoniae* (Mh) vaccination in piglets is a routine procedure, combined vaccines get advantage of a reduced piglet handling but still the alternative of a one or two injections products. Its use depends on the infectious pressure and production system, in some cases second doses applied in the weaning stage get challenge by other infectious like PRRSV circulation that could increase the problem and reduce the vaccine effectiveness. The use of an early vaccination program in the farrowing house could have the benefits of reduce stress, adverse reactions and be out from common PRRS viremia after 5 weeks age (1,2). The objective of this trial was to evaluate 2 combined vaccines (PCV2 and Mh) in a two dose scheme at 7 and 21 days age and a combined program at 21 days age in one dose.

Materials and Methods

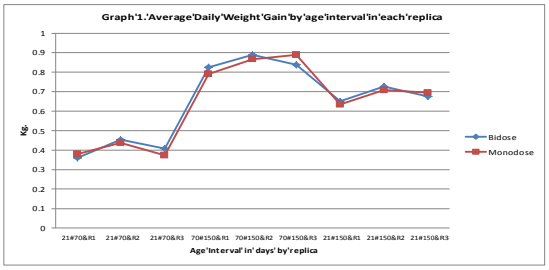
540 (180x3) just born piglets were ear tagged at three days age and assigned to two treatments, the bidose (2x2 ml.) and the monodose one (2x1 ml.) Bidose vaccine combine PCV2+Mh in the same formulation and monodose mix each antigen. Three consecutive production batches were enrolled in the same trial as the experimental replica. Piglets were weighted individually at 21, 70 and 150 days age, twenty pigs from each replica (ten by treatment) were bled at 7,21,70 and 150 days to perform individually ELISA test to PCV2 and Mh. (IDEXX Laboratories, Inc., Westbrook, ME, USA and BioChek B.V. Reeuwijk,The Netherlands).Weight and ADWG data was analized by a T-Student test using the SPSS software V.15.0

Results

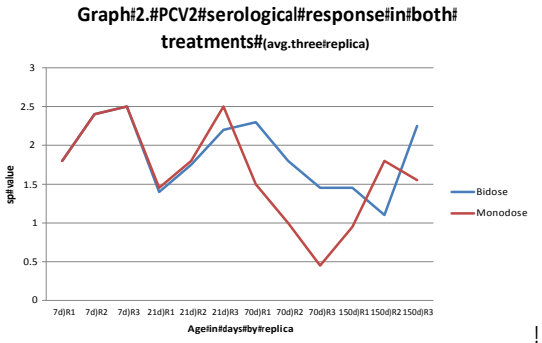
Mean ADWG by each replica are shown in Graph 1. Consolidate data from three replicas is indicated below, there were no an statistically significant effect. Graph 2 show the average sp ratio for PCV2 for both treatments in each sampled age and Graph 3. Show the weight distribution at 150 days age. One replica from the monodose treatment was rtPCR-PCV2 positive at 3.0 x 10^6 particles/ml

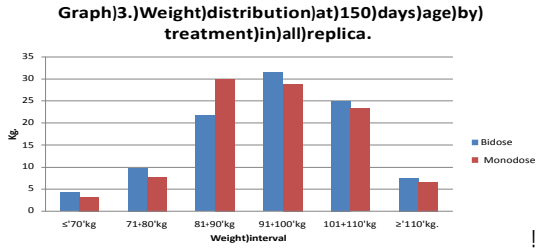
Conclusions and Discussion

Production performance in each treatment and replica was similar without statisticall differences, even the serologic response to PCV2 and Mh (data not showed) was higher in the bidose group there is no a relation between serology and productive performance. Viremia was detected in the second monodose replica with the higher ADWG. Weight distribution at market weight in the bidose group showed 7.6% ≥110 kg BW pigs vs 6.7% in the monodose one. Both programs perform similar, election from one or two dose will depend from the infectious pressure in the system, both applications in an early stage could have a management advantage.



Average'three'replicas Interval'(Age'days)	Bidose'(Media±'Stdev)	Var.'Coeff.'%	Monodose'(Media±'Stdev)	Var.'Coeff.'%
21#70	&0.407±&0.053)	13.12	&0.396±&0.062)	15.56
70#150	&0.850±&0.114)	13.47	&0.848±&0.123)	14.44
21#150	&0.683±&0.083)	12.15	&0.678±&0.086)	12.74





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Effects of transgenic tobacco and commercial attenuated live PRRSV vaccines on the field pigs

C-C Chang[1], T-Y Cheng[1], C-R Jeng[2], VF Pang[2], M-Y Chia[2], P-L Huang[3],
[1]*Department of Veterinary Medicine, National Chia-Yi University, Taiwan,* [2] *Graduate Institute of Veterinary Medicine, National Taiwan University, Taiwan,* [3]*Department of Horticulture, National Taiwan University, Taiwan,*
ccchang@mail.ncyu.edu.tw

Introduction

The development of anti-PRRSV strategies is constantly a challenge for pig researchers and veterinarians. Application of vaccines is believed to be one of principle ways to achieve the purpose. Our previous study proved that the transgenic tobacco plant expressing recombinant fusion protein of GP5 of PRRSV and B subunit of Escherichia coli (E. coli) heat-labile enterotoxin (LTB) has been established specific humoral and cell immune responses against PRRSV both mucosally and systemically in pigs(1,2,3,4). In this study, we further compare its efficacy with a comercial modified live PRRSV vaccine (Ingelvac PRRS MLV, Boehringer Ingelheim Vetmedica, Inc.) in the field pigs.

Materials and Methods

Two hundred and forty piglets were assigned into four groups, with 60 pigs in each group. Pigs in group GP5-T and W-T were orally given with GP5-T and wild-type tobacco respectively at days 0, 14 and 28 post experiment (dpe), while group MLV(C-V) intramuscularly inoculated with the MLV at 0 dpe, and group Con acted as unvaccinated control. Growth performance was timely recorded and evaluated. Antibodies specific to PRRSV were done using commercial ELISA Kit (IDEXX Laboratories, Inc., Westbrook, ME, USA) and concentrations of non-specific total globulin were determined by blood biochemistry test. Also, PRRSV loads in sera and lung tissues were evaluated by quantitative real-time PCR. Analyses of variance (ANOVA) were used to detect significant difference among all four groups over the entire experimental period.

Results

The results showed that pigs in group GP5-T obtained higher average body weight (Table 1) and total globulin than unvaccinated pigs, whereas the MLV vaccinated pigs showed a higher specific anti-PRRSV antibodies (Table 2) and lower viral loads in sera.

Conclusions and Discussion

The comparison between a transgenic tobacco plant and modified live vaccine in feasibility and efficiency was accomplished in naturally infected pigs. The former had advantages over the later of inducing systemic immunity and improving the growing performance in pigs, nevertheless, MLV was good at strengthening specific protection against virulent PRRSV in field by evoking anti-PRRSV IgG and downstream decreasing the viral load. Besides, the LTB seemed to provide a positive influence in eliciting non-specific immunity and should be regarded as a choice in the future of vaccine development and PRRS prophylaxes. We concluded that different PRRSV vaccine types and inoculation routes might lead to different, yet varying degrees of protection against PRRSV in the field.

Table 1. Comparison of growth performance among pigs in four groups at the end of the experiment (84 dpe).

Groups / Items	GP5-T (n=60)	W-T (n=60)	C-V (n=60)	Con (n=60)
Weaned(Kg)	8.2 ± 0.5	8.4±0.6	8.0±0.5	8.5±0.9
End (Kg)	53.9±4.2[a]	52.8±3.9	52.6±4.1	51.0±4.5[b]
ADG (Kg)	0.54±0.1	0.52±0.2	0.53±0.01	0.50±0.01
FCR	1.02±0.1	0.99±0.19	1.03±0.03	0.99±0.00

Different letters (a and b) indicate significant differences ($p \leqq 0.05$) between groups.

Table 2. Comparison of PRRSV-specific antibody responses from 0 to 84 dpe among pigs in four groups.

	GP5-T (n=10)	W-T (n=10)	C-V (n=10)	Con (n=10)
D 0	0.45±0.33	0.46±0.33	0.47±0.34	0.32±0.32
D 21	0.59±0.34	0.65±0.45	0.99±0.51[a]	0.46±0.29[b]
D 56	1.17±0.41	1.14±0.34	1.25±0.28[a]	0.81±0.42[b]
D 84	1.15±0.29	1.14±0.25	1.23±0.20	1.14±0.22

See footnote in Table 1.

Acknowledgments

Boehringer Ingelheim Taiwan Limited.
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Reproductive performance of sows and growth performance and anti-disease ability of pigs after attenuated live PRRSV vaccination in sows

C-C Chang, L-C Yang, T-Y Cheng, Y-T Yang, B-Y Lin
Department of Veterinary Medicine, National Chia-Yi University, Taiwan, ccchang@mail.ncyu.edu.tw

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most important diseases in Taiwanese pig farms. Vaccination with modified live vaccine is one of the solutions to control PRRS. The objective of this study is to investigate the effects of sow vaccinated with an attenuated live vaccine under different levels of antibody titers in field condition.

Materials and Methods

A total of sows (N=84) in a batch during their 75th -84th days of pregnancy were selected and their antibody titers were tested by a commercial ELISA kit (IDEXX herd check). Animals with either negative (S/P<0.4) or medium level of titer (S/P values between 1.2-1.5) were excluded in this trial. The rest of the sows (n=60) were then assigned into low titer group (L: S/P in between 0.4-1.2, n=30) or high titer group (H: S/P value between 1.5-3.3, n=30). Then, these 30 sows in each group were further divided into two treatment groups of vaccination (LV, n=15; HV, n=15) and non-vaccination control (LC, sows were weaned at 4 weeks of age, ear tagged and mixed into 4 nursery barns with the same feed and water supply. Reproductive parameters collected include farrowing rates, total pigs born, pigs born alive, average piglet bodyweight, number of stillborn, pigs weaned, average weaning weight, and pre-weaning mortality. The growth performance data collected include average daily gains, feed conversion rates, and mortalities. Positive rates of PRRSV and PCV2 in the serum and tonsil scrapings were also examined and detected by RT-PCR and PCR. Statistical significance was evaluated by t-test.

Results

A significant difference on the PRRSV-positive rates (data not shown) was seen in pigs between HV and other groups, leading a better performance in the categories of average weaning weight, pre-weaning mortality in the suckling pigs and average daily gain of nursery pigs was seen in the sows and their piglets in HV group.

Conclusions and Discussion

All of these results lead to a conclusion that keeping higher immune status with boosting vaccination against PRRSV in the sow population provides a better performance for their farrowing pigs.

Table 1. Reproductive performance of sows and growth performance and survival rate of piglets born from sows with low and high PRRS antibodies

Items	Sows with low antibody+		Sows with high antibody++	
	Con group (n=15)	Vac group (n=15)	Con group (n=15)	Vac. group (n=15)
Abortion	0	0	2	1
Farrow rate (%)	100	100	86.66	93.33
Piglets born alive	10.27	9.67	10.54	9.15
Piglets born dead	2.13	1.00	0.77	1.53
Piglets weaning	8.87	7.67	8.53	8.15
BW at birth (kg)	1.38	1.50	1.53	1.52
Weaned (kg)	6.95	6.50	6.20	7.7[a]
ADV (kg)	0.20	0.18	0.17	0.22[a]
Survival (%)	86.0	81.1	81.9	92.1[a]

+ indicates that sows with titer of PRRS S/P value between 0.4 to 1.2 and ++ between 1.5 to 3.3. Different letters indicate significant differences between groups.

Table 2. Growth performance of nursery pig born from sows with low and high PRRS antibodies

Items	Sows with low antibody+		Sows with high antibody++	
	Con group (n=80)	Vac group (n=80)	Con group (n=80)	Vac group (n=80)
Initial Bw (kg)	7.15	6.78	7.04	8.11
Final BW (kg)	25.5	26.8	26.2	29.6[a]
Daily gain	0.33	0.36	0.34	0.38[a]
Daily feed	0.67	0.69	0.68	0.74
FCR	2.02	1.91	1.95	1.89
Mortality (%)	7.50	3.75	2.50	2.50

See footnote in Table 1.

Acknowledgments

Boehringer Ingelheim Taiwan Limited.

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Circumvent® PCV M: *M. hyopneumoniae* standard efficacy and duration of immunity studies

M Allen, E Strait, A Lane, D Montgomery, P Funk, L Purtle, B Thacker
Merck Animal Health, DeSoto, KS, erin.strait@merck.com

Introduction

Porcine circovirus type 2 (PCV2) and enzootic pneumonia caused by *Mycoplasma hyopneumoniae (M. hyo.)* are infectious diseases of swine that continue to challenge the pork industry. Vaccination against both of these diseases has been shown to mitigate the negative effects of infection.[1] Circumvent® PCV M provides producers with a convenient tool for both PCV2 and *M. hyo.* protection. The following studies demonstrate the efficacy and duration of immunity (DOI) of the mycoplasma fraction of Circumvent® PCV M.

Materials and Methods

For each study, fifty-five 3- to 4-week-old commercial pigs were purchased. Fifty of the pigs were randomly assigned to one of two treatment groups, Circumvent® PCV M vaccinated (VACC) or placebo control (CONT). In addition, five pigs were designated as sentinels. Following an acclimation period of at least one week, pigs in the vaccinated group were given 2 mL of Circumvent® PCV M intramuscularly (IM). For the standard efficacy study, the pigs were 3 weeks of age at the time of first vaccination. For the DOI study, the pigs were 4-5 weeks of age at first vaccination. Pigs in each placebo control group were similarly injected with 2 mL IM. All pigs received a booster dose three weeks later with the same treatment. Just prior to challenge, the five sentinel pigs were euthanized and their lungs were evaluated to assess possible prior exposure to *M. hyo*. No lesions consistent with *M. hyo.* infection were found.

Pigs were challenged with *M. hyo.* intra-nasally on 3 consecutive days at either 9 or 27 weeks of age for the standard efficacy or DOI studies, respectively. Pigs were observed daily throughout the study for general appearance and signs of clinical disease. Four weeks post-challenge, pigs were euthanized and their lungs were examined for lesions typical of *M. hyo*. infection. The percentage of the surface area of the lung with lesions was recorded. In addition for the DOI study, coughing scores were recorded throughout the challenge phase and qPCR on bronchial swabs collected at necropsy was performed by the Iowa State University, Veterinary Diagnostic Laboratory.

Results

One pig in the placebo control group was removed prior to completion of the studies for non-vaccine related reasons. Similarly, 2 vaccinated and 4 placebo pigs were removed from the DOI study. The table below demonstrates for each treatment group, the percentage of the lung surface exhibiting lesions typical of *M. hyo*. In each study, pigs vaccinated with Circumvent PCV® M had fewer lung lesions typical of *M. hyo.* In the standard efficacy study, vaccinates had 4.7% pneumonia while pigs in the placebo control group had 12.8% pneumonia ($P<0.0003$). In the 20-week DOI, vaccinates had 2.5% pneumonia versus 8.0% pneumonia in the controls ($P<0.01$).

During the DOI study, coughing of individual pigs was monitored daily. In the placebo group, 17 of 21 pigs (81%) were observed coughing on at least one day post challenge versus 10 of 23 pigs (43%) in the vaccinated group. The average number of days coughing for the placebo group was 4.00 ± 4.15, whereas the vaccinated group had an average number of days coughing of 0.92 ± 1.56. The median number of days coughing of the placebo group was statistically ($P = 0.0008$) higher than that of the vaccinated group by 2.0 days. At necropsy, bronchial swabs were collected and quantitated for *M. hyo.* by qPCR. The average *M. hyo* $\log_{10}$genomic copy/mL for the placebo group was 4.53 ± 1.33, whereas the vaccinated group had an average $\log_{10}$genomic copy/mL of 3.60 ± 1.49. The median value of $\log_{10}$ genomic copy/mL of the placebo group was statistically ($P= 0.003$) higher than the median value of the vaccinated group by 1.1 $\log_{10}$genomic copy/mL.

Vacc. Age (wks)	Chall. Age (wks)	% Pneumonia		Percent Reduction
		VACC	CONT	
3 & 6	9	4.7	12.8	62.9
4 & 7	27	2.5	8.0	69.4

Conclusions and Discussion

These two studies demonstrate that the combined, ready-to-use product Circumvent® PCV M is an effective tool for long-term protection of at least 5 months, against disease caused by *Mycoplasma hyopneumoniae*.

Acknowledgments

Thank you to the Merck animal services department for their help with these studies.

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Circumvent® PCV M G2: *M. hyopneumoniae* standard efficacy studies with two different dosing regimens

M Allen, E Strait, A Lane, D Montgomery, P Funk, L Purtle, B Thacker
Merck Animal Health, DeSoto, KS, erin.strait@merck.com

Introduction

Porcine circovirus type 2 and enzootic pneumonia caused by *M. hyopneumoniae*(*M. hyo.*) continue to be important pathogens in today's swine industry. Circumvent® PCV M currently provides producers with a convenient tool for both protection against both PCV2 and *M. hyo*.[1] In the second generation, (G2) of this combination vaccine there are now 2 dosing options: either a single 2 ml dose as early as 3 weeks of age, or two 1 ml doses 3 weeks apart starting as early as 3 days of age. The following studies demonstrate the efficacy of the *M. hyopneumoniae* fraction of Circumvent® PCV M G2 for each dosing regimen.

Materials and Methods

For each study, 3- to 4-week-old commercial pigs were purchased. For the two dose regimen, a total of 55 pigs were included. Fifty pigs were randomly assigned to one of two treatment groups, Circumvent® PCV M G2 (VACC) vaccinated or placebo control (CONT). In addition, five pigs were designated as sentinels. For the one dose regimen, a total of 120 pigs were included. Fifty were randomly assigned to the Circumvent® PCV M G2 vaccinated group, 70 pigs were in the placebo control group and 10 pigs were designated as sentinels.

Pigs in the two dose vaccine group were given 1 mL of Circumvent® PCV M G2 intramuscularly (IM) at 3 days of age, and then again at 21 days of age. Pigs in the one dose vaccine group were vaccinated at 3 weeks of age with 2 ml of Circumvent® PCV M G2. Pigs in each placebo control group were similarly injected IM with either 1 ml twice, or 2 mL once. Just prior to challenge, the sentinel pigs for each study were euthanized and their lungs were evaluated to assess possible prior exposure to *M. hyo*. *M. hyo.* infection was not found in any pig.

Pigs in the two dose study were challenged with virulent *M. hyo.* 7 weeks post second vaccination. Pigs in the one dose groups were challenged with virulent *M. hyopneumoniae* at 33 or 47 days post vaccination. Pigs were observed daily throughout the study for general appearance and signs of clinical disease. Four weeks post-challenge, pigs were euthanized and their lungs were examined for lesions typical of *M. hyo.* infection. The percentage of the surface area of the lung with lesions was recorded.

Results

Two pigs in the sentinel group and one in the placebo group of the two dose study were removed prior to completion of the study for non-vaccine related reasons. The percentage of lung surface with lesions typical of *M. hyopneumoniae* for each treatment group is shown in the table below. In each study, pigs vaccinated with Circumvent PCV® M G2 had fewer lung lesions typical of *M. hyopneumoniae.* For the two dose regimen, vaccinates had an average of 6.7% pneumonia while pigs in the placebo control group had 16.1%. For the one dose regimen, vaccinates had an average of 3.2% pneumonia versus 10.1% pneumonia in the controls.

Vacc. Dose	Vacc. Age (days)	% Pneumonia		Percent Reduction
		VACC	CONT	
1 X 2mL	21	6.7	16.1	58.8
2 X 1mL	3, 21	3.2	10.1	68.6

Conclusions and Discussion

The second generation of the Circumvent® PCV M, Circumvent® PCV M G2 set out to maintain the high level of efficacy against both PCV2 and *M. hyo.* that is provided by the current Circumvent® PCV M, while adding the convenience of two dosing regimens that each allow for vaccination to occur prior to or at weaning. The results from these studies demonstrate that the combined, ready-to-use product Circumvent ® PCV M G2 is an effective tool for protection against disease caused by *M. hyopneumoniae*whether in a 1 ml, two dose, or a 2 ml, one dose regimen.

Acknowledgments

Thank you to Rural Technologies, Inc and the Merck animal services department for their help with these studies.

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Circumvent® PCV M G2: PCV2 standard efficacy and duration of immunity studies

B Thacker, L Purtle, F Roerink, M Allen, E Strait
Merck Animal Health, DeSoto, KS, brad.thacker@merck.com

Introduction

Our current PCV2 vaccines, Circumvent® PCV and Circumvent® PCV M, are highly effective at controlling viremia and PCV2-induced disease.[1] Our new vaccine reported here, Circumvent® PCV M G2, along with the monovalent Circumvent® PCV G2, will eventually replace the current products in the US market. The new G2 vaccines provide two dosing options: one, 2-mL dose administered at 3 weeks of age or older (Option 1) or an initial 1-mL dose as early as 3 days of age, with a second 1-mL dose three weeks later (Option 2). Four studies were performed for USDA licensure: the two dosing options by two challenge ages - standard efficacy (STD) at 10 weeks of age and duration of immunity (DOI) at 23 weeks of age.

Materials and Methods

The antigens and adjuvant in PCV M G2 are the same as in our current PCV2 vaccines. The formulation was adjusted to facilitate the single-dose and lower-volume, two-dose options.

All four studies were conducted in the same manner and were done in *Mycoplasma hyopneumoniae*- and PRRSV-free cross bred pigs. Vaccinated (VACC) and control (CONT) pigs were co-mingled throughout. The number of pigs per group for each study is presented in Table 2. Pigs were co-challenged with PCV2 and PRRSV at either 10 (STD) or 23 (DOI) weeks of age. Prior to challenge, pigs were bled periodically to assess serum antibody levels by IFA and for PCR testing to ensure they remained free of PCV2. Following challenge, blood was collected weekly for 5 weeks to test for PCV2 viremia by PCR and assess serum antibody levels by IFA. Nasal and fecal swabs were collected weekly to evaluate shedding by PCR. Lymphoid tissues were collected at necropsy 5 weeks post-challenge to evaluate PCV2 infection by immunohistochemistry (IHC). IFA and PCR tests were performed by Merck Animal Health Research and Development. IHC was performed at the Iowa State University Veterinary Diagnostic Laboratory. Data were analyzed by Merck Animal Health Research and Development according to procedures required by the USDA Center for Veterinary Biologics (CVB).

Results

The group geometric mean IFA titers for the DOI challenge studies are presented in Table 1. Both dosing options induced a PCV2 antibody response by 4 weeks post-vaccination with the Option 1 titers peaking at 8 weeks post-vaccination. By the time of challenge, titers in vaccinated pigs declined to low levels but were still greater than control pigs. At necropsy, the control pigs had significantly higher titers than the vaccinated pigs. The titer responses in the standard efficacy studies were similar to the DOI studies (data not shown.)

Viremia data is presented in Table 2. In all four studies, vaccinated pigs exhibited either no or a minimal level of viremia compared to controls. Most control pigs were viremic at each sampling time starting at two weeks post-challenge. In all four studies, vaccination reduced both nasal and fecal shedding approximately 100 fold and the duration of shedding was shorter in vaccinates compared to controls (data not shown). Vaccination reduced tissue infection rates by 48-69% and the intensity of staining in positive samples compared to controls (data not shown).

Table 1. IFA Geomean Titers by Weeks Post-Vacc.

Dosing	Group	0	4	8	20	25
Opt. 1:	VACC	222	1621	3112	216	596
1x2mL	CONT	197	35	25	23	4427
Opt. 2:	VACC	63	3694	2049	148	1202
2x1mL	CONT	49	31	50	21	4273

Table 2. Viremia

Dosing Option	Group	No. of pigs		% Viremic	
		STD Chall	DOI Chall	STD Chall	DOI Chall
Opt. 1:	VACC	25	24	0	16.7
1 x 2mL	CONT	26	25	100	96
Opt. 2:	VACC	25	22	4	4.5
2 x 1mL	CONT	24	23	96	95.7

Conclusions and Discussion

In total, these studies support the following label claims for at least 20 weeks post vaccination: 1) Aid in the prevention of PCV2 viremia; 2) Aid in the reduction of PCV2 virus shedding; and 3) Aid in the reduction of PCV2 lymphoid infection. In addition, Circumvent PCV M G2 provides an industry leading 5-month DOI, four weeks longer than competitor vaccines.

Acknowledgments

Thank you to the Merck animal services department for their help with these studies.

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P.617

Field comparison of two commercial vaccines for controlling mutant PCV2 viremia

B Thacker, J Lehman
Merck Animal Health, Desoto, KS, brad.thacker@merck.com

Introduction

Recently a new strain of porcine circovirus type 2 has been identified in the US based on genetic sequencing.[2] This virus has a similar sequence pattern to a virus previously identified in China and is often referred to as the "Chinese mutant" or mutant PCV2 (mPCV2). Some concern has been expressed regarding the ability of current US commercial vaccines to protect against this new strain. As part of a producer initiated PCV2 vaccine evaluation, we were provided the opportunity to monitor the PCV2 viremia and antibody status of pigs undergoing field exposure to mPCV2.

Materials and Methods

The pigs originated from a herd free of PRRSV and *Mycoplasma hyopneumoniae* (Mhp) and were part of a larger field evaluation comparing the performance between two commercial PCV2 vaccines: Fostera™ PCV (FOST) (Zoetis, Florham, NJ) and Circumvent® PCV (CVENT) (Merck Animal Health, Summit, NJ). This study used a "barn level" design. The pigs that were monitored for PCV2 viremia by PCR and PCV2 antibody by 4-dilution IFA were housed in two adjacent finisher barns on the same site. The FOST pigs were vaccinated once at weaning (3 weeks of age). For CVENT vaccination, the producer elected to administer the two vaccinations at processing (3 days of age) and at weaning. The pigs were tagged after arrival to separate nursery rooms and the same pigs were sampled at 4, 11, 16 and 19 weeks of age. Forty pigs from the source sow herd were sampled at 10 days of age. All laboratory testing was performed by routine methods at the ISU-VDL. Samples for PCR were tested in pools of 5. To confirm the presence of mPCV2 in each barn, oral fluids from 5 pens and blood from 10 non-tagged, light-weight pigs were collected at 19 weeks of age. Several positive samples were sequenced and all sequences indicated mPCV2. Based on serotesting at 19 weeks of age, the pigs remained free of PRRSV and Mhp. Statistical analysis was performed by ANOVA and a P value <0.05 was considered significant.

Results

The 10-day-old pigs from the source herd were not viremic and had a geomean IFA titer of 190.3 (Data not shown). The table below presents the PCR and IFA results from the tested serum. IFA titers were significantly higher in the CVENT pigs compared to the FOST pigs at 4, 11 and 16 weeks of age. At 19 weeks of age, the titers of the FOST pigs were significantly greater than the CVENT pigs.

The average cycle times (CTs) for the positive pools from the FOST pigs at 16 and 19 weeks of age were 27.90 and 23.84, respectively. For the light-weight pigs, the two FOST pools had CTs of 25.00 and 22.10. The one CVENT positive pool CT was 26.4. For the oral fluids, the average CTs for the FOST and CVENT barns were 23.54 and 33.53, respectively.

Age (wks)	PCR – Pools Pos./Tested		Geomean IFA Titers	
	CVENT	FOST	CVENT	FOST
4	0/5	0/5	498.7[a]	131.8[b]
11	0/5	0/5	560.8[a]	86.9[b]
16	0/4	5/5	266.6[a]	139.3[b]
19*	0/5	5/5	216.3[b]	844.3[a]
19**	1/2	2/2	139.3[b]	735.2[a]

[a,b] If different within a row, P < 0.05.
* Tagged pigs. ** Light weight pigs.

Conclusions and Discussion

The data presented clearly illustrates the ability of CVENT to protect against mPCV2 viremia and brings into question the ability of FOST to provide a similar level of protection. In addition, the IFA titers indicate a greater level of protection based on the declining titers of CVENT pigs and rising titers of FOST pigs at 19 weeks of age. This finding has been reported in a previous field study that compared non-viremic, CVENT vaccinated pigs (declining titers) to viremic, non-vaccinated controls (rising titers).[1] The oral fluid results indicate a lower level (approximately 1,000 times less) of virus shedding in the CVENT barn. This suggests that CVENT vaccinated pigs shed less virus than FOST vaccinated pigs. Accordingly, the benefit of vaccination may extend beyond controlling infection and disease in the pig to controlling the level of environmental contamination. The level of PCV2 in the environment may impact the onset of infection, disease severity and the performance of vaccines over time.

Aknowledgments

Thank you Dr. Amy Woods, Matt Harris and the farm staff for their assistance.

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Apparent absence of PCV2 exposure in a Circumvent® PCV M vaccination timing field study

B Thacker[1], D Miller[2], M Rodibaugh[2], J Spencer[3], J Lehman[1]
[1]Merck Animal Health, Desoto, KS, [2]Swine Health Services, Frankfort, IN, [3]JBS United, Sheridan, IN
brad.thacker@merck.com

Introduction

The timing of vaccination in a herd depends on several factors including onset of disease, labor availability, combining with other routine procedures and maternally-derived antibody (MDA) levels. The data reported here was part of a larger study that evaluated the impact of timing of Circumvent® PCV M vaccination on growth performance. Here we present the serological data that was generated from the study.

Materials and Methods

The study was conducted at the JBS United Burton-Russell Research Farm. Pigs from 4 consecutive weaning groups (every 4 weeks) were used. Pigs were allocated to treatment group at 1 week of age (WOA). Three treatment groups, as directed by the producer and herd veterinarian, were evaluated: 1) A - vaccination at processing (1 WOA) and weaning (3 WOA); 2) B - vaccination at processing and at 6 WOA; and 3) C - vaccination at weaning and 6 WOA (per label directions). Each treatment group contained approximately 475 pigs. Five pigs per weaning group per treatment (n = 20) were randomly selected for repeated blood sampling. Also, one pen in each of the first two weaning groups was left unvaccinated and 10 pigs from these pens were bled at each time point. All samples were tested for PCV2 by PCR. A subset of samples was randomly selected for determining serum antibody levels to PCV2 by the Four-Dilution (160, 320, 640 and 1280) IFA and to *Mycoplasma hyopneumoniae* (Mhp) by ELISA (IDEXX, Westbrook, ME). All testing was performed by Iowa State University Veterinary Diagnostic Laboratory.

Results

All PCV2 PCR assays were negative including the samples collected from the non-vaccinated control pigs.

Table 1 presents the PCV2 IFA results. Control pigs remained seronegative throughout the study with the exception that two pigs had titers of 160 and 320 at 17 WOA. In the vaccinated groups, the maximum IFA titer was 3 weeks after the second vaccination; 6 WOA for group A-1/3 and 9 WOA for groups B-1/6 and C-3/6. Titers declined thereafter and were low at 24 WOA.

The Mhp ELISA results are presented in Table 2. All groups had a moderate level of MDA at 1 WOA. The MDA had the greatest impact on the 3 week post-vaccination titers of group A-1/3. By 17 WOA, control pigs started to seroconvert and the antibody levels in the vaccinated pigs increased with the exposure to Mhp.

Table 1. PCV2 IFA Geomean Reciprocal Titers

Age (wks)	Para-meter	Treatment Group			
		CONT	A-1/3	B-1/6	C-3/6
1	Pos.	ND	0/10	0/10	0/10
	Geom.	ND	<160	<160	<160
3	Pos.	0/9	0/10	0/10	0/10
	Geom.	<160	<160	<160	<160
6	Pos.	0/9	10/10	7/10	8/10
	Geom.	<160	735.2	278.6	320.0
9	Pos.	0/9	10/10	10/10	10/10
	Geom.	<160	970.1	1114.3	1280.0
17	Pos.	2/9	4/10	8/10	10/10
	Geom.	100.8	121.3	183.8	367.6
24	Pos.	0/9	2/10	5/10	7/10
	Geom.	<160	98.5	130.0	171.5

Table 2. Mhp ELISA S/P Ratios

Age (wks)	Para-meter	Treatment Group			
		CONT	A-1/3	B-1/6	C-3/6
1	Pos.	ND	7/10	6/10	7/10
	S/P	ND	0.538	0.658	0.653
3	Pos.	0/9	1/10	3/10	3/10
	S/P	0.090	0.201	0.321	0.291
6	Pos.	0/9	6/10	0/10	0/10
	S/P	0.010	0.534	0.092	0.084
9	Pos.	0/9	5/10	10/10	6/10
	S/P	0.000	0.461	1.061	0.653
17	Pos.	6/9	10/10	10/10	10/10
	S/P	0.759	1.144	1.908	1.147
24	Pos.	7/9	10/10	10/10	10/10
	S/P	1.102	1.543	2.056	1.470

Conclusions and Discussion

Based on the negative PCV2 PCR results and the failure of the control pigs to seroconvert, the pigs in this study were not exposed to PCV2. This finding has implications for conducting field studies that rely on natural exposure; some method is needed to confirm exposure. In this study, non-vaccinated control pigs served that purpose. Oral fluid samples may also provide evidence of exposure. Mhp MDA appeared to decrease but not eliminate the antibody response to vaccination depending on the age of the pig at both the first and second vaccinations.

Comparative efficacy of a *Mycoplasma* and PCV2 vaccination program in a commercial farm in Japan

M Naito
Shokukanken Inc. naito@shokukanken.com

Introduction

Porcine Circovirus Type 2 (PCV2) and Mycoplasma hyopneumoniae (Mhyo) are two pathogens known to cause severe economic losses in the swine industry[1]. Both PCV2 and M.hyo infections are wide-spread in Japan and vaccination against both pathogens is common practice. The objective of this study was to compare the efficacy of two common PCV2 and M.hyo vaccination programs in a commercial farm in Japan.

Materials and Methods

The study was conducted in a 500 sow farrow to finish farm located in West Japan. 7-day old healthy piglets obtained from 18 sows were weighed and assigned to one of two groups. Group A (n=102) received a single 2ml shot of a PCV2/Mhyo carbomer adjuvanted vaccine at 21 days of age (vaccines mixed before use). Group B (n=102) received 2ml of an Mhyo vaccine (oil-based adjuvant) at 7 days of age, and 2ml of a PCV2 vaccine (oil-based adjuvant) at 21 days of age. Pigs were individually weighed on days 0 (7 days of age), 14, 92 and 144. Blood and fecal samples were collected on days 43, 92, 125 and 144 (20 pigs/group, followed till end of study). Weight gain was analyzed using Student's t-test, PCV2-PCR was compared by Fisher's exact test.

Results

Mortality was not significantly different between groups. 4 and 8 pigs died in groups A and B, respectively. PCV2 DNA was detected by PCR in serum and feces of both groups. In serum samples PCR was positive in both groups starting day 125. The positive rate was significantly lower in group B on days 125 and 144 (Fig. 1). PCV2 PCR of fecal samples was positive in both groups starting day 92. The positive rate was significantly lower for group B on day 125.

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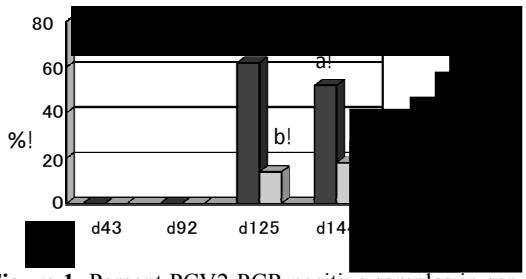

Figure 1. Percent PCV2-PCR positive samples in serum. *Values with different subscripst within same day differ significantly (p<0.05).*

Weight gain was not significantly different between groups on day 0 and 14. However, on day 92 and 144, average body weight of group A was significantly higher than group B (Fig. 2). At the end of the study, pigs in group A were 3.3kg heavier than those of group B. Weight distribution at study end (day 144) showed that 41% of pigs in group A had a weight range between 90-100kg, as opposed to only 23% in group B (Fig. 3).

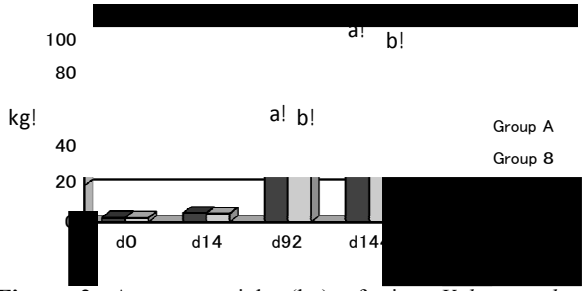

Figure 2. Average weight (kg) of pigs. *Values with different subscripts within same day differ significantly (p<0.05).*

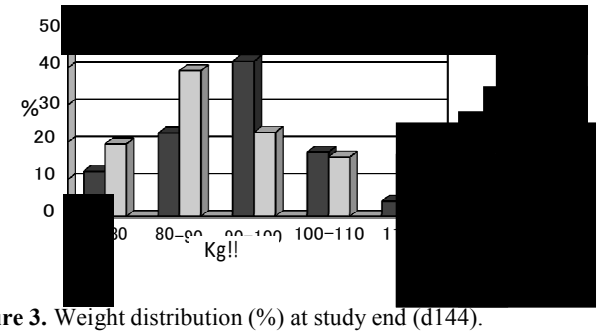

Figure 3. Weight distribution (%) at study end (d144).

Conclusions and Discussion

In this study, a single dose of a carbomer-adjuvanted PCV2/Mhyo vaccine resulted in a significantly higher weight gain compared to a PCV2/Mhyo vaccination program given separately at 7 and 21 days of age. Although pigs in the single dose program showed higher PCV2-PCR positive rates in serum and feces, weight gain was significantly improved, thus suggesting that PCR results after PCV2 vaccination may not correlate to performance.

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P.620

Compatibility of vaccines against Atrophic Rhinitis and neonatal *E. coli* diarrhea: A field approach

J Rouillier[1], S Mondy[1]
[1]*SELAS Vétérinaire de la Hunaudaye, Lamballe, France,* smondy@svh-vet.fr

Introduction

In modern swine production sows are vaccinated against several diseases, either to protect the sows or to protect the sows' progeny (or both). Sow vaccination schemes become more and more extended and complex. Moreover, the European rules impose the house-grouping of sows during gestation since January 2013.
To be able to work more practical and to reduce the number of injections for the sows, farmers are eager to look for options to combine vaccines without compromising on the efficacy.
Porcilis® AR-T DF [1] and Porcilis® Porcoli DF [2] are two vaccines that are given to pregnant sows to pass on passive immunity through maternal derived antibodies to their offspring to protect them against atrophic rhinitis and neonatal *E. coli* diarrhea, respectively. Porcilis® AR-T DF contains a subunit of the *P. multocida* toxin (PMT) in combination with *B. bronchiseptica* (Bb) cells and Porcilis® Porcoli DF contains purified fimbrial adhesins (F4ab, F4ac, F5 and F6) and the *E. coli* heat labile toxin (LT). Since both vaccines require the same vaccination schedule (2 primovaccinations 4 weeks apart and booster 4-2 weeks before farrowing) and both vaccine use Diluvac Forte (d,l-α-tocopheryl acetate) as the adjuvant, it has been validated inexperimentalm conditions on pigs that the injection of the vaccines after mixing has no bad influence the antibody responses against the individual vaccine antigens [3].
The aim of the present study was to transpose these results in a french conventional farm.

Materials and Methods

The gestating sows of the farm were used to be vaccinated with Porcilis AR-T DF (2ml) one week apart of Porcilis Porcoli (2ml) 3 to 4 weeks prior farrowing.
Two groups of sows were choosen :
18 sows were vaccinated with Porcilis AR-T DF (2ml) on one side of the neck, and with Porcilis Porcoli (2ml) on the other side of the neck the same day. (separated group)
9 sows were vaccinated with both products after mixing (4 ml). (mixed group)
The vaccination schedule was according to the use of the farmer.
The colostrum was collected at farrowing and freezed as soon as possible after collection.
Antibody titers were determined in a toxin-neutralization assay (PMT), or by ELISA (*E. coli* antigens).

Results

The colostral responses after vaccination with the two vaccines given individually or mixed are presented in Table 1.

Table 1. Antibody titers (log_2) against both vaccine antigens in colostrum at farrowing.

	groups	
	separated	mixed
number	18	9
rank	$3,4^a \pm 2,00$	$3,7^a \pm 1,12$
PMT	$11,0^a \pm 1,10$	$11,0^a \pm 1,58$
F4ab	$12,8^a \pm 0,96$	$12,5^a \pm 1,20$
F4ac	$15,6^a \pm 1,54$	$14,9^a \pm 1,76$
F5	$14,7^a \pm 1,34$	$14,2^a \pm 2,07$
F6	$12,4^a \pm 0,87$	$12,0^a \pm 1,38$
LT	$11,4^a \pm 0,93$	$10,4^a \pm 1,51$

For each antigen, groups with different superscripts are significantly different. ($p<0.05$, two-sample t-test)

Conclusions and Discussion

There was no significant difference between the antibody titers induced by the vaccines injected the same day or by the mixed products.
These results confirm in farm level the results obtained previously [3], and open a new way to facilitate work for farmers, in heavy vaccination program situation as well as in group-housed sows configuration.

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Demonstration of twenty-three week duration of immunity of an experimental inactivated chimeric PCV1-2 PCV vaccine

P Runnels, J Bubolz, L Taylor, T Ricker, D Slade, G Nitzel, J Allison

Zoetis Veterinary Medicine Research and Development, Kalamazoo, MI, USA , jim.allison@zoetis.com

Introduction

Currently, PCV2-associated disease (PCVAD) is one of the most economically important diseases of pigs worldwide, affecting unprotected pigs usually between 5 to 18 weeks of age with clinical signs of progressive weight loss, dyspnea, tachypnea, icterus, anemia and mortality up to 30%. Since the approximate age of a pig intended for the food supply is about 26 weeks, vaccination at 3 weeks of age followed by a 23 week duration of immunity would be desirable.

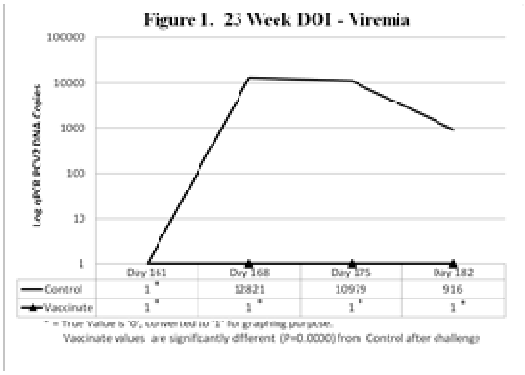
Materials and Methods

This study was conducted in the Zoetis Richland Farm facilities of Zoetis. Study conduct and use of swine were approved by the Zoetis Institutional Animal Care and Use Committee. Results reported here are a subset from a larger study. Thirty pigs, approximately 3 weeks old, PCV2 seronegative and viremia negative were vaccinated once, intramuscularly with 2 mL of an experimental formulation of an inactivated chimeric PCV1-2 vaccine adjuvanted with SLCD, which had previously been demonstrated to be sterile and potent. Thirty additional placebo-dosed pigs served as controls. Twenty-four pigs in each group were randomly selected to be challenged with PCV2 strain 40895 at a total dose of $10^{5.98}$ $FAID_{50}$, 23 weeks after vaccination. Pigs were monitored weekly after challenge for PCV2 antibody by ELISA and for viremia, nasal and fecal shedding by PCR. At necropsy, 3 weeks after challenge, three independent lymph nodes and tonsil were collected and assessed by immunohistochemistry (IHC) and for lymphoid lesions by histopathology.

Results

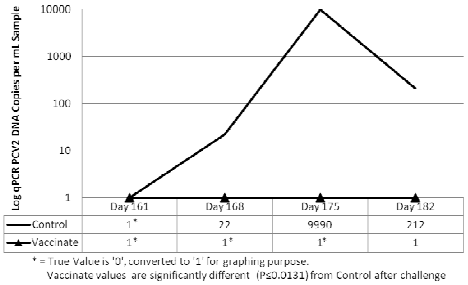
Viremia (Figure 1) was significantly reduced in both magnitude ($P<0.0001$) at each time point after challenge (peak geometric mean viremia, 12821 DNA copies per mL in controls vs 0 in vaccinates 7 days after challenge) and incidence ($P=0.0001$) across all time points. Fecal shedding (Figure 2) among vaccinates was significantly reduced in magnitude ($P\leq0.0131$) and incidence ($P<0.0001$) at all time points after challenge. Nasal shedding (Figure 3) among vaccinates was significantly reduced in magnitude ($P=0.0000$) at all time points after challenge and incidence ($P<0.0001$). Lymphoid tissue colonization (IHC) was significantly ($P=0.0003$) reduced among vaccinates. Lymphoid pathology (lymphoid depletion and histocytic replacement) was markedly reduced, but there was no significant treatment effect. ELISA S/P ratios were significantly ($P\leq0.0004$) increased among vaccinates from 3 weeks post-vaccination to necropsy.

Figure 1. 23 Week DOI - Viremia



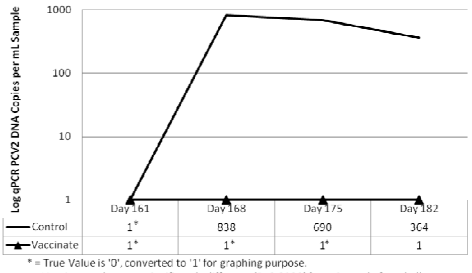
* = True Value is '0', converted to '1' for graphing purpose.
Vaccinate values are significantly different (P=0.0000) from Control after challenge

Figure 2. 23 Week DOI - Fecal Shedding



* = True Value is '0', converted to '1' for graphing purpose.
Vaccinate values are significantly different (P≤0.0131) from Control after challenge

Figure 3. 23 Week DOI - Nasal Shedding



* = True Value is '0', converted to '1' for graphing purpose.
Vaccinate values are significantly different (P=0.0000) from Control after challenge

Conclusions and Discussion

The experimental vaccine induced effective protection at 23 weeks after vaccination as evidenced by:
Reduced viremia
Reduced fecal shedding
Reduced nasal shedding
Increased antibody levels
Decreased lymphoid tissue infection (IHC)

P.622

Field efficacy of sow vaccination with AMERVAC® PRRS in a Vietnamese farm infected with PRRSV

Ha Thanh Huy[1], J Miranda[2], D Torrents [2]
[1]HIPRA VIETNAM, Ho Chi Minh City, Vietnam; [2]HIPRA., Amer, Spain daniel.torrents@hipra.com

Introduction

The emergence of highly virulent PRRSV (HP-PRRSV) strains in China in 2006 (1,2) was a milestone in the epidemiology of PRRS in Asia. Nowadays, genotype-II strains of different virulence coexist with genotype-I strains in Vietnam (3,4). At present, AMERVAC® PRRS (HIPRA, Spain), a genotype-I modified live vaccine (MLV), is licensed and marketed in many countries of Asia. Previously, AMERVAC®PRRS has demonstrated clinical efficacy in pigs experimentally infected with genotype-II HP-PRRS isolated from severe PRRS clinical outbreaks in Vietnamese pig farms (5,6). The present study was aimed to explore the field efficacy of AMERVAC® PRRS when applied in sows in a Vietnamese farm infected with PRRSV.

Materials and Methods

A 300 sows farrow-to-finish farm located in Binh Phuoc province (Vietnam) was selected for this study after being diagnosticated positive to PRRSV by PCR (7) and serology (CIVTEST® PRRS ES and AS, HIPRA, Spain). To evaluate the efficacy of AMERVAC® PRRS (VP-046 BIS strain; $\geq 10^{3.5}$ $TCID_{50}$/dose) we compared data about farrowing rate, abortion rate, percentage of scattered litters (<8 piglets/litter) and live born piglets per litter before (from June to November 2012) and after (from January to April 2013) the onset of the vaccination of the breeding herd. Initially, vaccination program was established as 2 mass vaccinations to all the breeders. First mass vaccination was applied in December 2012 and second mass vaccination to all sows as well in January 2013. For both mass vaccinations we applied the commercial dose (2 ml, IM) of AMERVAC® PRRS.

Results

Vaccination of sows with AMERVAC® PRRS significantly (*P<0.05*) improved all the reproductive parameters evaluated in this study as it is shown in the following table:

Table.1 Reproductive parameters before and after sow vaccination.

Reproductive parameters	Before vaccination	After vaccination
Farrowing rate (%)	81	87,12*
Abortion rate (%)	4	1,13*
Scattered litters (%)	17,6	14,61*
Piglets born live/litter	5,6	8,76*

(*) subscripts indicate statistically significant differences (*P<0.05*) between before and after vaccination results, *T-Student test.*

Conclusions and Discussion

Vaccination of sows with AMERVAC® PRRS improved the reproductive performance of the farm infected with PRRSV. Concretely, after 2 mass vaccinations of the entire breeding herd we observed an improvement of the farrowing rate and an increase of the number of piglets born alive per litter. At the same time, vaccination could reduce the abortion rate and the number of scattered litters.

Despite it was not established which genotype circulating in the farm, AMERVAC® PRRS showed a wide clinical efficacy under Vietnamese field conditions. Therefore, according to these results, we could consider AMERVAC® PRRS a very useful tool for the control of clinical reproductive PRRS in Vietnamese farms infected by PRRS pathogenic virus.

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Field study comparing two neonatal diarrhoea vaccines in Mexico

I Rodríguez-Ballarà[1], J Miranda[1], H Delgado[2], A Landa[3]
[1]Technical services, HIPRA, Spain, [2]HIPRA MEXICO, Mexico. [3] Granja la Joya, Mexico. isaac.rodriguez@hipra.com

Inroduction

Neonatal diarrhoea is an important and devastating disease to swine producer responsible of a substantial economic impact in farms worldwide (1).

In general, most neonatal infections can be prevented by passive calostral and lactogenic immunity obtained by vaccination of the sow. Neonatal diarrhoeas induced by E.coli are commonly prevented by vaccination of sow that are booster vaccinated 2-3 weeks before farrowing (2).

SUISENG® contains purified adhesion factors (F4ab, F4ac, F5 and F6) and the heat labile toxin (LT) of *Escherichia coli*, the β toxin of *Clostridium perfringens* type C and the α toxin of *Clostridium novyi*.

The aim of this study is to compare the production parameters in farrowing units during 2011 and 2012 in a commercial farm located in Mexico.

Materials and Method

The study was carried out in a 3000 sow's farm in Puebla State, Mexico. The vaccination protocol included a commercial Coli vaccine. However, the main concern in the farrowing units was neonatal diarrhoea appeared in piglets after 3 day of life. Crushed piglets, starvation and diarrhoea were the main causes of death. The Coli diagnosis of the diarrhoea was carried out by a multiplex PCR. This PCR was able to detect different Coli adhesins factors related with virulence (F4, F6, F5) and toxin β and α produced by different types of *Cl. Perfringens* (3). Sampling was performed using fecal samples from piglets showing acute signs of the diarrhoea.

Vaccination using SUISENG® was implemented at the beginning of January 2012 and the vaccination program used was one dose 8 weeks before farrowing and a revaccination 4 weeks later. A booster dose was administrated 4 weeks before the subsequent farrowing. In order to assess the field efficacy of SUISENG® data of piglet mortality, piglets weaned per litter, weaning weight and piglets with diarrhoea were reported during 2011 and 2012.

Results

The different parameters assessed are shown in the next figures (Figure 1, 2, 3 and 4). Statistical differences between 2011 and 2012 were observed in all the parameters evaluated. ($p<0,05$, t-test for independent samples).

Figure 1

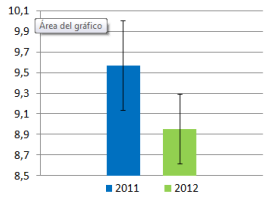

Figure 2

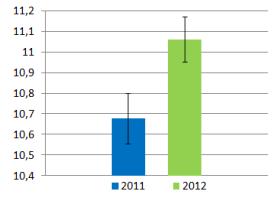

Figure 1. Mean piglet mortality rate in farrowing units (± SEM).

Figure 2. Mean piglets weaned per litter (± SEM).

Figure 3

Figure 4

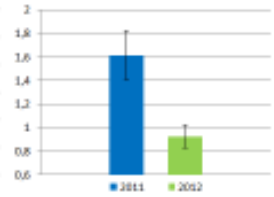

Figure 3. Mean body weight at weaning (± SEM).

Figure 4. Mean percentage of piglets with diarrhea (± SEM).

Conclusions and Discussion

Based on the data presented, SUISENG® vaccination program implemented in a farm affected by a chronic Colibacillosis was able to prevent the negative effects of E. coli infection in suckling piglets; thus improving pre-weaning mortality and percentage of piglets with diarrhoea, and consequently improving the weaning weight and piglets weaned per litter.

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Field efficacy of SUISENG® and RHINISENG® combined in a single injection

I Rodríguez-Ballarà[1], W Grieder[2], R Pinheiro[3]
[1]Technical services, HIPRA, Spain, [2]HIPRA SAUDE ANIMAL Brazil, [3]INTEGRALL®,Brazil,
isaac.rodriguez@hipra.com

Introduction

Neonatal diarrhoea can be prevented by passive calostral and lactogenic immunity obtained by vaccination of the sow (1). Besides, progressive and non-progressive atrophic rhinitis are commonly controlled by sow's vaccination worldwide (2). Therefore these two vaccines are commonly applied at the same time in sow farms.

SUISENG® is a vaccine against neonatal diarrhoea in piglets and sudden death in sows, and RHINISENG® is a vaccine for preventing progressive and non-progressive atrophic rhinitis of swine. Both vaccines contain a similar adjuvant, Hipramune-G®, and so are administered following the same vaccination schedule.

The aim of this study is to compare different productive parameters, when SUISENG® and RHINISENG® are mixed and injected in sows, in comparison with the injection of these two vaccines separately and with the injection separately of two more commercial vaccines.

Materials and Method

The study was carried out in a 800 sow's farm in Minas Gerais State, Brasil. This farm was selected due to the historical neonatal diarrhoeas problems and the high historical atrophic rhinitis index. 280 females were selected for the trial, 60 gilts and 220 sows, gilts were distributed in every batch of sows uniformly. All the females were divided in 4 treatments:

T1	PBS	4ml
T2	Suiseng® + Rhiniseng® Mixed	4ml
T3	Suiseng® / Rhiniseng® NOT Mixed	2ml /2ml
T4	Porcilis ART® / Littlegard®	2ml/2ml

The vaccination programs for every treatment were. T1 PBS at 6 and 3 weeks before farrowing; T2 and T3: Suiseng/Rhiniseng at 6 and 3 weeks before farrowing, T4: program recommended in the each leaflet.

In order to assess the performance of different treatments during the lactation period, two parameters at weaning were recorded and compared: Diarrhoea Mortality (DM) and Diarrhoea incidence (DI) per treatment. Besides, to evaluate the performance during the fattening period 3 parameters were selected and compared among treatments: cough index (CI), Final weight at slaughter (146 days) (FW) and nasal lesion score (NLS) using IRA index (3).

Results

Table 1. Selected lactation and fattening parameters of 4 treatments.

!	Lactation!!		Fattening!!	
	DM!	DI!	CI!	FW*!
T1!	12,6[a!]	21,22[a!]	1,78[a!]	100,78[a!]
T2!	3,58[b!]	10,02[b!]	0,65[b!]	103,99[b!]
T3!	3,1[b!]	6,99[d!]	0,87[b!]	103,11[b!]
T4!	10,3[a!]	14,3[d!]	1,65[a!]	104,94[b!]

a,b,c,d Different superscripts indicate statistical differences between treatment groups ($p < 0,05$, t-test for independent samples,*ANOVA).

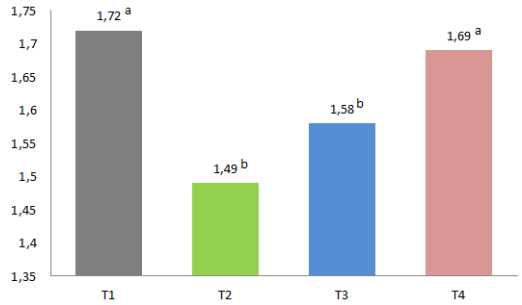

Figure 2. Mean Nasal Score lesion at slaughter (IRA)
a,b Different superscripts indicate statistical differences between treatment groups ($p < 0,05$, t-test for independent samples)

Conclusions and Discussion

Diarrhoea mortality and diarrhoea incidence in T2 and T3 groups were significantly different from the control group (T1) and from the group vaccinated with other commercial vaccines (T4). Therefore as SUISENG® mixed with RHINISENG® as injected separately was able to control the neonatal diarrhoeas in field conditions, moreover its performance was significantly better than other commercial vaccines.

The final weight at slaughter of control group (T1) was significantly lower than the vaccinated groups so the sow's immunisation through inactivated vaccines were able to control the atrophic rhinitis improving the weight at slaughter (4), besides the nasal lesion score was significantly better in the treatments 2 and 3, so RHINISENG® as mixed with SUISENG® as injected separately was reducing the atrophic rhinitis lesions significantly better than the other commercial vaccine.

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P.625

HIPRASUIS® GLÄSSER: Field efficacy in a Glässer disease case in Mexico

I Rodríguez-Ballarà[1], J Miranda[1], H Delgado[2], G Gomez[3]
[1]Technical services, HIPRA, Spain, [2]HIPRA MEXICO, Mexico, [3] Gigantes Tepa, Mexico. isaac.rodriguez@hipra.com

Inroduction

Haemophilus parasuis (Hps) causes Glasser's disease (GD) in pre-weaning and post-weaning pigs, is an important source of economic losses in pig production. Hps colonizes the upper respiratory tract of piglets early after birth and is pathogen under particular conditions. Hps can be found in the nose of healthy pigs, but those strains are often non-virulent or virulent but controlled by the immune system (1).

Disease may be seen in suckling piglets or in weaners when maternal immunity wears off before they become infected. It can also act as a secondary pathogen to other diseases particularly enzootic neumonia (*Mycoplasma hyopneumoniae*) and Porcine Reproductive and Respiratory Syndrome (PRRS) (2).

To prevent GD, commercial bacterins can be used directly in the affected piglets or, alternatively, in the sows. Vaccination of sows is less laborious and triggers an increase of the antibodies levels in colostrum. Sows produce strong maternal immunity which can persist in their offspring until 8 to 12 weeks of age(3).

The objective of this study is to measure the productivity rates in farrowing, nursery and fattening units when a specific vaccine program with HIPRASUIS® GLASSER vaccine (HIPRA) was applied in sows.

Materials and Method

The study was performed in a commercial multi-sites farm with 2300 sows located in Jalisco state (Mexico). Piglets were weaned at 21 days of age (mean weight 6,5 kg), then they were moved to nursery units until 10 weeks of age (mean weight 29 kg) and finally they went to fattening units until 24 weeks of age (mean weight 114,41 kg). During several months, post-weaned pigs were suffering high mortalities. Necropsy findings showed fibrinous poliserositis, arthritis and pneumonia. The diagnosis of Glässer disease was confirmed by bacteria isolation from respiratory and non-respiratory organs.

In order to reduce the losses due to Glässer disease a new vaccination program was applied using HIPRASUIS® GLASSER which contains serotypes 1 and 6. These serotypes give cross protection against serotypes 4 and 5 (4). Sows were vaccinated and revaccinated 6 and 3 weeks before farrowing, also gilts received the same vaccination program before mating.

Productivity rates were monitored 6 months after the onset of HIPRASUIS® GLASSER vaccination program and compared with the historical data of the farm.

Results

The different parameters recorded improved significantly after HIPRASUIS® GLASSER vaccination. (*P<0.05, t-test for independent samples*).

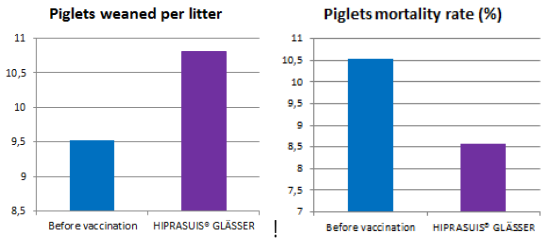

Figure 1 and 2. Parameters from farrowing units.

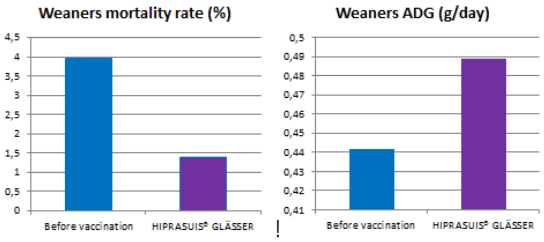

Figure 3 and 4. Parameters from Nursery unit.

Conclusions and Discussion

After 6 months vaccinating with HIPRASUIS® GLASSER vaccination program, data reported showed a clear improvement in the productive parameters of each one of the sites. Besides, clinical signs related to Glässer's disease disappeared. Therefore, in this field trial is demonstrated again that maternal derived antibodies (MDA) against *Hps* can protect piglets against *Hps* clinic disease (5).

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Influence of vaccination with an inactivated EU-PRRSV before vaccination with a live EU-PRRSV

H Schuh[1], M Seidenspinner[2], N Anthes[1], M Wendt[3], W Leibold[4]

[1]*Veterinary Practice (VP) H. Schuh,, Ipsheim,* [2]*VP M. Seidenspinner, Markt Neubrunn,* [3]*Clinic for Swine & Small Ruminants,* [4] *Inst. of Immunology, Univ. of Vet. Med., Hannover, Germany.* hermann.schuh@t-online.de

Introduction

Serum-titers of neutralizing antibodies (NAB) and/or lack of viremia are presently considered as indicators of protective immunity against PRRSV. Using exposure of piglets derived from sows with high and low titers of maternal NAB (mNAB) with live attenuated EU-PRRSV (LAV) as a model simulating "controlled infection" with PRRSV, the influence of vaccination with an inactivated EU-PRRSV (INV) pre "infection" with LAV was studied by means of NAB-development and viremia.

Materials and Methods

A total of 58 piglets derived from 6 sows, 3 with high (>= 4) and 3 with low titers (<4) of mNAB were randomly assorted to 2 groups (A & B) each comprising about 50% of piglets from sows with high and with low mNAB (see ref. 2&3). Group A received only LAV-"infection" at 11 weeks post natum (w.p.n). Group B was vaccinated with INV once 3 weeks before LAV at 11 w.p.n. Both groups received a LAV-"challenge" at 25 w.p.n. (comp. Fig. 1). Viremia was determined by means of nested PCR for PRRSV (performed by IVD GmbH, Hannover, Germany) and NAB were monitored (up to a limit of 11,2) against the live EU-PRRSV strain used for "infection" (performed by Bavarian Animal Health Service, Poing, Germany). Fishers two-sided test for small n was used for statistics.

Results

By 7 to 13 w.p.n. mNAB had dropped below detection level (1.7). Between 16 to 21 w.p.n. piglets started to develop NAB (Fig.1). By 25 w.p.n. 54 of 58 piglets had developed detectable levels (>=2) of NAB without significant differences between neither groups A & B nor the origin from sows with high or low mNAB.

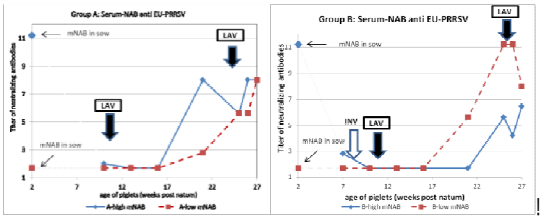

Figure 1. Influence of mNAB in sows and of the treatment on NAB development in piglets. *(Titration up to 11.2, median values)).*

Monitoring of viremia by means of EU-PCR 2, 3, & 10 weeks post LAV-"infection" (w."p.i.") and evaluating the earliest and consistent control of the virus (neg. PCR) the treatment and the origin of the piglets were of importance: Almost all of the piglets derived from sows with low mNAB had detectable viremia 2 w."p.i." disappearing consistently 5 & 10 w. "p.i.", irrespective of the treatment (Fig.2A). In contrast some piglets derived from sows with high mNAB were able to control the LAV consistently already from 2 w."p.i." on (Fig. 2B). Significantly more piglets were able to control the virus at 2 w."p.i." having received INV vaccination three weeks before LAV (group B = INV & LAV) than those without INV pretreatment (group A = LAV only). The mechanism of early virus control enhanced by INV prevaccination is unknown. There was no correlation with detectable NAB.

Fig. 2A **Fig. 2B**

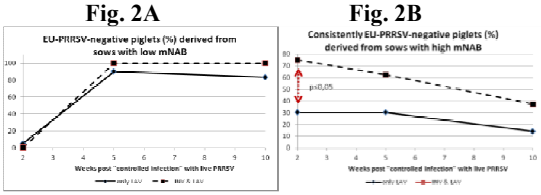

Figure 2. Influence of prevaccination with INV on the early and consistent PRRSV control- (PCR negative) by piglets after LAV-"infection". **2A:** Piglets from sows with low mNAB. LAV (n=6 to 20), INV&LAV (n=7) **2B**: From sows with high mNAB. LAV (n=7 to 23), INV&LAV (n=8). LAVat 11 w.p.n. Week 2, 5,& 10 "p.i." correspond to 13, 16 & 21 w.p.n., respectively. Significant difference only at 2 w."p.i."

A challenge with live PRRSV (LAV) 25 w.p.n. was well controlled by all piglets 1 & 2 weeks post challenge: None of them became EU-PCR positive.

Conclusions and Discussion

Simulating "PRRSV infection" with live attenuated EU-PRRSV (LAV) in piglets derived from sows with high or low titers of mNAB against EU-PRRSV we found that vaccination with inactivated EU-PRRSV (INV) had no significant influence on the development of NAB when their titration was limited to 11.2. No differences regarding virus control after LAV could be seen in piglets from sows with low mNAB with and without INV prevaccination. However, when piglets from sows with high mNAB received INV-vaccination 3 weeks before LAV more piglets were able to control LAV-PRRSV already by week 2 and consistently up to week 10 "p.i." than those without INV-prevaccination.

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Comparative field efficacy of two commercial PCV2 vaccines in the North of Mexico

J Jaime-Villafaña, H Perez-Leaño, R Gonzalez-Martinez
Zoetis de México, *México, City*, D.F. abrahamraul.gonzalez@zoetis.com

Introduction

Porcine Circovirus 2 (PCV2) is an infectious agent that has led to dramatic economic losses in pig production worldwide, through both obvious PCV2-associated diseases and reduced growth performance in subclinically affected pigs. Vaccination is now widespread and losses have been markedly reduced, but pigs remain at risk and vaccination against PCV2 is likely to remain a standard management practice. It is therefore of interest to investigate different vaccination protocols to maximize efficacy, while minimizing labor and pig disturbance.[1-3]

Materiasl and Methods

The study was conducted on 2070 growing pigs from a farm in the region of Sonora, México. Pigs were allocated to one of two experimental groups: Group A received 1 dose of 2 mL of a single dose vaccine at 6 weeks of age (Fostera PCV®, Zoetis) and Group B received 2 doses of 2 mL, at 3 and 6 weeks respectively, of a two dose vaccine (Circumvent® PCV, MSD). The study used 8 batches of pigs, started one week apart and reared in different buildings. Each batch included both treatments, randomly allocated by pen, to give a final total of 1044 pigs in Group A (one dose) and 1026 in Group B (two doses). Pigs were slaughtered at a cut-off age of 24 weeks and classified as either first or second class animals, being the later those that failed to achieve the target weight range. Pigs and feed were weighed allowing calculation of Average Daily Gain (ADG) and Feed Conversion. The pigs were weighed at 21, 80 & 170 days of age and feed every day in the feeder. 15 blood samples were taken randomly from each group at 3, 6, 10, 14, 18, 21 and 24 weeks of age for measurement of PCV2 viremia by rt quantitative PCR.

Results

The overall production results can be found in Table 1.

Table 1. Production parameters

	Group A	Group B	P
Av. weight in S2 (kg)	7.11	7.22	0.57
Av. weight in S3 (kg)	37.27	36.94	0.68
Av. weight out S3 (kg)	123.80	123.85	0.971
ADG (kg)	0.814	0.809	0.799
FE (feed/gain)	3.12	3.26	0.12
% First Class	91.69	90.62	0.507
% Second Class	8.31	9.38	0.507
Culls (%)	2.88	3.25	0.543
Mortality (%)	2.75	3.62	0.321

There were no statistically significant differences in performance between the groups (P>0.05 for all parameters).
The level of PCV2 viremia for the two groups also showed no statistical difference between them. (Figure1)

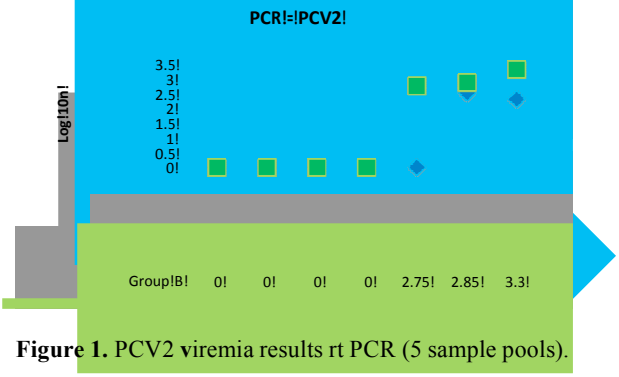

Figure 1. PCV2 viremia results rt PCR (5 sample pools).

Conclusions and Discussion

The results show no difference between the two vaccine protocols in growth and overall productive performance. The level of PCV2 challenge is unknown as there was no negative control group. Nevertheless, a period of PCV2 viremia did occur and clinical experience is that unvaccinated pigs are likely to suffer clinical evidence of PCVAD and/or reduced performance. A single dose administration protocol minimizes labor requirement and reduces the pig stress, resulting in important practical advantages.

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Efficacy of a PRRSV NA and EU strains combined inactivated vaccine in specific pathogen-free pigs

M Yeom[1,4], B Kang[2], H Kim[3],H Moon[2], J Kim[2], S Kim[1,4], D Song[1,4], B Park[5]

[1]!*Viral Infectious Disease Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-806, Republic of Korea,* [2]!*Research Unit, Green Cross Veterinary Products Co. Ltd., Yongin 449-903, South Korea,* [3]! *Research Evaluation Team, Institute for Basic Science, Daejeon, 305-811,South Korea,* [4] *University of Science and Technology, Daejeon 305-350, Republic of Korea,* [5] *Department of Veterinary Medicine Virology Laboratory, College of Veterinary Medicine, BK21 Program for Veterinary Science, Seoul National University, Kwanak-gu, Seoul 151-742, Republic of Korea,* songdaesop@gmail.com; parkx026@snu.ac.kr

Introduction

Acute outbreaks of porcine reproductive and respiratory syndrome virus (PRRSV) were reported in a pig farm in Korea in 2008. The virus was isolated from lung samples, and an inactivated homologous vaccine was applied on the farm. The inactivated vaccine was effective in reducing clinical disease, viremia, and mortality. In Korea, commercial vaccines comprise 1 PRRSV strain. However, NA type PRRSV of Korea are separated into 4 clusters based on the ORF5 gene. In particular, these viruses have high variations in the ORF5 epitope region(1). With the expected results through a previous experiment of an inactivated PRRSV vaccine, an inactivated cocktail vaccine was prepared, which contains PRRSV of both European (EU) and North American (NA) type strains. Therefore, 1 PRRSV strain (GCEU0907) of type 1, and 3 strains (GC4019, GC6262 and GC0412) of type 2, all highly prevalent in Korea, were selected for development of an inactivated cocktail PRRSV vaccine.

Materials and Methods

To evaluate the efficacy of the vaccine, 3 NA-type PRRSV strains and 1 EU-type strain were selected that had been prevalent in Korea. Specific pathogen-free (SPF) pigs (n = 24) were randomly assigned to 2 groups. One group of 8 pigs was not vaccinated; the remaining 16 pigs were vaccinated twice at 4 and 6 weeks of age. Vaccinated pigs were randomly assigned to 4 groups (n=4) and were challenged with each strain of PRRSV at 2 weeks after second vaccination. Non-vaccinated pigs were challenged to check viral pathogenicity and 2 pigs of a group were challenged with each strain. All pigs were monitored with clinical signs, gross lung lesions, microscopic lesions, viremia, and virus neutralization (VN) titer.

Results

Mean PRRSV ELISA S/P ratios are shown in Table 1. The two main effects (collection material, sampling order) had a statistically significant effect on the PRRSV ELISA S/P response. The interaction between material and order was not statistically significant (p = 0.52).

Conclusions and Discussion

These results demonstrates that the NA/EU combined BEI-inactivated vaccine may be an excellent option for the development of a protective vaccine against PRRSV infection in Korea

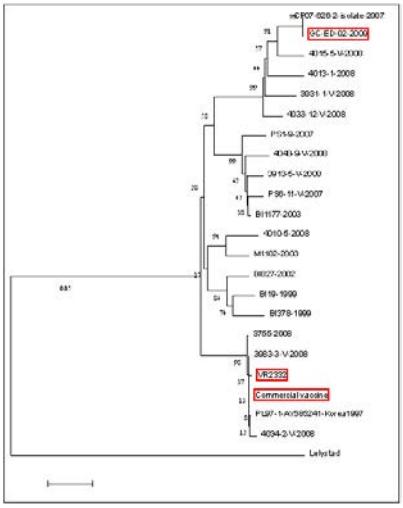

Figure 1. Phylogenetic tree of isolated porcine reproductive and respiratory syndrome virus based on ORF5 sequence.

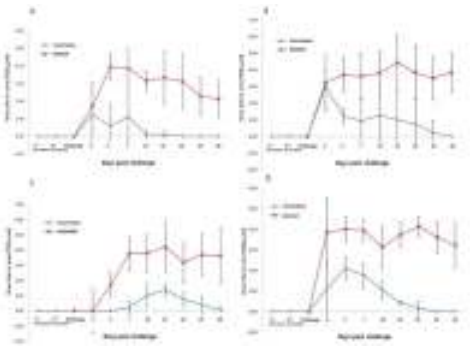

Figure 2.!Viremia in vaccinated and non-vaccinated pigs after virulent PRRSV challenge

Acknowledgments

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P.629

Serological response to vaccination against Aujeszky's disease with a needle-free injector

C Tonelli[1], F Ostanello[2]
[1]DVM, Mantova; [2]Department of Veterinary Medical Science, Bologna University, Bologna, Italy; tonelli66@libero.it

Introduction

Aujeszky's disease or pseudorabies is still present in Italy. One of the major tools to control and eradicate the disease is the systematic vaccination of swine with gE-deleted modified live vaccines (1). Needle-free injection of vaccines prevents residual needle fragments and associated meat defects from the injection site. AKIPOR® 6.3 (Merial, Lyon, France) is a freezed dried modified live vaccine (gE-deleted Bartha strain) with an oily adjuvant against Aujeszky's disease. This study aimed to compare the serological response to different vaccination programs either combining the recommended intra-muscular (IM) administration route of AKIPOR® 6.3 to intra-dermal (ID) vaccine administration using a needle-free injection device or combining only ID vaccine administrations.

Materials and Methods

The trial was conducted in a 400-sow multi-site farrow-to-finish operation known to be gE-negative and raising fatteners for Parma ham until 270-300 days of age. The vaccination program against Aujeszky's disease in force on the farm includes a two-shot primo-immunization at 70 and 90 days of age in the pre-fattening period followed by a booster injection at 180 days of age in the fattening period.

A total of 36 pigs divided in 3 groups of 12 pigs was vaccinated against Aujeszky's disease according to the treatment groups described in Table 1.

Table 1. Vaccination protocols

Group		Vaccination route		
		70 days	90 days	180 days
G1	n=12	ID	ID	ID
G2	n=12	ID	IM	ID
G3	n=12	IM	ID	ID

Vaccine IM administrations were performed under a 2.0-mL dose following reconstitution according to the manufacturer recommendations (i.e. mixing 100 mL O/W adjuvant mixed with a 50-dose freezed-dried virus pellet).

Intra-dermal administrations were performed using a needle-free injection device (VALERY® device, equipped with a nose-piece adapted for 0.2 mL ID injection, Giordano Poultry-Plast, Caraglio, Italy) under a 0.2-mL dose prepared by mixing 10 mL O/W adjuvant mixed with 50-dose freezed-dried virus powder.

Serum samples were collected from each pig before primo-immunization, one month later and one month following booster injection. Routine gE and gB ELISA assays were conducted at IZSLER (Brescia, Italy).

Results

No adverse event was observed following vaccine administration whatever the vaccination protocol. The average gB titers observed in each experimental group over the monitoring period are depicted in Figure 1.

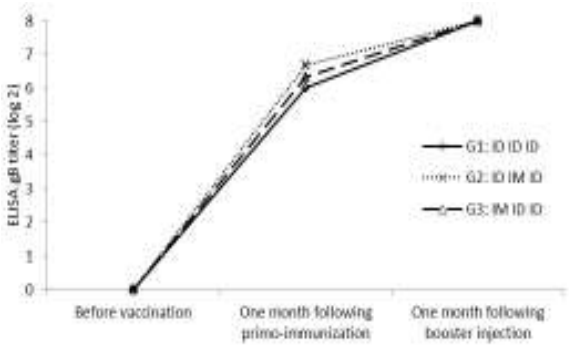

Figure 1. Average ELISA gB titres (log 2) in the 3 experimental groups. For calculation, titres < 16 and ≥ 256 were replaced respectively by 0 and 256.

No seroconversion against gE protein did occur thus confirming the absence of viral circulation in the pigs in the conditions of the study. In this context, a clear seroconversion against gB protein following primo-immunization was evidenced following each vaccination protocol with no difference evidenced between groups (Kurskall-Wallis test, $p>0.27$). A clear increase in gB antibody titer was also observed following the ID booster whatever the vaccination protocol applied for the primo-immunization.

Conclusions and Discussion

The vaccination programs against Aujeszky's disease tested in this study included 2 to 3 ID injections with AKIPOR 6.3 at an adjusted dose in 0.2 mL O/W adjuvant and appeared to be similarly potent to elicit a satisfying serological response. These findings corroborate previous results obtained in similar conditions where only the booster injection was administrated intra-dermally (2).

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PCV2 vaccination in sows: A review of the benefits of CIRCOVAC® on the number of weaned pigs per sow per year

T Vila, O Merdy, F Joisel
MERIAL S.A.S., Lyon, France; thais.vila@merial.com

Introduction

PCV2 has been associated with reproductive disorders (1,2) and is a causative agent of foetal death in swine (2,3). CIRCOVAC is an inactivated adjuvanted PCV2 vaccine which was shown to increase prolificacy (4,5,6) and to reduce mortality rate in the suckling phase (7,8). The aim of this paper is to review the studies that investigated the beneficial impact of CIRCOVAC on the final product of the breeding sow herd, i.e. the weaned piglets expressed as weaned piglets per sow per year.

Materials and methods

The review was performed in the proceedings of international and regional meetings focusing on porcine veterinary science. Data records for which the criteria "total weaned piglets per sow and per year" was reported were selected. This criteria is representative of both fertility and prolificacy performance. In all studies the CIRCOVAC vaccination was performed according to the recommendation of the manufacturer: 2ml per dose, IM, primo-vaccination: twice 3 weeks apart by mass vaccination or before insemination (for gilts) or farrowing (sows). Booster vaccination was performed before farrowing.

Results and discussion

Results are summarized in Table 1. All the publications reviewed were historical data collections comparing performance before and after/during sow vaccination with CIRCOVAC. Interestingly, in the 277 German farms study, the regional evolution (Low Saxony) of the weaned piglets/sow/year parameter for the same period of time only showed a much lower improvement (21.4 to 21.7 = +0.3) thus confirming the contribution of CIRCOVAC vaccination in the improvement by 1.13 piglets weaned per sow per year in the vaccinated farms.
Following the introduction of sow vaccination with CIRCOVAC, an improvement by 0.5 to 1.8 piglets weaned per sow per year was reported when introduced in a variety of reproduction performance level cases ranging from 16.0 to 26.5 weaned piglets per sow per year.
The financial impact of sow performance improvement of 1 weaned piglet per sow per year was estimated to be around €57 per sow (assuming 2012 French technical and economical parameters, and excluding vaccine and labor costs).

Table 1. Comparison of number of weaned piglets/sow/year before and after sow vaccination with CIRCOVAC (2 ml).

Reference (year of publication)	Number of farms	Total weaned piglets/sow/year		
		Before	After	Delta*
Delisle *et al.* (2008)	27	25.4	26.1	+0.7
Joisel *et al.* (2008)	253	21.2	22.4	+1.13 (p<0.001)
Kunstmann *et al.* (2008)	34	24.65	25.88	+1,23 (p<0.05)
Kmiec *et al.* (2008)	1	26.5	27	+0.5
Kmiec *et al.* (2008)	1	23.3	25	+1.7
Tang *et al.* (2009)	1	16.0	17.6	+1.6 (p<0.05)
Tee *et al.* (2011)	1	18.9	20.7	+1.8 (p<0.01)
Tee *et al.* (2011)	1	18.9	20.4	+1.5 (p<0.05)

*Difference in total weaned piglets/sow/year between "After" and "Before" periods; p-value between brackets when available.

Conclusion

These studies performed in 319 farms concluded that CIRCOVAC in breeding herds is improving substantially the sow technical and economic performance.

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Field experience of the use of CIRCOVAC® as a whole herd solution on Russian farms

H Smits[1], V Pedchenko[2], V Plioplys[2], S Ermilov[2], O Merdy[1], T Vila[1], F Joisel[1]
[1]*Merial S.A.S., Lyon, France,* [2]*Merial, Moscow, Russia,* Han.Smits@Merial.com

Introduction

Since the mid-nineties, Porcine Circovirus (associated) Diseases (PCV(A)D) have spread to all parts of the world. CIRCOVAC (Merial, Lyon, France) was the first PCV2 vaccine registered in the world and still now the only one registered for piglets and sows and has been extensively shown to improve herd performances (1,2). The aim of the present paper is to report 3 different field experiences of PCV2 vaccination with CIRCOVAC under Russian conditions.

Case description

Case 1. On a 1.500 sows farm mortality rate and the average daily weight gain (ADWG) in the weaners and finishers was below expectations. Of one week production 3 groups of piglets were weaned at 3 weeks of age and randomly assigned to one of 3 groups. Group A = 1.250 piglets, CIRCOVAC 0.5 ml IM, Group B = 1.250 piglets, vaccine B 2.0 ml IM, Group C = 1.000 piglets, no vaccination. ADWG, mortality rate and age at slaughter presented covered February 2012 till August 2012.

Case 2. Two Russians farms, both PRRS positive. Farm 1 of 700 sows and farm 2 of 2.500 sows.
Farm 1 is weaning at 25 days of age. On the whole farm sows were vaccinated since March 2012 with CIRCOVAC, 2 ml IM as follows: * Gilts received 2 vaccinations 3 weeks apart, the second 2-3 weeks before first insemination. Sows received their primo-vaccination twice 3 weeks apart, second vaccination 2-3 weeks before farrowing. Re-vaccination every 2-3 weeks before expected farrowing. * The smaller piglets were vaccinated with 0.5 ml IM, at 21 days of age, at weaning. The data included in this study covered March 2012 till October 2013.
Farm 2 vaccinated piglets since April 2012, once with 0.5 ml IM of CIRCOVAC at weaning, i.e. at 21 days of age. The data recorded cover in total 50.000 piglets over a period of 1 year and are compared with the year before. On both farms production data were collected: mortality, weaning weight (only on farm 1) and ADWG.

Case 3. To identify the best piglet vaccine a Russian swine producer with 15.000 sows performed a large trial on one of their 5.000 sows units with 4.645 piglets. The piglets were assigned at random to one of three groups and vaccinated at weaning at an age of 21-25 days with one of three commercial vaccines:
- Group A = 1.654 piglets, 0.5 ml IM CIRCOVAC
- Group B = 1.506 piglets, 2.0 ml IM vaccine A
- Group C = 1.485 piglets, 1.0 ml IM vaccine B

Production data were recorded with the focus on ADWG and mortality in the different age groups for the period of March 2013 till October 2013.

Results

Case 1. In the weaners from 21 days till 10 weeks as well as in the finishers the ADWG was clearly the highest in the group A (CIRCOVAC). In post-weaning the ADWG was 405, 385 and 360 g/day and in the finishers 800, 730 and 700 g/day in groups A, B and C, respectively. Mortality in post-weaning was 3.5%, 4.4% and 6.5% and in the finishers 2.5%, 2.8% and 3.5%, in groups A, B and C, respectively. Slaughter weight of 110 kg was reached in group A soonest, due to the higher ADWG. Age at slaughter was reached for the 3 groups at 190, 197 and at 210 days of age, respectively.

Case 2.
Farm 1: with CIRCOVAC vaccination, weaning weight increased from 7.5 to 8.3 kg, ADWG in the weaners increased from 397 to 426 g/day and in the finishers from 765 to 804 g/day; total mortality decreased in the weaners from 4.0 to 3.0% and in finishers from 2.6 to 2.3%.
Farm 2: ADWG increased from 498 to 538 g/day and from 649 to 712 g/day in the weaners and in the finishers; total mortality decreased from 9.4 to 6.3% in the weaners and from 5.9 to 3.2% in the finishers.

Case 3. ADWG in post-weaning was 420, 420 and 423 g/day in groups A, B and C, respectively. ADWG in the finishers was 680, 648 and 651 in groups A, B and C g/day, respectively. Post-weaning mortality was 6.1%, 6.3% and 6.0% in groups A, B and C, respectively. Finisher mortality was 4.0%, 4.0% and 3.9%in groups A, B and C, respectively.

Conclusions and Discussion

These field case reports confirm previous results (1,2) that CIRCOVAC used in piglets or in sows and the smallest piglets improve production data of the weaners and finishers.

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Efficacy against erysipelas conferred by ERYSENG® PARVO

M Fontseca, M Roca, A Camprodon, R March, M Sitjà
HIPRA, Amer (Girona), Spain, agusti.camprodon@hipra.com

Introduction

Swine erysipelas, when uncontrolled, is an economically significant disease capable of affecting all stages of pork production (1). Classic clinical signs such as pyrexia, lethargy, inappetence, abortion, death, and rhomboid urticarial (diamond skin) lesions are easily recognized. The aim of this study was to determine the efficacy elicited by ERYSENG® PARVO, a new inactivated Porcine Parvovirus (PPV) and *Erysipelothrix rhusiopathiae* vaccine after challenge with pathogenic swine *E. rhusiopathiae* strains in gilts and boars.

Materials and Methods

Fifteen six-month-old gilts and fifteen six-month-old boars were randomly assigned to group 1 (n=10 gilts), group 2 (n=10 boars) or group 3 (n=10, 5 gilts and 5 boars). Groups 1 and 2 were immunised intramuscularly with a 2 ml dose following the primary vaccination scheme (two doses three weeks apart). Group 3 (placebo) received PBS using the same immunisation strategy as groups 1 and 2. On day 43, all animals were challenged with separate dorsal and intradermal injections (10^6 cfu/dose) of pathogenic *E. rhusiopathiae* BRP belonging to serovars 1 and 2. Body temperature and the diameter of the skin erythema at the injection site were recorded daily until the end of the trial (day 50). Serum samples were obtained on days 0, 21, 35 and 43. Serum antibodies to *E. rhusiopathiae* (IgG) were titrated using a commercially available ELISA assay. Samples are considered positive when IRPC≥40. Antibody titres and temperatures were compared by means of a T-test ($p<0.05$), and skin lesions were compared using a Chi-square test.

Results

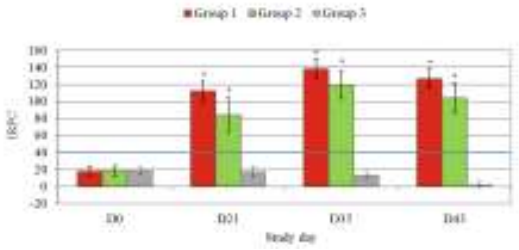

Figure 1. Mean antibody levels against *E. rhusiopathiae*. **Statistically different within the same day (T-test; p<0.05).*

Vaccinated animals showed seroconversion after priming vaccination, with a significant increase in antibody titres from day 21 to challenge day, with significant differences with group 3.

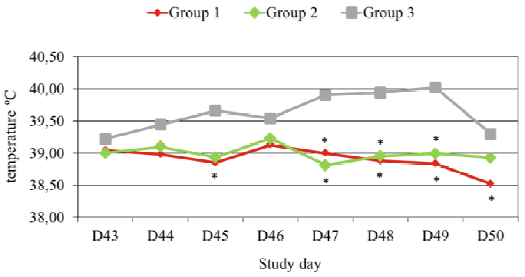

Figure 2. Average body temperature (ºC) post-challenge. **Statistically different within the same day (T-test; p<0.05).*

The differences in the mean body temperature between the vaccinated and the placebo animals after challenge were statistically significant on days 45, 47-50 (group 1) and on days 47-49 (group 2), rising to a peak of 40.02 ºC in group 3. No increase of body temperatures was observed in the vaccinated groups ($p>0.05$).

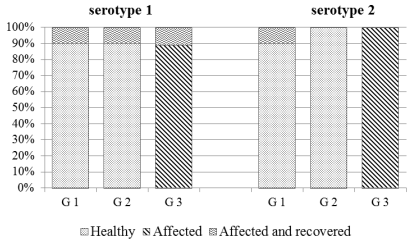

Figure 3. % of animals with skin lesions by serotype.

100% of the placebo animals challenged with serotype 1 displayed typical skin lesions after infection (10% were recovered at the end of the study) and only 10% of the vaccinated animals, groups 1 and 2, were affected and recovered. The challenge with serotype 2 showed 10% affected and recovered in group 1, 0% affected in group 2 and 100% affected in group 3 ($p<0.01$).

Conclusions and Discussion

According to these results, ERYSENG® PARVO is effective for active immunisation of gilts and boars against *E. rhusiopathiae*. The humoral immunity elicited by the *E. rhusiopathiae* fraction of the new vaccine remained high throughout the trial. Revaccination increased antibody titres and allowed animals to handle the experimental infection, showing no fever and only sporadic skin lesions that recovered after challenge.

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Field experience of the use of PROGRESSIS® on two Russian farms

H Smits[1], V Pedchenko[2], V Plioplys[2], S Ermilov[2], O Merdy[1], T Vila[1], F Joisel[1]
[1]*Merial S.A.S., Lyon, France,* [2]*Merial, Moscow, Russia,* Han.Smits@Merial.com

Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) has been provoking losses in pig farms for more than 20 years all over the world. Its impact differs on the situation on the farm including management, internal and external biosecurity and the presence of other pathogens. PRRSV is still regarded as the pathogen with the highest economic impact in the swine industry in the world. The impact in the US was recently estimated around $ 580 million per year. As in most parts of the world also in Russia the virus is present in most of the pig farms.

The aim of this paper is to show the positive effect of the use of PROGRESSIS (Merial, Lyon, France), an inactivated adjuvanted PRRS vaccine (KV vaccine), under Russian condition.

Materials and Methods

Case 1. On a 2.000-sow farm in Russia (Farm 1), reproduction performance was poor and mortality in the pre-weaned piglets was high. Many abortions in late gestation, a high amount of repeat breeders and a low farrowing rate were observed. The reproductive disorders were seen as a seasonal problem. A MLV PRRS vaccine was used in sows prior to the trial without clearly improving the situation.

Case 2. On a Russian farm with 700 sows (Farm 2), farrowing rate was low due to a high frequency of sow abortions, especially in late gestation and of repeat breedings. These reproductive disorders were seen mainly seasonally as well. Mortality rate in the pre-weaned piglets was also high.

The following vaccination schedule was implemented on the whole breeder herd in the 2 farms using PROGRESSIS, 2 ml per dose, IM :

Gilts received 2 injection 3 weeks apart, the second 3-4 weeks before the first insemination.

Sows were mass vaccinated twice also 3 weeks apart. In all females, re-vaccination was done every 60-70 days of gestation.

The results were evaluated by comparing the production parameters focusing on reproduction performance and pre-weaning mortality between the period with the use of a MLV vaccine (Farm 1) or without any PRRS vaccination (Farm 2) and the one following the implementation of PROGRESSIS.

Results

The results observed are summarized in Tables 1 & 2.

Table 1. Reproductive parameters and pre-weaning mortality results in Farm 1.

	Before: MLV vaccination *Year 2011*	After: KV vaccination *Year 2012*	Variation
Abortion rate	2.33%	1.70%	-0.63%
Farrowing rate	82.2%	85.2%	+3.00%
Pre-weaning mortality	6.78%	5.52%	-1.26%
Number of piglets weaned/sow/ year	22.1	23.8	+1.7

Table 2. Reproductive parameters and pre-weaning mortality results in Farm 2.

	Before: No vaccination *2011/03 to 2012/02*	After: KV vaccination *2012/03 to 2013/02*	Variation
Abortion rate	1.5%	0.8%	-0.7%
Farrowing rate	83%	87%	+4%
Pre-weaning mortality	9%	6%	-3%
Number of piglets weaned/sow/ year	23.3	23.8	+0.5

After implementation of PROGRESSIS, the reproductive performance of the herds were improved: both the abortion rate and the piglet pre-weaning mortality decreased. The farrowing rate increased as well. Consequently, the number of piglets weaned per sow per year was increased by 1.7 and 0.5 in Farm 1 and Farm 2, respectively.

Conclusion and Discussion

The implementation of PROGRESSIS vaccination on these two Russian farms clearly improved the reproductive results. These results were in coherence with previous reports showing a stabilization of the sow herd due to the implementation of the PROGRESSIS vaccination (1-3).

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P.634

HIPRAMUNE®-G^d: An ally in the duration of immunity against *E. rhusiopathiae*

M Fontseca, MC Moreno, M Roca, A Camprodon, R March, M Sitjà
HIPRA, Amer (Girona), Spain, agusti.camprodon@hipra.com

Introduction

Swine erysipelas caused by *E. rhusiopathiae* continues to affect pigs worldwide causing major economic losses due to farm outbreaks and animals being condemned at slaughter. Therefore, it is important to induce proper seroconversion against *E. rhusiopathiae* in order to provide stronger protection.

HIPRAMUNE®-G^d is composed of three elements with known immunological properties. Aluminium hydroxide that promotes formation of aggregates that can be more easily phagocytised (1). DEAE-dextran reduces hepatic up-take of the antigen, thereby increasing the amount of antigen reaching the APCs (2). And finally Ginseng extract from *Panax ginseng* that contains immunomodulators named ginsenosides, which enhance the antibody response to viral and bacterial antigens in pigs (3).

This study intends to investigate whether the combined adjuvant, HIPRAMUNE®-G^d, is essential for providing lasting seroconversion and, therefore, longer protection against *E. rhusiopathiae*.

Materials and Methods

Nineteen gilts clinically healthy and free from antibodies against *E. rhusiopathiae* were randomly assigned to group A (n=3), group B (n=8) and group C (n=8) were vaccinated twice intramuscularly with a 2 ml dose three weeks apart (days 0 and 21 of the study) with one of the following formulations:

Vaccine*	Adjuvant composition
A	Aluminium hydroxide
B	HIPRAMUNE®-G^d
C	Placebo

*Vaccines A and B contained porcine parvovirus and *E. rhusiopathiae* at the same concentration and vaccine C contained PBS.

Serum samples were obtained on days 0, 21, 41, 60, 80 and 93, and antibodies to *E. rhusiopathiae* (IgG) were titrated using a commercially available ELISA assay. Results were expressed in IRPC (relative index x 100) value. IRPC≥40 were considered positive samples. Serology was analysed by means of a One Way ANOVA. Statistically significant differences ($p<0.05$) are indicated by asterisk.

Results

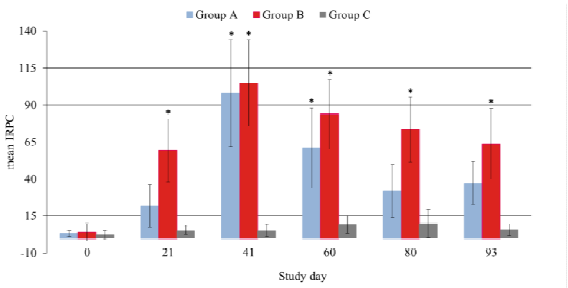

Figure 1. Serology against *E. rhusiopathiae.* **Statistically different within the same day (Anova 1F; $p<0.05$).*

On day 21 of the study, the mean of the serological response of group B (HIPRAMUNE®-G^d as adjuvant) showed significant differences from the placebo group. No statistical differences were observed in group A (aluminium hydroxide as adjuvant).

On day 41 and 60 both vaccinated groups showed significant differences from the placebo group.

On day 80 and 93 (approximately 3 months after vaccination) the mean of antibodies in group B (HIPRAMUNE®-G^d) remained significantly higher than control group, while group A did not show significant differences with the control group.

Conclusions and Discussion

The humoral immune response against *E. rhusiopathiae* in the group of animals vaccinated with vaccine containing HIPRAMUNE®-G^d as adjuvant is faster and longer than the humoral immune response developed by the vaccine containing only aluminium hydroxide as adjuvant.

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Impact of PCV2 vaccination around weaning on nursery weight gain

M Miyashita, R Steens, PH Rathkjen
Boehringer Ingelheim Vetmedica,

Introduction

PCV2 vaccination is widely used in pig production all over the world. Most commonly vaccination is administered at or around weaning time. For some vaccines it has been reported that vaccination around weaning can have a negative impact on the feed intake and growth in the period following vaccination, resulting in significant weight differences at the end of the nursery (Potter et al., 2012). The present abstract describes the results of a customer initiated, field observation focusing on the impact of two different PCV2 vaccines on the nursery weight gain.

Materials and Methods

The field observation was carried out on a farrow-to-nursery farm in Southern Germany after a Veterinary Practitioner wanted to evaluate PCV2 vaccine options. Healthy litters from one sow batch (46 sows) were randomly allocated at 3 weeks of age to two different treatment groups balanced according to health status (average, above average, very good). Non-healthy litters (4) were excluded from the observation. Allocation resulted in two treatment groups of 230 pigs each. The treatment each group received was decided by tossing a coin. Group 1 received Suvaxyn PCV (Zoetis), 2 ml i.m., group 2 was treated with Ingelvac CircoFLEX® (Boehringer Ingelheim), 1 ml i.m. Pigs in both groups were vaccinated on the same day with Ingelvac MycoFLEX® on the opposite neck side of the PCV2 vaccine.

Pigs were weighed in groups of 1-8 animals selected randomly at inclusion and 40 days post vaccination (ahead of moving out of nursery). 20 pigs from each group were bled at 4 weeks post vaccination and then again every 3 weeks until slaughter. Blood samples were tested for PCV2 antigen and antibodies by qPCR, IgM and IgG (Ingenasa ELISA) and PRRSV by PCR, both type 1 and 2. (All testing was done at BIVRC, Hannover).

End weight differences between the groups were analyzed using ANOVA procedure considering start weight as covariate (GLM Pocedure, SAS V9.2, Cary, NC)

Results

Average inclusion weight of Group 1 was 6.7 kg and group 2 was 6.8 kg, this difference was not statistically significant. End of the nursery average weight and weight gain of both groups is shown in table 1. The nursery end weight was statistically different (P≤0.05) between the 2 groups. No PCV2 antigen was detected in any of the 2 groups throughout the study. PRRSV type 1 was detected in both groups 4 weeks post vaccination but not later on. PRRSV type 2 was detected in both groups 7 weeks post vaccination but not later on.

Table 1. Summary of weight results (kg)

	1 (#43) Suvaxyn PCV	**2 (#44) Ingelvac CircoFLEX**
Start weight (3w)	6,7[a]	6,8[a]
End Weight	19,36[a]	20,42[b]
Weight Gain (40d)	12,66[a]	13,62[b]
Difference (40 d)		0,96

Means with different superscripts are significantly different at P≤0.05.

Conclusions and Discussion

As no challenge with PCV2 virus was found in the nursery period, the differences in weight gain can only be due to different features of the vaccines. As feed intake data was not collected, we can only speculate that the superior growth for Group 2 vaccine was due to its proven excellent tolerance in pig vaccinated around weaning (Fangman et al., 2011). Reduced feed intake after application of other vaccines has been shown in previous studies (Potter et al., 2012, Johnson et al., 2013). A calculated difference of 960 grams by end of nursery means a lower price of about 1 Euro per pig sold (market value 7-2013), which should be taken into consideration when making a value based vaccine choice.

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Efficacy against porcine parvovirus infection after vaccination of gilts using ERYSENG® PARVO

M Fontseca, M Roca, A Camprodon, R March, M Sitjà
HIPRA, Amer (Girona), Spain, agusti.camprodon@hipra.com

Introduction

Porcine parvovirus (PPV), a ubiquitous virus that affects swine reproductive performance all over the world, has been identified as a major cause of embryonic and foetal death in pigs (1,2). When embryos are infected before their immunological competency is activated (prior to 70 days of gestation), embryonic and foetal death may occur (3).

The aim of this study was to assess the efficacy in naïve young breeding stock to PPV conferred by ERYSENG® PARVO, a new inactivated bivalent vaccine against PPV and *Erysipelothrix rhusiopathiae*. The study design sought to test the "prime immunization strategy" by means of an experimental infection in gilts during gestation.

Materials and Methods

Twelve six-month-old gilts, clinically healthy and free from antibodies against PPV were randomly assigned to group 1 (n=7) or group 2 (n=5). Group 1 was immunised intramuscularly with a 2ml dose following the primary vaccination scheme (two doses three weeks apart, three weeks before mating). Group 2 (placebo) received phosphate buffered saline using the same prime immunisation strategy as the vaccinated group.

Animals in both groups were challenged intravenously and intranasally on day 40 of gestation (day 85) with 4 ml $10^{5.8}$ $CCID_{50}$ of a pathogenic PPV strain. Blood samples were obtained on days 0, 73 and 85 and antibody titres against PPV in serum were determined by the haemagglutination inhibition (HI) assay. All animals were humanely sacrificed to perform a necropsy on day 90 of the gestation (day 135). The appearance of the foetuses was evaluated, and blood samples as well as lung, liver and intestine tissues were collected for virus detection (by haemagglutination, HA) and antibody detection (by HI). The differences in antibody titres between groups were assessed using the T- test ($p<0.05$) and the differences in reproductive parameters between groups were assessed using the Chi-square test.

Results

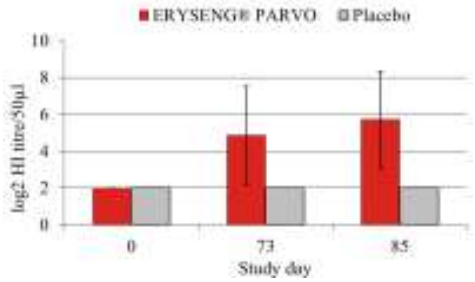

Figure 1. Mean log_2 HI titres against PPV.

Although statistically significant differences were not observed, from day 73 until day 85, the titres of PPV-specific HI antibodies were higher and increased in the vaccinated group.

Table 1. Aspect of foetuses and detection of PPV.

	% foetuses normal aspect	Average number piglets / litter	% foetuses infected with PPV
ERYSENG® PARVO	95.00*	11.00*	0.00*
Placebo	10.28	1.20	92.72

Regarding the appearance of the foetuses, the percentage of normal foetuses per litter was 95.00% in the vaccinated group and 10.28% in the placebo ($p<0.05$). The average number of piglets per litter was 11 in the vaccinated group and 1.20 in the placebo group ($p<0.05$). Whereas 92.72% of the foetuses in the placebo group were infected by PPV, no infection was detected in any of the foetuses from the vaccinated group ($p<0.05$).

Conclusions and Discussion

The regime of vaccination (primary schemes) effectively protects the progeny against transplacental infection caused by PPV. Therefore, it can be affirmed that the immunity against PPV conferred by the prime immunisation scheme of ERYSENG® PARVO covers the most critical phase of the gestation period.

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Protective immunity against porcine parvovirus and Erysipelas infection after vaccination of gilts using ERYSENG® PARVO

A Puig, M Simon, E Perozo, M Noguera, C Casado, M Fontseca, A Camprodon, R March
HIPRA, Amer (Girona), Spain, agusti.camprodon@hipra.com

Introduction

Swine erysipelas (SE) and Porcine Parvovirus (PPV) are diseases found all over the world that have an important economic impact on pig farming (1,2,3,4).

ERYSENG® PARVO is a new inactivated PPV and *Erysipelothrix rhusiopathiae* vaccine for active immunization of sows and gilts as an aid in controlling SE and for the protection of embryos and foetuses against PPV infection.

The objective of the present study was to evaluate the development of antibodies against PPV and *E. rhusiopathiae* in gilts vaccinated with ERYSENG® PARVO.

Materials and Methods

A randomized, double-blinded, positive controlled field trial was conducted on five commercial European farms.

348 gilts were randomly distributed into two groups: group 1 was vaccinated with a commercially available vaccine adjuvanted with aluminium hydroxide (positive control group) and group 2 was vaccinated with ERYSENG® PARVO, which is adjuvanted with HIPRAMUNE®-G^{d}. Both groups were immunized twice with a 2ml dose three weeks apart (D0 and D21), three weeks before mating.

To evaluate the protective immunity against PPV and *E. rhusiopathiae,* serum samples from all animals were collected on D-1, D20 and at partum. Serological analysis against PPV of sera samples was performed by using the haemagglutination inhibition (HI) technique. Samples were considered positive when $\log_2$ HI/50µl>2. Serum antibodies to *E. rhusiopathiae* (IgG) were titrated using a commercially available ELISA assay. Samples are considered positive when IRPC≥40.

Results

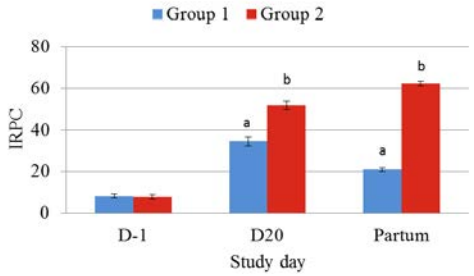

Figure 1. Mean antibody levels against *E. rhusiopathiae* in gilts. [a,b] *The difference between the mean of the E. rhusiopathiae antibodies in the ERYSENG® PARVO group compared to the positive control group was significant (p value <0.05).*

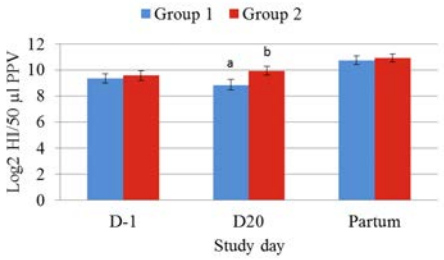

Figure 2. Mean antibody levels against PPV in gilts. [a,b] *The difference between the mean of the Porcine Parvovirus antibodies in the ERYSENG® PARVO group compared to the control group was significant (p value < 0.05).*

Conclusions and Discussion

The mean antibody levels against *E. rhusiopathiae* before vaccination (D-1) indicate the seronegative status of the animals. On D20 of the study (just before the administration of the 2nd dose of vaccine), the mean antibody levels observed in group 2 were higher than group 1, with statistically significant differences between groups. At partum, the mean antibody levels observed in group 2 were also higher than group 1, with statistically significant differences between groups. The mean antibody levels observed in group 2 (ERYSENG® PARVO) at partum were higher than the mean observed on D20 and the IRPC≥40 which indicates that the humoral immunity, that plays a significant role in host defense against *E. rhusiopathiae* infection, remained high throughout the study.

The mean antibody levels against PPV before vaccination (D-1) indicate that the animals had been in contact with the virus (PPV is prevalent in the pig population and highly stable in the environment (4)). On D20 of the study, the mean antibody levels of group 2 were higher than group 1, with statistically significant differences. At partum, the mean antibody levels observed in group 2 were higher, but without significant differences with group 1.

References

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Characterization of *E. rhusiopathiae* antigen: A key component for ERYSENG® PARVO

M Fontseca, MC Moreno, A Camprodon, M Sitjà
HIPRA, Amer (Girona), Spain, agusti.camprodon@hipra.com

Introduction

Erysipelothrix rhusiopatihae is a facultative, non-spore-forming, non-acid-fast, small, gram-positive bacillus (1). The disease is worldwide in distribution and of economic importance throughout Europe, Asia, and the Australian and American continents (2).

It has been described that the 64-66 KDa cell surface SpaA protein of *E. rhusiopathiae* is responsible for eliciting highly protective antibodies (3), and is considered to be the major immunogenic antigen of the species (4).

The characterization of the *E. rhusiopatihae* antigen was done in order to determine its immunoprotective properties and its immunologic structures.

Materials and Methods

Two vaccines were prepared with aluminium hydroxide gel with two different concentrations of *E. rhusiopathiae* antigen for the study of protection in mice: vaccine A with $8x10^8$ BT/dose and vaccine B with $8x10^7$ BT/dose.

Thirty ICR mice weighing approximately 17-20g were vaccinated with one of the following formulations:

Group	Vaccine	No. animals/group
Group 1	Vaccine A	10
Group 2	Vaccine B	10
Group 3	Placebo	10

Immunoprotective properties were evaluated by immunizing mice subcutaneously with 0.2 ml of each vaccine on day 0. All the animals were challenged intraperitoneally with 0.2 ml of *E. rhusiopathiae* serotype 2 on day 21. The mice were observed for one week and the responses were determined by a quantitative (live-dead) method. Mortality results were statistically analysed using the chi-square method.

The antigen was characterized by SDS-PAGE and Western blotting from the bacterial cell surface as follows. The broth culture was centrifuged at 12.000 g for 20 minutes. The bacterial cells were washed 3 times with PBS. SDS-PAGE was performed by applying 60µg of total protein to 10% polyacrylamide slag gel. The approximate molecular masses were determined by comparing the migration pattern. SDS-PAGE solubilized cell surface proteins of bacteria were subsequently transferred to a PVDF membrane by electrophoresis at 100V for 2 hours.

Results

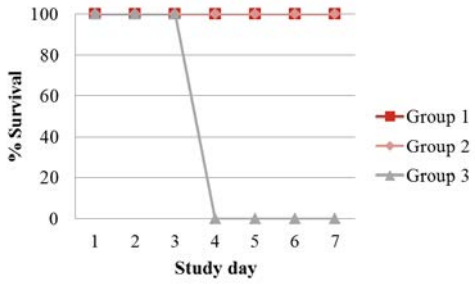
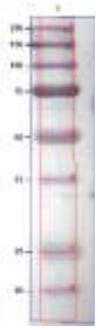


Figure 1. Survival in mice.

There was no mortality in groups 1 and 2 throughout the 7 days post infection. Group 3 showed mortality of 100% at four days post infection. There were statistically significant differences between vaccinated groups and the placebo group ($p<0.01$).



The membrane strip was blocked by 0.5% bovine serum albumin in PBS buffer O/N. It was treated with *E. rhusiopathiae* polyclonal antibody and was diluted 2000 times and incubated at room temperature for 1 hour. After washing, the strip was incubated for 1 hour with Anti porcine IgG HRP Mab. The coloring of the strip was developed by HRP Kit 4CN (Bio rad). An immunodominant band was detected at 65KDa compatible to SpaA protein.

Figure 2. Western blot.

Conclusions and Discussion

Immunoprotective properties of the antigen were demonstrated by the challenge conducted in mice. On the other hand, Western blot analysis of the antigen revealed an immunodominant protein band at 65KDa. All these data showed that the immunoprotective properties of the *E. rhusiopathiae* antigen from ERYSENG® PARVO could be related with the presence of SpaA protein in its composition, which is recognized as the major immunizing antigen of *E. rhusiopathiae*.

References

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Serological and virological evaluation and performance of pigs vaccinated with two protocols of PCV2-Mhyo ready-to-use combination vaccine

C Feronato[1], JTT Fritzen[1], V Saporiti[1], AK Novais[2], CP Dias[2], CA Silva[2], AA Alfieri
[1]*Department of Veterinary Preventive Medicine and* [2]*Department of Animal Science, Universidade Estadual de Londrina, Paraná, Brazil.* cesar.feronato@gmail.com

Introduction

The etiology of the porcine respiratory disease complex (PRDC) involves bacterial and viral agents. *Mycoplasma hyopneumoniae* (Mhyo) is the most important bacterial agent and causes enzootic pneumonia (EP), a chronic respiratory disease that affects mainly growing and finishing pigs[1]. The porcine circovirus type 2 (PCV2) causes respiratory signs and is the principal agent of post-weaning multisystemic wasting syndrome (PMWS), a multifactorial disease with a severe impact on pig production worldwide[2]. On farms where mycoplasma infections occur earlier in life, vaccination is attempted during the first week of life (off label)[3]. The aim of this study was to compare two different protocols (early and label recommendation) with a ready-to-use combination vaccine of PCV2 and Mhyo.

Materials and Methods

The study was performed in a Brazilian single-site pig herd with 2000 sows using 900 piglets. The test was performed using a randomized, non-blinded and controlled design (to control for sow and litter effects), with three groups and an unequal number of repetitions/ treatment. Multiple pens (*n*=9-11) containing 20 animals/pen were used for evaluation of performance parameters. Up to 63 days of age, serological and viremia response were determined by ELISA and qPCR, respectively, on 60 pigs/group per repetition. After that age, up to 30 animals per group were tested. The treatments were:; T1 - vaccinated with Circumvent PCV M on 21 and 42d (Label recommendation); T2 - vaccinated with Circumvent PCV M on 7 and 21d (Off label – as recommended by the herd veterinarian); T3 - Control group, inoculated with saline on 21 and 42d. The animals were bled at 0, 7, 21, 42, 63, 90, 115, and 145 days of age for serological and viral load analyses. The performance and the mortality rate were analyzed during the growing and finishing phases with ANOVA followed by Tukey Test.

Results

The performance results are shown in Table 1.

Table 1. Performance of growing and finishing phases according the treatments (weighed at 63 and 145 days).

Groups	n (pen)	ADFI (g)	ADG (g)	FCR
T1	9	2.235[b]	0.862	2.60[a]
T2	11	2.420[ab]	0.874	2.78[ab]
T3	10	2.588[a]	0.863	3.01[b]
VC %		9.71	6.69	11.50

The vaccinated groups had better PCV2 and Mhyo serological responses than the control group (Figure 1). At 145 days of age, all the groups had a similar PCV2 titer. These results were correlated with the PCV2 viral load at 115 and 145 days, the T3 group had the highest viral load at 145 days; all the groups had no significant difference on viral load at 115 days (Figure 2).

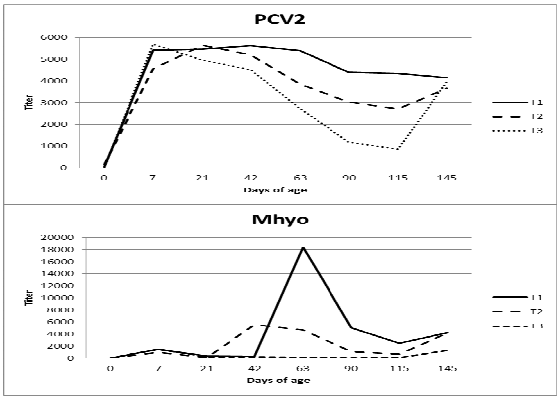

Figure 1. PCV2 and Mhyo antibodies by indirect ELISA (Biochek Inc., NL).

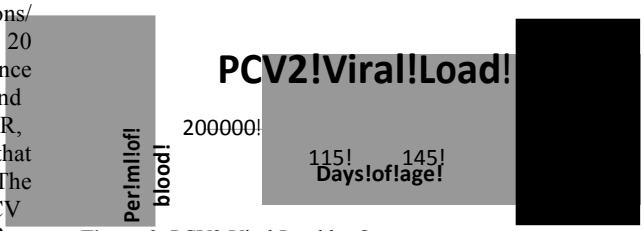

Figure 2. PCV2 Viral Load by Qpcr.

Mortality rate was not different between the various treatments.

Conclusions and Discussion

The T1 group had better serological results than the other groups and had improved feed conversion, an important index for pig production, compared to the control group. In addition, vaccinated pigs in comparison with controls had a lower viral load at 145 days of age which corroborates with a higher immune response. These results support the positive impact of PCV vaccination on return of investment.

References

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Control of PCV2 viral circulation by CIRCOVAC® vaccination in piglets in Danish slaughter pigs throughout the fattening period

P Mortensen[1], R Soegaard[1], T Vila[2], F Joisel[2]
[1]*MERIAL Norden, Copenhagen, Denmark;* [2]*MERIAL SAS, Lyon, France;* preben.mortensen@merial.com

Introduction

The objective of this study was to assess the extent of control of viral PCV2 circulation in several Danish farms throughout the fattening period from 30 – 100 kg of body weight provided by CIRCOVAC following one 0.5-mL dose administration at 3 weeks of age.

Materials and Methods

Eight Danish swine farms rearing pigs vaccinated with 0.5 ml CIRCOVAC at the age of three weeks for at least 6 months were included. The 8 farms included in the study either started PCV2 vaccination or changed from the use of a competitor PCV2 piglet vaccine 6 to 14 months before the study.

In each farm, a cross sectional sampling of 4 sets of 5 blood samples was done for a total of 20 samples per farm. The 4 groups were defined by weight intervals: "30 to 35 kg", "40 to 50 kg", "60 to 70 kg" and "80 to 100 kg". The 5 serum samples from pigs in the same weight interval from the same farm were pooled and assayed for PCV2 viral load by a q-PCR technique and for PCV2 antibody titration by a PCV2 ELISA. The analyses were conducted by DTU-Veterinærinstituttet, Fredriksberg, Denmark (www.vet.dtu.dk).

PCV2 DNA quantitation was expressed in $\log_{10}$ copies. Cut off was below 3 $\log_{10}$, 3 to 5 was low, 5 to 7 was moderate and above 7 was a massive number of DNA copies. Less than 3 is returned as the value 0 indicating a negative finding. The ELISA technique used in the trial gives anti-PCV2 antibody titre results ranging between 0 and 781250. Natural infections usually trigger a 6-digit titre peak for approximately a month contrary to vaccination (unpublished data).

Results and Discussion

The virological and serological results are reported in Tables 1 & 2.

No PCV2 DNA was found in any of the 32 pools of serum assayed thus strongly in favor of the absence of PCV2 circulation in any of the 8 test sites. The serological results confirmed as well the absence of PCV2 circulation since the anti-PCV2 antibodies titres were very low, under the 6 digit titre positivity threshold.

Table 1. PCV2-qPCR values of pooled serum samples in a cross sectional survey in slaughter pigs.

Group	30-35 kg	40-50 kg	60-70 kg	80-100 kg
Farm A	0	0	0	0
Farm B	0	0	0	0
Farm C	0	0	0	0
Farm D	0	0	0	0
Farm E	0	0	0	0
Farm F	0	0	0	0
Farm G	0	0	0	0
Farm H	0	0	0	0

Table 2. PCV2 antibody titres of pooled serum samples in a cross sectional survey in slaughter pigs.

Group	30-35 kg	40-50 kg	60-70 kg	80-100 kg
Farm A	10	10	0	0
Farm B	0	50	50	10
Farm C	0	50	50	0
Farm D	50	50	10	50
Farm E	50	250	50	50
Farm F	50	0	1250	1250
Farm G	250	50	50	10
Farm H	1250	6250	0	50

Conclusion

This survey demonstrated both on virological and serological parameters that an active immunization by 0.5 ml CIRCOVAC at the age of three weeks provides an excellent control of PCV2 circulation throughout the fattening period, thus providing protection against PCVDs.

References

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P.641

Vaccine efficacy against PCV2-related reproductive pathology in gilts

C Bianco[1], S Panarese[1], ML Bacci[1], M Dottori[2], P Bonilauri[2], D Lelli[3], F Ostanello[1], G Leotti[4], T Vila[5], F Joisel[5], G Sarli[1]
[1]*Department of Veterinary Medical Science, Bologna University, Italy; IZSLER Reggio-Emilia[2] and Brescia[3],* [4]*MERIAL SpA, Milano, Italy;* [5]*MERIAL SAS, Lyon, France;* giuseppe.sarli@unibo.it

Introduction

PCV2 is involved in reproductive failure in swine that includes clinical and subclinical forms (1). Subclinical PCV2 *in utero* infection is diagnosed by the detection of PCV2 DNA or specific antibodies in foetal tissues, pre-suckling serum or foetal thoracic fluid without the presence of microscopic lesions or indication of reproductive failure (1). We have evaluated the efficacy of two vaccines against subclinical PCV2-related reproductive pathology and detected modifications in the classic target (lymphoid tissues) of the infected gilts.

Materials and Methods

Specific Ab titres in serum, viraemia, foetus and foetal membranes/fluids samples positivity to PCV2 were compared in four groups of conventional gilts. Six gilts (group VAI) received 2mL, IM of a commercial inactivated PCV2 vaccine licensed for sows and piglets and 6 gilts (group VBI) received 1 mL, IM of a commercial vaccine based on an ORF2 capsid protein expressed in a baculovirus system, and licensed for use in piglets. Both vaccines were administered in gilts at 120 and 150 days of life. Nine additional gilts (group NVI, n=6 and group CTR, n=3) were kept unvaccinated. All animals received Regumate® for 18 days followed by an oestrus synchronization and superovulation protocol. Gilts in VAI, VBI and NVI groups were inseminated with a double (24 h apart) dose of PCV2-negative semen spiked with a PCV2b (0.2 ml of suspension containing $10^{3.9}$ $TCID_{50}$/25 µl of virus) strain isolated in a PMWS outbreak in Italy. CTR gilts were fecundated with a double dose of PCV2-free semen. Necropsies were performed 30 days or 54 days following insemination depending on the gestational status of the gilts: samples of lymph nodes (Lfns: superficial inguinal, mesometrial, tracheobronchial and mesenteric), tonsils and spleen in the dam as well as placenta, amniotic fluid and tissues (heart, liver, spleen) from foetuses were collected for histology, immunohistochemistry to PCV2 and its quantitation by real time PCR. Lymphoid tissue was graded according to a previous system (2). Pearson Chi-square test was used for statistic.

Results

No statistically significant differences in antibody titres nor viraemia were revealed among groups during the trial. Mainly mesometrial (3/12 VAI; 4/12 VBI; 5/12 NVI; 0/6 CTR) Lfns, and in a lesser extent superficial inguinal (1/12 NVI) Lfns, showed grade 3 (depletion and presence of giant cells) while the other lymphoid tissues were normal or at least with grade 1 depletion. Four out of 6 gilts were pregnant in the VAI group, 3 out of 6 in VBI, 3 out of 6 in NVI and 2 out of 3 in CTR, from which were collected 23, 19, 33 and 15 foetuses, respectively and the corresponding placenta membranes and amniotic fluid. See table 1 for results.

Table 1. PCV2 prevalence (real time PCR)

Sample	VAI	VBI	NVI	CTR
Fetuses (%)	21.7^{ab}	36.8^{bc}	48.5^{c}	0.0^{a}
Placentas (%)	73.9^{b}	100^{c}	72.7^{b}	13.3^{a}
Aminiotic fluids (%)	17.3^{a}	68.4^{b}	33.3^{a}	13.3^{a}

CTR group showed the lowest positivity proportion of all sample types compared to the other groups. In the experimentally infected animals, the percentage of positive fetuses was significantly lower in the 2 vaccinated groups compared to NVI, while the percentage of PCV2 positive placentas and amniotic fluids was significantly lower in VAI compared to VBI. Immunohistochemical stain to PCV2 was found in one foetus of the VBI group in placenta, heart and liver and in the superficial inguinal lymph node of a gilt of the NVI group.

Conclusions and Discussion

From the results of the trial it was successfully produced a subclinical form of PCV2 reproductive failure, in which it was possible to demonstrate the presence of PCV2 specific lesion mainly in loco-regional Lfns (those mesometrial). In the present experimental conditions with a very severe challenge, vaccination with a commercial inactivated PCV2 vaccine licensed for sows and piglets (vaccine A) did not eliminate but significantly reduced the risk of foetus infection. Considering the most common way of foetus infection through the foetal membranes/fluids, the protective role of the two vaccines against the subclinical form of PCV2-associated reproductive failure does not seem to be similar. The lowest proportion of placentas and amniotic fluid infected in VAI compared to VBI groups may explain the lowest foetal positivity to PCV2 in this group of gilts compared to NVI animals.

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Characterization of porcine Clec12a gene and expression in lymph nodes tissues

AJ Burgara-Estrella[1], MG Reséndiz-Sandoval[1], IC Rodríguez-Hernández[2], E Rascón-Castelo, V Mata-Haro, J Hernández

[1] *Centro de Investigación en Alimentación y Desarrollo A.C. (CIAD), Hermosillo, Sonora, México.*

[2] *Universidad de Sonora, Hermosillo, Sonora, México*

jhdez@ciad.mx

Introduction

The antigen presenting cells (APC) are essential for the initiation of immune response, since APC expressed a gamma of surface proteins for capture and presenting antigens (1). Type C lectin receptors of the antigen presenting cells have been used as a target to increase the immune response (2). The aim of the present study was to characterize by PCR amplification and sequencing the CLEC12a gene from several nodes tissues of swine.

Materials and Methods

CLEC12a primers were designed based on GenBank sequence XM_003126487. RNA was extracted from swine tissues using Trizol and following the manufacturer´s procedure. The RT-PCR was done with SuperScript II kit from Invitrogen and following the manufacturer´s condition. PCR conditions were: one cycle at 94° C per 3 minutes and 33 cycles a 94° C per 30 seconds, 60° C per 30 seconds and 72° C per 1 minute. One final cycle at 72° C per 10 minutes. Samples were kept at 4° C. The PCR products were electrophoresed on a 2% agarose gel. The PCR product (800 bp) was sequenced in the GATC sequence service from the University of Arizona (USA). The sequence alignment was carried out with the BioEdit program, while the phylogenetic tree was constructed with the MEGA4 software.

Results

CLEC12a gene was amplified from several tissues nodes: submaxilar, inguinal, tonsil, mesenteric. Figure 1 shows PCR product of CLEC12a gene of 800 pb and other product of 650 pb probably an isoform of the gen. The PCR products were purified and sequenced in the University of Arizona. The obtained sequence was analyzed and compared with CLEC12a of others species. CLEC12a gene shows an 80% of identity with *Bos taurus* and *Equus caballus* and 70% and 64% of identity with *Homo sapiens* and *Mus musculus*, respectively. Figure 2 shows the phylogenetic reconstruction of CLEC12a of swine and other species.

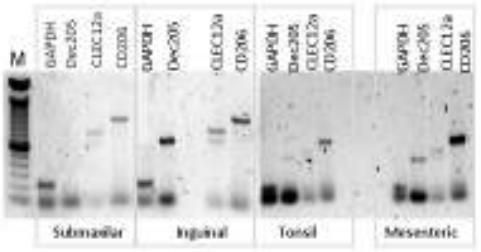

Figure 1. Amplification of CLEC12a from several nodes tissues of swine. Dec205 gene fragment, CD206 gene fragment and GAPDH were included as control. M: DNA ladder.

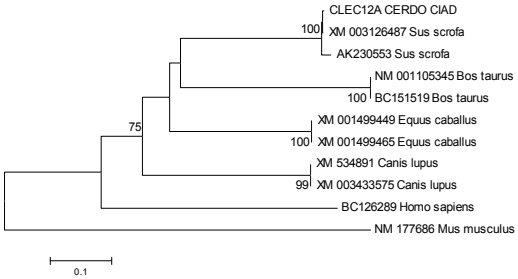

Figure 2. Evolutionary relationship of CLEC12a gene among swine and other vertebrates. Evolutionary history was inferred by Neighbor-Joining method with 1000 bootstrapping. Phylogenetic analysis was conducted in MEGA4 software

Conclusions and Discussion

The gene CLEC12a was amplified and sequenced from several tissues nodes of swine. Some isoforms of this gene have been previously reported; in this study a potential isoform was amplified but it was not possible to obtain the sequence. From previous reports it is known that type C lectin receptor can be used as target to increase the immune response. The confirmation of expression in swine, unlock the possibility to be used as a target receptor in swine vaccination strategies.

Acknowledgements

This study was supported by ANR Francia-CONACYT grant No. 160315.

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Effects of management strategies on abortion episodes and PRRSV circulation in an endemically infected breeding farm

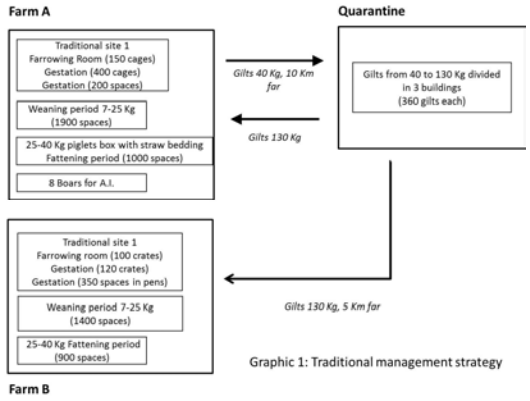
G Veronesi[1], S Faccini[2], I Barbieri[3], F Cominotti[4], S Rosina[4], M Beccalossi[4], AD Nigrelli[2]
[1]*Veterinary Practitioner, Mantova, Italy ;* [2]*IZSLER Diagnostic Section of Mantova, Italy;* [3]*IZSLER Genomic Department of Brescia, Italy;* [4]*MSD Animal Health Italy,* mauro.beccalossi@merck.com

Introduction

Since its appearance, in the first '90, Porcine Reproductive and Respiratory Syndrome (PRRS) has caused huge losses in pig farms. Nowadays, despite all developed efforts and research, PRRS remains a frustrating disease. Furthermore, the real understanding of the pathology is not completely clear. This field study describes an endemically infected breeding farm, before, during and after a significant management change, aimed to decrease the abortions in late gestation stages.

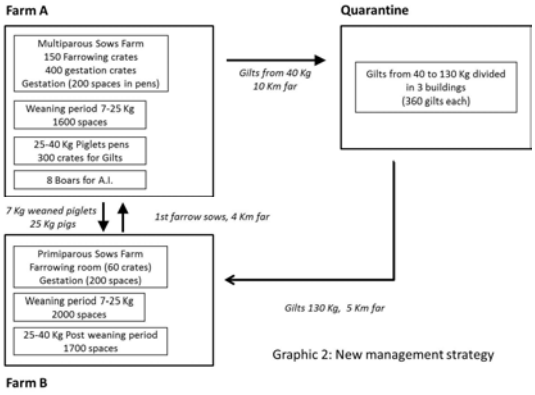
Materials and Methods

The farm was a farrow-to-nursery of more than 700 productive sows, located in a high density swine area of northern Italy. The farm (A) was part of a group, of the same owner, formed by another farrow-to-nursery facility (farm B), and a quarantine unit, both placed at more than 5 Km. The study covers the period from the beginning of 2011 to September 2013. The reorganization, which began at the end of 2011, was completed in the second half of 2012. The purpose of the change was to better separate animals of different age, immune condition, parity and care necessities, a measure known to be useful for improving both production performance and PRRS control. In practice: sows were segregated from gilts (sows in farm A and gilts in B), reducing in the meantime the number of the latter, and weaned pigs were decreased in farm A by transferring them to farm B.



Graphic 1: Traditional management strategy

Periodic blood samplings were performed (>600 samples) for serological and virological analyses. About 95% of sows, in farm A, were found PRRS seropositive (mean S/P±SD 2.2±0.98), but none were demonstrated viremic by Real-Time PCR.



Graphic 2: New management strategy

In contrast, 59% of blood samples collected from sows at time of abortion, during outbreaks, were PCR positive. PRRSV isolates were sequenced (orf5 and orf7) in order to acquire epidemiological information.

Results

The proportion of abortions over farrowings, either in farm A or in the whole group, was significantly ($P<0.01$) reduced after the introduction of the new management system (92/2011 – 58/2012 – 44/2013). No significant change could be reported for other production and reproductive parameters.

Production and Reproduction Data

FARM	TIME	AVERAGE N° PRUDUCTIVE SOW	N° WEANED PIGLETS / PRODUCTIVE SOW / YEAR	N° FARROWS	N° BORN ALIVE PIGLETS / FARROW	N° WEANED PIGLETS / FARROW	N° ABORTION	TOTAL N° WEANED PIGLETS
A	1/1/11 - 8/31/11	751,6	21,55	1134	10,61	8,78	92	9.960
A	1/1/12 - 31/8/12	723,3	23,53	1079	11,48	10,52	58	11.346
A	1/1/13 - 31/8/13	710,6	22,13	1099	10,76	9,54	44	10.483

Conclusions and Discussion

The study demonstrates the effectiveness of the new management strategies in reducing abortions.

Acknowledgments

MSD Animal Health Italy

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Development of a PRRS outbreak investigations program

DJ Holtkamp[1], S Radke[1] C Mowrer[1]; RB Baker[1]; J McKean[1]; R Main[1]; D Polson[2]; JP Cano[2]
[1]*Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA*
[2]*Boehringer Ingelheim Vetmedica Inc., St. Joseph, Missouri, USA* holtkamp@iastate.edu

Introduction

Despite almost three decades of research and experience with the porcine reproductive and respiratory syndrome virus (PRRSV), the frequency of herd-to-herd transmission and consequent outbreaks remains stubbornly high. A 2011 estimate of the cost of productivity losses attributed to PRRSV is over $664 million annually (1). Approximately $300 million (45%) of the 2011 estimate is attributed to reproductive losses in the breeding herd. Furthermore, breeding herds in which PRRS outbreaks occur serve as a major source of virus because they typically wean PRRSV positive pigs for several weeks after the outbreak. The pigs are transported to other locations which very effectively spreads the virus within and between geographic regions. Therefore, the objective of this project was to establish a PRRS outbreak investigations program for breeding herds with the aim of improving biosecurity and reducing the geographic spread of the virus and the duration of time breeding herds remain positive unstable (AASV category I) (2) following outbreaks.

Materials and Methods

The program consists of 1) collecting pre-outbreak data, 2) development of a pre-outbreak investigations report and 3) development of a post-outbreak investigations report. An outbreak investigations coordinator was hired to manage the gathering and reporting of information. A pilot was initiated to further develop and test the PRRS outbreak investigations program.

The pre-outbreak data includes site information, PRRS status history, history of outbreaks and elimination events, historical diagnostic reports, immunization history, historical production records and a current Production Animal Disease Risk Assessment Program (PADRAP) survey. This information must be collected well ahead of time so that it is available when outbreaks occur. The pre-outbreak investigations report functions as a summary of the pre-outbreak data that was collected before the outbreak investigation, including weather data, and as a data collection form for information learned during the outbreak investigation. The information is then pulled from the pre-outbreak investigations form to prepare a post-investigation report that the veterinarian may provide to the producer.

A key element of the PRRS outbreak investigations program was the hiring of a PRRS outbreak investigations coordinator. The coordinator completes the time consuming task of compiling all of the information and generating reports for the herd veterinarians greatly increasing the likelihood that the outbreak investigations will be done in a timely manner.

Results

The program is being piloted in the Buchanan County (13 herds) and Southeast Iowa (6 herds) regional PRRSV projects in Iowa (USA). Data collection began June 1, 2013 and included five years of historical information on immunization protocols, PRRSV elimination events, PRRS outbreaks, PRRSV status changes, diagnostic and virus sequencing information, and PADRAP surveys. As PRRS outbreaks occur, weather data four weeks prior to the outbreak will be compiled and summarized. The pre-investigation report that includes all of the relevant historical information on the herd and the weather data will be compiled by the PRRS outbreak investigations coordinator for the herd veterinarian who will conduct the outbreak investigation.

Conclusions and Discussion

Outbreak investigations help veterinarians and producers better understand how the failure in bio-exclusion occurred. The investigations provide an opportunity for immediate feedback after an outbreak to identify areas of weakness, improve biosecurity and reduce the frequency of PRRS outbreaks in the future. The PRRS outbreak investigations program enables veterinarians and producers to systematically observe and gather information to see associations and patterns when bio-exclusion failures and PRRS outbreaks occur and to more rapidly "learn from our mistakes." Outbreak investigations done in the context of a regional PRRSV project to control or eliminate PRRSV are even more valuable since producers and veterinarians have agreed to communicate and share information with each other. Success of the program will be measured by comparing the historical frequency of outbreaks in breeding herds in the pilot project to the frequency of outbreaks after the outbreak investigation program was implemented.

Acknowledgments

Funding for this study was provided by the Iowa Pork Producers Association (Clive, Iowa USA).

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Two cases report of PED in different states in Mexico

RC Fajardo[1], A Alpízar[1], AC Martínez[2], V Quintero[3], F Diosdado[2], D Córdova[2], S Cuevas[2], AE Díaz-González[1], JL Zamora[1], B Valladares[1], JS Martínez[1]

[1]*Centro de Investigación y Estudios en Salud Animal. CIESA-FMVZ-UAEMex.* [2]*Laboratorio de virología y laboratorio de Epidemiología del CENID Microbiología*. [3]*Práctica privada*, raul_fajard@hotmail.com

Introduction

The virus of porcine epidemic diarrhea (PEDV) causes acute outbreaks of vomiting and severe diarrhea in pigs of all ages, there is a high morbidity (100%) and elevated mortality in young pigs (50-100%). It is transmitted by direct or indirect fecal-oral pathway. Histopathology: It revels an enteritis with atrophy of the villi of the small intestine. The final diagnosis must be made by PCR, as the clinicopathological sings are very similar to those of the porcine transmissible gastroenteritis (PTGE) (2,3). Although PEDV exists in other parts of the world, in May 2013, the first detection of PEDV outbreak was reported in the United States, but it remains unclear how PEDV entered the country and nearly 1,000 confirmed cases of PED was reported in 18 states (3). The DEP has not been reported in Mexico, until July 2013 in the middle area of the country where pigs were symptomatic, and later they were DEPV positive by PCR (1). Here, two outbreaks of PED are described; one was in the La Piedad region and later another in the Estado de Mexico This indicates the presence of this new disease and how it is spreading in Mexico.

The outbreak of the La Piedad was between July and August 2013 on a farm of 200 sows, where greenish diarrhea, vomiting and anorexia in pregnant sows and in growing pigs was observed. Three days later, the piglets showed vomiting and lumpy yellow diarrhea. In 24 hours subsequently, piglets showed severe dehydration, hypothermia and death. Mortality was 100% of the newborn piglets and those born during the following two weeks. In the third week mortality in piglets was 80% and decreased to 30% in the fourth week. The outbreak in the Estado de Mexico was on September 8, 2013 with anorexia, diarrhea, vomiting and dehydration of 2-3 days, with a morbidity of 67% and 27.5% mortality in piglets.

Materiasl and Methods

Necropsy of piglets was performed; frozen tissues for PCR and tissues for histopathology were collected. Tissues for histopathology were fixed in buffered 10% formalin for 24 hours and were treated by conventional histochemistry techniques.

Results

At necropsy, in both cases, severe dehydration, perianal irritation and diarrhea attached to the skin of the body was observed. The mesentery and mesenteric lymph nodes were found very congested, catarrhal enteritis with yellowish diarrhea and intestinal distension was observed. The stomach contained coagulated milk. Histopathology of the small intestine: jejunum and ileum showed severe villus atrophy, characterized by the presence of short, broad and round appearance villi was found. Mild nonsuppurative enteritis with mononuclear infiltration composite in the *lamina propria* was also observed. Molecular tests were positive to PEDV and negative about the porcine transmissible gastroenteritis virus (PTGEV).

Conclusions and Discussion

Due to the great similarity of the clinical and pathological alterations between PTGE and PED, the differential diagnosis between both diseases should include additional tests to demonstrate the presence and/or absence of these etiological agents. This work confirms the presence of the DEPV in farms from two states of Mexico. In addition, it demonstrates how the PED is spreading in Mexico since the first outbreaks in the region of La Piedad and then to the nearby states such as the Estado de Mexico in a short time. It is likely that further outbreaks of PED in other states of Mexico begin to appear. So it is necessary to take urgent measures to prevent and control new outbreaks of PED.

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PEDV surveillance at the Minnesota pork congress

J Ertl, M Culhane, A Rovira, M Torremorell
College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota

Introduction

The 2013 introduction of Porcine Epidemic Diarrhea virus (PEDV) into the United States has put producers and swine professionals on high alert and increased biosecurity measures are in place to minimize the spread of the virus. PEDV is reported to have a long inactivation time at room temperature[1], leading to concerns about the presence of PEDV on fomites outside of swine populated areas as sources of contamination. Hundreds of producers and exhibitors gathered in January at the 2014 Pork Congress held at the Minneapolis Convention Center. Many were concerned that PEDV could be tracked into the convention center by attendees[2]. To address these concerns, environmental samples were collected during the congress from areas of high foot traffic in an attempt to ascertain the presence of PEDV outside the farm environment.

Materials and Methods

Five areas of high foot traffic were identified, including the base of the escalators in front of the showroom, the vehicle tracks at the back vehicle loading dock entrance, the showroom floor at the main entrance, and the tile floor in the men's and women's bathrooms. The environmental samples were taken over the course of three days: the first sampling event at the end of exhibitor load in the day before the congress, and the following two events at the end of activities on both days of the two day congress. Three samples from different points at each high traffic area were taken using sterile 2x2 gauze dipped into a tube of sterile phosphate buffered saline (PBS). An area of approximately 1 square foot was wiped for 20 seconds with the gauze. The gauze was then pressed against the inside of a 50ml tube to release the remaining PBS along with the material collected from the surface. The 50 ml tube containing the environmental material and PBS was submitted for testing. New nitrile gloves were worn for collecting each sample. A negative control (sterile PBS not used to collect samples) was collected before and after the completion of collecting the samples each day. The samples were stored on ice and submitted to the University of Minnesota Veterinary Diagnostic Lab for individual PEDV PCR testing.

Results

There was no PEDV detected in any of the 50 samples, including 44 environmental surface collections and 6 negative controls. One environmental sample was discarded at the lab due to the tube breaking in transit.

Conclusions and Discussion

These negative results suggest the likelihood of PEDV on the floors of high traffic areas at the 2014 Minnesota Pork Congress was minimal. More surveillance studies are needed to better understand PEDV in the environment and to identify other areas of high risk.

Acknowledgements

The authors would like to thank Dave Preisler, executive director of the Minnesota Pork Board, for his support for this study and Drs. Rovira, Torremorell, and Culhane at the University of Minnesota for their assistance in developing an environmental surface testing protocol.

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Serological and molecular overview of swine influenza virus in pigs from Northwestern Mexico (2008-2009)

MG López-Robles[1], M Montalvo-Corral[1], A Burgara-Estrella[1], M Reséndiz-Sandoval[1], H Ramírez-Mendoza[2], J Hernández[1]

[1]*Laboratorio de Inmunología, Centro de Investigación en Alimentación y Desarrollo A.C (CIAD), Hermosillo, Sonora, México.* [2]*Departamento de Microbiología e Inmunología. FMVZ-UNAM, México, D.F.*, jhdez@ciad.mx

Introduction

Swine influenza virus (SIV) belongs to the *Orthomyxoviridae* family; it is easily spread among pigs and it has world-wide prevalence. The virus has a high genetic and antigenic variability due to the elevated rate of mutation (1). The main subtypes circulating in pigs are H1N1, H3N2 and H1N2. Outbreaks on farms are characterized by high morbidity with less than 5% of mortality. In addition, SIV represents a zoonotic risk for people in close contact with infected pigs (2). For this reason, epidemiological surveillance of influenza viruses on swine farms is a valuable tool for public and veterinary health. The aim of the present study was to provide a serologic and molecular overview of SIV in pigs from Northwestern Mexico.

Materials and methods

A cross-sectional study was conducted in 15 commercial farms selected from major producers in Sonora, Mexico, during October 2008 to March 2009. The sample size (n=150) was estimated considering a 30% prevalence of swine influenza infections in farms and a confidence level (CL) of 95%. Nasal swabs and blood samples were collected from unvaccinated pigs. Commercially available ELISA kits (IDEXX) were used for the detection of SIV-specific antibodies. Viral RNA was extracted from each nasal swab sample and rRT-PCR assay targeting the M gene was performed. Positive samples were further subtyped with conventional RT-PCR using previously described primers (3). Also, sequencing and phylogenetic analyses were conducted.

To estimate statistically significant differences between SIV prevalences, we conducted proportions for hypothesis test, and squared Chi (X^2) to infer differences for virus detection with a CL of 95%.

Results

Serological testing showed that 55% of the samples were positive for H1N1, 59% for H3N2 and 38% for both. Seropositivity was observed in all farms with at least one positive sample. The seroprevalence for H1N1 showed an age-dependent decreasing trend ($p < 0.05$), while the H3N2 subtype demonstrated a decrease from 1 to 12 weeks, and a slight rise through the 13th week.

The molecular analyses revealed that 25 out of 150 samples were positive to type M gene (16.6%). Six of them could be subtyped by RT-PCR: four samples were positive for H1 and two were of the H3 subtype. Sequence analysis was done in four samples (three H1 and one H3). Results showed that the influenza viruses H1 circulating in Sonora shared 97-100% of identity at the nucleotide level among them. In the comparison with other sequences, were phylogenetically closer to North American (NA) strains and grouped into cluster α, also were phylogenetically distant to the A/H1N1p2009 viruses. Furthermore, the Sonora strains showed the highest nucleotide identity with A/SW/NE/123/77(H1N1) (89%); and the H3 gene with A/SW/MN/SG-00234/05 (H3N2) (97%).

Conclusions and Discussion

Serological studies have documented that the occurrence of SIV is higher for H1 than H3 subtype (4). The opposite has been observed in Mexico (5) and our results are in agreement with these findings. For H3N2 subtype, no differences are found among age, this could mean that we are detecting seroconverted pigs due more active circulation of H3N2 subtype on the farm than H1N1. However, we detected positive samples for H1 at 3 and 7 weeks-age by RT-PCR. Therefore, as has been described, the method for antibody detection employed in this study has limitations to detect antibodies against all circulating strains of H1 subtype (6). The phylogenetic analyses of the viruses showed that the H1 strains from Sonora were grouped into cluster α following the same genetic analysis previously described (7). However, more sequences of influenza virus from Sonora and other regions of Mexico are necessary, to know the genetic diversity of influenza virus in Mexico.

In conclusion, we confirmed the high circulation of strains similar to the North American lineage among commercial farms in Northwestern Mexico, involving a progenitor virus different to the influenza pandemic of 2009. This study highlights the importance of survey activities for detecting the occurrence and genetic modifications of SIV.

Acknowledgements

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Investigation of the prevalence of *Ascaris suum* infections in Danish finishing herds using a new serological test

B Ellegaard[2], E Vandekerckhove[1], J Vlaminck[1], P Geldhof[1], J Haugegaard[2]
[1]*Laboratory of Parasitology, Faculty of Veterinary Medicine, Ghent University, Belgium;* [2]*MSD Animal Health, Copenhagen, Denmark,* bjarne.ellegaard@merck.com

Introduction

The SERASCA® test is a newly developed serological test that can be used to determine the exposure to both larval and adult *Ascaris suum* in pigs. The test result is an average of 10 blood samples and reflects parasite exposure levels in that specific group or batch of pigs (reported in no/low infection (OD-value < 0,5) or positive for *A. suum* infection (OD-value ≥ 0,5)).

As antibodies persist for several months, a positive reaction reflects that pigs have been infected with *A. suum* during their fattening phase. This information is important, as diagnosis of *Ascaris suum* can be difficult due to the relatively short lifetime of white spots on the liver and the immune systems ability to control intestinal adult worms and shedding of eggs.

In Danish slaughterhouses, no reduction in payment is made for pigs delivered with white spots on their livers. Even though the migration and presence of *A. suum* has a negative impact on daily growth and feed conversion rate[1,2] and seems to reduce the ability to mount an adequate immune response after vaccination against *Mycoplasma hyopneumoniae*[3], there is very limited focus on this infection under Danish conditions. This is also reflected in a very low treatment rate with anthelmintics. Based on an estimation made from prescriptions recorded in vetstat[4], only about 250.000 of the 30 million piglets (1,7%) and 20 million finishers (2,5%) produced are treated with an anthelmintic.

The aim of this study was to use the SERASCA® test to determine the prevalence of positivity in batches of fattening pigs delivered for slaughter.

Materials and Methods

Blood was sampled shortly after killing of pigs at the slaughter line in a large abattoir in Denmark. None of the tested farms were organic farms. Individual farms were identified by their specific number and coded with a running number for future blinded handling and analysis of the samples by the investigator. A total of 174 sets of 10 blood samples were collected and screened with the SERASCA® test at the Laboratory of Parasitology, Faculty of Veterinary Medicine, Ghent University in Belgium. From these 174 sets of samples, 34 sets originated from farms that were sampled more than once and were not included in the calculation of prevalence.

Results

Prevalence of *A. suum* was calculated using serological test results of 140 different farms.. Of these 140 farms, 54 (39%) farms tested positive for exposure to *A. suum* (see figure 1).

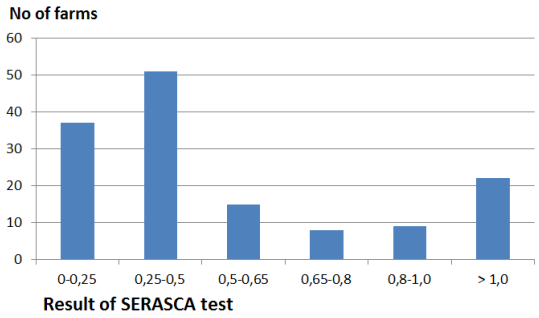

Figure 1. Number of farms in different OD-value levels. OD-values ≥ 0,5 are positive

Conclusions and Discussion

The results of this prevalence study imply that there is a general underestimation of the *A. suum* infection level in Danish fattening farms. In this investigation, 39 % of the investigated finishing farms had *A. suum* infected pigs. Since it has been previously shown that the infections with *A. suum* can cause production losses[1,2] and reduced vaccination efficacy[3], the presence of these roundworm infections in Danish swine industry possibly causes a yet unknown but substantial economical deficit.

The occurrence of *A. suum* infections should be part of a veterinary practitioners' consideration in cases where lack of vaccine efficacy or poor production results cannot be explained.

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Analyzing effects of Aujeszky's disease seropositivity on swine herd productivity

I Yamane[1], H Yamazaki[1], S Ishizeki [2]
[1]National Institute of Animal Health, Tsukuba, Japan, [2]Summit Veterinary Services, Gunma, Japan,
iyamane@affrc.go.jp

Introduction

Despite a series of control programs, Japan remains endemic for Aujeszky's disease virus (ADV). A three-year ADV eradication program was started in 2013 by the animal health section of the Japanese Ministry of Agriculture, Forestry, and Fishery. To motivate farmers to continue with this program, evaluation of the effects of ADV status on herd productivity is crucial. Therefore, we investigated the association between ADV and herd productivity using the benchmarking system PigINFO, developed in 2011[1].

Materials and Methods

The target population was 85 farrow-to-finish herds, all of which were clients of veterinarians of the Japanese Association of Swine Veterinarians. Production variables of these herds from 2011 were calculated using PigINFO. The variables used in this study were marketed pigs per sow per year (MP), postweaning mortality (POWM), preweaning mortality (PRWM), pigs weaned per mated female per year (PWMFY), farrowing percentage (FP), and drug cost per marketed pig (DC). From the targeted herds, at least 3 serum samples were obtained individually from fattening pigs at approximately 60, 90, 120, and 150 days, as well as from low, medium and high parity sows. All sera were tested for antibodies to gI of the wild-type ADV virus using a commercially available competitive ELISA (IDEXX Laboratories, Inc., ME, USA). Herds with at least one positive serum sample were defined as positive. Herds where all serum samples were ADV negative were defined as negative. The effects of ADV status on productivity was determined by comparing means of the study variables between both groups using the Student's t test.

Results

Out of the 85 tested herds, 12 were ADV positive and 73 were negative. ADV positive herds had 2 fewer MP and 2.11% higher POWM compared to ADV negative herds. ADV positive herds also had a 5.11% lower FP and $4.7 higher DC compared with ADV negative herds. No significant difference was observed in PRWM and PWMFY (Table 1).

Table 1. Comparisons of mean production variables between ADV negative and positive herds

	AD status	
Production variables	Negative	Positive
Marketed pigs per sow per year	22.1	20.1**
Postweaning mortality, %	4.73	6.84***
Preweaning mortality, %	10.11	9.72
Pig weaned per mated female per year	23.4	22.3
Farrowing percentage, %	85.8	80.7**
Drug cost per marketed pigs, $	13,43	18,13***

**P<0.05, ***P<0.01

Conclusions and Discussion

These results indicate that having ADV seropositive pigs on a farm may have significant detrimental effects on farm productivity, particularly MP, POWM, FP and DC. This may be because of the direct effects of ADV on reproductive and growth performance, or may be caused by farm differences in factors such as hygiene, resulting in an apparent association between ADV status and productivity. Further analysis after controlling for potential confounding effects would be necessary to confirm these findings. However, this is consistent with previous studies that found similar results when comparing herd PRRSV status to productivity[2]. While single infection is problematic, the synergistic effects of multiple infectious agents may be of greater concern. We are now increasing the number of herds participating in studies using the PigINFO system, which will allow for evaluation of more complex effects of multiple infectious agents on productivity variables.

Acknowledgments

We appreciate the participation of the farmers and the assistance of the Japanese Association of Swine Veterinarians.

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P.651

Serology of trichinellosis in pigs under different husbandry systems in Southwest Nigeria

O Adediran[1], E Uwalaka[1], O Abiola[2]
[1]Department of Veterinary Microbiology and Parasitology, [2]Department of Veterinary Medicine, University of Ibadan,
oa.adedokun@mail.ui.edu.ng

Intoduction

Trichinellosis a disease caused by the parasite, *Trichinella*, has been a major public health problem and has been reported in many parts of the world including Africa (6). It is a zoonotic disease considered as a re-emerging disease in developing countries (3). Focus on control has been to eliminate *Trichinella* from the food chain especially in pigs.

Materials and Methods

Sera form pigs raised under intensive, semi-intensive and extensive management systems between January and December 2010 were analysed using commercial ELISA kit (Prionics Lelystad B. V.Netherlands), Statistical analysis was done using 1-way ANOVA and Student's *t*-test.

Results

Forty- nine pigs were seropositive to *Trichinella* out of the 450 pigs (10.89 %).

Table 1. Seroprevalence of trichinellosis in pigs under different management systems

Farming systems	No. of samples examined	No. positive (%)
Extensive	180	23(12.80)
Semi-intensive	170	17(10.00)
Intensive	100	9(9.00)

Adults had 12.44%, growers 8.28% and weaners 12.50% prevalence while females and males were 9.55% and 12.17% respectively. The results were not significantly different ($p>0.05$)

Conclusion and Discussion

Molyneux *et al* (2011) observed that zoonotic diseases in the tropics have been neglected more than other tropical neglected diseases and indeed trichinellosis in Nigeria especially in pigs have not received much attention. Apart from (2), the authors also carried out a seroprevalece study on slaughtered pigs (1) . ELISA adds a significant level of sensitivity and specificity to the detection of *Trichinella* infection in pigs (7) hence the higher prevalence compared with the 5.21% by (2) who used the pepsin digestion technique. *Trichinella* infection in pigs is influenced by housing systems, (5) identified faulty hygienic conditions in husbandry practice as the main epidemiological factor. Intensive management system should preclude the risk of *Trichinella* infection in pigs, however, the result of this study calls for concern and confirms that pigs in Nigeria cannot be considered to be entirely *Trichinella* free.

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Degradation of swine residues by composting I: Rate of C/N change

A Vargas[2], ME Trujillo[2], LB Reyes[1], A Ciprián[1], E Hernández[1], C Moreno[1], S Valdés[1], E García[1], S Mendoza[1]
[1]*Facultad de Estudios Superiores Cuautitlán-UNAM,* [2]*Facultad de Medicina Veterinaria y Zootecnia- UNAM, México,*
cachemira3@yahoo.com.mx

Introduction

Swine industry generates organic residues like dung, wastewater, stillborn, afterbirth and mortality which can be infected with pathogens (4). Composting animal mortalities, has multiple advantages in comparison with burning and burial of the carcasses, but for adoption of the process, is necessary to prove it´s degrading capacities in an easy to understand way. The main way to monitor the process is measuring the temperature inside the piles along a period of time (3). The carbon to nitrogen ratio (C/N) can be used to know the usage of the macromolecules by degraders microorganisms (2). Finally, if the composting process is in the right course, the direct analysis of the carcasses allow to evaluate the loss of mass ocurred (1).

Materials and Methods

A pilot compost pile was built of 116 kilograms (k). The raw material was wastewater from a pig farm, sawdust and mature compost as amendment. Two pig heads were co-composted. The temperature inside the piles was monitored using an infrared digital thermometer (STEREN:100). Sixteen samples were taken from specific degradation zones inside the pile at 0, 14, 28, 42 and 56 days of the mixing of the raw materials. This composite sample was dried (105 ^{0}C x 72 h), passed through a metal 0.5 inch sieve and stored at 5 ^{0}C. The analysis performed to samples were: total nitrogen Kjeldahl (NKT) using standardized technique (H_2SO_4-digestion; H_3PO_3-destilation; HCl-titration) and volatile solids (520 ^{0}C x 6 h). To obtain the total organic carbon (COT), the volatile solids were divided by 1.73 (5). During sampling, pig residues were collected and weighted using an analytical scale (SC4010; OHAUS).

Results

The rise of temperature (55 ^{0}C) inside the pile was achieved in 5 days and the thermophilic temperature match the environmental temperature in 56 days. The greatest percentage of loosed weight was achieved in the early stage of the process (Fig 1). The evolving C/N rate, showed a decreasing tendency along time (Fig 2).

Conclusions and Discussion

The temperature trends indicate the level of activity by microbial populations inside the piles. The loss of mass evidence the oxidization-mineralization process of the macromolecules (solids, sawdust and residues) by microbial activity. The reduction in C/N ratio indicate the loss of carbon by emission of the carbon dioxide and the maintenance of the level of nitrogen for biomass production. This findings together showed the degradation inside the piles since the begining of the process. However, to obtain this performance is necesary to mix the raw materilas in an apropiatte ratio (25:1) to secure a bioavaliability of nutrients for microorganisms.

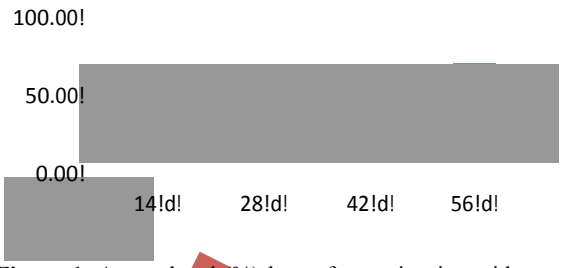

Figure 1. Acumulated (%) loss of mass in pig residues. *Initial weight: 6,015 g ; final weight 768 g*

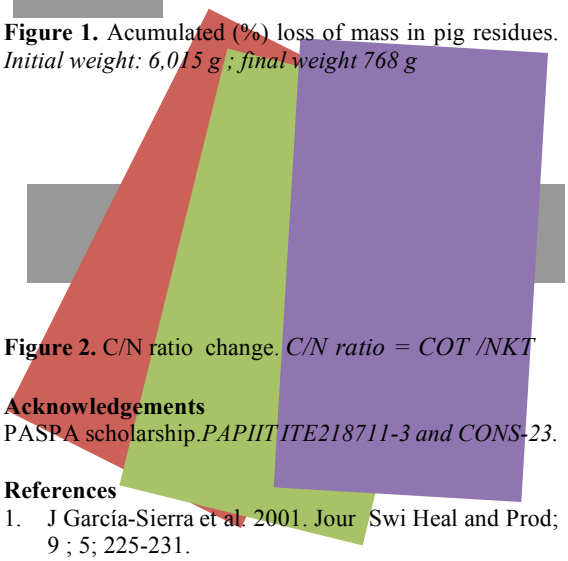

Figure 2. C/N ratio change. *C/N ratio = COT /NKT*

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Degradation of swine residues by composting II: Use of bioassays

A Vargas[1,2], ME Trujillo[2], LB Reyes[1], S González[1], E Hernández[1], E García[1] A Ciprián[1], S Mendoza[1]
[1]*Facultad de Estudios Superiores Cuautitlán-UNAM,* [2]*Facultad de Medicina Veterinaria y Zootecnia- UNAM, México.*
cachemira3@yahoo.com.mx

Introduction

Swine farms generate mortality and organic residues which impact the environment if they are not managed properly. Composting transform them in mineralizable forms with high bioavailability to producer organisms, however, during the initial phase of the process, intermediate products, as ammonium salts, are generated. These products are toxic for animals living in soil and some seeds if the immature compost added as an amendment. During the composting, there is a transformation of the protein contained in raw material (pig carcasses and organic matter) into useful forms of nitrogen (N) for the plants, but the presence of intermediate products, indicate that the process is happening in the right way but it is the beginning of the decaying of the proteins. Measuring direct or indirectly the presence of nitrogen forms is the key strategy to know the maturity of a heap of compost.

Materials and Methods

One compost pile of 116 kilograms was built with sawdust, wastewater solids of a pig farm and mature compost in a rate of 1:1:1 (w:w:w). Tap water was added to adjust the relative humidity (RH) in 60%. Additionally, two pig heads were co-composted. A composite sample was conformed from samples obtained (*n=16*) in specific zones inside the pile (1) in days 0, 14, 28, 42 and 56 of the mixture of the raw material. The pH (CONDUCTRONIC pH10;USA) and the electrical conductivity (EC; HANNA;USA) were monitored. To demonstrate the toxicity of compost during the early phase of the process, were used 6 mature *Eisenia andreii* earthworms, randomly assigned to one of two treatments: T1 consisted in addition in plastic boxes of 50 grams (g) of mature compost (MC) plus 200 g of composite sample. T2 or control received just 250 g of MC (45 days of composting plus 45 days of vermicomposting). The earthworms were weighted in days 7 and 14. The RH and the environmental temperature, inside the containers were controlled.

Results

The results showed a high mortality of the earthworms in the 14 d compost sample. After this time, the mortality was not seen (Table 1). The pH trend along the time showed an increase just in the 14 d compost, after this time, the pH decrease to a neutral level. The EC begin to increase in the 14 days sample, afterwards, the level was maintained to the final of the trial.

Table 1. Survival test and characteristic of composite samples

	Days of composting				
	0	14	28	42	56
Worm survival %					
Pig residues compost	100^a	0^a	100^a	100^a	100^a
SD	0	0	0	0	0
Control compost	100^a	100^b	100^a	66.6^b	66.6^b
SD	0	0	0	16.66	16.66
Physicochemical characteristics					
pH	7.2	8.0	6.9	6.8	6.6
SD	0.02	0.00	0.01	0.00	0.01
EC (µS/cm)	441.3	590.3	568.0	658.0	630.6
SD	9.01	21.45	27.87	31.09	7.09

a, b. Superscripts indicate statistically significant differences in columns. (p<0.05) t-test. SD = Standard deviation of three replicates.

Conclusions and Discussion

The results indicate the presence of N salts (3) as factors affecting the viability of the worms during the early stage of the degrading process. After this period, during the nitrite formation, and ammonia assimilation by microorganisms (4), the survival increased, due to the presence of N forms free of risk to them. The apparently better survival of worms of T1 in 42 and 56 days samples was a natural process due to de depletion of nutrients in MC (2). So, the above results induce to continuing with additional research to recognize the precise forms of N evolved inside a compost heap and the usage of vegetable seeds to evaluate the phytotoxicity of the compost in early stages of the composting process.

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The likelihood and consequences of PRRSV introduction into Australia

E Neumann[1], W Hall[2], R Morris[3], BO'Leary[4]
[1]EpiCentre, Massey University, Palmerston North, NZ, [2]William Hall and Associates, Googong, NSW, Australia,
[3]MorVet Ltd, Masterton, NZ, [4]EpiSoft Ltd, Palmerston North, NZ, e.neumann@massey.ac.nz

Introduction

New Zealand, a country free of PRRSV, recently modified their Import Health Standards (IHS) for fresh pig meat to allow importation of fresh, untreated pork originating from PRRS positive countries. In addition to traditional risk mitigation steps such as cooking and curing, the IHS authorized use of a novel risk mitigation measure *'PREPARATION as consumer-ready cuts packaged for direct retail sale, not including minced (ground) meat, not including the head and neck, and not exceeding 3 kg per package'* (1).

Australia is also free from PRRSV. As New Zealand and Australia historically consult closely in biosecurity matters, there was interest in determining the likelihood of PRRSV introduction into Australia if similar IHS were applied to pork imports into Australia. A project was undertaken to investigate the risk and consequences of PRRSV introduction into Australia through fresh pig meat imported under conditions similar to those described in the New Zealand IHSs.

Materials and Methods

A quantitative stochastic model was constructed using @RISK 5.7 to determine the frequency with which 'the first pig holding in Australia' was likely to become infected with PRRSV through the importation of virus contaminated pig meat. The model described a chain of sequential events and their associated probabilities, beginning with importation of contaminated raw pig meat and ending with consumption of an infectious dose of PRRSV in food waste by a backyard pig (BYP). The model considered amount and type of pork consumed by Australians, estimated the amount of fresh pork scrap that would be generated by those households, added a variable amount of fresh pork swill generated by food service and fed to BYPs, then estimated the total annual number of swill meals that would be consumed by Australian BYPs. By further estimating the number (and size) of BYP herds in the country and the likelihood of infection occurring when a PRRSV containing swill meal was consumed by a BYP, the annual number of PRRSV incursions onto BYP herds was estimated. Outbreak simulation modelling (2) was then undertaken in order to estimate the likelihood and consequences of a multi-farm outbreak of PRRSV occurring in Australia.

Results

As a result of the approximately 200,000 'swill meals' containing untreated, PRRS-infected pork that were estimated to be fed to BYPs each year, the median interval between PRRSV incursions was estimated to be 1.14 years (interquartile range = 0.15 to 6.20 years. Once the likelihood of an incursion of PRRSV had been established, information about the pattern and frequency of contact between different classes of pig farms was combined with actual locations or near-estimates of all commercial and BYP herds to estimate the frequency and spatiotemporal characteristics of a potential multi-farm outbreak of PRRS. Separate outbreaks originating in each of NSW, QLD, VIC, SA, TAS, and WA were simulated.

In approximately 25% of the simulations, the outbreak did not spread beyond the primary BYP outbreak site. However, in most of the simulations the outbreak was transmitted to multiple commercial and BYP farms, and frequently spread into a neighbouring State. The median sizes of the uncontrolled outbreak simulations ranged from 76 herds (TAS) to 323 herds (QLD) with total outbreak size being approximately proportional to the number of pig herds in each State (Table 1).

Table 4. Estimated number of infected herds by farm class, for 99 simulated outbreaks of PRRS in each State.

	NSW	QLD	SA	TAS	VIC	WA
Median	260	323	82	76	182	90
75th	438	629	322	115	526	210
25th	51	3	2	2	3	8
Mean	277	388	181	76	302	124

Conclusions and Discussion

On-going investment by the industry in biosecurity compliance, identification of both commercial and BYP pig herds, and consultation with government import policy makers will be important in managing the potential risks associated with importation of animal-related risk goods such as fresh pork.

Acknowledgments

The authors wish to acknowledge the assistance of Australian Pork Limited for assisting in describing farm locations.

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Descriptive and temporal analysis of post-mortem lesions recorded in New Zealand slaughtered pigs in New Zealand from 1999-2010

E Neumann[1], W Hall[2], M Stevenson[1], R Morris[3], JLM Than[1]
[1]*EpiCentre, Massey University, Palmerston North, NZ,* [2]*William Hall and Associates, Googong, NSW, Australia,*
[3]*MorVet Ltd, Masterton, NZ, e.neumann@massey.ac.nz*

Introduction

Programmes have been established in many countries around the world to collect data at abattoirs that describe the presence of lesions or disease agents in pig carcasses associated either with poor meat quality or that present a risk to human health. Less frequent are standardised programmes to collect information from abattoirs that can inform producers about the presence or frequency of production diseases (1, 2, 3, 4).

In 1997, the New Zealand Pork Industry Board (NZ Pork) developed a formal national programme called 'PigCheck' to survey for the presence of important production limiting diseases in market weight pigs delivered to commercial abattoirs.

The objective of this project was to complete a retrospective analysis of the existing data from the PigCheck recording system with the specific aims of establishing the prevalence of 20 post-mortem disease lesions, describing any long-term trends in the prevalence of these lesions, and identifying the percentage of the monthly variation in lesion prevalence that could be attributed to farms versus abattoirs.

Materials and Methods

Slaughter lesion data were collected and reported at the lot level (a cohort of pigs delivered from one farm, at one time). Data on the prevalence of lesions between January 2000 and December 2010 were aggregated by month, and time-series analysis of the data for each lesion was conducted. The time series pattern for each lesion was described with an autoregressive integrated moving average (ARIMA) model; seasonality of lesion occurrence was assessed separately. To determine the proportion of variance in lesion prevalence that could be attributed to farms relative to that attributed to abattoirs, a hierarchical binomial generalised linear mixed model was created incorporating two random effect levels, at the farm (within abattoir) and abattoir levels.

Results

A dataset comprised of 124,407 lots (6,220,664 pigs, 279 farms, five abattoirs) was compiled for analysis. The most prevalent conditions across the 11-year time series were antero-ventral pneumonia (7.6%), pleuropneumonia (11.4%), and milk spots (9.2%). Of the 15 lesions shown to have a significant annual change in prevalence, 10 decreased over time and five increased. The variance in prevalence that was observed for pyogenic lesion (92%), mange (73%), and ileitis (62%) was attributed primarily to variation between abattoirs (Figure 1). By contrast, the farm of origin explained the greatest percentage of variance in prevalence for rectal prolapse (98%), pneumonia (97%), and antero-ventral pneumonia (96%).

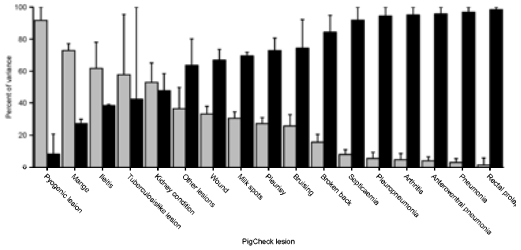

Figure 5. Percentage of variance in lesion prevalence attributable to farm (black bars) or abattoir (grey bars).

Conclusions and Discussion

The overall prevalence of most lesions recorded in PigCheck for the period was low relative to published data from other countries. Common lung pathologies contributing to lesions such as antero-ventral pneumonia and pleuropneumonia were primarily a function of farm management and were not likely due to variability in lesion recording at different abattoirs.

Based on the low frequency of lesions in pigs at commercial abattoirs, the health status of pigs in the New Zealand pig industry is considered to be very good. Pneumonia, pleurisy, and ascariasis are some of the most prevalent conditions that should be focussed on through development of herd health management plans.

Acknowledgments

The authors wish to acknowledge the assistance of AsureQuality in accessing data for this study and the New Zealand Pork Industry Board for negotiating confidential access to the PigCheck data of its registered members.

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Canadian swine health intelligence network

C Byra[1], J Berezowski[2], E Brockhoff[3], D Hurnik[4], C Klopfenstein[5], H Kloeze[6], L Bergeron[7], G Charbonneau[8], F Cardinal[9], T Herntier[10], Iqbal Jamal[11]

[1]*Canadian Swine Health Board, Chilliwack, BC, Canada,* [2]*Veterinary Public Health Institute, University of Bern, Bern Switzerland,* [3]*Prairie Swine Health Services, Red Deer, AB, Canada,* [4]*Atlantic Veterinary College, University of Prince Edward Island, Charlottetown PEI, Canada,* [5]*Le Centre de développement du porc du Québec, Québec, PQ, Canada,* [6]*Canadian Food Inspection Agency, Owen Sound, ON, Canada,* [7]*Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, Québec, Canada,* [8]*South Western Ontario Veterinary Services, Stratford, ON Canada,* [9]*Consultants Avi-Porc, Drummondville, Québec, Canada,* [10]*FD Solutions, Winnipeg MB, Canada,* [11]*AQL Management Consulting, Edmonton, AB, Canada,* byra@cshin.ca

Introduction

The Canadian Swine Health Intelligence Network (CSHIN) is a national swine surveillance network that was designed primarily to help veterinarians deal more effectively with diseases on their clients' farms, while at the same time providing other benefits such generating information to support trade.

Materials and Methods

The CSHIN is made of two integrated networks:

1. Swine Veterinary Network (SVN), a network of veterinarians and swine specialists
2. Practice Based Surveillance (PBS) network, a web-based veterinary practice data collection, analysis and reporting system

These two networks work together to produce and communicate information to swine veterinarians, producers, governments, researchers and other stakeholders.

The SVN is made up of three regional networks that form one national network. The three regional networks are: 1) Western Provinces, 2) Ontario-Maritimes and 3) Quebec (Réseau porcin). Three regional meetings and one national meeting are held every three months to discuss changing health issues. Participants in regional meetings include a small group of veterinarians plus a pathologist, epidemiologist and other experts (as needed). Meetings are web-hosted and all three regional meetings occur within a one week period. Prior to each meeting a web-based clinical impression survey is filled out by members of the regional networks. The survey data is collated, diagnostic laboratory data is added and information is prepared for each meeting. Participants are allowed to nominate for discussion, health issues that they feel are most important. Following the completion of the regional meetings, representatives from each region meet with members of the national network to discuss disease problems at a national level and international level. Regional reports are produced for distribution to veterinarians within each region and a national report is produced for more widespread distribution.

The PBS system collects data through a web-based practice management software application. Practicing veterinarians enter data collected during interactions with their clients. Data includes clinical estimates of the prevalence of each of 13 clinical syndromes (coughing, lameness, morbidity, scours etc.) within each of 7 possible subpopulations (boars, sows, nursery, grower etc.). Also included are veterinarians' clinical diagnoses and the results of any laboratory testing, which are received at a later time. The data is transported electronically to a secure CSHIN server every night. To protect the confidentiality of farmers, there is no farm identifying data sent to the CSHIN server. At the CSHIN server the data is automatically processed into reports that include National and regional tables, charts and maps. A daily interactive report is sent to CSHIN managers and more detailed reports are available on the CSHIN server.

Results

Participation in the SVN has been strong during the last meeting there were 51 veterinarians from across Canada that completed the last clinical impression survey. Topics discussed in previous and current meetings included infectious diseases such as Lawsonia intracellularis, Swine Influenza, Strep. suis, Swine dysentery, and Porcine Circovirus 2 (PCV2), as well as other health issues such as chest adhesions and head demerits seen at processing plants.

Participation in the PBS network has increased steadily and data is being collected from all swine producing regions of Canada. Many reports are being produced and distributed including: disease syndromes, and clinical diagnoses by production type aimed at setting benchmarks and identifying emerging problems.

Conclusions and Discussion

At this time, practicing veterinarians from most parts of Canada are supportive of the CSHIN, as evidenced by their participation. However, continued support will require the production of information that is meaningful to them. This is a critical on-going task for the CSHIN.

Acknowledgements

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Wild boars as a reservoir of *Leptospira* in Poland

A Jablonski[1], J Zmudzki[1], D Borowska[1], Z Arent[2], S Zebek[1], Z Pejsak[1]
National Veterinary Research Institute, Swine Diseases Department, Pulawy, Poland[1], Leptospirosis Reference Laboratory, Agri-Food and Biosciences Institute, Belfast [2], artur.jablonski@piwet.pulawy.pl

Introduction

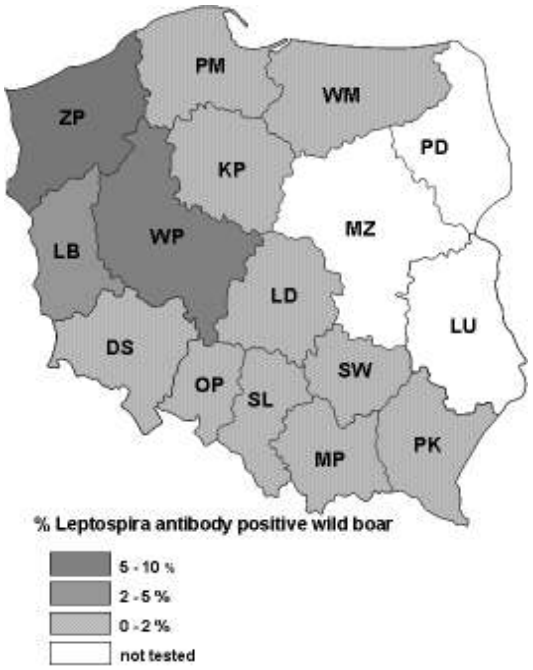
Leptospirosis is widely distributed zoonotic disease caused by pathogenic serovars of the genus Leptospira. Important reservoir animals of leptospirosis for humans transmission are rats and mice, domestic animals such as cattle and swine and companion animals. In wild boar (*Sus scrofa*), antibodies against leptospires have been detected. Previous survey on leptospirosis in wildlife in Europe have been limited to some coutries and regions. An early study in Poland reported overall seroprevalences of 24% in one region (northtern Poland) but based on limited number of serum samples (4). The purpose of this study was to investigate the seroprevalence of *Leptospira sp*. infections in wild boars.

Materials and Methods

A total of 1000 serum samples were collected from wild boars. The wild boar sera were obtained during classical fever monitoring program between 2012 and 2013. Serum samples were taken from 44 to 148 wild boars from each of 13 Polish provinces. Sera were screened against a panel of 7 leptospiral serovars using the microscopic agglutination test (MAT). The panel represented the following serovars of Leptospira interrogans: Bratislava (strain Jez Bratislava), Icterohaemorrhagiae (RGA), Pomona (Pomona), Grippotyphosa (Moskwa 5), Sejroe (M84), Tarrasovi (Perepelicyn), Canicola (HondUtrecht4). The minimum sera dilution was 1:30.

Results

Overall, 19 (1.9%) serum samples were positive against *Leptospira* spp. Of these 19 samples, 9 demonstrated cross-reactivity with antigens of other serovars. Among the samples without cross-reactivity, *Leptospira* spp. serovar Bratislava (n=5), serovar Icterohaemorrhagiae (n=2), serovar Pomona (n=1), serovar Tarrasovi (n=1), and serovar Sejroe (n=1) were identified. Titers of leptospira-positive serum samples varied from 30 through 300. The samples with anti-serovar Bratislava antibodies (9 samples at 1:30 and 5 at 1:100) and anti-serovar Pomona (3 samples at 1:30, 2 at 1:100 and 2 at 1:300) were the most frequently identified. From the panel of 7 leptospiral serovars, no seropositive reactions to serovar Canicola were detected. The highest percentages of seropositive wild boars of 7.7%, 5.5% and 2.1% were found for Greater Poland (WP), West Pomeranian (ZP) and Lubusz (LB) provinces, respectively (north west).



Conclusions and Discussion

The results of our study show the presence of seropositive samples tested for serovars of Leptospira in wild boars in Poland. However a lower prevalence and lower antibody titers, especially compared with reports from continental Europe (Italy - 6% (1), Spain - 14.6% (2) Germany - 18% (3), were detected. Presented data based on relatively small number of serum samples are only the preliminary study and will be continued with much more samples, serovars and using wild boars density data.

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Kernel spatial analysis applied to management of health protection of swine in Minas Gerais, Brazil

J Gonçalves[1], M Oviedo[2], J Haddad[2]
[1]*Instituto Mineiro de Agropecuaria,* [2]*Department of Preventive Veterinary Medicine, University Federal of Minas Gerais, Belo Horizonte,MG, Brazil* juniapmg@yahoo.com.br

Introduction

In this work a spatial analysis by the method of Kernel on commercial and subsistence farms of pigs from an official database of the state government of Minas Gerais, Brazil was performed. The objective was to verify the existence of cluster of farms containing pigs to improve the management of health suidea with planning actions and prevention.

Materials and Methods

The secondary database of Instituto Mineiro de Agropecuária - IMA was utilized. 1300 commercial farms and 2000 subsistence farms of pigs was analyzed. Spatial analysis by the Kernel method for verification of clusters was performed in ArcGIS V9.3

Results

Initially was obtained a distribution of points of geographical location of commercial farms and subsistence farms of pig (Figure 1). There was a concentration of farms with pigs mainly in the south, southeast and southwest of the state.

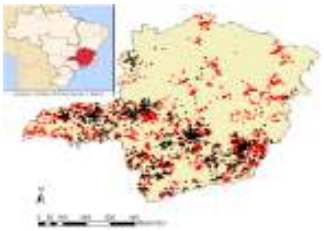

Figure 1. Distribution of points of geographical location of commercial farms (black) and subsistence farms (red) of pig in Minas Gerais, Brazil.

The areas of the cluster using ArcGIS been identified. The spatial autocorrelation by Local Moran Index - LISA and Kernel function was used to determine the intensity of the concentration of the number farms pigs. The geographic coordinates of latitude and longitude were transformed into UTM zone 23 South.
In these cases, the search radius (bandwith) of 32 km was taken. The method of classification of intervals of categories was natural breaks. The Kernel density map for the distribution of farms and subsistence farms of pigs was obtained for the state of Minas Gerais (Figure 2)

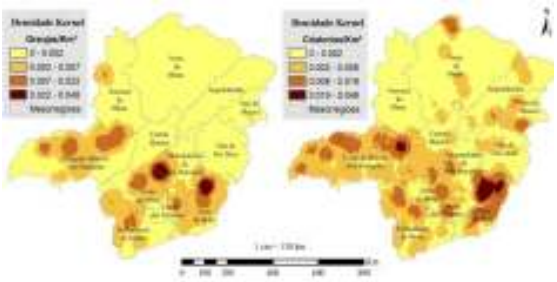

Figure 2. Kernel density map for the distribution of commercial farms (granjas) and subsistence farms (criatorios) of pig in Minas Gerais, Brazil.

Conclusions and Discussion

This spatial study allowed a preliminary assessment of data from commercial farms and non-commercial farms. The spatial analysis power [to be used to correlate other variables in the database by Kernel method and improve the management of health protection for the flock of pigs

Acknowledgments

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Serological study of influenza viruses in veterinarians working with pigs in Mexico

M Saavedra-Montañez[1], H Castillo-Juarez[2], F Rivera-Benitez[1], ME Manjarrez[3], I Sánchez-Betancourt[4], H Ramírez-Mendoza[1]

[1]Departamento de Microbiología e Inmunología, FMVZ-UNAM. [2]Departamento de Producción Agrícola y Animal, UAM Xochimilco. [3]Instituto Nacional de Enfermedades Respiratorias. [4]Departamento de Medicina y Zootecnia de Cerdos FMVZ-UNAM. Correspondence: Dr. Humberto Ramírez-Mendoza, betosram@yahoo.es

Introduction

Occupational exposure to pigs considerably increases the risk of infection with the swine influenza virus.[1] On the other hand, despite the fact that positive serology and sporadic isolations of the swine influenza virus have been reported in humans in several countries. [2,3,4] The seroprevalence in swine specialist veterinarians remains unclear in Mexico. The objective of this study was to determine the seroprevalence of human influenza viruses pH1N1 (pandemic) and hH1N1 (seasonal), as well as of the swine influenza viruses swH1N1 and swH3N2, in swine specialist veterinarians in Mexico.

Materials and Methods

We processed a total of 85 sera samples obtained from veterinarians. The following influenza subtypes were used as antigens: seasonal human influenza (hH1N1) A/Mexico/INER1/2000 (H1N1), pandemic influenza (pH1N1) A/Mexico/LaGloria-3/2009 (H1N1), classical swine (swH1N1) A/swine/New Jersey/11/76 (H1N1) and a triple-reassortant swine influenza virus (swH3N2) A/swine/Minnesota/9088-2/98 (H3N2). We used the procedure established by the WHO,[5] with the following modifications: hemagglutinating units were adjusted to 8 and the titers of sera were considered positive if they were ≥ 1:80. Statistical analysis: Each subjet who provided a sample also answered a questionnaire that was used to analyze variables. A generalized linear model was used to assess seropositivity and its association with different factors.

Results

The recorded seroprevalence against viruses pH1N1, hH1N1, swH1N1, and swH3N2 is presented in Table 1.

Table 1. Seroprevalence and seropositivity against human and swine influenza viruses in swine specialist veterinarians.

Variable	n	pH1N1	hH1N1	swH1N1	swH3N2
Total number of analyzed veterinarians	85	-	-	-	-
Seropositivity	-	10	65	38	10
Seroprevalence (%)	-	11.7	76.4	44.7	11.7

Statistical analyses revealed that vaccination had a significant association with pH1N1seropositivity in all veterinarians ($P < 0.05$). Age was significantly associated with hH1N1 seropositivity in all veterinarians ($P < 0.05$). In the immunized veterinarians, sex, years of veterinary practice with pigs, region, and biosafety level were significant factors ($P < 0.05$). When analyzing only non-vaccinated veterinarians, sex and region were statistically significant factors ($P < 0.05$).

Conclusions and Discussion

In the present study, we detected antibodies against subtypes pH1N1, hH1N1, swH1N1, and swH3N2 in veterinarians working with pigs in Mexico, in titers similar to those reported in other countries,[6,3,7] the average antibody titers being the highest for subtype hH1N1 followed by subtype swH1N1. In this serological evaluation, we used lineages that circulate in Mexico, in contrast to European lineages considered in other publications.[8,9] Vaccination was significantly associated with pH1N1 seropositivity. Similarly, age, sex, number of years of veterinary practice with pigs, region, and biosafety level were significantly associated with hH1N1 seropositivity; years of veterinary practice with pigs was significantly associated whit swH1N1 seropositivity; and finally, sex and region were significantly associated with swH3N2 seropositivity. The generated information could prove instrumental determining the seroprevalence of the swine influenza virus among veterinarians in Mexico and establishing the associated risk factors.

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Acknowledgments

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Impact on farm management of the use of buserelin (PORCEPTAL®) in a single fixed time insemination program in sows

E Sallé [1], M-A Driancourt[2], P Baldwin[2], M Collell[3]
[1] MSD Santé Animale, Beaucouzé, France, [2] MSD Animal Health Innovation, Beaucouzé, France, [3]MSD Animal Health, miquel.collell@merck.com

Introduction

The trend for increasing the size of modern swine herds is still continuing. Use of the batch management system offers farmers the opportunity to concentrate specialized work on specific days of the week, or in a specific week. In particular it allows tasks such as estrus detection, performing AI and assisting farrowing to be planned so that skilled people dedicated to these tasks can be available at the right time.

But some parameters are still not predictable, e.g. the exact time of ovulation after estrus is a naturally variable parameter loosely related to the weaning to estrus interval. Optimal fertility occurs when AI is performed approx. 8h before ovulation [1]. Consequently 2 or more AIs are routinely done in the hope that one falls at the right time. This is an inefficient part of swine breeding.

MSD Animal Health has developed a single Fixed Time Insemination program (sFTI) using a GnRH agonist, buserelin (Porceptal®) injected 86+/-3 hours post weaning to induce ovulation, followed by a single AI 30-33hours later. The technical results between classical breeding programs (estrus detection + 2 AI) and the sFTI program with Porceptal® were statistically equivalent for fertility and prolificacy [2].

The present study aims to clarify the interest of a sFTI program in farm management, at breeding time as well as at farrowing.

Materials and Methods

The clinical field trial was run in 6 commercial herds in France, Spain and Germany. At weaning, 441 sows (20% primiparous and 80% multiparous) were randomly assigned to a treated or control group based on their parity and number of piglets born alive. The treated group received the Porceptal® sFTI program, whilst the control group was bred classically with 2 AIs. The day of weaning and 1st estrus detection as well as the dates of AI and farrowing were recorded for each sow.

Results

Ninety percent (90%) of sFTI program sows showed estrus on day 5 post weaning and were inseminated on day 5 (i.e. weaning on a Wednesday, AI the following Monday). A further 4.7% of sows displayed estrus and were inseminated on day 6. The 2 AIs of the control group were spread between day 3 and day 8 post weaning (Sunday to Thursday) (Figure 1).

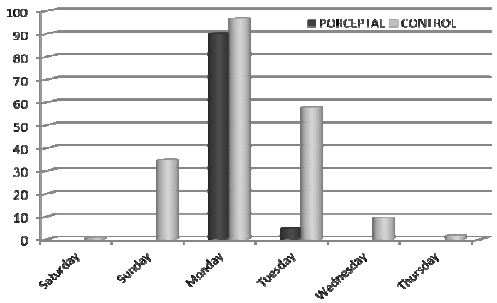

Figure 1. Number of AI performed per day per 100 adult sows in the 2 groups (all data based on weaning day fixed on Wednesday).

Farrowing occurred over a 13 day period, from the week before the scheduled time to a week after. It took place between Monday to Friday in 95.8% of the sows in the sFTI group *vs.* 88.6% in the control group ($p<0.01$). While 5 sows of the control group and 6 sows of the sFTI group farrowed the weekend before, only one sow (0.6%) farrowed during the weekend after in the sFTI group versus 15 in the control group (8.6%). In addition, 42.3% of the farrowings were induced in the control group versus 36.1% in the sFTI group.

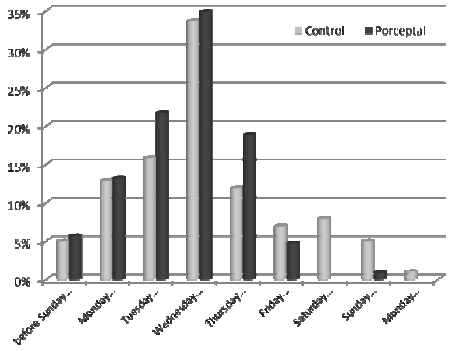

Figure 2. Spread of farrowing during the week, for the 2 groups.

Conclusions and Discussion

Even without counting the substantial time-saving of not detecting estrus before AIs, it is clear that the sFTI program optimizes the work of the farmer by allowing AI and farrowings to be done within the working week (Monday to Friday). Furthermore, if a stricter farrowing window is needed, the sFTI program can be combined with farrowing induction.

In addition, concentrating the farrowing in a shorter time period facilitates cross-fostering of piglets at birth, which allows creating more homogeneous litters at the beginning of lactation. This in turn leads to more homogeneous piglets at weaning which benefits dietary and health management.

The improvement of farm management provided by the sFTI program is tangible and deserves further attention.

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Ovulation variability in sows and gilts

E Sallé[1], M Le Jeune[2], Y Huang[2], S Boulot[2], M Collell[3]
[1]MSD Santé Animale, Beaucouzé, France, [2]IFIP Institut du porc, Le Rheu, France [3]Merck AH Summit NJ, USA
Miquel.collell@merck.com

Introduction

To achieve good reproductive performance, adequate timing of insemination close to ovulation is needed (1). At farm level, multiple inseminations (>2 semen doses) are often recommended to compensate for variable and unknown ovulation time. Efficient prediction or control of ovulation could improve labor costs (Soede et al 2002). The objective of this work was to investigate factors associated with variability of ovulation in different herds and possible impacts on reproduction.

Materials and Methods

The study was performed in 4 conventional Breton farms having from 300 to 1000 sows, weaning at 3 weeks, doing 2 to 4 insemination/sow, herd fertility >85%. Measurements were performed on several batches from (?) a total of 314 gilts and weaned sows. They included daily recordings of estrus and ovarian status using transcutaneous ultrasound technique (3.5-5 Mhz probe, Exago®, ECM) (2). Information about number and timing of inseminations (AI), backfat (BF) at AI, weaning-to-estrus (WTE) or last altrenogest-to-estrus intervals in gilts (ATE), parities, previous litter size or lactation duration, health status, treatments, and subsequent performances was collected. Results were analyzed using GLM or LOGISTIQ procedures (SAS 9.2) for quantitative or qualitative data respectively.

Results

Within 8 days after weaning, 97.5% females exhibited estrus and ovulated, 2.5 % remained in anestrus and one ovulated silently. Ovulation occurred at 76 ± 8 % of estrus duration, 44.1 ± 18.7 h after the onset of estrus, with large individual variations (-3 h to +105 h).
Gilts had shorter estrus and ovulated earlier ($p<0.01$). Late weaning-to-estrus interval was associated with earlier ovulation, and shorter estrus. Weaning to estrus or last altrenogest-to-estrus intervals where the best predictors of estrus duration and ovulation time ($p<0.01$) in 3 of 4 farms. Table 1

		WTE or ATE (days)			
		<4	5	6	≥7
Sows	N	101	90	38	3
	estrus/ovulation (h)	49,7a	43,1b	34,5c	21,1c
Gilts	N	-	14	39	14
	estrus/ovulation (h)	-	45,3a	30,6b	21,5c

[a,b] values with different superscript within a row were significantly different (?)

Previous litter size was unrelated to ovulation criteria but lactation length impacted weaning to-ovulation interval. BF at AI had no effect on sows but was related to last altrenogest-to-ovulation interval ($p<0.05$) in gilts. Fertility was high (83.6 to 96 % according to farms) and poorly related to ovulation criteria. However, it increased ($p<0.05$) with the number of AI falling into the interval of [-24;+12 h] around ovulation. Low BF at AI (≤13 mm) was associated with lower fertility in gilts.

Conclusions and Discussion

Results confirmed variability of ovulation and the importance of good estrus detection procedures to adapt AI protocols. Impact of parity, BF and previous lactation should be further investigated.

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Optimizing management of gilts: Concentrating AI and farrowing to specific times

E Sallé [1], M-A Driancourt[2], P Baldwin[2], M Collell[3]
[1] *MSD Santé Animale, Beaucouzé, France,* [2] *MSD Animal Health Innovation, Beaucouzé, France,* [3]*MSD Animal Health,*
miquel.collell@merck.com

Introduction

Although replacement gilts are the 'future of the farm' and should receive more attention to ensure optimal productivity, the increasing size of modern swine herds means that the time per animal is actually decreasing.

Batch management systems offer farmers the opportunity to concentrate specialized work on specific days of the week. Batch management of gilts is clearly linked to the use of altrenogest (Regumate®) [1], as it groups estrus in treated animals. Farrowing induction is another way to help farmers to concentrate work within a dedicated week. But, to date, whilst the use of Regumate usefully allows estrus to be grouped, the exact timing of ovulation (and hence the optimal time for AI) is unknown. Farmers therefore put in a lot of time and effort for heat detection, and then need to perform 2 or more AIs.

MSD Animal Health has developed a single Fixed Time Insemination program (sFTI) using a GnRH agonist, buserelin (Porceptal®) injected 115 to 120 hours after the last dose of a 18 days-long distribution of Régumate®, followed by a single AI 30-33hours later, with equivalent technical results to classical breeding programs [2].

The present study aims to clarify the interest of a sFTI program in farm management, at breeding time as well as at farrowing in the particular case of gilts.

Materials and Methods

The clinical field trial I involved 5 commercial herds in France. A total of 229 gilts from the 5 farms were randomly assigned to a treated or control group. The treated group received the Porceptal® sFTI program, whilst the control group was bred classically with 2 AIs. The day of 1st estrus detection as well as the dates of AI and farrowing was recorded for each gilt.

Results

A total of 97.4% of sFTI program gilts displayed estrus at the time of AI and insemination took place on day 6. In the control group, 98.3% gilts showed estrus and the first of the 2 AIs were spread between day 5 and day 11 after the last dose of Régumate® (from Sunday to the next Saturday – see figure 1), followed by a 2nd AI 12 to 24 hours after, and a 3rd one in 2 cases.

Farrowing occurred over an 11 day period, starting the week before the scheduled time to a week after in the control group (Figure 2), whilst it only took 7 days to have all gilts farrowed in the Porceptal® group. The use of the Porceptal® sFTI protocol allowed 97,8% gilts to farrow between Monday and Friday in the Porceptal® group vs 90.4% in the Control group.

Only 38.9% of the farrowings were induced in the Porceptal® group versus 50.0% in the Control group.

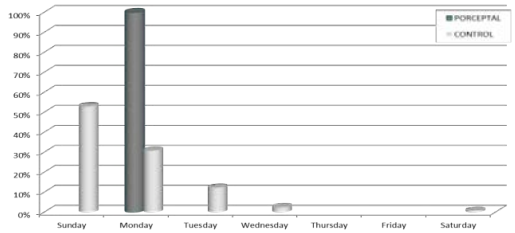

Figure 1. Time of the 1st AI in the 2 groups (all data based on last Régumate® day set on Tuesday).

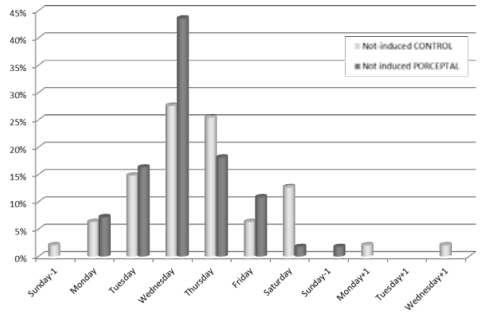

Figure 2. Spread of farrowing during the week, non-induced farrowings.

Conclusions and Discussion

The management of gilts can be improved by using a combination of the Porceptal® sFTI protocol and farrowing induction therefore helping to increase the results of the farrowing unit: the sFTI program helps ensuring that mature piglets are farrowed during working days of the week. The viability of the piglets is also likely to be increased via the knowledge of the exact fertilization day, such that induction before 114 days of gestation should no longer happen. Furthermore, cross-fostering of gilts' piglets should be easier to manage during the first hours of life, and should benefit the health status of the herd.

The improvement of farm management provided by the sFTI program is tangible and deserves further attention.

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Correlation between dynamic sperm DNA fragmentation and acrosome structure in Mong Cai pigs of Vietnam (*Sus Scrofa ssp. domestic*)

LY Parra-Forero[1], Y de Loera[2], LA Cruz[3], J Guevara[2], AC García - Contreras[1]
[1]*Universidad Autónoma Metropolitana. Unidad Xochimilco,* [2] *Universidad Autónoma Nacional de México. FES Cuautitlán,* [3] *Red de Estudios Moleculares Avanzados Instituto de Ecología, A.C. (INECOL).*
adelfa@correo.xoc.uam.mx, lyparraf19@gmail.com

Introduction

Several research groups have sought a parameter that has a high correlation with DNA fragmentation, this in order to establish exclusive use of sampling methods which, being altered their genetic information is less likely to reach a conception to term. The technique for determining the rate of sperm DNA fragmentation index (DFA) is a static test that directly responds to a physiological event of direct DNA damage. The sperm cell has become the measure of excellence for cytotoxic response to exposure to pollutants, nutritional supplementation, use of alkylating agents, among others (1, 2). The objective of this study was to determine the rate of sperm DNA fragmentation Vietnamese pork and determine its correlation with the damage found in the acrosome.

Materials and Methods

Six ejaculates of Vietnamese pork were obtained one week apart. T0: Freshly collected, T2: 15 minutes (min) after, T3: 30 min, T4: 60 min, T5: 4 hours (hrs), T6 8 hrs, T7: 12 hrs, T8: 24 hrs in which a dynamic freezing 8 times was performed as follows.

Samples were fixed in a solution of 2% formaldehyde; the aliquot was treated with PBS for subsequent mounting on Poly - L - Lysine 0.1 %. They were incubated with FITC - PSA for 30 minutes and the acrosomal damage was evaluated in a fluorescence microscope with phase contrast at 400X (3.4). To obtain the spermatic DNA fragmentation index (DFA) the technique used was SCD (Sperm Chromatin Dispersion) with Halotech ® kit for pigs.

Results are expressed as mean standard error followed by the average EEM. Significant differences between groups were analyzed with ANOVA followed by LSD test. Significant differences between two variables were determined by Student's t test. The relationships between the parameters were measured by the Pearson correlation.

Results

There were significant differences (P <0.01). Rates of sperm DNA fragmentation were, mean and SEM: T0: 5,67 (2,08), T1: 6,67 (2,16), T2: 7,66 (2,5), T3: 12 (2), T4: 15 (1), T5: 22 (2,64), T6: 29,67 (2,09), T7: 34,67 (3,51). The mean percentages of sperm with altered acrosomes T0: 8 (2), T1: 13,33 (1,53), T2: 15,67 (0,58), T3: 20 (2,64), T4: 22,67 (1,53), T5: 31,67 (3,21), T6: 34,33 (1,52) y T7: 37,33 (3,21). IFA positive correlation was found with the age (r = 0.949 P = 0.000), also with the DFA and the% of altered acrosomes (r = 0.974 P = 0.000). Figure 1.

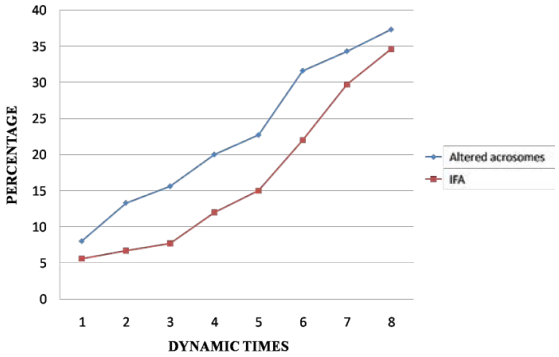

Figure 1. Comparison of percentages IFA and altered acrosome

Conclusions and Discussion

There is correlation between the IFA and the damage acrosome, however the percentage of damage is greater in these last, assuming a level of damage that is not measured by the test of SCD, we believe that the evaluation of acrosome indicate also capacitated sperm but still has not been damaged DNA. Another hypothesis is that these sperm are in the process of apoptosis and DNA fragmentation has not occurred (5,6). In conclusion the sample should be extended to define these correlations. Provision should be the realization of other studies to define the total spermatic DNA damage and correlate directly with espematobioscopy parameters in this species.

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Value of health and production performance improvement resulting from the treatment of sows exhibiting post-farrowing vaginal discharge

M Turner[2], E Nemechek[3],
[1]*North Carolina State University College of Veterinary Medicine.* [2]*Prestage Farms.* [3] *Zoetis.*
blmclam2@ncsu.edu

Statement of the problem

Vaginal discharges are considered normal in sows within the first 3 days post-farrowing and are an attempt to clear placental remnants and uterine debris. Normal vaginal discharge is often difficult to differentiate from pathological discharge by farm personnel. Historically discharging sows have been treated with antibiotics to reduce the perceived adverse effects of discharges on sow lactation, litter performance, and post weaning reproductive performance. Due to the potential for antibiotic residues and the lack of information on treatment efficacy, it has been difficult to justify the use of antibiotics consistently for the treatment of vaginal discharge.

The objective of this study is to evaluate the value of treatment of sows with post-farrowing vaginal discharge.

Methods

Rectal temperatures and vaginal discharges were recorded for all sows on a 4000 head farrow to wean farm on days 0-3 post-farrowing. Discharges were scored as light, medium, or heavy. Sows with a vaginal discharge were enrolled in the treatment protocol, assigned to either a treatment or control group, and blocked by parity. The treatment group received an injection of 1.37 mg/kg Naxcel (Zoetis, Madison, NJ) IM for three days. The control group received no treatments. Sows with rectal temperature exceeding 104º F post-farrowing and sows that were off feed were excluded from the trial and treated according to the herd veterinarian's recommendation. Piglets were administered routine vaccinations and litter weight and piglet numbers were recorded at weaning. Following weaning, sows will be tracked for post-weaning discharge, return to service, and subsequent farrowing information**.**

Results

Over the duration of the study 1480 sows were observed. In the control and treatment groups there were 325 and 324 sows, respectively. The average temperature among all sow groups was 102º F and the average weaning age was 18 days. Both the normal non-discharging and control (discharging non-treated) groups weaned an average of 11 pigs per litter, while the discharging, treated group weaned 11.5 pigs per litter. Additionally, the treatment group demonstrated a 5 pound increase in weaned litter weight (126 lbs) over that of the non-discharging and control groups (121 lbs). The treatment group had a pre-weaning mortality (PWM) of 15.6%, demonstrating a reduction of 1.4% less than the 17% PWM in the control group.

Discussion

The cost of treating sows for 3 days with Naxcel was $15.65/sow. Looking at an increase in 0.5 pigs per litter, the economic value to the producer was $4.35 ($20/.5 pig value - $15.65 treatment cost). Treatment with Naxcel also reduced the risk of antibiotic residues compared to other alternatives due to its 4 day withdrawal time, making it a valuable treatment option for practitioners and producers. Further value of treatment of sows with vaginal discharge will be evaluated by comparing subsequent conception and farrowing information.

Effect of insulin-like growth factor-I (IGF-I) and follicular fluid addition from ovarian follicles with different diameters on porcine oocyte fertilization *in vitro*

G Oberlender[1], AC Silva[2], MG Zangeronimo[2], LDS Murgas[2], TA Menezes[2], TP Pontelo[2]
[1]*Department of Veterinary Medicine, Federal University of South Frontier (UFFS), Realeza Campus, Paraná, Brazil,*
[2]*Department of Veterinary Medicine, Federal University of Lavras (UFLA), University Campus, Lavras, Minas Gerais, Brazil,* guilherme.oberlender@uffs.edu.br

Introduction

Procedures currently used for *in vitro* maturation (IVM) and *in vitro* fertilization (IVF) for *in vitro* production (IVP) of porcine embryos frequently result in low rates of embryonic development (2,3); and the composition of the culture medium is one factor that may limit the efficiency of these technologies. Incomplete maturation of oocytes is an important factor contributing to the low success rates of IVP, suggesting that culture media for current IVM systems may be deficient in factors that facilitate cytoplasmic and nuclear oocyte maturation equivalent to that which occurs *in vivo*. Therefore, with the goal of improving efficiency of IVF, the objective of the present study was to determine the effects of IGF-I (0, 60, 120, 180, and 240 ng/mL) and follicular fluid derived from 2 to 5 and 6 to 10 mm diameter follicles (SpFFs and LpFFs, respectively) added during IVM of porcine oocytes on IVF results.

Materials and Methods

Ovaries from prepubertal Landrace × Large White crossbred gilts were collected immediately after slaughter and transported to the laboratory. After cumulus-oocyte complexes (COCs) from follicles 3 to 6 mm in diameter were aspirated.

Afterwards, 900 COCs were matured in NCSU-37 medium supplemented with SpFFs or LpFFs and various IGF-I concentrations. The COCs were cultured for 44 hours, and then fertilized *in vitro*. The IVF results (percentage of degenerated oocytes, penetration rate, monospermy rate, fertilization performance, number of penetrated sperm per oocyte, and pronuclear formation rate) were recorded 18 hours after insemination.

All variables obtained were modeled according to the binomial model of parameters. Data were analyzed by ANOVA and, when significant were submitted to regression analysis and compared by Tukey test. A significance level of 5 % was considered to indicate a statistically meaningful difference. All statistical analyses were performed using the statistical package *SPSS for Windows*, version 17.0 (5).

Results

Regarding IVF results, at all IGF-I concentrations, the percentage of degenerated oocytes was higher in COCs matured in SpFFs than in LpFFs. Penetration (%) did not differ ($p > 0.05$) between COCs matured with SpFFs or LpFFs when 60 (66.8 ± 9.4 vs. 72.7 ± 11.3) or 180 ng/mL of IGF-I (75.7 ± 10.4 vs. 73.8 ± 13.2) were used. Monospermy (%) was similar between SpFFs and LpFFs only with 120 ng/mL IGF-I addition. IVF performance (%) did not differ between COCs matured with SpFFs or LpFFs when IGF-I concentrations of 120 (28.5 ± 8.8 vs. 38.5 ± 8.3) and 180 ng/mL (24.3 ± 10.2 vs. 30.12 ± 8.2) were used. There was no effect of IGF-I concentration or of FF type on the number of penetrated sperm per oocyte and on male pronuclear formation. For COCs matured with SpFFs, there was a quadratic relationship between IGF-I concentration and penetration, monospermy, and IVF performance (peak results at IGF-I = 179, 122, and 135 ng/mL, respectively).

Conclusions and Discussion

In this study, as also shown by other authors, fertilization rates without IGF-I addition were significantly higher when LpFFs were used in the IVM compared with SpFFs (1,4). On the other hand, the presence of IGF-I in the IVM medium potentiated the beneficial effects of SpFFs on IVF results.

On the basis of the results of quadratic regression analysis, we concluded that the optimum concentrations of IGF-I needed to exert positive beneficial effects on penetration, monospermy, and fertilization performance were 179, 122, and 135 ng/mL, respectively. Therefore, when SpFFs are used in porcine oocyte maturation, an IGF-I concentration between 122 and 179 ng/mL in the IVM medium can result in oocyte fertilization rates similar to those obtained with the use of LpFFs.

In conclusion, the addition of IGF-I to the IVM medium supplemented with SpFFs improved IVF results. Alternatively, IGF-I had no effect on IVF when used with LpFFs.

Acknowledgments

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Adequacy of insemination protocols with weaning day in pig farms

S Boulot[1], E Sallé[2], F Cade[1], B Badouard[1], M Collell[3]
[1.]IFIP Institut du porc, Le Rheu, France, [2.]MSD Santé Animale, Beaucouzé, France, [3.]Merck AH Summit NJ, USA
Miquel.collell@merck.com

Introduction

Labor constraints, are strong determinants of sow herd management (1). Changing weaning day is a frequent option to shift major week-end tasks, from farrowing to insemination (2). The aim of this study was to record recent weaning day choices in pig farms and to assess the adequacy of their insemination practices.

Materials and Methods

A survey was conducted among 214 pig herds randomly selected from the French National Pig Management database (metropolitan production indoor herds >150 sows). The questionnaire was e-mailed to farms for self-completion using Sphynxonline®. Questions (n=90), addressed five topics: weaning and breeding practices, pregnancy management, lactation and gilts. Average fertility at first service (TF1) and Total Born (TB) in 2012 were calculated for the 120 participating farms. Relationships between weaning day, insemination and performances were investigated through univariate analysis, using Chi^2 or GLM procedures, respectively for qualitative and quantitative data (3).

Results

Weaning is more frequent on Wednesday (60%) than on Thursday (37%), with only 3% on other days (4 herds excluded). Large herds and weaning at 21 days are significantly associated with Wednesday weaning (Table 1). More estrus detection and inseminations (AI) are declared on Sunday for Wednesday weaning. Though AI protocols are adjusted to weaning to estrus interval (WEI), for a given interval, position of 1st AI depends on weaning day. When WEI=4 days it is more frequently delayed over 18 hours for Wednesday weaning (Figure 1). Rate of multiple AI is similar (45% sows with 3 AI). TB (14.5 ± 0.67) and TF1 (90.0 ± 4.7) do not depend on weaning day, but practicing AI on Sunday is associated with better results (p=0.07 for TF1 and p=0.13 for TB).

Conclusions and Discussion

This study confirms overall French preference for weaning on Wednesday, with subsequent requirements for efficient estrus detection and insemination during the week-end. Most of the farms have coherent management, but data show variable or potentially detrimental insemination practices (no week-end AI or delayed first AI) in some herds, which can result in worse reproductive results.

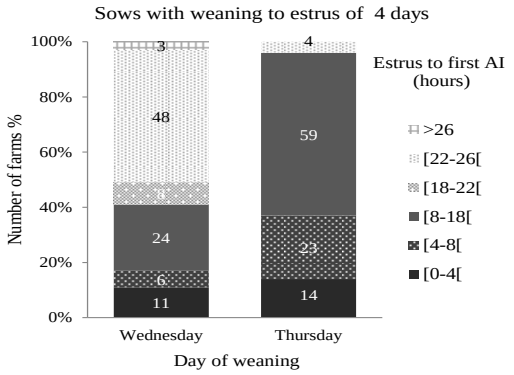

Figure 1. Distribution of farms according to day of weaning and position of expected 1st insemination for weaning to estrus = 4 days (116 farms, p<0.001, Fisher exact test).

Table 1. Main herd characteristics according to day of weaning. *(1) χ^2 or Fischer exact test, N=116 farms*

Number farms (%)		Day of weaning		p values (1)
		Wednesday	Thursday	
Herd size (sows)	<200	19	78	0.003
	200-400	60	45	
	>400	21	7	
Weaning at 21 days		65	48	0.06
Sunday detections		96	75	0.002
Sunday AI		80	41	0.001
Different AI protocoles		46	18	0.003
Specialized staff	detection	79	89	0.21
	AI	75	84	0.25

Acknowledgements

To farmers and pig producers organizations for participation, and to MSD-Santé Animale and France Agrimer, for financial support.

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Weaning management associated with reproductive performances in French pig farms

S Boulot[1], E Sallé[2], F Cade[1], B Badouard[1], M Collell[3]
[1.]IFIP Institut du porc, Le Rheu, France, [2.]MSD Santé Animale, Beaucouzé, France, [3.]Merck AH Summit NJ, USA
Miquel.collell@merck.com

Introduction

Though multi-factorial determinants of reproductive performances are well established, few studies investigated weaning management at farm levels (1, 2), with more frequent focus on insemination and semen (3). The aim of this study was to make an inventory of the different weaning practices in farms and to investigate possible relationships with fertility and litter size.

Materials and Methods

A survey was conducted among 214 pig herds randomly selected from the French National Pig Management database (metropolitan production indoor herds >150 sows). The questionnaire consisted of 90 mainly closed-ended questions, addressing five topics: weaning, breeding, pregnancy management, lactation and gilts. It was e-mailed to farms for direct self-completion, using the Sphynxonline® module: 120 answers were collected. Average fertility at first service (TF1) and Total Born (TB) in 2012 were calculated. Relationships between weaning management and TF1 or TB were investigated through univariate analysis, using SAS® and GLM procedures (4).

Results

Herds were representative of average French pig farms: 313 ± 224 sows, 59% weaning at 3 weeks. Fertility (89.8% ±4.8) and litter size (14.5 ±4.8) varied within a large range, with TF1≤85% and TB≤13 for 15% and 23% farms respectively. Significant moderate associations with TF1 or TB were found for various weaning practices (Table 1). Large herds and weaning on Wednesday were associated with high fertilities, with no effect of timing of separation (morning=86% farms, sows leaving farrowing house first =81% farms). *Ad libitum* water delivery, total restriction of water and dry-off treatments negatively impacted TF1 and TB. Partial feed restriction at weaning (<3 days), or specific dietary supplements were both associated with higher prolificacies ($p<0.05$), with variable associations, precluded evaluation of single products (oligo-vitamins, cod liver oil, and sugar for 79%, 26% and 19% farms respectively). Weaning allotment (various condition criteria and parity) was positively correlated with TB. Early boar stimulation and systematic light schedules were associated with high fertilities while mixing of sows at weaning showed negative but low correlation with fertility ($p=0.15$).

Conclusions and Discussion

This study supports that weaning practices exhibit large variation according to farms, with both favorable and detrimental impacts on fertility or litter size. Data confirm structural benefits in large herds with specialized staff (1), and also emphasize the importance of specific feeding and stimulation (2). Peri-weaning period is crucial for ovarian activity (estrus, ovulation) and embryo quality, but practical recommendations require updating. Therefore, this study and further multi-factorial approach could contribute to update guidelines and improve farm management.

Table 1. Weaning practices associated with herd fertility (TF1) or litter size (TB) (n=120 farms, factors with p values p<0.20 only).

Weaning management practices	Farms %	P-values[1]	
		TF1	TB
Sow herd size (<200,200-400,>400)	32/53/15	**0.007**	NS
Day of weaning (We/Thu/Others)	60/37/3	0.06	NS
Feed <2.5 kg (Yes/No)	80/20	NS	**0.04**
Water supply (Ad lib/Limited/No)	23/61/16	0.08	0.17
Dietary supplements (Yes/No)	74/26	NS	**0.03**
Dry off treatment (Yes/No)	41/59	0.19	NS
Weaning allotment :			
number criteria (0,1,>1)	26/28/46	NS	**0.03**
by parity (Yes/No)	37/63	NS	**0.02**
1st boar contact (weaning/day1/later)	19/44/37	0.08	NS
Sow mixing (Yes/Sometimes/No)	66/12/23	0.15	NS
Light schedule (Yes/No-sometimes)	78/22	0.10	NS

Acknowledgements

To farmers and pig producers organizations for participation, and to MSD-Santé Animale and France Agrimer, for financial support.

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Concentration of Zn in liquid follicular pigs female. Introduction to the oovogenesis metabolomics

LY Parra-Forero[1], G Vela-Correa[1], O Cano-Flores[1], G Mendoza[1], S Romo[2], AC García-Contreras[1]

[1]*Universidad Autónoma Metropolitana. Unidad Xochimilco, Ciudad de México. 2Universidad Autónoma Nacional de México, FES- Cuautitlán,* adelfa@correo.xoc.uam.mx, lyparraf19@gmail.com

Introduction

Metabolomics assays of cellular processes are now major biochemical studies to understand processes at the cellular level. One of the main applications of this branch of genomics is creating culture media that allow the oocyte to mature when purchasing quality characteristics to apply some technique of assisted reproduction. Zn is an essential component of cellular signaling as well as being the cofactor 300 enzymes and be intrinsically linked to the dynamic functionality of the DNA. These studies are critical to its application in human cells. Therefore, the objective of this study was to determine the concentration of Zn in the follicular fluid of sows in three different stages of oocyte maturation

Materials and Methods

Active ovaries of 20 slaughtered females pigs, 16 of these were selected. Were classified by size F3 ≥ 4mm were protruding from the parenchyma 2-4 mm F2, F1 <2 mm does not project the parenchyma. Number of oocytes per ovary was determined and the volume was measured by rating group. The concentration of Zn was done in graphite furnace Perkim Elmer P316 ®.

The results were statistically analyzed used a completely randomized design with factorial arrangement using the statistical package SAS version 9.3, the data were considered statistically significant when P <0.001

Results

There were significant differences in the results of the concentrations of Zn in different follicles (P <0.001), the mean for the group were 28.58 ± F1 10.25 pg /µL, 46.82 ± 17.82 pg/µL group F2 and the group F3 32.87 ± 2.1 pg /µL, these results can be seen in Table 1, as the results of the total concentration by volume and follicle

Conclusions and Discussion

The estimate we report, is a rough measure of the concentration of Zn and / or necessary for an egg to mature and be ovulated , 8.29 pg is the average amount of Zn found in F3 follicles, preovulatory these are considered . The distinction was made in this study could give an estimate of the mineral requirements of the oocyte to mature, essential for culture media.

The effects of Zn supplementation on in vitro embryo production are varied, a study of effects of magnesium supplementation, found that this inhibited the formation of Metafase II inhibiting Calmudulina interfering with kinase activity in oocytes and because of its similarity to Zn, a study done with this phase Zinc also inhibited by the addition of 10mg/ml in cows (1). Other reported effects are due to antagonism with Calcium that is located at the junctions of the cell clusters, if the Zn was not in appropriate concentrations these inhibit or hinder the entry of the sperm at the time of fertilization (2, 3).

Table 1. Concentration of Zn in follicles of pigs females

GRUPO	Concentración de Zn (pg/µL) (MEDIA/EEM)	Volumen (µL) (MEDIA/EEM)	Concentración total por folículo (MEDIA/EEM)	SIGNIFICANCIA ESTADISTICA
F1[a]	28,58 (10,25)	146 (43,27)	3,62 (0,78)	0,001
F2[b]	46,82 (17,82)	189 (50,93)	5,54 (1,35)	0,001
F3[c]	32,87 (11,71)	112,67 (28,84)	8,29 (2,1)	0,001

Different literals are significantly different

In conclusion each maturity level has different level of Zn, studies should be made with females pigs controlling diet and mineral premix, to determine the role of this in the follicular maturation in vivo and correlate with hatching rates after IVF or other assisted reproduction technique.

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The research of swine seminal quality indicators: The relationship between seminal abnormalities and seminal motility parameters

S Balasch, T Pérez, R Salvans
Alea! Technological Centre of the Animal Life, Gepork, Masies de Roda - Barcelona, Spain, sbalasch@gepork.es

Introduction

When seminal quality is evaluated, the only parameters tested are *motility* (subjective evaluation) or *an objective evaluation*, based on the use of computer-assisted motility analysis methods, together with the evaluation of seminal abnormalities.

The combination of the 2 parameters will be used to validate or reject an ejaculate. However, there are primary anomalies that cannot be observed in a subjective or objective evaluation by using only a motility test. These abnormalities could suffer seasonal variations throughout the year.

Therefore, the aim of the study is discovering a pattern of the evolution of these abnormalities which are hidden in a routine analysis; and also comparing the semen quality parameters (anomalies) with the motility values obtained with CASA system for seminal analysis.

Materials and Methods

The study includes with boars from 2 insemination centers with similar location and conditions.

When the ejaculates arrive at our laboratory at 32ºC (prediluted 1:1), a phases-contrast microscope equipped with CASA system is used for analyzing the processed ejaculates. CASA system evaluates exclusively the seminal concentration of each sample and the progressive and total motility. At the same time, a subjective evaluation is done and if the sample has a viability of 80% or more, an Eosin–Nigrosin stain is prepared in order to obtain the exact % of acceptable spermatozoid. This evaluation is corroborated with the record of the seminal quality of the boar.

The good semen quality ejaculates (with more than 80% viability) and the bad ones (dismissed) are not included in the study. The selected ejaculates are those with an initial subjective evaluation of viability between $\leq$ 70% and $\geq$ 80%.

The morphoanomalies of 3773 ejaculates are analyzed during 21 months, and also the type of movement from all the ejaculates, specifically referring to the following parameters: DAP (Average path distance); DCL (Curvilinear distance); DSL (Straight line distance); VAP (Average path velocity); VCL (Curvilinear velocity); VSL (Straight line velocity); STR (Straightness - VSL/VAP); LIN (Linearity - VSL/VCL); WOB (Wobble - VAP/VCL); ALH (Amplitude of lateral head displacement); BCF (Beat cross frequency).

Results

First of all, observing how the % of anomalies are distributed throughout the year, based on VLA (the limit value analyzed of each anomaly), it can be stated that there is not a standard distribution.

The FAT anomalies (Total abnormal forms) reach the minimum values in winter (from November to January), whereas the maximum values are reached in April (spring) and July (middle summer).

Referring to the "hidden" anomalies, specifically CA (Head anomalies) and AA (Acrosome anomalies), it can be observed:

* CA anomalies: maximum values are reached in January (lower FAT) and in July (higher FAT); and the minimum values appear in May and June (higher FAT).
* AA anomalies: maximum values are obtained in April and May; and the minimum values, in February and September.

In order to analyze the anomalies by taking into account the average movement of the ejaculates, the 50 worst values are compared with the 50 best ejaculates:

Among the different values of Velocity, (VSL, VCL & VAP), the best results are the ones from ejaculates with a higher % of normal spermatozoids.

Among the different values of Distance (DSL, DCL & DAP), the best results are the ones from ejaculates with a higher % of normal spermatozoids.

Among the different values of Distance (DSL, DCL & DAP), the worst results are the ones from ejaculates with propulsion problems: Tail anomalies & Distal Drops anomalies (GCD), as well as problems with flagellum beating frequency (BCF).

A high STR indicates good seminal quality, whereas if the value is low, it indicates a high % of GCD.

A high % of CA anomalies affects directly to the values of Velocity and curvilinear-linear Distance, as well as the ALH parameter.

AA anomalies are unperceived referring to the values of Route and Velocity.

Conclusions and Discussion

The % of anomalies does not follow a standard distribution throught the year and the CA & AA anomalies do not have a parallel evolution with the % of total FAT.

There are specific parameters of spermatic Velocity and Route –and parameters mixing both elements– that can demonstrate the presence of unperceived anomalies in a routine test. This may be a new line of study for the improvement of CASA systems.

It is very important to make a complementary test of evaluation of seminal quality, apart from the routine motiliy evaluation with CASA systems.

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Boar semen supplementation using a novel insemination device reduces the negative effects of seasonal infertility

J van Leeuwen-Ibarrola[1], A Echegaray[2], D Reicks [3], A Cervantes[4], O Carion[1], E Schmitt[1]
[1]*R&D department, IMV Technologies, l'Aigle, France,* [2]*Humeco, Huesca, Spain,* [3] *Swine Vet Center, St Peter, Mn, USA,* [4]*Agromex Importaciones SA de CV, Tepatitlan, Jalisco, Mexico,* Jessika.vanleeuwen@imv-technologies.com

Introduction

Seasonal infertility is estimated to cause a yearly economic loss of 0.5 billion USD to the US pork industry. The sow contributes to this infertility (Hughes and van Wettere, 2010), but also the boar's fertility is affected during the summer months. Semen quality is reduced with lower motility, higher percentages of abnormal cells and higher agglutination rates (Murase et al., 2007). Attempts have been made to keep sperm motility high during storage by the addition of caffeine to the semen preservation media (Yamaguchi et al., 2013), but this was associated with reduced survival rates (Funahashi et al. 2000; 2001). The addition of both caffeine and $CaCl_2$ to boar semen immediately before AI improved fertility (Yamaguchi et al., 2009). The current study used a novel insemination device to investigate the effect of supplementation with caffeine and Ca_{2+} at the moment of insemination (without increasing the labor input) on subsequent reproductive performance of commercial sows.

Materials and Methods

Two field studies were conducted. The first trial (1) was conducted in July 2011 (summer) in 2 commercial swine herds in Minnesota, USA. The second trial (2) was conducted in July-August (summer) 2013 in a commercial swine herd in Spain. After weaning sows (Trial 1: n = 414; PIC and Trial 2: n=550; Topigs 20) were randomly assigned to be: **1.** Inseminated with a novel insemination device (patent WO 2008/ 152366A1) either containing both caffeine and calcium (GoldenGel; n=209) or with a control catheter containing the same gel carrier but no caffeine and calcium (Control; n=205). **2.** Inseminated with a novel insemination device (WO 2008/ 152366A1) containing both caffeine and calcium (GoldenGel; n=271) or with a standard AI catheter (Control; n= 279). The GoldenGel catheter deposits a gel into the cervix which is mixed with semen in the genital tract. Data were analyzed using SAS (SAS Inst. Inc., Cary, NC, USA). Data were corrected for farm effect, where relevant. Data presented are means ± SD unless stated otherwise.

Results

Trial 1: Average farrowing rate was 84.1% and did not differ between treatments. Litter size was higher (live born) for GoldenGel compared to Control (12.4±0.3 vs. 11.6±0.3 piglets, respectively, $P<0.04$; Table 1).

Trial 2: Average farrowing rate was 79%. GoldenGel AI resulted in a farrowing rate of 82% versus 76% for Control (P = 0.08). Litter size was not affected by insemination method (14.8±3.4 vs 14.9±3.8, respectively; $P > 0.1$).

Table 1. Reproductive performance in Trial 1 and Trial 2

Item	Treatment		
	GoldenGel	Control	P-value
Trial 1			
No assigned	209	205	
Farrowing rate (%)	82	86	0.2
Litter size (LB)	12.5±3.1	11.8±3.4	0.04
Trial 2			
No assigned	271	279	
Farrowing rate (%)	82	76	0.08
Litter size (TB)	14.8±3.4	14.9±3.8	0.95

Conclusions and Discussion

Sow fertility improved after insemination with GoldenGel shown by an increase in litter size (trial 1) and in farrowing rate (trial 2). This could be attributed to an increase in sperm motility as caffeine is known to stimulate sperm motility (Harrison et al.,1980_{ab}; Imoedemhe et al., 1992) with a faster sperm transport through the reproductive tract to the sperm reservoir (Matthijs et al., 2003) and reduced back flow. Also, calcium stimulates capacitation (Neild et al., 2005), which could result in more sperm cells reaching the oocytes in a capacitated stage.

In conclusion, supplementation of semen during AI using the GoldenGel catheter improves sow fertility during summer heat stress.

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Effect of synthetic progesterone on motility sperm from boar ejaculate

L Becerril[1], R Huerta[2], M Méndez[2], CL Morales[1], JM Palacios[1], A Aragón[3]
[1]*Centro Universitario UAEM Temascaltepec, Universidad Autónoma del Estado de México,* [2]*Benemérita Universidad Autónoma de Puebla, Facultad de Medicina Veterinaria y Zootecnia,* [3]*Universidad Autónoma Metropolitana-Iztapalapa,* rubenhuertac@live.com.mx

Introduction

Estrogen receptors are reported in sperm pork, (4) these may be associated with sperm motility (5). Ejaculates, present sperm subpopulations, which differ by their movement patterns, functional characteristics and cooling abilities (3). Objective of the present study was to determine the progesterone (P_4) effect addition over sperm motility in pork semen, after a conservation process at 17 ° C.

Materials and Methods

Four 1.5 years age boars were confined in individual pens and fed with the same diet, two ejaculates were obtained per week from each animal for two consecutive weeks, using the gloved hand technique. Sperm motility was evaluated with the House system (UltiMate Sperm Analyzer ™, Hamilthon Thorne Biosciences, Beverly MA, USA), before adding the hormone (MF). Subsequently the P4 was diluted in dimethyl sulfoxide (DMSO, Sigma) at two concentrations; 0.333 µg/ml and 0.033 µg/ml. Absolute control (CL-R) and a control with DMSO (CL-DMSO) were used. A multivariable analysis with chernoff faces to identify differences between the descriptors of mobility and an ANOVA analysis were used to determine statistically differences among treatments on the number of sperm with fast motion ($P < 0.05$), using the STATISTICA V8 Software (StatSoft, Inc. 2007).

Results

Motility descriptors were modified between treatments and animals in each ejaculate (Figure 1), each facial figure was formed as a descriptor, analyzing the literals in the columns that identify one of the animals and their repetition, the legends of the right side identifies treatments. The number of sperm with fast movement does not present significant differences (Figure 2), point inside the box indicate median and standard error, the whisker values circles and ends outliers. Figure 1. Describes high face = MOT, to centre of mouth = VCL, to face higher eccentricity = MPR, to curvature of the mouth = ALH, to lower eccentricity of VAP, long's mouth face = BCF, to long nose = VSL and to eye height = STR

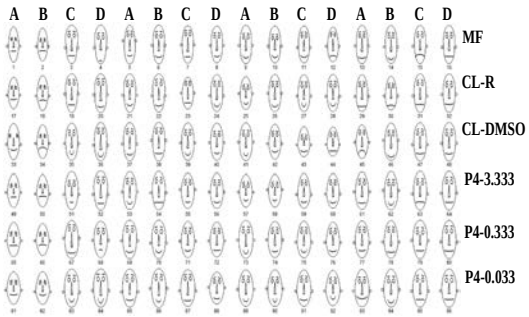

Figure 1

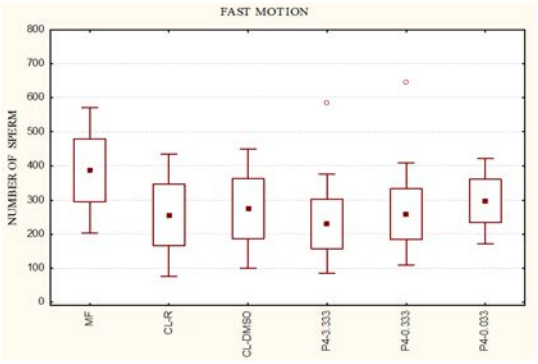

Figure 2. The number of sperm with fast movement 4 µ/s, to MF and the treatments, CL-R- CL-DMSO, P_4 3.333 µg/ml, P_4 0.333 µg/ml y P_4 0.003 µg/ml.

Conclusions

Progesterone used doses did not alter significantly the scroll speed, however it can continue developing effect studies with lower than those employed.

Acknowledgments

CONACYT, FMVZ-BUAP, Super-Gen

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P.674

Knowledge and practices of biosecurity among pig farmers in South Western Nigeria

JO Abiola
Department of Veterinary Medicine, University of Ibadan, Ibadan. Dnk12_day@yahoo.com

Introduction

Biosecurity measures are important for the herd's protection against diseases and also to provide nationwide protection against the introduction of exotic/foreign diseases. The adequate application of biosecurity measures largely depends on farmers' attitude towards and comprehension of contagious diseases and their prevention.(12) Some studies carried out in Great Britain among cattle and sheep farmers have shown that farmers frequently have a negative attitude towards biosecurity and are unaware of the effectiveness and economic benefits of adopting biosecurity measures (6). Several reasons can be attributed to farmers' lack of confidence in biosecurity: lack of knowledge, the economic cost of implementing these measures, the additional work implied and the dislike of mandatory rules (5, 6, 8). Veterinarians play a key role in training and educating farmers and, for most of them, they represent the main source of information (6).

In recent years, the importance of implementing a biosecurity programme has been recognised, and several studies have investigated the biosecurity measures put in place on pig farms (1,2,3, 4, 7, 9, 10, 11). Of late, some studies carried out in Belgium showed that farm type and size bear an influence on the degree of biosecurity applied (11). The perceptions and attitudes of farmers and veterinarians towards biosecurity have also been explored (3, 5, 6 8).

The objectives of this study were to assess the biosecurity practices currently reported on Nigerian pig farms, as well as to describe the attitudes of pig farmers towards biosecurity. Furthermore, the effect of the farm type on the application of biosecurity measures was also examined.

Materials and Methods

Two hundred and ninety four pig farmers in the South Western Nigeria were interviewed coupled with farm visit to determine the biosecurity measures currently applied, as reported by farmers, and to investigate the importance awarded by farmers to each of these measures. Data was gathered by means of a questionnaire administered to farmers and farm visits to assess the level of the biosecurity measure puts in place. Biosecurity measures were reported based on two scenarios: in the presence (Small holder farms) and in the absence of a highly contagious disease (High density farms), with some farmers feigned the ignorance about biosecurity measure.

Results

Farmers awarded significantly higher scores to their farms' level of biosecurity. According to the farmers, the most important biosecurity measures were those aimed at minimising the risk of disease introduction by visits and vehicles. Biosecurity practices seeking to reduce the risk of disease introduction by breeding stock were not applied on a considerable number of farms as there are free movements of breeding boars among or between the farms. The findings also revealed that medium-sized to large farms with high pig density reported higher biosecurity measures than small herds with low pig density with some having backyard piggery without any biosecurity measure in place..

Conclusions and Discussion

The aspects of biosecurity examined mainly concern the application of measures to prevent the transmission of new infectious diseases to herds, and also to contain the spread of infections already present in different production phases. The biosecurity level of Nigerian pig farms may be insufficient to prevent the transmission of highly contagious diseases given the high pig density in the south west of the country and the considerable extent of pig movement. For this reason, veterinarians should collectively revisit or discuss biosecurity measures that are important in order to provide the same message to swine farmers. Moreover, there is a clear need to raise farmers' awareness as regards the economic benefits yielded from implementing biosecurity measures, and to dispel the perception of these measures as an additional cost. Finally, the deficiencies in biosecurity in low pig density farms highlight the need for devoting special attention to this farm type that could pose a risk to other types of farms.

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Effect of ractopamine and arginine for gestating sows on the reproductive performance

A Silva[3], CAP Garbossa[1], FC Morais[1], LF Rocha[1], A Resende[2], L Mendonça[1], RHR Moreira[1], R Betareli[2], H Silveira[2], LGM Amaral[2], L Catelli[3], VS Cantarelli[1]

[1]*Department of Animal Science*, [2]*Department of Veterinary Medicine, Federal University of Lavras, Lavras, MG, Brazil*, [3]*Ourofino Saúde Animal LTDA., Cravinhos, SP, Brazil, e-mail:* amilton.silva@ourofino.com

Introduction

The nutrition programs for sows have been developed in a great way in the last years, mainly because it had to be changed to achieve the requirements of the "new" sows that have a large number of piglets born. The hyper-prolificity that considering the number of the piglets born per sow is interesting, but it is not interesting if we consider the lower weight at birth and higher variability of these animals. The increase in the variability can compromise the performance of the litter because the post-natal growth of the muscle of light weight piglets is limited by the lower hypertrophy capacity of the muscle fibers (1) besides that, piglets with low weights have a lower capacity of milk intake. In this way two promising technologies are ractopamine (2) and L-arginine (3). Therefore, the aim of the present study was to evaluate the effect of ractopamine (RAC) and L-arginine (Arg) in the reproductive performance of sows.

Materials and Methods

One hundred sows were used for the trial, being each sow one replicate. The sows were blocked by the parity and were inseminated with the same group of boars, they were divided in four treatments (25 replicates per treatment), the treatment groups were control, (A) – 1,0% of L-Arginine, (R) – 20ppm of Ractopamine and, (AR) – treatment (A) + (R), the treatments were made on-top from day 25 to day 53 of gestation. On day 25 and 53 of gestation and at the farrowing the backfat thickness were evaluated by Renco Lean Meater Series 12. The sows were transferred to the farrowing room with 110 days of gestation. The number of total birth, stillbirth, mummified, piglets, and placenta weight were recorded at farrowing.

The effect of the treatments were evaluated using PROC MIXED ANOVA model followed by Tukey-Kramer adjusted t-test (SAS® Enterprise Guide 4.3, Cary, NC, USA).

Results

The results for total born, mummified, stillborn, and born alive piglets are shown in Table 1, the results for Individual weight, litter coefficient of variation and litter weight are shown in Table 2. The treatment effect had a statistically significant effect on the individual weight of piglets at birth ($p=0.0101$) and percentage of stillbirth ($p=0.0173$).

Conclusions and Discussion

A greater percentage of stillborn were observed in the piglets from the sows that received the treatments when compared to the control it could be related with the higher individual birth weight of the piglets, being harder for the sows to deliver them, but considering the number of born alive piglets there wasn't any influence of the treatments. This shows that the treatments did not had an deleterious influence in the sow performance.

Table 1. Total born, mummified, stillborn, and born alive piglets from sows treated with RAC, Arg or both.

Treatment	Total Born	Mummified (%)	Stillborn (%)	Born alive
Control	13.33	1.93	1.04[a]	12.87
Arg	14.63	4.89	3.96[ab]	13.17
RAC	13.36	2.04	3.59[ab]	12.68
Arg+RAC	13.80	4.83	5.55[b]	12.32

Different superscripts letters indicate statistically significant differences (p <0.018)

The sows receiving RAC or RAC+Arg had piglets with higher weight this should be related with a increased number of the muscle fibers in these piglets.

Table 2. Litter weight, individual piglet weight and Coefficente of variation of the litter from sows treated with RAC, Arg or both.

Treatment	Litter weight (kg)	Individual weight (kg)	Coefficient of variation
Control	17.721	1.368[b]	19.88
Arg	19.748	1.426[ab]	20.58
RAC	19.973	1.542[a]	19.16
Arg+RAC	19.465	1.513[a]	20.64

Different superscripts letters indicate statistically significant differences (p <0.011)

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The effect of Lianol Ferti-T® to the current sow reproductive performance after weaning and following parity

W Nusupa[1], AYuenyaw[1], W Thongmak[1]
[1]*Live informatics co., ltd, Thailand,* arayan.liveinfo@gmail.com

Introduction

During the lactation period, sows are often in a state of negative energy balance (NEB). This catabolic state is a key factor limiting colostrum and milk production, fertility and embryonic survival (1,2). IGF-1 (insulin-like growth factor 1) is a likely candidate to mediate the effects of a NEB (3,4). After many years of development, Lianol® is introduced as a complimentary feedstuff based on highly digestible fermented potato protein. Former research has demonstrated positive effects of this product on plasma IGF-1 levels in sows (5). This study focuses on the effect of supplementing this product on pre-weaning piglet survivability, weaning weight, weaning – first service interval and litter size.

Materials and Methods

174 (LR X LW) sows were divided based on parity, into two groups; a control group of 82 sows and a treatment group of 92 sows. All sows were housed in the same house at the same time. In a first part of this trial, sows were treated around farrowing. The treated sows were fed 10 grams of Lianol ferti-T® per sow per day during feeding from four days pre-farrowing until 1 day post-farrowing (a 6 days treatment). During this part of the trial, litter size (live born, death born and mummified), birth weight and weaning weight was recorded. In a second part of the trial, the selected sows in the treatment group were fed 10 grams Lianol Ferti-T® per sow daily from 2 days before weaning until the date of first service. In this part of the trial, the weaning to first service interval (WFSI) was observed and the litter size (live born, death born and mummified) and farrowing rate was noted.

Results

In the first part of the trial where sows were treated around farrowing, the number of weaned piglets was significantly higher in the group Lianol® supplemented group (see table 1). The number of live born piglets, the percentage of stillborns and mummies was equal in both groups. No effect on daily litter weight gain was observed.

Table 1. Effect of Lianol Ferti-T® supplementation around farrowing. Ns: not significant

	Treatment	Control	Δ	P-value
Number of sows	92	82	10	-
Parity	1.84	2.00	-0.16	-
Litter size	12.18	12.32	-0.14	ns
Live born/sow	11.72	11.76	-0.04	ns
Birth weight (kg)	1.47	1.46	0.01	ns
% stillborns	2.85	2.57	0.28	ns
% mummies	0.98	1.78	-0.80	ns
Weaned per litter	10.00	9.62	0.38	<0.05
Weaning weight	6.82 kg	6.83 kg	-0.01	ns
Litter gain (kg/d)	2.26 kg	2.18 kg	0.08	ns

In the second part of this trial, when treated sows received Lianol Ferti-T® around weaning, the standard deviation of the WFSI was significantly reduced. Furthermore, the number of live born piglets increase significantly from 11.01 in the control group to 11.99 in the treated group.

Table 2. Effect of Lianol Ferti-T® supplementation around weaning. Ns: not significant; SD: standard deviation

	Treatment	Control	Δ	P-value
Number of inseminated sows	89	77	12	-
WFSI (day)	4.42	4.86		
SD of WFSI	1.16	2.75		
Parity	1.96	2.20	-0.24	ns
Farrowing rate (%)	95.50	92.20	3.3	ns
LItter size	12.60	11.73	0.87	ns
Live born/litter	11.99	11.01	0.98	<0.05
% stillborns	3.99	4.31	-0.32	ns
% mummies	1.48	2.04	-0.56	ns

Conclusions and Discussion

When sows were treated with Lianol Ferti-T® around farrowing, the number of weaned piglet per sow significantly increased from 9.62 in the control to 10.00 in the treated group.

If the product was supplied around weaning, the variation of the WFSI and the number of live born piglets improved significantly.

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Early gestation feeding: Effects on litter size and farrowing rate

G Sørensen, LU Hansen, J Vinter
Danish Pig Research Centre, DK 1609 Copenhagen V, luh@lf.dk

Introduction

High feeding levels during the first four weeks after mating affects the reproductive performance of sows[1,2,3]. Hoving (2012) found that second parity sows fed 3.25 kg of feed per day for the first four weeks after mating led to significantly more total born piglets in the subsequent litter compared to second litter sows receiving 2.5 kg per day.

Legislation in Denmark states, that from 2015 sows in new built must be kept loose housed from weaning to farrowing. Unless using electronic sow feeding (ESF) with the opportunity to feed individually, it will be a large challenge for the farmers to meet different requirements of each sow. In other feeding systems with competition between the sows in the critical four weeks after mating the reproductive results may be affected. One way to reduce aggressive behaviour is to increase the daily feed intake.

The hypothesis tested in the trial was that feeding sows with a high energy intake during the first four weeks of gestation would reduce farrowing rates but not affect litter size.

Materials and Methods

In two herds a total of 4,594 gilts and sows in normal heat 3-7 days after weaning was mated and included in the trail. The sows were housed individually in crates after mating and received a set ration of feed twice daily for 28 days. Afterwards the sows were moved to gestations pens with ESF and fed individually.

After mating the sows were allocated to one of three energy levels blocked by parity: Low (28 MJ digestible energy (DE/d); Medium (42 MJ/d) and High (56 MJ/d). The same diet was used for all sows (13 MJ/kg and 12.2 % protein). Sows were weighed and scanned for backfat depth at P2 site at mating and four weeks after mating.

Litter size was analyzed using the mixed procedure of SAS. Week was included as a random effect whereas parity, herd and treatment were included as fixed effects. Farrowing rate was analyzed by logistic regression using the glimmixed procedure of SAS. Week was included as a random effect whereas parity, herd and treatment were included as fixed effects.

Results

As expected, the extra energy during the first four weeks resulted in an increased weight for gilt and sows given Medium and High level of energy. Gilts and sows at Low energy level on the other hand lost weight (Table 1).

Table 1. Effect of extra energy during the first four weeks of gestation on litter size and farrowing rate

	Energy level (DE/d)		
	2.3	3.6	4.6
Number of sows	1,503	1,542	1,549
Average parity	3.6	3.8	3.6
BW at mating (kg)	228±43	235±42	233±42
BW gain, 4 wk (kg)	-5.0±14	1.7±14	13.3±12
P2 at mating (mm)	13.0±3.1	13.3±3.3	13.1±3.1
P2, 4 wk (mm)	0.1±1.5	0.6±1.5	1.4±1.5
Total born per litter (P=0.22)	17.3±3.8	17.2±3.9	17.3±4.0
Farrowing rate (%) (P=0.26)	86	87	88

Table 2. Effect of extra energy on the distribution of total born per litter (%)

	Energy level (DE/d)		
Total born per litter	2.3	3.6	4.6
0-12	20	20	19
13-15	17	15	17
16-18	29	30	28
18-30	34	35	36

Conclusions and Discussion

There were no significant differences in litter size (P=0.22) or farrowing rate (P=0.26) between the groups. Also there were no differences between groups on the distribution of total born per litter (Tabel 2).

This is in agreement with results by Athorn *et al.* (2011) who also found no differences, but are in contrast to findings by Hoving (2012).

Acknowledgments

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Performance and carcass traits from finishing pigs fed with fibrous diets

B Berenchtein[1], A Abdalla[2], H Louvandini[2], A Abdalla Filho[2], P Lima[2], D Danashekaran[2], M Sbardella[3], P Santos[2], A Souza[2], E Santos[2], H Correa[1], F Simas[1]

[1]*Institute of Social Sciences, Education and Animal Science, Federal University of Amazonas, Laboratory of Studies and Researches of Poultry and Swine Nutrition and Production (LEPPNAS), Parintins, AM,* [2]*Centre of Nuclear Energy in Agriculture, University of São Paulo, Animal Nutrition Laboratory, Piracicaba, SP,* [3]*ESALQ,University of São Paulo, Piracicaba, SP,* bernardob@ufam.edu.br

Introduction

The possibility of using forages and other hays as feedstuffs in swine production had already been theorized (1). However, further studies on the potential of various fibrous feedstuffs in swine production through the identification, quantification and evaluation of interactions between physiological and associative effects on digestibility, animal performance are needed. Therefore, the purpose of this study was evaluated the effects of fibrous diets, with inclusion of 8% of NDF, on performance and carcass traits of finishing pigs.

Materials and Methods

Eighty commercial hybrid pigs were used (120 days of age and initial live weight of 71 ± 2.5 kg), spread across 40 stalls according to sex and body weight, being a castrated male and a female by pen (experimental unit). The animals had access to isoenergetic and isoproteic diets (basal diet and Citric pulp, Tifton hay, Soybean hull, with inclusion of 8% of NDF) and water for the entire trial period. To reach 145 days old, the animals were slaughtered and the carcasses evaluated for carcass length, backfat thickness, loin eye area and muscle-to-fat ratio according to (2). The analysis of variance was performed using PROC GLM of SAS (3) and the means was compared by Tukey test (P<0.05).

Results

The final body weight (FBW), average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), carcass length (CL), backfat thickness (BT), loin eye area (LEA) and muscle-to-fat ratio (RF/M) are shown in Table 1. Pigs fed with Soybean hulls showed significant higher (p<0.05) ADG and ADFI. For carcass traits (CL, BT, LEA and RF/M) no significant difference (p> 0.05) between treatments was founded.

Conclusions and Discussion

Soybean hulls (inclusion of 8% NDF) will be used like feedstuff in finishing pigs diets. (4) do not observed changes in performance of finishing pigs when using Soybean hulls supplemented with some energy source. However, it is important to note that to maintain energy levels, it is necessary to include some energy source.

Table 1. Growth performance and carcass traits of finishing pigs fed with Basal Diet (BD), Tifton Hay (TH), Citric Pulp (CP) and Soybean hull (SH), and Coefficient of Variation, % (CV).

Variable	BD	TH	CP	SH	CV
Inicial BW, kg	70.52	71.12	70.27	72.13	-
Final BW, kg	93.59^{a}	88.44^{a}	81.53^{a}	90.43^{a}	8.09
ADG, kg/day	0.91^{b}	0.88^{b}	0.10^{c}	1.22^{a}	21.98
ADFI, kg/day	2.63^{b}	2.34^{c}	1.88^{d}	2.84^{a}	8.51
FCR	2.85^{ab}	2.90^{ab}	3.80^{b}	2.37^{a}	37.94
HCY[1], %	81.72^{a}	80.54^{a}	80.30^{a}	80.24^{a}	3.70
CL[1], cm	90.65^{a}	90.04^{a}	88.80^{a}	87.78^{a}	3.23
BT[1], cm	2.13^{a}	2.30^{a}	2.39^{a}	2.42^{a}	14.92
LEA[1], cm^2	35.25^{a}	35.13^{a}	34.11^{a}	34.12^{a}	9.44
RF/M[1]	0.38^{a}	0.39^{a}	0.39^{a}	0.41^{a}	15.31

ADG-Average daily gain; ADFI-Average feed intake; FCR-Feed conversion ratio; HCY- Hot carcass yield; CL- Carcass length; BT- Backfat thickness; LEA- Loin eye area; RF/M- Relation Fat/Meat;

[1]Adjusted means by covariance for the slaughter weight of animals;

(a,b) Superscripts indicate statistically significant differences within main effect ($p \leq 0.05$)

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CNPq, Centre of Nuclear Energy in Agriculture, University of São Paulo (CENA-USP) and Federal University of Amazonas (UFAM), Parintins Campus.

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Morphometry of the duodenal mucosa of pigs fed with different diets supplemented with multienzimatic complex

RL Silveira[3], TP Bonaparte[1], RTRN Soares[2], RP Araujo[2], ECQ Carvalho[2], RM Medina[2], RB Ribeiro[2], MA Silva[2], AO Carvalho[2], JG Vargas Júnior[1]

[1]*Department of animal science, Espírito Santo Federal University,* [2] *LMPA, North Fluminense State University,* [3]*PPGMVCR, MMO, MZO - Fluminense Federal University,* talitabonaparte@gmail.com

Introduction

Early weaning of piglets maximizes pigmeat production through the increase in the number of births per array/year. Weaners early lack the digestive system able to digest all nutrients found in foods provided. Weaning diarrhoea occurs can be generated by the power exchange with modifications in the intestinal epithelium, reduction in the absorption of nutrients and increased occurrence of enteric problems (1). The objective of this study was to evaluate the use of the Allzyme SSF and different diets on the duodenal mucosa of pigs Morphometry of 30 kg.

Materials and Methods

The experiment was of 10 to 30 kg liveweight, with randomized blocks design, factorial arrangement (2 x 2 x 2), composed by factors: Mutienzimatic Complex: 0 and 0.02%; Metabolizable energy + digestible Lysine: 3375 Kcal/Kg + 1.33% and 3300 Kcal/Kg + 1.23%; Wheat bran: 0 and 7% in the diet, totaling eight treatments and six replications. Portions of the duodenum were collected from 48 pigs for morphometric analysis of the intestinal epithelium. The samples were kept in neutral buffered formalin solution to 10%, and processed and evaluated. Histotécnico processing was for inclusion in paraffin for routine staining. The cut of the material was performed with 5 µm thickness, the sections were placed on slides and stained with hematoxylin and eosin. The reading was held in Microscope Nikon Eclipse 80i, no Software Imaging Software, no Programa NIS – Elements BR, Advanced Solutions for your imaging World Basic Research (Uenf, Campos, RJ, Brazil). The parameters studied were villus height (HV, µm), depth of crypts (DC, µm) HV measures were from the upper base of the crypt until the apex of the villi and the measures of DC were taken between the villi of the lower base until the upper base of the crypt. The data were analyzed by using the mixed models procedure (PROCMIXED) of SAS (v.9, SAS Systems, Inc., Cary, NC, USA), using ANOVA followed by Tukey-Kramer adjusted t-test (SAS® Enterprise Guide 4.3).

Results

In relation to the DC was effect of wheat bran factor (P = 0.005), deepening of the Crypts to introduce this food in the diet. The values observed for PC were, 413.0 ± 26.8 (µm) in the absence of wheat, and 468.3 ± 26.8 (µm) with the inclusion of 7% of wheat bran in the diet. Was observed weak evidence of effect of interaction between the enzyme factor and the wheat bran (P = 0.077) for both DC and HV (P = 0.084), however, due to inherent variability measurement of these variables has been chosen by the unfolding of these effects (table 1).

Conclusions and Discussion

The introduction of wheat bran in diets containing enzyme complex promoted increase in DC, while for AV, no significant effect was found after scrolling. The result may be related to a difference in diet consumption with inclusion of wheat bran, and due to the high value of this food fiber irritate the intestinal mucosa by mechanical abrasion, leading to increased endogenous secretion of mucus and water and in the renewal of the cells of the intestine (3).

Table 1. Effect of interaction between wheat bran and use of enzymes in the DC (µm) and HV(µm) of the duodenal mucosa of pigs.

Wheat bran	Depth of crypts		Villus height	
	Multienzimatic Complex			
	0 %	0,02%	0 %	0,02%
0 %	444,0 ± 37	382,0 ± 37^{A}	478,1 ± 44	444,7 ± 44,
7%	465,1 ± 37	471,5 ± 37^{B}	441,5 ± 44	485,6 ± 44

Medium followed by equal letters uppercase the column did not differ among themselves by Tukey test at 5% probability.

The greater nutrient absorption occurs due to greater surface area in the mucosa of the small intestine caused by the villi. The reduction in height of the villi and the increase in depth of crypts are often observed as a function of weaning, exchange diet and lower consumption, reducing the absorption capacity (2) and the use of food.

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P.680

Effect of L-carnitine in sow diets on performance of sows and piglets

SL Kinzinger[1], CMC van der Peet-Schwering[2], T Ihnen[1], J Willamil[1], E von Heimendahl[1]
[1]*Lohmann Animal Health GmbH, Germany,* [2]*Wageningen UR Livestock Research, The Netherlands*
joseane.willamil@lohmann.de

Introduction

Several studies have shown that L-carnitine supplementation of sow diets increases reproductive performance and performance of piglets. The aim of the present study was to investigate the influence of carnitine in high performance sows and their offspring.

Materials and Methods

50 postpuberal gilts (Dutch Landrace x Dutch Large White) were allotted to two treatments and monitored over two parities for a 9-months period. They received diets containing either no supplemental carnitine during gestation and lactation (Control) or 50 mg L-carnitine/kg feed during gestation and lactation (LC). All sows had free access to drinking water. Piglets were given free access to a commercial creep feed from d20 after birth until weaning at d26. Body weight (BW) and backfat thickness (BFT) were measured at the day of transfer to the mating room, at the day of transfer to the farrowing room and at weaning in parity 1 and 2. BFT was measured ultrasonically at the last rib, 5 cm left and right of the median. Feed intake of the sows also was measured. Number of total born piglets and weaned piglets, and weights of live born piglets at parturition, at d12 and 19 and at weaning were also determined. Furthermore, weaning-to-oestrus interval and number of sows that returned to oestrus were recorded.

Results

Feed intake and BW development of sows during all production stages were not affected by carnitine. Backfat loss during lactation was numerically less in the LC group in both parities. Parity 1 sows receiving carnitine had a significantly shorter weaning-to-oestrus interval (5.2 vs. 7.7 days) while in parity 2 this interval was similar in both treatment groups.
The number of weaned piglets per litter was numerically higher in parity 1 sows receiving carnitine (11.4 vs. 10.7) while the percentage of culled piglets was lower in the carnitine group (8.9 vs. 15.2%). Especially the number culled with reason "not viable" was reduced by carnitine supplementation to the sows (2 vs. 11).

Table 1. Effect of L-carnitine in sow diets on performance of sows

	Parity 1		Parity 2		*p*-value	
	C	LC	C	LC	Treat	Parity
Carnitine in milk at day 12 (mg/L)	23.7^{c}	31.1^{b}	27.8^{b}	49.1^{a}	0.05	0.10
Weaning-to-oestrus interval (days)	7.7^{a}	5.2^{b}	5.2^{b}	5.2^{b}	<0.001	0.01

(a, b) Superscripts indicate statistically significant differences ($p \leq 0.05$)

Discussion

Aim of this study was to test the effect of carnitine in modern, high performance sows. In contrast to previous studies with lower reproductive performance (1) there was no influence of the treatment on the number of piglets. Carnitine plays an important role in fat and carbohydrate metabolism (2). This might explain the greater effect of the supplementation in first parity sows as they need energy for both, own body weight gain and reproduction and therefore experience greater metabolic stress. An improved fat metabolism, indicated by the numerically reduced backfat loss during lactation, could also be the reason for the reduced weaning to estrus interval in the LC supplemented group. This was also found in lactating cows experiencing reduced metabolic load due to LC supplementation (internal data, not published).
The higher number of weaned piglets in the LC supplemented group is mainly a result of reduced culling of non-viable piglets. Carnitine has been shown to not only improve prenatal (3) but also early postnatal myofiber formation (4). As the milk of the supplemented sows contained significantly more carnitine than that of the unsupplemented sows, this could be a reason for the better performance of piglets from LC sows.
In conclusion, L-carnitine has been shown to be a suitable ingredient to improve sow reproductive performance and piglet development, especially in first parity sows experiencing greater metabolic stress.

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Use of chromium yeast in diets for finishing pigs

D Baffa[1], M Hannas[1], H Rostagno[1], F Silva [1], M Chizzotti, F Rutz[2], C Pereira[1], M Almeida[1],
[1]*Department of Animal Science, Universidade Federal de Viçosa, Viçosa, MG,* [2]*Universidade Federal de Pelotas, Departamento de Medicina Veterinária.* melissa.hannas@ufv.br

Introduction

In view of the high cost of production in the finishing phase of pig rearing, research has been conducted aiming to evaluate additives that can be used as animal-performance enhancers without acting as chemotherapeutics and or antibiotics. Chromium Yeast (CrY) is a source of chromium in the form of organic mineral developed by the industry for use in animal nutrition. Chromium can affect the protein metabolism because the insulin-sensitive cells capture greater amounts of glucose and convert it to energy, besides the greater stimulation of the uptake of amino acids[1]. An additional amount of energy serves as fuel for the protein synthesis[2]. A meta-analysis of experiments revealed effect of the Cr supplied in the diet on fat reduction, increase in the carcass protein deposition and improvement in the feed efficiency of pigs[3]. However, in other studies, the results of Cr supplementation were inconsistent and varied according to differences in the source and time of supplementation of Cr in the diet. The present study was conducted to evaluate the inclusion of CrY as an additive in diets for finishing pigs on the performance parameters of these animals.

Materials and Methods

Ninety-six commercial hybrid barrows with initial body weight (BW) of 75.07 ± 3.9 kg. were distributed into a randomized blocks design composed of two treatments, eight blocks with three replicates and two animals per pen. The treatments consisted of a control diet (C) and a diet with inclusion of 400 ppb of Chromium Yeast (CrY). The experimental diets were formulated so as to meet the nutritional requirements of the animals[4]. Throughout the experimental period, feed and water were available *ad libitum*. The evaluated variables were final BW (Kg), average daily gain (ADG, Kg/day), average daily feed intake (ADFI, Kg/day) and feed conversion (F:G). The effects of the nutritional plans were analyzed using a mixed effect two-way ANOVA by the F test (SAS® Enterprise Guide 4.3, Cary, NC, USA).

Results

Inclusion of CrY in the diets for the pigs in the finishing phase promoted an improvement in the parameters ADG ($P<0.04$) and ADFI ($P<0.03$), of 5.3 and 4.81%, respectively. In contrast, Cr did not affect ($P>0.05$) the final BW or F:G of the animals.

Conclusions and Discussion

Because the F:G was not affected by the treatments, the effect of CrY on the performance of pigs in the evaluated phase is directly related to the increase in ADFI and consequently greater nutrient uptake for the weight gain. The increase in the ADG in pigs obtained with addition of CrY in the diet provided animals with a daily weight gain close to that described in the growth tables of male pigs with high genetic potential and high performance[4]. The results of the present study confirm that inclusion of 400 ppb of Chromium Yeast in diets for pigs with 75 to 90 kg of body weight acts as an additive enhancing animal performance.

Table 1. Growth performance of barrows fed control (C) or Chromium Yeast (CrY) diets in the finishing phase

Parameters	C	CrY	SEM	CV (%)
Initial BW (Kg)	75.17	74.96	0.02	5.06
Final BW (Kg)	89.95	90.79	1.69	4.39
ADG (Kg/day)	1.05 b	1.11 a	0.21	7.33
ADFI (Kg/day)	2.96 b	3.12 a	0.05	6.17
F:G	2.824	2.805	0.04	5.31

[a,b] - Means followed by different letters in the same row differ ($P<0.05$) by the F test.

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Alltech INC and Alltech Brazil.
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Effect of total replacement of inorganic minerals by organic minerals on growth performance, fecal excretion, hemoglobin concentration and hematocrit in weaning pigs

L Hernández[1], R Sahagún[1], M Forat[2], V Navarro[2]
[1]*Alltech de México*, [2]*Instituto Internacional de Investigación Animal*, lhernandez@alltech.com

Introduction

Minerals are important for maintaining correct growth in growing animals. The current work is concerned with the comparison of feeding different forms of manganese, iron, copper, zinc, selenium and chromium to weaned pigs. These minerals are recognized as necessary for correct and efficient growth in animals. Minerals are available, broadly, in two forms: inorganic (such as oxides and sulphates) and organic (chelated to small peptides or amino acids via chemical or biochemical means). Various experiments have shown improvements in productive performance in animals receiving minerals that have been chelated to certain length peptides, due to improved uptake from the digestive tract and better distribution and utilization within the body (1). Feeding these chemically organic forms may result in higher circulating plasma concentrations (2) or increased storage in tissues (3).

Materials and Methods

Two hundred sixteen weaning pigs, hybrid product of white females x black male, approximately 6 kg of weight at the beginning of the experiment were used.

They were assigned to 3 treatments with 72 pigs per treatment, 12 replicates per treatment and 6 pigs per replicate. The pigs received the experimental diets from day 21 to 56 of age, which were isoenergetic and isonitrogenous.

The treatments were: T1. Inorganic minerals, T2 and T3 Organic Minerals with a lesser dose in T3 (Table 1). The variables evaluated were weight gain, feed intake, feed conversion, copper and iron levels in feces, serum hemoglobin and hematocrit at day 35 and 56. The data was analyzed by the t-test and GLM Procedures of the SAS system for Windows, and confidence limits for significance at 5%.

Results

There was no statistical significant difference between treatments in the variables weight gain, feed intake, feed conversion, hemoglobin concentration and hematocrit. There was an increased excretion of Cu and Fe in the T1 (inorganic minerals) compared with treatments T2 and T3 (organic minerals) at day of age 35 (Fe $p < 0.01$ - Cu $p < 0.001$) and 52 (Fe $p < 0.07$ - Cu $p < 0.12$).

Conclusions and Discussion

Despite the inclusion of minor minerals in T2 and T3 there was no difference in weight gain or feed conversion, probably due to increased bioavailability of organic minerals (1), this matches a greater excretion of Fe and Cu in T1. These results also indicate that it is possible to make a total replacement of inorganic minerals by organic ones without affecting these variables. An additional advantage is the reduction of the excretion of minerals in the environment.

Table 1. Concentration of minerals in the treatments.

	Treatments		
MINERAL	1	2	3
Zn	100	37.5	24.7
Cu	6.0	3.75	2.47
Mn	40	7.5	4.95
Fe	100	30	19.8
Se	0.30	0.11	0.07
Cr	-	0.07	0.04

Table 2. Concentration of Fe and Cu in the feces of pigs at 35 and 56 days old (mg/kg).

	FE		CU	
	Day 35	Day 56	Day 35	Day 56
T-1	2543a	4021a	262	391
T-2	2247b	2627b	67	151
T-3	2206b	2580b	90	285

Means not sharing a letter differ significantly ($P < 0.05$).

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Essential oil of *S. terebinthifolius* Raddi (Brazilian red pepper) on intestinal microbiota and pH of digestive content of weanling pigs

FD Gois[1], PL Cairo[1], MRC Amaral[2], C Andrade[2], ELB Costa[2]
[1]*Department of Agricultural and Environmental Sciences – UESC,* [2]*School of Agricultural Sciences and Veterinary Medicine – PUCPR, Brazil,* batista.leandro@pucpr.br

Introduction

Essential oils as growth promoters have been showed positive effects on performance of broilers (1) and intestinal microbiota of weanling pigs (2). *In vitro* studies, essential oil of *Schinus terebinthifolius* Raddi showed antimicrobial effect (3) and *in vivo* studies should be performed. Thus, the objective of this study was to evaluate the essential oil of *Schinus terebinthifolius* Raddi as replacement to antimicrobial on intestinal microbiota and pH of digestive content of weanling pigs.

Materials and Methods

Ninety 21-d weaned male pigs (5.6 ± 0.78 kg BW) were used in a randomized complete block design experiment with six treatments, six replications per treatment and three animals per experimental unit (pen). The treatments were basal diet (BD) with 120 mg/kg of chloro-hydroxyquinoline (ANT), and BD with 0, 500, 1,000 or 1,500 mg/kg of the essential oil. At the end of the experimental period (35 days), one animal from each pen was slaughtered, after 8h fasting, for measurements of pH of digestive content (stomach, jejunum and cecum). For intestinal microbiota, the intestine was cut longitudinally and the contents of the jejunum and cecum were collected by scraping with a spatula. Afterwards, the samples were placed in identified plastic bags and sent to Microbiology Laboratory of UESC, Ilhéus, Brazil, for quantification of Total bacterium, *Enterobacterium* and *Lactobacillus* spp, by (4). These results were expressed in colony-forming units per gram of sample (cfu.g^{-1}). Data were submitted to analysis of variance using the "R" 3.00 for Windows (5). When ANOVA was significant ($p < 0.05$), differences between treatments means were compared using Friedman test ($p < 0.1$).

Results

No effects ($p > 0.05$) of the treatments were observed on pH of digestive content of weanling pigs (data not shown). For intestinal microbiota of jejunum, there was effect ($p = 0.06$) of essential oil on *Lactobacillus* spp. count (Table 1).

Conclusions and Discussion

Pigs fed diets with 0 (negative control) or 500 ppm of essential oil had higher ($p = 0.06$) *Lactobacillus* count in the jejunum than those fed diets with antimicrobial or 1,000 ppm of essential oil. (2) also demonstrated positive effect on intestinal microbiota, decreasing the *E. coli* count in the feces of weanling pigs. No effects ($p > 0.05$) of treatments were observed on pH of digestive content of weanling pigs, corroborating with (6). Therefore, 500 ppm of essential oil of *Schinus terebinthifolius* Raddi was more efficient than antimicrobial to improve the intestinal microbiota, but without affecting the pH of digestive content of weanling pigs.

Table 1. The intestinal microbiota of weanling pigs fed with antimicrobial or different levels of essential oil (*Schinus terebinthifolius* Raddi) for 35 days post-weaning

Treatments[1]	Item		
	Bacterium count, cfu.g$^{-1,2}$		
	Total	*Enterobacterium*	*Lactobacillus*
	Jejunum		
ATN	17.0	15.0	13.0^{b}
0	21.0	20.0	22.5^{a}
500	18.5	17.5	23.0^{a}
1,000	12.5	17.0	12.0^{b}
1,500	21.0	20.5	19.5^{ab}
EPM[3]	21.05	16.66	19.47
	Cecum		
ATN	11.3	10.9	11.3
0	11.1	10.1	11.0
500	11.3	10.9	11.0
1,000	11.1	11.3	11.0
1,500	11.2	11.0	11.2
EPM[3]	20.25	20.48	20.31

[1]ANT=Antimicrobial; 0; 500; 1,000 and 1,500 mg/kg of the essential oil of *Schinus terebinthifolius* Raddi; ²Colony-forming units per gram of sample; [3]The standard error of the mean; Within a column, means without a common superscript differ (p =0.06) by Friedman Test.

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Evaluation of different ractopamine feeding programs on growth performance and carcass characteristics of finishing pigs in Yucatan, México

E Ramírez[1], D Solis[1], A Jordan[1], C Feoli[2] , A Alfonso[1],
[1]*Grupo Porcícola Mexicano-Keken, Mérida, YUC, Mexico.*[2] *Cargill Animal Nutrition, Umán, YUC, México.*
esteban.ramirez@keken.com.mx

Introduction

Ractopamine HCL is widely used in swine industry to improve growth and carcass traits of finishing pigs (1,2,3). Although different RAC feeding strategies have been studied, data are not consistent on the ideal approach between a constant or step-up feeding method (2). The majority of work evaluates RAC at levels of 5 and 10 ppm, however no publications for step-up using 5 and 7.5 ppm are known.

Therefore, the objective of the present study was to determine the effect on growth performance, carcass characteristics and economic impact of 3 different RAC-feeding programs.

Materials and Methods

The experiment was conducted in a commercial finishing farm in Yucatan, Mexico. One barn of gilts and one barn of barrows were used. The barns were identical, naturally ventilated and curtain sided, every two pens shared a WF9 Crystal Spring Feeder to form an experimental unit.

A total of 864 gilts and 864 barrows (136 d old), were used in this 29-d experiment, with 36 pigs per pen, 16 pens or 8 replicates per treatment. Pens were randomly assigned to 1 of 3 treatments balanced by average initial BW.

Treatments were a basal corn-soybean diet containing 0.9% of digestible lysine with a) 5 ppm RAC from d 0 to 14 and 7.5 ppm from d 15 to 29 (Step-up 1), b) no RAC from d 0 to 7 and 7.5 ppm from d 8 to 29 (Constant), and c) 5 ppm RAC from d 0 to 14 and 10 ppm from d 15 to 29 (Step-up 2).

Feed was measured daily and offered manually to the pigs. Pigs were weighed as a group at the beginning and end of the trial. ADG, ADFI and F/G were determined. On d 29, HCW was collected and Fat-O-Meter was used to determine muscle, backfat, loin depth, and carcass lean at the processing plant.

Statistical analysis was performed using the PROC MIXED procedure of SAS. Data were analyzed as a randomized complete block design. Location within the barn and sex were the blocking factors.

Results

Economic analysis, growth performance and carcass characteristics are shown in Tables 1 and 2.

There were no statistical differences between Step-up programs. Statistical differences between Constant and Step-up programs were observed in ADG, F/G, feed cost, feed/kg gained and HCW.

Table 1. Effect of different RAC feeding programs on growth performance of finishing pigs and economic impact.

	Feeding program		
Item	*Step-up 1*	*Constant*	*Step-up 2*
Average initial weight, kg	94.21	94.23	94.21
Average final weight, kg	124.57	122.03	123.89
ADG, kg	1.047[a]	0.959[b]	1.023[a]
ADFI, kg	2.69	2.65	2.69
F/G, kg	2.57[a]	2.77[b]	2.63[a]
Feed cost, $/ kg gained	1.04[a]	1.09[b]	1.08[ab]
Income, $/kg gained[1]	63.76[a]	58.38[b]	62.33[a]
Income over feed cost , $/pig	32.05[a]	27.96[b]	30.37[ab]

[abc] Means on the same row with different superscripts differ (P <0.05).
[1] $2.10/ kg live wt.

Table 2. Effect of different RAC feeding programs on carcass characteristics of finishing pigs and economic impact.

	Feeding program		
Item	*Step-up 1*	*Constant*	*Step-up 2*
HCW, kg	101.94[a]	100.61[b]	102.37[a]
Muscle, %	52.93	52.88	52.95
Lean, %	54.25	54.21	54.19
Loin Depth, mm	71.33	70.78	70.79
Backfat, mm	15.53	15.58	15.40
Income, $/kg gained[1,2]	68.14	64.67	69.23
Income over feed cost , $/pig	36.41	34.23	37.27

[abc] Means on the same row with different superscripts differ (P <0.05).
[1] Calcualted based on $2.56/kg carccass.
[2] kg carcass gained= HCW-(Initial weight*0.8).

Conclusions and Discussion

Step-up programs resulted in better F/G and ADG which is shown in the lower feed cost/kg gained. Income over feed cost, at the farm, was better for Step-up 1 program vs Constant program, while Step-up 2 was intermediate.

HCW was better in Step-up programs, but no differences in carcass characteristics were observed between the 3 treatments. As the price of carcass kg is given by carcass quality, no differences could be observed in carcass income over feed cost; however, volume of meat commercialized could be affected with the Constant program.

Step-up programs were similar in performance, but feed cost/ kg gained was lowest in Step-up 1, which is why the use of RAC step-up feeding programs containing 5 and 7.5 ppm is recommended. Under the conditions of this production system, no benefit was observed with the use of a Constant program containing 7.5 ppm of RAC.

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Effect of the addition of fresh avocado paste in feeding pigs on productive performance traits

SH Hernández[1], JG Rodríguez-Carpena[1], C Lemus[1], F Grageola[1], J Galindo[2], F Loya-Olguín[3]
[1]*Autonomous University of Nayarit,* [2]*University of Guadalajara,* [3]*Promin group SPL of RL*
silviahdezlopez@hotmail.com

Introduction

In nutrition a great effort to achieve increasingly efficient and economical pig diets with high nutritional requirements is made. One strategy is the incorporation of unconventional foods in the diet. Avocado antecedent has focused on numerous studies as a source of fatty acids or sterols of biological interest (4), in the presence of phenolic compounds and pigments with antioxidant activity (1,5). In recent reports the pulp (3) and fresh avocado paste (pulp, seed and peel) (2) as unconventional food in the feeding of pigs was used, with 20 % inclusion to a sorghum-soy bean basal diet, reporting the 86.91 % digestibility (3) and there were no signs of rejection on feed intake (2,3). The importance of using avocado pulp in the feed of pigs (high oleic ingredient) is to improve feed efficiency, change to unsaturated fatty acid profiles and improve the quality of animal protein, because during the physiological processes of growth and fat deposition, these could be modified and cause beneficial changes in meat quality. Therefore, the objective is evaluate the productive performance traits of growing pigs by adding fresh avocado pulp in the diet.

Materials and Methods

Eight hybrids York-Landrace pigs, castrated males, in growth stage were allotted at a randomized experimental double Latin square design (4 x 4) to study the influence of the addition of different levels of fresh avocado paste (Table 1) on growth performance traits and digestibility percentage.

Table 1. Formulation of the diets used in the experiment

INGREDIENTS	Fresh Avocado Paste, %[1]			
	0	**10**	**20**	**30**
Sorghum meal	83.7	74.3	65.1	55.7
Soybean meal	12.9	12.6	12.2	11.9
Fresh avocado paste	-	10	20	30
$CaHPO_4H.2H_2O$	1	0.9	0.8	0.7
$CaCO_3$	1.2	1.1	1	0.9
NaCl	0.2	0.2	0.1	0.1
Premix[2]	1	0.9	0.8	0.7
Analysis calculated				
N x 6.25	14	14	14	14

[1] *The paste was composed of the entire, discarded and ripe fruits. Percent dry matter.* [2] *Vitamins and trace elements.*

The amount of consumption in matter dry was of 10% of metabolic weight (0.10 kg $DM/W^{3}/_{4}$).

Results

Pigs successfully accepted diets with different percentages of avocado paste. It is clearly seen as pigs fed the diet with 30% of avocado paste had a better productive performance compared to the other levels (Table 2).

Table 2. Productive performance traits in pigs fed diets with fresh avocado paste

	Fresh Avocado Paste, %			
	0	**10**	**20**	**30**
WWG (Kg)	2.511	2.931	2.966	4.041
FC	3.926	3.462	3.53	2.353
FE (CP/WG) (Kg)	0.55	0.485	0.494	0.329
Digestibility (%)	87.33	87.39	86.63	86.25

WWG: weekly weight gain, FC: Feed Conversion, FE: feed efficiency (crude protein diet / weight gain).

Conclusion and Discussion

Pigs fed the diet containing 30% of avocado paste showed higher WWG, better FC and better FE, this is possibly due to having more energy available by lipid rich diets (specially by the high amount of unsaturated fatty acids from avocados) what resulted in better utilization of dietary protein (isoproteic diets). Percentage of digestibility four diets had a similar result, this is encouraging to indicate that diets with inclusions of up to 30% of avocado paste are efficient. The use of avocado paste as unconventional food, proved to be a good alternative in the feeding of pigs to improve their productive performance and could be used as a strategy to improve the quality and oxidative stability of the meat through the modification of lipid profile and the use of phenolic compounds reported in the peel and seed.

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Performance enhancement and feed efficiency of Donmany® in Korea

H Cho[1], E Cho[1], J Min[1]
[1]*Techinical Institute, KBNP Inc., Korea,* albatross1@lycos.co.kr

Introduction

Lots of risk-inducing factors such as continuous growth of feed cost, disease outbreaks and manure problems have been influencing in managing pig farms in Korea. Particularly, the part of feed purchase cost has occupied more than 40~50% of whole pork production cost, so that the elevation of feed efficiency is a key point in increasing farm incomes. To decrease the feed cost, many pig farms have been utilizing feed additives for the growth enhancement in growing to fattening period and have been updating farm facilities. To maximize farms sales revenue by providing more pigs to the market, it has been crucial points to produce a large number of piglets with less mortality in pre-weaning and weaned stage and transfer the pigs to growing to fattening houses with the maintenance of healthy state as many as possible. And also pig survival rate and health conditions of 7-13 weeks old pigs give a significant impact on the number of pigs sent to the market.

Donmany® is the feed additive with an active ingredient of *Toxicodendron vernicifluum*(Stokes) extract that is called "Ott tree" in Korean. "Ott" is a caustic, toxic sap, called urushiol, is tapped from the trunk of that tree to produce lacquer. The resin of the "Ott tree" has been used in Korean traditional medicine for the recovery after diseases and for stopping bleeding. Donmany® was evaluated to increase the feed efficiency and survival rate during this period of 7-13 weeks of age.

Materials and Methods

A total number of 106 pigs that are 7 weeks old were used for the experiment in two commercial farrow-to-finish pig farms. In A farm with 330 sows, 79 pigs of 7 weeks old (the average bodyweight = 13.5 kgs) were allocated into 2 groups: Control-A(Donmany® free feed), Treatment-A (Added Donmany® to the feed at 2kg/MT). In B farm with 120 sows, 27 pigs of 7 weeks old (the average bodyweight =13.2 kg) were consisted of 2 groups: Control-B(Donmany® free feed), Treatment-B (Added Donmany® to the feed at 2kg/ton). Donmany® has been provided until 13 weeks old. The efficacy of Donmany® was evaluated by comparing feed consumption, body weight gain and mortality between two groups. Results were analyzed by T-test using SAS® 9.2 software.

Results

In A farms, the average BW was 13.5kgs at 7 weeks of age in all groups but the final average BW at 13 weeks of age were 41.5 and 42.6 kg respectively. In B farm, the average BW was 13.2kg at 7 weeks of age in all groups but the final average BW at 13 weeks of age were 40.3 and 41.9kg respectively. The mortality during the period was 8.0% and 4.0% respectively in A farm and 7.0% and 3.0% in B farm. A total of average feed consumption per pig was 72.9 and 65.1kg respectively in A farm and was 70.5 and 64.9kg in B farm (Table 1).

Table 1. Results of the weight gain, mortality and feed consumption between treatment and control group

	A farm		B farm	
	Control	Treatment	Control	Treatment
Initial avr. BW/pig(kg)	13.5	13.5	13.2	13.2
Final avr. BW/pig(kg)	41.5	42.6	40.3	41.9
Weight gain(kg)	28.0	29.1	27.1	28.7
Mortality(%)	8.0	4.0*	7.0	3.0*
Total feed consumption /pig(kg)	72.9	65.1*	70.5	64.9*
Feed efficiency**	2.60	2.23	2.60	2.26

* p<0.05, ** = Total feed consumption(kg)/weight gain(kg)

Conclusions and Discussion

We found the weight gains had increased, the survival rate had enhanced and total feed consumption had decreaed in both Treatment groups with the supplement of Donmany® at the rate of 2kgs/MT. Especially, in the treatment group mortality % was much lower than the control group($p<0.05$) and in the experiment period, total feed consumption amount showed a significant difference beween the control and the treatment group($p<0.05$). In order to obtain more accurate information about the effects of Donmany®, additional samples will be necessary. However, the results so far show that Donmany® would give more performance enhancement and feed efficiency on the 7-13 week aged pigs.

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Defining the nutrient requirements of male pigs immunized with Improvest®

D Nelson, PJU Moraes, MA Mellencamp, J Allison
Zoetis Inc, Florham Park, NJ, daniel.nelson@zoetis.com

Introduction

Physical castration (PC) of male pigs has been a common practice for centuries. However, research has shown that intact male pigs produce more muscle (e.g. lean tissue) and are more efficient in conversion of feed to muscle than PC barrows. Nevertheless, production of intact males has negative implications including off-odor pork. Immunological castration (IC) using Improvest® (*gonadotropin releasing factor analog-diphtheria toxoid conjugate*), an FDA approved veterinary-prescription product, can be used in place of PC to reduce off odors in pork. Physiologically, IC barrows are similar to intact males until 7-10 days after the second Improvest® dose. After this transition, IC show a substantial increase in feed intake and body lipid deposition (Ld). Also, IC barrows exhibit slight reductions in protein deposition (Pd) and maintenance energy requirements, as their metabolism changes to resemble that of PC barrows. These changes in feed intake and growth performance must be considered when developing feeding programs.

There have been very few studies exploring nutrient requirements of intact males and IC barrows with contemporary US genotypes. As an alternative to conventional nutrient requirement studies, nutrient requirements may be estimated based on a factorial (modeling) approach. Modeling requires that the main determinants of energy and nutrient requirements (Pd, Ld, feed intake and BW) are characterized. Using the NRC (2012) pig growth model, performance data from 6 US commercial research trials were used to estimate Pd and Ld curves, which provided the basis for estimating nutrient requirements of IC barrows.

Materials and Methods

The NRC (2012) publication 'Nutrient Requirements of Swine' includes a model that can be used to generate Pd and Ld curves for pig groups from their observed feed intakes and growth rates. This information can be used to estimate their nutrient requirements. Lean tissue growth is closely associated with Pd. Pd is an objective measurement and key determinant of nutrient (lysine) requirements. Analyses were conducted that compared the observed performance of gilts, PC and IC barrows from 6 Zoetis-sponsored trials to that predicted by the NRC (2012) model, assuming 5% feed wastage and a diet ME content of 3300 kcal/kg. Key assumptions were (1) no impact on gut fill; (2) no impact on nutrient partitioning other than energy intake, maintenance energy, and Pd (and thus Ld); and (3) simultaneous and linear changes in feed intake, maintenance energy requirements, Pd (and Ld) during the transition period.

Results

In PC barrows and gilts, the observed ADFI were 3% lower, but actual growth rates were identical to predicted default NRC (2012) values. In intact males (up to 2nd Improvest® dose), actual ADFI were 3% lower and actual growth rates were 3% higher than predicted default NRC (2012) values. After the 2nd Improvest® dose, actual intakes were 2% higher and actual growth rates were 5% higher than predicted default NRC (2012). To more closely fit the predicted performance estimates with the observed results from the 6 trials, inputs in the NRC (2012) model were adjusted. For each of the genders, ADFI and mean lean tissue growth potentials were adjusted until the predicted ADFI and ADG matched the observed mean values from across the 6 trials. The resulting adjusted Pd curves were used as the basis for estimating the dietary lysine requirements of IC barrows (Table 1). Values are expressed as a percent of the gilt lysine concentrations that a production system currently uses.

Table 1. Recommended dietary lysine and other nutrients[a] for IC barrows.

Gender	NRC 2012 lysine table values (kg)			
	25-50	50-75	75-100	100-130
Gilts	100%	100%	100%	100%
IC barrows	103%	103%	107%	95%
	NRC 2012 – Adjusted model values (kg)			
	25-50	50-75	75-100	100-130
Gilts	100%	100%	100%	100%
IC barrows	104%	108%	112%	98%

[a]Adjust levels of other amino acids, minerals (phosphorus, vitamins) in proportion to lysine. After 2nd immunization, increase threonine:lysine to reflect increased feed intake (61-66% of lysine).

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Effects of dietary zearalenone contamination on histological parameters of epithelial cell layer of uterus, vagina and endometrial glands of sexually immature gilts

JPH Sato[1], M Sbardella[2], AGS Daniel[1], MP Gabardo[1], EM Gloria[2], VS Miyada[2], RMC Guedes[1]

[1] *Veterinary School, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brazil;* [2] *Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ), Universidade de São Paulo (USP), Piracicaba, SP, Brazil,*

guedesufmg@gamil.com

Introduction

Alterations of estrogenic hormone balance during swine development can lead to serious problems in the mature reproductive performance. Zearalenone (ZON) is produced by *Fusarium* sp. fungi and acts as a non-steroidal estrogenic mycotoxin (6). The presence of this toxin in sexually immature animals results in several histological alterations on reproductive tract (1). The purpose of this study was to evaluate the effects of dietary ZON contamination on histological parameters of epithelial cell layer of uterus, vagina and endometrial glands of sexually immature gilts.

Materials and Methods

Twenty 28 day-old gilts (9.18 ± 0.61 kg BW) were fed diets with or without 1,000 ppb of ZON contamination during 28 days. At the end of the experiment, all animals were slaughtered and samples of vagina (mid portion), uterus body (mid portion) and uterus corns (proximal and distal portion) were collected and fixed in 10% buffered formalin phosphate for evaluation of epithelial cell height of vagina, uterus and endometrial gland. Then, samples were processed according to routine histological techniques and stained with hematoxylin and eosin (2). Different fields of epithelial surface layer of each segment were photographed with a magnification of 400x, and thirty areas were measured with the program "Image-Pro plus® Version 4.5.0.29". Data were submitted to analysis of variance, considering a randomized block design experiment (blocked by initial BW), using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA).

Results

ZON feed contamination increased (P<0.001) epithelial cell height of vagina, uterus and endometrial glands (Table 1). In gilts fed diets without ZON contamination, epithelial surface of vaginal mucosa was regular stratified with uniform and regular epithelial cells. The epithelial overlayer of endometrial uterus was simple columnar, while the endometrial uterus corns had small number of glands. In contrast, gilts fed diets with 1,000 ppb of ZON had irregularities on cell proliferation and on the stratified epithelium of vagina, and thickening and proliferation of the uterine epithelial cells, in addition to squamous metaplasia of the uterus. Endometrial layer and glands of gilts fed diets with ZON contamination had hyperaemia, stimulated glands and hyperplasia with proliferation of epithelial cells with irregular increase of epithelial layers.

Conclusions and Discussion

Pigs are particularly sensitive to ZON mycotoxin, which might be attibuted to its metabolic products, predominantly α-zearalenol (3). Morphological changes caused by ZON have been observed mainly on female reproductive tract, as a result of the competition between ZON and its hepatic biotransformation products with estradiol for estrogen cell receptors (4).

Table 1. Effects of dietary contamination with or without 1,000 ppb of zearalenone (ZON) on epithelial cell height of vagina, uterus and endometrial glands of sexually immature gilts[1].

	ZON contamination		SEM	CV	P-value
	Without	With			
Vagina	18.03	23.54	0.68	17.20	<0.001
Uterus	20.43	45.28	3.07	49.23	<0.001
Endometrial Glands	14.96	17.94	0.33	11.37	<0.001

Coefficient of variation (CV); Standart error of the mean (SEM).
[1]Values are LSMeans of 10 replications.

The morphologycal changes observed due to feed ZON contamination in this study are consistent with the findings of other studies and suggest that ZON is a phytoestrogen with powerful effect on the epithelialium of vagina and uterus and on the number of endometrial glands (1, 5).

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Sexual behavior of young boars supplemented with two Zn source

Y De Loera-Ortega[1,2ab], C García[1a], J Guevara[2b], A Palomo[1a], B Isabel[1a], A García-Contreras[3a]
[1]*Facultad de Veterinaria, Universidad Complutense de Madrid;* [2]*Facultad de Estudios Superiores-Cuautitlán-UNAM;*
[3]*Laboratorio de Imagenología, UAM-Xochimilco.* [a]*España,* [b]*México.* yasdlomvz@gmail.com

Introduction

Puberty is a period of accelerated reproductive development culminating in functional fertility. This process involves not only growth of reproductive organs and increased spermatogenesis, but also development of sexual behavior[1]. Key aspects of the external environment may affect the sexual behavior of the male including factors nutritional. Nutrition plays a very important role in maintaining the good health of individuals and, at the same time, has a favourable effect on reproductive functions[2]. Zn play an important role in the male reproductive system, especially in the differentiation and function of sperm. Zn deficiency leads to dysfunction of the gonads, decreased weight of testes. Reproductive output includes evaluations of libido, mating ability and semen quality[3]. However the scientific literature did not described a standardized detailed procedure to evaluate the sexual behavior of boars, so that the objective of this study was to determine the effect of two sources of zinc in sexual behavior of young boars in training.

Table 1. Sexual behavior of young boars supplemented with two Zn source.

TREATMENTS	NTS	IBT	FM	EM**	DE**
BD (25ppm Zn)	2.95	3.25[b]	3.19	21.64	5.87
BD+$ZnSO_4$ 150ppm	2.67	3.31[b]	4.82	20.97	5.27
BD+ZnMET 150ppm	2.69	5.44[a]	3.13	22.86	6.49
P=F*	0.68	0.013	0.137	0.97	0.38
EEM	0.26	0.48	1.91	5.63	0.54
			P=F*		
Treatment x Time	0.064	0.54	0.87	0.36	0.72
Time	0.0001	0.0001	0.29	0.0012	0.96

BD=basal diet; NTS = Number of training session; IBT=Interval between training (days); FM= Number false mounts (mounting dummy but dismounting before allowing a collection of semen); EM=Effective mounts (mounting dummy and complete collection of semen); DE=Duration of ejaculation. *Probability;**min.

Materials and Methods

Fifteen young male (York-Landrace), 6.8-months-old with 120 Kg body weight (BW) were randomly assigned to three treatments. Boars received a basal diet (BD=T1) corn-soybean without addition of Zn (the analyzed Zn content of the BD was 25 ppm). The BD were supplemented according to their required concentrations in each treatment (T2=BD+ZnSO4 150ppm and T3=BD+ZnMET 150ppm). Were trained to mount a dummy located in an isolated pen ($6m^2$). The boars were trained 30 min per day to mount and mate dummy in a special pen for four weeks, during which sexual behavior characteristics were evaluated: NTS = Number of training session; IBT=Interval between training (days); FM=Number false mounts (mounting dummy but dismounting before allowing a collection of semen); EM=Effective mounts (mounting dummy and complete collection of semen); DE=Duration of ejaculation in minutes. Data were analyzed using the Mixed Model procedure (SAS, 2003).

Results

During the evaluation of boars, the reaction time and duration of ejaculation were measured, finding that there was no effect (P>0.05) between the observed sexual behavior variables (NTE, FM, EM, DE; P>0.05). However, the use Zn-Met 150ppm increase the IBT (P=0.013), also a longer DE (6.49min) (Table 1). Significant effect in time was observed for variables NTS,IBT, ME, that induced a positive effect on the behavior of boars (P>0.0001) by facilitating its handling, best answer and faster to exposure to dummy sow and the operator, thereby reducing training time and the time of ejaculation. Treatment x Time interaction (P>0.064) in the variable NTS, decreased when using ZnMET 150ppm.

Conclusions and Discussion

The results of our experiment demonstrate a positive effect when using ZnMET 150ppm on sexual behavior and some variables assessed during training boars. The effect to feeding can be decisive for the sexual behaviour of the boars (libido, difficulties during mountings, longevity, amount and quality of the semen) nevertheless the welfare of pigs can be affected by a number of factors related to their diet and the way that diet is presented[4]. However, the studies reported to the present day do not respond completely the questions on what are all the significant factors that can influence in the sexual behavior of boar. However, other features would you value to discard deficiencies or toxicities caused by use of an organic source of Zn.

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Evaluation of mycotoxin binder KLIN SIL on productive behavior in piglets fed with prestarter diet contaminated with Zearalenone

J Higareda[1], M Manzanares[1], LB Hernández[2], CM Flores[2]
[1]*Helm de México S.A.*, [2]*Biogeoquímica, UBIPRO, FESI, UNAM*, mmanzanares@helm-mexico.com.mx

Introduction

Zearalenone (ZEA) is a mycotoxin that causes a hyperestrogenism syndrome and reduction of production parameters in pigs (Zinedine *et al*, 2007; Flores *et al* 2011a). Studies of mycotoxins natural occurrence, have allowed recognizing the incidence of contamination of ZEA in different grains and raw materials in México since 1999, with incidence rates ranging from 10 to 30%. In more recent years, the incidence of contamination with ZEA has decreased and the highest values were recorded in products and byproducts of corn and soybean meal (Flores, *et al* 2006, Flores *et al*, 2011b). The use of binder materials is most commonly strategies to reduce ZEA intoxication, therefore, the objective of this study was to evaluate the effect of KLIN SIL, a mycotoxins binder product to analyze productive performance of pigs in presence of ZEA.

Materials and Methods

The effect of ZEA on growth performance of piglets was done using an experimental design of 4 treatments with twelve piglets each. Treatment 1 Prestarter Control Diet; Treatment 2 Control Diet + KLIN SIL 0.3%; Treatment 3 Control Diet + ZEA 1000 ppb; Treatment 4 Control Diet + KLIN SIL 0.3% + ZEA 1000 ppb.

The experiment was started at 21 days of age piglets and lasted 14 days (35 days old). The conditions of production and sanitary handling procedures were in accordance with commercial farm. Piglets were housed in individual cages with controlled temperature, 60% relative humid and access to food and water *ad libitum*.

During the bioassay productive parameters of feed intake, weight gain and feed conversion index were evaluated. Additionally, clinical records of lesions, diarrhea and mortality were made.

Results

The results of performance parameters of body weight for 21 to 35 days old piglet are shown in Table 1, where we can see that the presence of ZEA has a significant negative effect on body weight from 9.521 kg for control Treatment 1 decreased to 8,738 kg for Treatment 2 with 1000 ppb ZEA. ($P < 0.05$). The Table 1, shows that the presence of the KLIN SIL material has significant recovers of the value of the body weight to 9.071 kg. Furthermore, Table 2 shows the effect of ZEA on weight gain and feed conversion, in this Table we can see that the presence of ZEA causes less feed efficiency, since the gain in weight reduced from 167 g/day in Treatment 1 to 109 g/day in the Treatment 4 with ZEA 1000 ppb. Additionally, feed conversion increased from 1.341 to 2155 for Treatment 1 and Treatment 4, respectively. In addition, the presence of KLIN SIL material recovers significantly the weight gain and the conversion to a value of 1.748 ($P < 0.05$)

Table 1. Corporal weight at 21 and 35 days.

	Weight kg	
Treatment	21 days	35 days
1	7.177	9.521a
2	7.189	9.308ab
3	7.210	8.738c
4	7.280	9.071b

Table 2. Feed Conversion.

	Parameters	
Treatment	Weight Gain g/day	Feed Conversion
1	167	1.341a
2	151	1.536a
3	109	2.155c
4	131	1.748b

Conclusions and Discussion

The addition of ZEA produces significant reductions in the productive performance of piglets, mainly about weight gain, a decrease of 34.7%, also, feed conversion is 60.7% less efficient. No significant clinical effects or mortality with the inclusion level and duration of treatment with ZEA in this study was recorded. When KLIN SIL material was used, significantly recover of the production parameters with a 20% gain in weight and 18% in feed conversion were observed.

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Effect of type of antibiotic on postparturient disorders and backfat loss in tropical sows

P Pearodwong, P Tummaruk
Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Pathumwan, Bangkok, 10330, Thailand, Padet.t@chula.ac.th

Introduction

Postpartum dysgalactia syndrome (PDS) in sows is characterized by inadequate and insufficient colostrum and milk production during the first few days after farrowing. This symptom cause high economic loss due to an increase mortality rate and decrease growth of the newborn piglets (1). Baytril®100 is a fluoroquinolones antibiotic commonly used in veterinary medicine. This antibiotic is a concentration dependent, i.e., the rate and the extent of micro-organism killing increase as the concentration increase. It has been recommended that a single dose of a high concentration enrofloxacin (100 mg/ml, Baytril®100) is enough to eliminated most of pathogenic bacteria in pigs. Generally, postpartum sows had a high risk of getting bacterial infection due to stress and a physiological dilatation of cervix and the teat cannel (2). The objective of the present study was to investigate the effect of a single dose of antibiotic treatment on postparturient disorders and backfat loss in sows under tropical climates.

Materials and Methods

In total, 81 sows were carefully determined for the duration of parturition and postpartum clinical signs. The sows were categorized according to the type of antibiotic used postpartum into 2 groups: control (n=36) and treatment (n=45). The control group received a control antibiotic (generic enrofloxacin 100 mg/ml, 7.5 mg/kg) 3 days postpartum and the treatment group received a single dose of injected Baytril®100 antibiotic (enrofloxacin 100 mg/ml, 7.5 mg/kg) postpartum. The rectal temperature, the presence of abnormal vaginal discharge, PDS, appetite of the sow were determined on Days 0, 1, 2 and 3 postpartum. Backfat thickness was measured before farrowing and at 21 days postpartum. The data were analyzed by Chi-square test (postpartum disorders) and general linear models (litter size and backfat loss). $P<0.05$ was considered as statistically significant.

Results

The effects of type of antibiotics used postpartum on postparturient disorders are presented in Table 1. It was found that the incidence of abnormal vaginal discharge syndrome was less frequent in the treatment group than the control group ($P=0.046$). Likewise, the incidence of PDS in the treatment group tended to be lower than that in the control group ($P=0.057$). In addition, primiparous sows lost backfat during lactation more than multiparous sows (15.7% and 4.8%, $P=0.004$).

Conclusions and Discussion

An improvement on the incidence of abnormal vaginal discharge and PDS in sows that used a high quality of enrofloxacin postpartum was found. Nevertheless, a proportion of sows with postpartum disorders remain in both groups. Therefore, not only the antibiotics but also supportive treatments and effective postpartum care of sows should be carefully performed (3). In conclusion, type of antibiotic influenced the incidence of abnormal vaginal discharge and PDS. A single dose of Baytril100® is adequate to control postpartum disorders compare to three doses of generic enrofloxacin.

Table 1. Postparturient disorders in sows treated with Baytril®100 compared with a control (LS means±SEM)

Items	Control (n=36)	Baytril®100 (n=45)
Performance		
BF at farrowing (mm)	21.0±0.5[a]	21.5±0.4[a]
Total born	11.5±0.7[a]	11.3±0.6[a]
Born alive	10.2±0.9[a]	9.9±0.8[a]
Stillborn (%)	16.2±3.9[a]	13.9±3.3[a]
Mummy (%)	1.5±3.7[a]	4.5±3.1[a]
Farrowing duration (min)	178±6.5[a]	182±5.5[a]
Backfat at 21 days (mm)	18.2±0.8[a]	19.8±0.7[b]
Backfat loss (mm)	2.8±0.8[a]	1.7±0.7[a]
Relative backfat loss (%)	12.9±3.8[a]	7.7±3.2[a]
Postpartum disorders		
Fever D0	77.8[a]	75.6[a]
Fever D1	55.6[a]	55.6[a]
Fever D2	36.1[a]	46.7[a]
Fever D3	28.1[a]	41.5[a]
Discharge D0	11.1[a]	15.6[a]
Discharge D1	55.6[a]	55.6[a]
Discharge D2	80.6[a]	60.0[b]
Discharge D3	52.8[a]	48.9[a]
Low appetite D0	38.9[a]	37.8[a]
Low appetite D1	52.8[a]	46.7[a]
Low appetite D2	33.3[a]	40.0[a]
Low appetite D3	22.2[a]	33.3[a]
PDS D0	41.7[a]	28.9[a]
PDS D1	66.7[a]	55.5[a]
PDS D2	80.6[a]	66.7[a]
PDS D3	83.3[a]	64.4[a]

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Farrowing duration, postparturient disorders and backfat loss in primiparous and multiparous sows in the tropic

P Tummaruk, P Pearodwong
Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Pathumwan, Bangkok, 10330, Thailand, Padet.t@chula.ac.th

Introduction

During the last decade, the management of sows in the lactation period has changed dramatically due to many reasons such as genetic improvement, health status and nutrition (1). Studies on the management of tropical sows to minimize the incidence of post-partum dysgalactia syndrome (PDS) need to be explored. The objective of the present study was to determine the incidence of postparturient disorders and backfat loss in primiparous and multiparous sows in the tropic.

Materials and Methods

The study was conducted in a swine commercial herd in the eastern part of Thailand during May to July 2013. In total, 81 sows were included. The duration of parturition and postpartum clinical signs were carefully determined. The sows were categorized according to parity number into two groups: primiparous (n=42) and multiparous sows (n=39). The rectal temperature, the presence of abnormal vaginal discharge, PDS, appetite of the sow were determined on Days 0, 1, 2 and 3 postpartum according to our previous study (4). Backfat thickness was measured at farrowing and at 21 days postpartum. The data were analyzed by Chi-square test (postpartum disorders) and general linear models (litter size and backfat loss). $P<0.05$ was considered as statistically significant.

Results

Reproductive performances, postpartum disorders and backfat loss in primiparous and multiparous sows are presented in Table 1. The duration of farrowing was 114.5±60.2 min (range 30 to 373 min). The duration of farrowing did not differ significantly between primiparous and mutiparaous sows ($P>0.05$). Stillborn piglets in the sows with a long duration of farrowing (≥4 h, mean 287.9 min) was significantly higher than those with a short duration (≤2 h, mean 85.5 min) of farrowing (29.2% and 7.9%, respectively, $P<0.044$).

Conclusions and Discussion

During recent years, a number of researches are trying to determine factors influencing the sow's ability to produce adequate milk for their offspring in order to enhance piglet growth and reduce piglet pre-weaning mortality (1-4). In the tropic, high temperature and humidity may cause suboptimal feed intake in lactating sows and resulted in negative energy balance conditions. In the present study, backfat loss in primiparous sows was higher than multiparous sows. This may lead to inferior subsequent reproductive performances. Therefore, special care needs to be emphasized in primiparous sows. The present study also found that nearly 50% of primiparous sows still have fever until Day 3 postpartum (Table 1). Therefore, postpartum medications, e.g., antibiotic, anti-inflammatory drug and vitamins, using a high quality of medicine are specially recommended in primiparous sows.

Table 1. Postparturient disorders in primiparous sows compared with multiparous sows (LSmeans±SEM)

Items	Primiparous sows (n=42)	Multiparous sows (n=39)
Performance		
BF at farrowing (mm)	20.8±0.5[a]	21.6±0.4[a]
Total born	11.3±0.7[a]	11.6±0.6[a]
Born alive	9.5±0.9[a]	10.5±0.7[a]
Stillborn (%)	16.3±4.1[a]	13.7±3.2[a]
Mummy (%)	5.2±3.8[a]	0.8±2.9[a]
Farrowing duration (min)	175±6.7[a]	185±5.2[a]
Backfat at 21 days (mm)	17.4±0.8[a]	20.5±0.7[b]
Backfat loss (mm)	3.4±0.9[a]	1.1±0.7[b]
Relative backfat loss (%)	15.7±3.9[a]	4.8±3.1[b]
Postpartum disorders		
Fever D0	85.7[a]	66.7[b]
Fever D1	59.5[a]	51.3[a]
Fever D2	50.0[a]	33.3[a]
Fever D3	48.8[a]	18.8[b]
Discharge D0	19.1[a]	7.7[a]
Discharge D1	57.1[a]	53.8[a]
Discharge D2	57.1[a]	82.0[b]
Discharge D3	38.1[a]	64.1[b]
Low appetite D0	52.4[a]	23.1[b]
Low appetite D1	57.1[a]	41.0[a]
Low appetite D2	40.5[a]	33.3[a]
Low appetite D3	33.3[a]	23.1[a]
PDS D0	28.6[a]	41.0[a]
PDS D1	59.5[a]	61.5[a]
PDS D2	69.1[a]	76.9[a]
PDS D3	64.3[a]	82.1[a]

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Productive and economic analysis of pigs raised in "wean-to-finish" and conventional production systems

J Cristani[1], W Consoni[1], PM Arruda[1], F Klaumann[1], G M Preis[1], AT Zimmermann[1], RG Lorenzetti[1], SD Traverso[1], A Thaler Neto[1]
[1]*Santa Catarina State University- UDESC;*cristani@cav.udesc.br

Introduction

The "wean-to-finish" production system (WF) where weaned pigs are held in the same barn until slaughter (2), currently used in the United States, Canada (5), Chile (4) and Mexico (3), began its implementation in Brazil in 2008. This study compared the productive and economic outcomes of the WF and conventional production system (CC).

Materiasl and Methods

Two groups of pigs from the same origin, born and weaned in the same week, were evaluated. One was housed in a conventional production system and the other in a WF barn. At the end of the nursery phase, part of the CC group was transferred to a CC grow/finish barn along with a part of the WF group, and the other part of the CC group was transferred to the WF barn in order to form 4 groups (WF, with 410 animals; CC, with 390 animals; and CC-WF and WF-CC with 160 animals each). Each pig was individually weighed between changes of diet and then the average feed intake, daily weight gain (DWG) and feed conversion ratio (FCR) were calculated. The cost of feed per kilogram of liveweight gain was also determined. Analysis of variance (SAS 9.1., SAS Institute, Cory, NC, USA) was performed on the recorded data, and the groups means compared with the Tukey test (5%).

Results

Productivity results are found in Tables 1 and 2. Economically, the WF group had the lowest costs, with a difference of up to R$ 0.09 per kilogram of liveweight gain compared to the other groups.

Table 1. Productivity results in the nursery phase, in Kg.

Group	WEIGHT	DWG	FCR
WF	24.140 a	0.468 a	1.48 a
CC	23.550 b	0.449 b	1.42 b
P	*0,0174*	*0,0024*	*0,0068*

Table 2. Productivity results in the grow-finish phase, in Kg.

Group	WEIGHT	DWG	FCR
WF	119.932 a	0.893 a	2.31 a
CC-WF	118.64 ab	0.897 a	2.31 a
CC	116.453 b	0.874 ab	2.32 ab
WF-CC	116.028 b	0.854 b	2.38 b
P	*0,0002*	*0,0003*	*0,0167*

Discussion and Conclusion

The results seen in the nursery phase point to a better final average weight and DWG for the WF group while the FCR was better in the CC group. This is due to the higher feed intake of the WF group in the end of the nursery phase. The results were similar for the WF and CC-WF group in the grow-finish phases, which were better than the remaining groups. On the economical side, there was a significant cost difference in the CC group due to higher drug expenses during the nursery phase. Moreover, the WF group had no expenses with transportation, cleaning and disinfection after the nursery phase. This is considered one of the main advantages of this system, along with the animal welfare and ruling out the stress caused by transportation (1). The productive outcomes associated with the economic gains shows that the WF system can be a viable alternative for the pork industry.

Acknowledgements

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Life-cycle environmental benefits derived from immunological castration of pigs as compared to physical castration: From a global perspective to a United States specific model

PJU Moraes, MA Mellencamp, J Allison
Zoetis Inc, Florham Park, NJ, paulo.moraes@zoetis.com

Introduction

As the world's population grows, global meat consumption will also increase. There is pressure from all sectors of society to produce food more sustainably. This will mean further intensification and industrialization of livestock production and adoption of technology that improves production efficiencies while also accounting for animal welfare issues. Improvest® (*gonadotropin releasing factor analog-diphtheria toxoid conjugate,* Zoetis, Florham Park, NJ) reduces boar taint and it is a safe and effective alternative to physical castration and is approved for use in 63 countries, including European Union and Japan. This product works with the pig's immune system. Boars grow to their full potential with all the inherent advantages of intact males; improved feed conversion, less manure, and carcasses with a greater percentage of lean meat than barrows. These efficiencies and resource savings provide significant life cycle environmental benefits (1). This life cycle assessment (LCA) quantified the potential environmental benefits of using Improvest in US pork production.

Materials and Methods

During 2009-2011, a global study was conducted using life cycle burden data collected from modern farms with intensive pig production where pigs were physically castrated (PC) and compared to data collected from the same/similar farms in the same countries where pigs were immunologically castrated (IC). The study was conducted using LCA ISO compliant guidelines. Data were collected by direct interviews in modern farms and abattoirs in many countries and an Environmental Product Declaration was published in early 2012 (2). When Improvest was introduced in the US in 2011, the global LCA model was adapted to the US specific inputs according to the University of Arkansas LCA model (3).

Results and Discussion

The GWP (Global Warming Potential) contributions for 2 doses of immunological product manufacturing are negligible and represented only 0.01% of the total GWP for one kg of pig live weight. The main contributions to the GWP are related to the production of feed given to pigs and pig manure management. The US LCA model key input was feed conversion and came from results from 8 trials conducted for product approval; the improvement in feed conversion for IC pigs compared to PC pigs was 8.4% which resulted in feed savings of 26 kg/pig. Based on USDA statistics for crop yields during the years 2009 - 2011 (2012 yield data was excluded due to drought conditions), a land savings (devoted to crop production) of 31 m^2/pig is realized. Reduction in manure, in the absence of direct measurement, was assumed to be proportional to the reduction in feed intake. IC pigs had a 6.1% lower GWP than PC pigs when comparing live weight results and 3.8% for carcass weight results (Table 1). Potential reductions in carbon footprint with increasing Improvest adoption are shown in Table 2.

Conclusions

For a 124 kg of pig (live), the use of the Improvest over the baseline scenario of physical castration results in a reduction of GWP of about 28.6 kg CO_2 equivalents per pig. If only 33% of the 53.3M male pigs (2011 data) raised annually in the US were IC, that is equivalent to approximately 508,000 mt of avoided GHG emissions per year, equivalent to removing emissions of 99,579 passenger vehicles/year or the carbon sequestered by 164,850 hectares of pine forests.

Table 1. Carbon footprint of immunological castration compared with physical castration.

Global warming potential	PC kg CO_2e	IC kg CO_2e
Live wt, per kg	3.91	3.66
Carcass wt, per kg	5.21	4.79

Table 2. Potential reductions for impact categories for 3 levels of adoption of Improvest in US pork production.

Parameter	Improvest adoption rate		
	33%	66%	100%
CO_2 emision reduction, millions kg CO_2e	553	1,110	1,622
Feed use reduction, tons	522,000	1,0444,000	1,567,000
Energy use reduction, million BTU	1,850	3,700	5,550
Water use reduction, billion gallons	2.3	4.6	6.9
Land use reduction, acres	137,000	274,000	411,000

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Optimizing long-term feeding and building decisions on farms using immunological castration

B Cowles, PJU Moraes, MA Mellencamp, S Stanford
Zoetis Inc, Florham Park, NJ, paulo.moraes@zoetis.com

Introduction

Improvest® (*gonadotropin releasing factor analog-diphtheria toxoid conjugate*) is an FDA-approved veterinary prescription product to manage unpleasant aromas that can occur when cooking pork from some male pigs. It is a safe and effective alternative to physical castration. Male pigs receiving Improvest® grow intact at substantial feed efficiency improvements compared to physical castrated barrows while maintaining the same meat quality characteristics. We wanted to understand the longer run optimization questions which face today's Improvest adopters. We gathered information necessary to examine how producers adopting Improvest might be incented to alter current building and feeding structures as well as pig flow to more fully realize the benefits of adoption over time. Four basic facilities and management options were examined over a 20 year period for a baseline 5,000 sow farrow-to-finish operation.

Materials and Methods

The analysis of Improvest ® adoption was accomplished by constructing a stochastic simulation model for the wean-to-finish facilities required to support a 5,000 head sow herd. The model was programmed in Matlab with 4 modules:

The four facility and management options were:

1. A mixed-sex barn with animals fed a mixed-sex diet. This was the baseline model without Improvest® as Improvest® use is disqualified when males are not separated for Improvest® administration.
2. A mixed-sex barn with animals separated by sex on each side of the barn, so that a split-sex diet could be utilized.
3. Separate-sex barns fed split-sex diets
4. A double-stocked, wean-to-finish building flow with females (or males) removed at the feeder pig stage to separate-sex finishing. Split-sex diets were fed after the move.

Each options (except as noted, the baseline option) were run assuming no Improvest® was utilized and then with Improvest® adoption leading to a total of 7 scenarios.

We evaluated which of these several proposed long run adaptations is likely to produce the greatest present value net returns under conditions of risky input and output prices. We evaluated whether one strategy dominates others when taking into account the riskiness of returns. In addition, the payback period or liquidity outcome of each choice was determined. Because building costs differ widely throughout the United States, each alternative was estimated without upfront costs of facilities or facility changes specified. This generates a breakeven type of analysis which reveals how much a producer *could* spend under each option to reconfigure the farm for greater returns.

Results and Discussion

Compared to Mixed-Sex Barn & Mixed-Sex Diet Without Improvest®

Switching to split-sex feeding in separate barns with Improvest® yielded the greatest increase in the present value of net returns. Such a switch is also likely to require the fewest modifications to facilities. Split-sex feeding in mixed-sex barns with Improvest® was second in terms of an increase in the present value of net returns and would likely require more facilities modifications in order to provide a second feed source and supply system if one does not already exist and to separate pigs by sex. Switching to the double-stock option with Improvest® decreased the present value of net returns due to the additional cost of moving feeder pigs to finishing barns. However, this option also substantially reduced the number of barns that would be required to accommodate the pigs, which would reduce the facilities costs not included in these net return calculations.

Without Compared to With Improvest®

For all three facilities and management options, switching to using Improvest® increased the present value of net returns without substantially changing facilities requirements. This result is attributable to increased feed efficiency, higher optimal marketing weights, and the potential for higher packer premiums. The increase in the present value of net returns was highest for switching to Improvest® in the split-sex feeding and mixed-sex barns system, followed by the double-stock barns system, and then split-sex feeding with separate-sex barns system.

Conclusions

Adoption of Improvest increases net returns compared to physical castration.

Split-sex feeding in separate barns with Improvest yielded the greatest increase in net returns and is likely to require the fewest modifications to existing facilities.

Effect of management practices on infection dynamic of PRRSV in vaccinated farrow-to-finish herds

E Czyżewska[1], A Dors[1], M Pomorska-Mól[1], K Podgórska[1], M Porowski[2]
[1]*Department of Swine Diseases, National Veterinary Research Institute, Pulawy, Poland,* [2]*Veterinary Clinic Vet-Com, Pobiedziska, Poland,* arkadiusz.dors@piwet.pulawy.pl

Introduction

Porcine reproductive and respiratory syndrome (PRRS) has caused a significant economic impact on the global swine industry (1). The control of the infection is difficult and requires a combination of different measures including management, biosecurity and vaccination.

Vaccination belongs to the most predominant strategies allowing for stabilization of the breeding stock and production of virus negative weaners (2). However, biosecurity and management practices including all-in-all-out pig flow are the fundamental parts of PRRS control at the farm level (3).

The aim of this field study was to compare the PRRSV serological profile in two vaccinated herds with different pig flow management.

Materials and Methods

The study was conducted in two 1000 sows farrow-to-finish PRRS positive pig herds with history of acute outbreaks of PRRS (reproductive disorders in sows). To control PRRS outbreak herd A and B started vaccination program against PRRS, in July 2011 and November 2011, respectively. In both herds mass vaccination program was initiated in sows using commercial modified live vaccine (MLV) as follows: one mass vaccination followed by a revaccination 4 weeks later. After two months break sows were vaccinated at every 60 day of gestation. In both herds replacement gilts were vaccinated twice using MLV. Four weeks after second vaccination gilts were entering to the sow herd.

In herd A high biosecurity program including all-in-all-out pig flow have been established. In herd B all-in-all-out practice was abandoned.

At the time of the study reproductive disorders in breeding stock in both herds were not observed. However, respiratory distress in weaners and fatteners were noted in herd B.

Serum samples were taken from 10 animals per age category about two years since the introduction of vaccination program in breeding stock. For detection of specific antibodies against PRRSV commercial ELISA were used: PRRS X3 ELISA test kit (IDEXX).

Statistical analyses were carried out with Statistica 8.0 using Mann-Whitney *U* test. Differences with $p < 0.05$ were considered as significant.

Results

The prevalence of positive animal per age category in herd A and B is shown in Figure 1.

Comparison of the mean S/P ratio values in pigs from different age group in herd A and B is presented in Table 1.

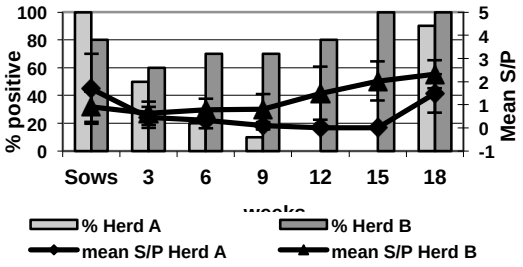

Figure 1. Serum profile of PRRSV in herd A and B

Table 1. Comparison of mean S/P ratio value per age category in herd A and B

Age group	mean S/P		p value
	A	B	
Sows	1,685	0,912	0,473
3 weeks	0,455	0,628	0,427
6 weeks	0,318	0,786	0,037
9 weeks	0,098	0,813	0,000
12 weeks	0,005	1,496	0,000
15 weeks	0,007	2,024	0,000
>18 weeks	1,490	2,315	0,045

Discussion

In the present study, regular sows vaccination in herds A and B stabilized the situation in breeding stock (clinically stable). However the clinical symptoms from respiratory tract were still observed in weaners and finishers in herd B. Clinical situation in herd B was connected with significantly higher S/P ratios compared to those observed in the herd A. In herd B where continuous pig flow was implemented, the horizontal spread of PRRSV from older, infected pigs to younger, susceptible animals took place (increase in prevalence of positive animals and S/P ratio over time). In herd A the S/P ratio decreased over time and no seroconversion were noted in weaning house. Seroconversion occurred just in the end of fattening units (probably indirect contact).

In conclusion obtained results demonstrated that MLV vaccine limits reproductive disorders in sows but circulation of PRRSV in weaners and fatteners depends on biosecurity measures including all-in-all-out production flow.

Acknowledgements

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Productive evaluation and economic analysis of a swine production scheme: 1st parity-elimination of sows

G Ordaz[1], A Juárez[1], A García[2], RE Pérez[1] , R Ortiz[2]
[1]*Instituto de Investigaciones Agropecuarias y Forestales-Universidad Michoacana de San Nicolás de Hidalgo,*
[2]*Facultad de Medicina Veterinaria y Zootecnia-UMSNH,*
ordazog@gmail.com

Introduction

One of the main problems that cause improductivity in swine production systems is the policy of replacement of sows, it has been established based on the age of the females (2). However, longevity does not currently ranks first in the elimination of sows; because, gilts 1st to 3rd parity, are eliminated in a higher percentage due to a higher incidence of reproductive problems (5), impacting on the population structure, the productive efficiency and the economic results of the herd. Therefore, the objective of this study was to determine the productivity, production costs and profitability of two schemes of elimination: 1st parity-elimination schemes (100% replacement) against conventional elimination scheme (30% replacement).

Materials and Methods

Analyzed 100 parity, from 80 hybrid sows (Landrace x Duroc x York), divided into two groups (G): G1 (n = 17 sows / 45 parity's) with replacement rate of 100% (EPE) and G2 (n = 17 sows / 65 parity's) with conventional replacement rate of 30% (EEC). Both Gs were monitored for 24 months and were subjected to the same husbandry practices. Both the G1 and G2 was evaluated: litter size (TC), piglet born alive (NV), weaned piglets (LD), interval weaning-estrus (IDE), percentage of repeated services (PSR), and non-productive days (DNP). The analysis of biological variables was performed by the method of Generalized Linear Models (GLM), followed by testing least square means (LSMEANS) to determine the difference between means (SAS ®). For economic analysis was used the methodology proposed by Muñoz Ruco (1) amended by Bobadilla et al. (2).

Results

The elimination scheme, didn't impact (P > 0.05) TC, NV, LD, IDE and PSR. However, it impacted (P < 0.001) to the DNP (Table 1). Likewise, the benefit / cost ratio (B / C) was higher for the 1st parity-elimination scheme (Table 2).

Conclusions and Discussion

The structure of parity in the EEC can not compensate reproductively, productively and economically the decreased reproduction and production of the sow's 2nd and 3rd parity. Likewise, in the EEC, the DNP increase of 2nd to 4th parity for increase of the IDE and PSR, reflected in higher production costs (Table 2).

Table 1. Least squares means for reproductive and productive indicators of the sow according to the elimination scheme

	EPE		EEC	
Indicator	Mean	E.E.	Mean	E.E.
TC	9.8[a]	1.39	10.4[a]	0.42
NV	9.5[a]	0.45	10.0[a]	0.39
LD	8.6[a]	0.44	9.1[a]	0.38
IDE	--	--	7.1[a]	0.57
PSR	--	--	13.8[a]	0.04
DNP	9.9[a]	2.76	39.0[b]	2.40

[a, b] different literals indicate statistical differences (P < 0.05) within row.

Table 2. Análisis of production costs, incomes and revenues per weaned piglet UDS

Concept	EPE	EEC	DIF.
Fixed costs	9.20	10.76	1.56
Variable costs	23.92	25.30	1.38
Total incomes	41.35	41.35	--
Net gain	8.24	5.52	2.94
Deadlock (Nº LD)	200.52	217.75	17.23
Relationship B/C	1.25	1.15	0.10

The implementation of EPE is economically more profitable because revenues from the sale of the sow and her litter, a smaller number of DNP, the increase in the number of parity's/year and increased number of weaned piglets/year.

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Application of knowledge management as a tool for pork production companies in Venezuela

M Tovar[1], C Rojas[2]
[1]*Inversiones Porcinas C.A. Yaritagua, Venezuela,* [2]*Teacher of Mastery in "Fermín Toro" University Barquisimeto, Venezuela,* mv.marcost@gmail.com

Introduction

In a world where technology plays a fundamental role in all changes, pork production companies in Venezuela, need to find strategies that allow them to complete in saturated market where knowledge and information are essential elements of differentiation. This is critical for generating profit, achieving results, improving the use of technology, meeting customers needs and strengthening relationships with suppliers.

The achievement of these goals is fundamental for competition and productivity (1,2). Therefore, the goals of this study were to describe the use of knowledge management in Venezuelan pork production companies and evaluate the application of this tool to transform current companies in smart organization.

Materials and Methods

A descriptive study, supported by a field investigation was performed (3) to analyze the use of knowledge management as a tool to transform Venezuelan pork production companies in smart organizations. Direct observation, literature review, non-structured interviews and a survey were used to collect data from the company. The survey, validated through the expert panel method with a result of Cronbach alpha equal to 1, was applied to 40 farm managers. Results were organized in tables and histogram allowing for descriptive analysis.

Results

The findings of this study demonstrate the need to initially develop a consistent and continuous approach with defined strategic planning of knowledge management, (Shown in Figure 1). The implementation of the propose vision will allow the development of a competitive company and possibility for the transformation in to a smart organization (4).

Conclusions and Discussions

Pork production companies as many other livestock production or manufacturing companies are facing challenging times not only in Venezuela but globally.

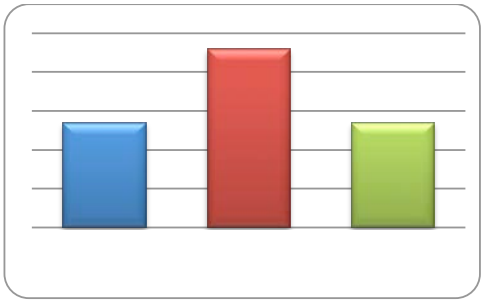

Figure 1. Principal actions in the strategic planning for knowledge management in the organization.

The situation demands the development management tools to support high productivity levels with a competitive approach using the model, of smart organizations as the engine to generate the changes. The program needs to be based in the development of the people as the main goal, to maximize their contribution to the organization.

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Control of water consumption in swine barns; one step-closer to real time management

C Piñeiro, P Castro, J Morales, G Montalvo
PigCHAMP Pro Europa, Spain; carlos.pineiro@pigchamp-pro.com

Introduction

Providing enough quality water is essential for good livestock husbandry. Given that drinking water needs are farm -and management- specific, water-metering equipment's to obtain accurate measurements of water use should be applied in each location.

Currently, to check if the use of water by the animals is within the appropriate limits, it is usual to consult water consumption tables in the bibliography. These contain water intake patterns for a given age and productive activity. Nevertheless, these tables turned out to be somewhat controversial, due to the fact that the rates given by authors differ significantly (1). The aim of this study was to use new technologies to monitor and predict daily water consumption of fattening pigs with higher accuracy.

Materials and Methods

In order to monitor water consumption, a real-time sensor monitoring system was installed and set in the fattening unit of one Spanish commercial swine farm. The fattening unit consisted of two buildings, both including about 1000 pigs. Each building was filled with one-only batch of pigs, 60 days of life at the entrance. Pigs were allotted in pens of 10 pigs, with a stock density of 0.70 m^2/pig, natural ventilation, partly slatted concrete flooring and one hopper and cup drinker per pen. Feed and water were available *ad libitum*.

The metering system consisted in two VTH25 flow sensors with an optimal measure range of 200 to 10.000 liters per hour and two PT100 ceramic temperature sensors, connected to a processing unit which polled the data from these devices, calculated the hourly and daily averages and uploaded it real-time to the server database through ethernet. Four batches of pigs were followed up in each building, registering water intake, room temperature and daily control of mortality and health. Water consumption was daily calculated per pig from 60 to 150 days of life. Simple linear regression was conducted to predict water intake in L per animal and day from age in days using the REG procedure of SAS.

Results

In the figure 1, the relationship between water consumption and age is presented. As expected, water consumption was highly correlated to age (r square = 0.72). In the experimental farm where this measurement was conducted, the most statistically valid equation to predict water consumption was y=0.0324x – 0.2081, where y is individual daily water intake (L) and x is age in days. Therefore, an average water consumption range of 1.73 to 4.65 (at 60 to 150 days of age) L per animal and day was obtained. Values found in bibliography differ significantly among different authors and are higher than the mean values obtained in the present study, being the average difference 22.4% with the lower interval reviewed in the bibliography and 48.9% with the upper one.

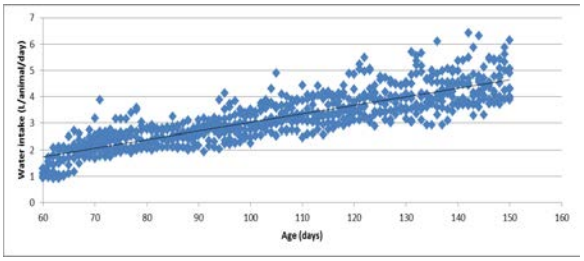

Figure 1. Linear regression of water consumption versus age of fattening pigs

Conclusions and discussion

This system allows monitoring water consumption in a particular facility and then detecting in an early stage any significant deviation of water intake from the expected range. In addition, knowing water consumption in detail also allows ensuring proper dosing rates of medication provided through the watering system.

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Evolution of sow productivity in a Brazilian farm during the last twenty years

M Aparicio[1], MA de Andrés[1], J Morales[1], N Lisboa[2], C Piñeiro[1§]
[1]PigCHAMP Pro Europa SL, Segovia, Spain; [2]Consuitec, Paulinia, Brasil, joaquin.morales@pigchamp-pro.com

Introduction

Improvements in sow productivity have been described progressively in last years in many countries. Producing 30 pigs weaned per sow per year is now within the reach of many progressive pig units in Europe, North- and South-America. Genetic selection has been the key driver of increased sow productivity, but also improvements in housing, management and a more exhaustive analysis of data have been important. In this sense, current swine production is everyday more linked to a proper analysis and monitoring to maintain competitiveness. Despite of the fact that every farm has to improve taking into account its own results, in many cases general standards can be useful to set a quick reference in order to help to define farm objectives and to compare performances through benchmarking processes.

The objective of this study was to analyse the evolution of sow productivity data in the last 20 years in a large swine herd from Brazil.

Materiasl and Methods

The present study analyse key performance indicators from a large Brazilian swine herd. Reproductive data have been collected since the origin of the farm, in 1995, registered with PigCHAMP® software and monitored by PigCHAMP Pro Europa (Segovia, Spain). The current number of reproductive sows in the farm is about 5,200. Total born (TB), born alive (BA), weaned per litter (WP), farrowing rate (FR), pre-weaning mortality (PWM), weaned piglets per sow and year (WSY) and individual body weight (BW) at birth and at weaning were monitored and analysed using the statistical process control chart with Minitab package software (v.16).

Results

Total born, BA, WP and FR evolutions are presented in figure 1. All of them showed an increasing evolution with time (P time<0.05). Prolificacy showed a linear increase since 2003, reaching the maximum value in 2013 (12.7 TB and 11.6 BA). However, number of WP increased until 2007 and was almost constant since then (about 10.4 per litter). Farrowing rate showed an increase until 2008, and was also constant since then. Individual BW and litter weight at birth and at weaning are presented in figure 2. Both showed a clear decrease in 2004 to 2007, correlated with the main increase observed in BA, and could be associated with the change in sows's genetic line in the farm at that moment. Since 2007 BW at birth was stabilized, with a slight decreasing trend also associated with the increase observed in BA. Improvement of Sow productivity was also proven in the increasing WSY evolution (figure 3), although it was maintained constant for the last 7 years. On the other hand, PWM did not show a clear pattern with time (figure 3).

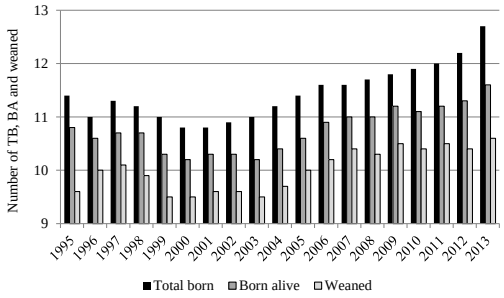

Figure 1. Evolution of total born (TB), born alive (BA) and weaned piglets per litter in 1995 - 2013.

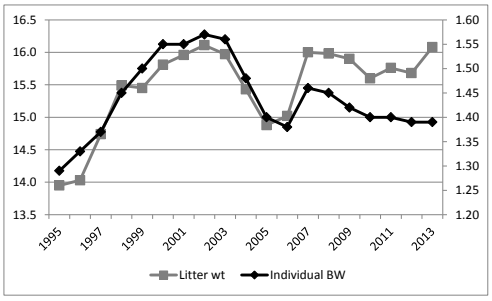

Figure 2. Evolution of individual body weight and litter weight (kg) at birth 1995-2013

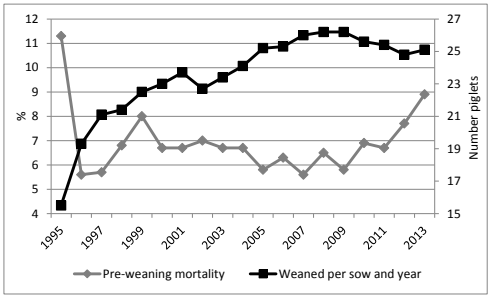

Figure 3. Evolution of pre-weaning mortality and number of piglets weaned per sow and year in 1995-2013

Conclusions

Improvement of sow productivity (TB, BA and FR) in last 20 years is clear. Improvement in TB is the main cause of the increase of BA in these last years, because the ratio between them has been kept almost exact through this time. Consequently, litter weight at birth is also higher nowadays, but individual weight of newborns has been kept constant.

The Individual Pig Care (IPC) management program helps to reduce the percentage of mortality of nursery pigs

E Vizcaíno[1], I Díaz[1], J Morales[1], P Doncecchi[2], A Dereu[2], C Piñeiro[1]
[1]*PigCHAMP Pro Europa SL, Segovia, Spain;* [2]*Zoetis – EuAFME, France,* joaquin.morales@pigchamp-pro.com

Introduction

Individual pig care (IPC) is a management program based on daily individual observation of pigs allowing early detection of health problems that occur in animals leading to a prompt and proper treatment of the disease. The use of the IPC program delivers a good return on investment, by reducing the medication costs, mortality rate and improving productive performance, delivering healthier meat for the consumers.

The objective of this study was to evaluate the clinical and economic benefits of the IPC program application in a medium-low health status Spanish farm, where only in-feed mass treatments were used to control health problems.

Materials and Methods

This study was conducted in the nursery phase of a commercial farm located in Valladolid, Spain. Every 3 weeks a batch of about 600 weaners (28 days age) was allotted in 5 nursery rooms (12 pens per room; 10 piglets per pen). The farm was PRRS positive, with a high incidence of arthritis and respiratory problems in the nursery phase, causing high percentage of mortality and wasted pigs.

Historical clinical data in nursery phase were collected from June 2012 to September 2013. In Sep2013 workers taking care of the nursery were trained under IPC guidelines and the IPC program started to be applied from October 2013 onwards.

According to the IPC guidelines, sick pigs were scored and symptoms were quantified according to the severity (A-mild signs of disease; B-medium; C-serious and D-very serious or dying). Type of disease was evaluated and classified: respiratory, enteric, lameness, neurological, biting or other signs. Clinical signs and mortality were monitored in each batch from weaning at 28 d of age to 77 d of age (about 26 kg BW). The effect of IPC was assessed analyzing the evolution of the percentage of mortality using the statistical process control of the Minitab software (v 16).

Every sick pig was treated with injectable antibiotics and, when necessary (B- and C- cases) were removed from their pens and allotted in hospital pens. More serious cases (D-cases) were humanely euthanized.

Results

Results showed that mortality rate in the nursery phase was significantly reduced after IPC program application (figure 1). In the historical data (June 2012 – September 2013) the average percentage of mortality in nursery phase was 3.85%, and this percentage was reduced to 1.01% since October 2013.

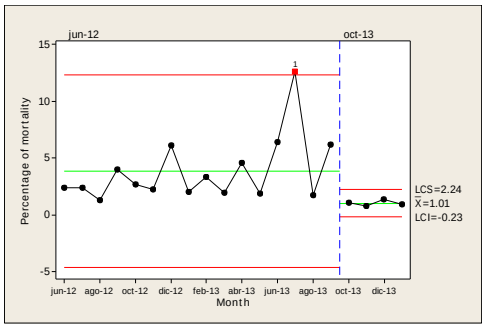

Figure 1. Percentage of mortality in nursery phase before and after the IPC program application.

Percentage of mortality was reduced based on an early detection of clinical signs, which allowed farm caregivers applying the right antibiotic at an early stage of disease, which increases probability of success. Figure 2 shows the number of piglets treated with individual antibiotic interventions since IPC program is used, depending on severity of disease.

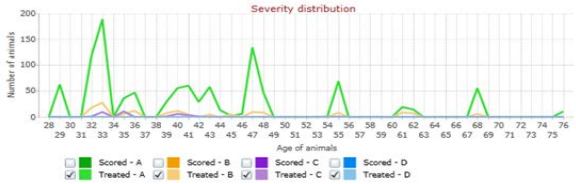

Figure 2. Number of piglets treated through the nursery phase depending of severity of disease (A-mild; B-medium; C-serious; D-dying)

Most of cases were treated for mild signs of disease (A-cases), indicating that detection of disease is correct in an early stage.

Conclusions and Discussion

These results show that implementation of the IPC program was performed properly at an early stage, increasing treatment success and reducing mortality rate from 3.85 % to 1.01 %. In conclusion, IPC program promoted better health control in a nursery phase with a more judicious use of antiinfectives.

Reducing improper handling during farrowing improves performance in lactation period

I Díaz, E Vizcaíno, L de Frutos, A Manso, J Morales, C Piñeiro
PigCHAMP Pro Europa SL, Segovia, Spain, carlos.pineiro@pigchamp-pro.com

Introduction

The perinatal phase is a particularly sensitive phase in piglet production. The sows are stressed physiologically and also by behavioral restriction imposed by the farrowing crate system. Inappropriate handling can aggravate the situation increasing stress in sows. Thus, actions carried out the first few days after the farrowing, such as excessive movement of the workers in the farrowing´s rooms or too many treatments applied to the sows to promote uterine contractions can generate this situation.

Maternal glucocorticoids induced by prenatal stress have been shown to lead to a deficient supply of colostral milk to the piglet, translated as a higher pre-weaning mortality and lower growth rate during lactation (1).

The aim of the present study was to assess the productive impact of causing stress to sows in the peripartum days due to improper management.

Materials and Methods

The study took place in a 540-sow facility with conventional farrowing crates in the lactation barn. A problem of aggression in sows was detected towards both their litters and the workers, associated with a high number of stillborns per farrowing and a high percentage of pre-weaning mortality.

Data from 220 farrowings were collected: number of total born, born alive and stillborn, pre-weaning mortality (including age of piglet and cause of death) and number of weaned piglets per litter. Management of sows during the farrowing was also observed, recording if sow was assisted during the farrowing and treatments applied (product and reason of treatment).

A plan of personal training was done, relative to the handling during the farrowing. After the training, a total of 124 farrowings were followed up, collecting the same data.

Data were analysed by GLM models of SAS, including workers training as main factor. .

Results

After the training of workers, the assistance during the farrowing was clearly reduced (33.5% vs 9.7% in pre- and post-training, respectively; P<0.01). In addition, percentage of sows which required an antibiotic intervention to treat mamitis was also reduced after training (38.6% vs 25.0%; P<0.05).

Results of prolificacy and pre-weaning mortality are presented in tables 1 and 2, respectively.

Table 1. Farrowing data (Number per farrowing) before and after training of workers

	Born alive	Stillborn	Total born	Weaned piglets
Before	12.97	1.01	14.10	9.06
After	12.29	0.90	13.37	9.92
SEM[1]	*0.315*	*0.155*	*0.346*	*0.166*
P[2]	*t*	*NS*	*t*	*****

[1]Standard Error of Mean; [2]Probability: NS, P>0.10; t, P<0.10; ***, P<0.001

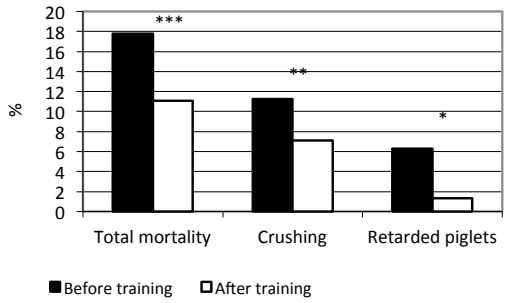

Figure 1. Total % of pre-weaning mortality and of the main causes before and after training of workers

Total number of born pigs and of born alive tended to be higher after the training of workers. However, no differences were found in number of stillborn.

Pre-weaning mortality was reduced when the intervention during the farrowing was lower (17.08 vs 11.05 and p<.0001) related to both causes, crushing and retarded piglets (figure 1). Consequently, the number of weaned piglets (table 1), was higher after training.

Conclusions and Discussion:

In conclusion, the study showed that an extensive peripartum intervention causes stress, which promotes a negative impact in performance during lactation: higher pre-weaning mortality and poorer growth rate of piglets.

We can conclude that a correct management in the prenatal period contributes to improve the productivity of the farm.

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Impact of Improvac® vaccination of entire male pigs on carcass quality under field conditions in China

M Linatoc[1], J Mei Jiang[1], D Hennessy[2]
[1]Zoetis International Trading (Shanghai) Co., Ltd, Shanghai China, [2]Private Consultant, Melbourne Australia, marlon.linatoc@zoetis.com

Introduction

A key economic indicator of the efficiency and profitability of meat production is lean meat yield which is often reflected in the "carcass grading" applied post slaughter. Slaughterhouses generally reward producers with a better price for a better grade, creating an incentive scheme that reflects the higher value of such carcasses and their attractiveness to the meat industry. Improvac (Zoetis, Florham Park, NJ, USA) is a vaccine used for the immunological castration of male pigs. The 2nd dose of Improvac is timed to occur in the late finishing phase, and allows producers to maximize the production and carcass benefits of raising entire boars without the issue of boar taint. The aim of this trial was to compare the carcass grade assigned, by commercial slaughterhouses, to Improvac vaccinated entire male pigs with the grade assigned to physically castrated pigs.

Materials and Methods

A series of 24 controlled field evaluations were conducted in various regions of China. The trials were conducted in commercial pig farms in 11 provinces of China, involving a total of 3,670 physical castrates and 3,733 litter and age matched male pigs immunized with Improvac. At birth pairs of male pigs within litters were randomly assigned to either castrate or Improvac treatments. Castration was performed at a few days of age according to the practices for that particular farm. The Improvac males were left as intact and later vaccinated with Improvac. Two, 2 mL doses of Improvac were given by sub-cutaneous injection the first at around 16 weeks of age and the second 4 weeks later All pigs were slaughtered at around 24 to 26 weeks of age or 4 to 6 weeks after the 2nd dose of Improvac. Both treatments were fed the same rations according to the normal farm practices, meaning that both groups received diets formulated to meet castrate requirements. In 7 of these studies a randomly selected sub-set of the trial pigs were used to collect carcass grading data at slaughter. The assessments were made at 7 commercial slaughterhouses across 6 provinces of China. In total 368 castrate carcasses and 403 Improvac carcasses were graded at the slaughter plants. Unfortunately the scales used by the plants varied and the data could not be fully pooled for analysis. Five plants used a 4-point scale where 1 was the highest grade. The data from these 5 plants was pooled for analysis; 1 plant used an 8-point scale and 1 used a 10-point scale. Differences in carcass grading were assessed by a series of Chi-square tests.

Results

When the data from the 5 slaughter plants using the 4-point scale were pooled a statistically significant ($P<0.004$) shift in distribution of grading to more superior grades was found for the Improvac vaccinated pigs. The results are shown in Figure 1. The total number of castrates and Improvac pigs graded in the 5 plants was 225 and 255 respectively. A similar statistically significant ($P<0.005$) shift in distribution in favor of better grades for Improvac vaccinates was also found with the plant that used a 10-point scale. That plant assessed 128 Improvac pigs and 123 castrate pigs. There was no change in distribution in the plant that used the 8-point scale ($P=0.57$). However, there were only 20 pigs of each treatment in that plant.

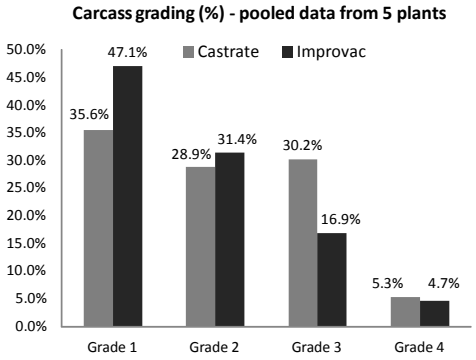

Figure 1. Percentage carcass grading on a total of 225 castrate and 255 Improvac vaccinated pigs processed in 5 plants in China.

Conclusions and Discussion

In the majority of carcass grading schemes the higher the grade the higher the lean meat yield and lower the fat content and hence the better the value. In the current study the Improvac pigs had better carcass grades than physically castrated pigs.. The findings of a shift in distribution to more high-value grade carcasses in the Improvac vaccinated pigs confirms the findings from a 10 country, 13 study report by Allison et al, 2011.

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Effects of unsaturated lipid rich diet on carcass composition and fatty acids profile in pork

JG Rodríguez-Carpena[1], C Lemus[1], A Sánchez-Escalante[2], T Sumaya[1], S Hernández, P Fránquez[1]
[1]*Autonomous University of Nayarit*, [2]*CIAD-Hermosillo*, germencillo@yahoo.com.mx

Introduction

Amongst other factors, animal fat and particularly, saturated fatty acids (SFA), have been recognized as influential factors in the health associated to meat consumption. In recent years, great efforts have been exerted in order to improve the nutritional quality of meat products and regain consumer's trust in meat. For instance, the replacement of animal fat with vegetable oils in meat products (5). Another strategy is to modify the fatty acids profile through animal feeding (2,4). In contrast with what occurs with other vegetable sources of fat such as canola meal, palm oil or corn oil, among others, there are scanty information regarding the feeding and nutritive value of diets rich in vegetable fats for pigs, particularly using avocado (*Persea americana* Mill.) byproducts (1,3). In this context, the use of the entire discarded avocado fruit should be an interesting alternative for animal feeding, since it is not practical the separation of the fruit pulp from seed and peel.

Therefore, the objective of the present study was to report experimental data concerning to carcass composition and fatty acids profile of fattening pigs fed avocado paste as a source of unsaturated fatty acids.

Materials and Methods

Sixteen Yorkshire x Landrace pigs, castrated males and females in equal proportion and averaging 67.0 kg initial weight were allotted at random into two treatments consisting in cereal based diets formulated to contain 0 and 21% fresh avocado paste. The paste was composed of the entire, discarded and ripe fruits. The diets were given *ad libitum* to the animals. After nearly 49 days on test, the pigs were slaughtered following Mexican regulations for commercial practice in municipal abattoirs (Official Mexican Regulation NOM-033-ZOO, 1995). Assessments of carcass composition were conducted according to the methodologies described in the NPPC. Fatty acid methyl esters (FAMEs) were prepared by trans-esterification *in situ* following the method described by Rodríguez-Carpena (5). FAMEs were analyzed by gas chromatography using a HP-6890 gas chromatograph, equipped with an on-column injector and a flame ionization detector. Data were processed by the analysis of variance technique, in accordance with a general linear model.

Results

No statistical differences ($p>0.05$) were found amongst treatments for the variables of carcass yield, pH, carcass length and loin muscle area. However, animals fed fresh avocado paste in the diet, the backfat thickness at the level of 10-11th rib, was considerably less ($p<0.05$) than the animals fed the control diet (Table 1).

Table 1. Effect of fresh avocado paste on carcass characteristics of finishing pigs.

	Fresh Avocado Paste, %			
	0	**21**	**EE±**	**p-value**
Carcass yield, %	48.802	50.014	0.39	0.132
backfat thicknes, mm	35.874^{a}	27.706^{b}	1.46	0.002
carcass length, cm	76.313	74.325	0.76	0.201
pH Loin	5.741	5.929	0.07	0.248
Loin muscle area, cm^2	69.54	64.84	5.30	0.673

EE, error standard.

There was a highly significant ($P<0.001$) dietary influence on fatty acids profile in the pigs (Table 2).

Table 2. Summations obtained for the fatty acids in pork according to diet.

	Fresh Avocado Paste, mg/g	
	0	**21**
∑SFA	32.59^{a}	23.081^{b}
∑MUFA	64.46^{b}	69.36^{a}
∑PUFA	2.113^{b}	5.214^{a}

SFA, saturated fatty acid; MUFA, Monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. Values with a different letter ($^{a-b}$) within a row are significantly different ($p<0.05$).

Conclusions and Discussion

The fatty acid composition of muscle foods has a great impact on the nutritional value. Hence, the large differences found in the present study amongst diets would certainly influence relevant quality traits of pork. Regarding nutritional aspects, SFA are known to increase low density lipoproteins (LDL) and hence, blood cholesterol levels whereas unsaturated fatty acids exhibit the opposite effect. Amongst unsaturated fatty acids, MUFA display more beneficial effects because, unlike PUFA, do not decrease high-density lipoproteins (HDL) which protects against CHD.

It is suggested that 21% of discarded fresh avocado paste in the diet, lead to products with enhanced nutritional properties as a result of a more favorable fatty acid profile in pork.

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Comparison of immunological castration and physical castration for efficiency of boar taint reduction in male pigs

MA Mellencamp, C Calhoun, J Allison
Zoetis Inc., Florham Park, NJ, marnie.mellencamp@zoetis.com

Introduction

Physical castration (PC) of male pigs is performed usually within the first week of life to reduce aggressive behavior and improve pork quality by reducing the incidence of boar taint. Two compounds contribute to boar taint in pork. Androstenone (5a-androst-16-ene-3-one) is a testicular pheromone with a urine-like odor. Skatole (3-methylindole) is a gut derived metabolite of tryptophan metabolism with a fecal odor. These compounds accumulate in fat of intact male pigs at puberty. Both are reduced in the fat of PC pigs.

Immunological castration (IC) using Improvest® (*gonadotropin releasing factor analog-diphtheria toxoid conjugate*, Zoetis) is an alternative to PC that uses the pig's immune system to reduce the boar taint compound production. IC occurs near the time of slaughter, when immunized animals temporarily become like PC, with a similar control of boar taint and objectionable behavior. However, the difference in timing allows IC pigs to grow as intact males for most of their life, benefitting from the naturally induced improvements in feed conversion and carcass composition. This report summarizes 56 global studies of the effects of Improvest on chemical and sensory assessment of boar taint.

Materials and Methods

Two types of boar taint assessments were conducted and aggregate results are reported. Chemical analysis (HPLC with mass spectrophotometry detection) of fat from PC, IC and intact males for androstenone and skatole was determined in 35-56 global studies. Results are given as the percentage of pigs below established thresholds for both compounds. These thresholds were 1.0 and 0.2 µg/g of fat for androstenone and skatole, respectively. Sensory evaluations by trained and/or consumer panels were conducted in 35 studies.

Results

Global and US studies by trained sensory panels, consumer panels and chemical analysis of boar taint compounds have repeatedly demonstrated that immunological castration is effective in reducing boar taint to levels of PC barrows. The aggregate results of chemical analysis studies are shown in Table 1 and 2. While chemical assays are reliable indicators of boar taint, boar taint is complex and is, by definition, a human sensory perception. Therefore, sensory evaluation by trained panels and particularly consumer panels can provide a "real life" assessment of the efficacy of immunological castration. A total of 35 global and US studies confirmed that the eating quality of pork from immunologically castrated animals was as good as pork from physically castrated barrows or females (1). US FDA registration studies showed that trained panelists were able to identify meat from intact males (boars) but they were unable to differentiate pork from physically castrated or immunologically castrated pigs. Consumers who were representative of the general public did not find pork from immunological castrates inferior to pork from physically castrated barrows (2).

Conclusions and Discussion

Taken together, these results show that immunological castration is effective in reducing boar taint to levels at or below physical castrates. These data were generated in well-controlled field trials where protocols eliminated any cryptorchid or abnormal pigs from the study. Each individual study was designed for statistical evaluation, although the aggregate results are not suitable for comparison across groups.

Table 1. Effects of Improvest on androstenone

Treatment	# Studies	# Pigs	% Pigs with androstenone
Physical castrates	39	2544	0.1
Immunological castrates	56	4941	0.5
Intact males	35	2212	40.3

Table 2. Effects of Improvest on skatole.

Treatment	# Studies	# Pigs	% Pigs with skatole
Physical castrates	39	3083	0.2
Immunological castrates	56	5875	0.6
Intact males	35	2225	15.4

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The effect of different environmental enrichment materials on behavior and skin lesions of weaner pigs

A Scollo[1,2], B Contiero[1], E Scalzolaro[1], C Mazzoni[2], F Gottardo[1]
[1]*University of Padova, Department of Animal Medicine, Production and Health, Italy,* [2]*Suivet snc veterinary, Italy,*
mazzoni@suivet.it

Introduction

EU welfare legislation requires that weaner pigs are provided with environmental enrichments that satisfy their needs for investigation and manipulation. Although a variety of different suitable materials is used in commercial pig farms, the benefits to the animals throughout the weaning phase have not always been clear.

The aim of the present work was to determine the extent to which provision of separate enrichment materials gives additive improvement in occupation time and skin lesions, and whether there is consistency in the use of different enrichment materials over time.

Materials and Methods

The study involved a total of 300 weaner pigs from 28 to 56 days of age, housed in 12 slatted pens in a controlled environment building. Four enrichment materials hanging to the wall at the same height were separately provided (3 pens for each material): sisal rope, metal chain, stump and straw in a metal rack. Time interacting with each enrichment material, percentage of recumbent pigs and frequency of aggressive interactions and skin lesions were determined 6 times over the 28-day test period. Skin lesions were scored as 0 (absence of lesions), 1 (mild lesions) or 2 (serious lesions) for each anatomical area (head, ears, neck, shoulders, body and tail).

Behavioral data, expressed as percentage of animals involved in each activity, were processed using a chi-square analysis, testing the effect of each environmental enrichment within time of observation. Skin lesions were processed using the non-parametric Kruskal-Wallis analysis.

Results

The results showed that pigs spent a greater proportion of time interacting with sisal rope up to 42 days of age (0.07, $P < 0.001$). However, getting on the end of the weaning phase this percentage drastically decreased and was replaced by a greater interest in the straw (56 days of age: 0.14, $P < 0.001$). Pigs showed the lowest interaction with the chain and the stump throughout the test period (Figure 1). Although the number of aggressive interactions decreased for all the groups over the test period, the greater interest in sisal rope and straw was associated with higher frequency of skin lesions close to the end of the weaning phase ($P < 0.01$). In particular, the body areas most affected were ears and neck (0.43 and 0.17 respectively, $P < 0.05$), with a greater number of severe lesions ($P < 0.05$). No significant difference was shown for recumbency.

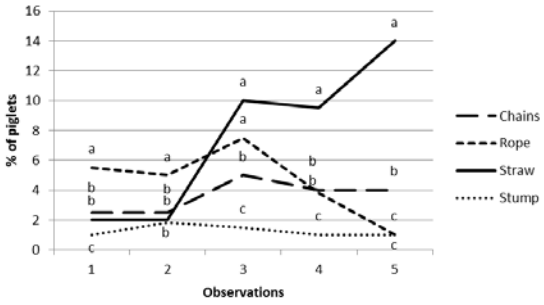

Figure 1. Percentage of piglets exploring each environmental enrichment within observation.
[a,b,c]: different letters shown significant statistical difference ($P < 0.05$).

Conclusions and Discussion

Sisal rope and straw in the rack, among the materials used in the current study, better stimulate pigs investigation and manipulation; the first in the initial part of weaning and the latter in the second part. Efficacy of straw confirms results reported in literature (1, 2), but seems useful to replace it with another material like the rope in the initial stages.

However, it is important to considerate the risk of skin lesions, probably due to hierarchical competitive interactions among pigs (3). A limited amount of suitable material might be responsible of detrimental resource-related aggressiveness.

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The effect of immobilization stress of sows on selected immunity parameters in piglets in the early postnatal period

M Kulok[1], K Wojtas[1], M Porowski[2], Z Pejsak[3], R Kołacz[1]

[1]*Wroclaw University of Environmental and Life Sciences, Department of Environmental Hygiene and Animal Welfare, Chelmonskiego Str. 38C, 51-630 Wroclaw,* [2]*Veterinary Clinic, ul. Kościuszki 1, 62-010 Pobiedziska,* [3]*Department of Swine Diseases , National Veterinary Reserch Institute, Partyzantów 57, 24-100 Pulawy,* kolacz@gmail.com

Introduction

Practice of movement restriction of pregnant sows is usually discussed in terms of animal welfare (1). The health aspect of this housing system is often neglected despite the evidence that prolonged stress has a negative effect on immune functions of animals (3,4). In case of gestation crates the issue concerns pregnant animals which means that stress can affect not only sows but also newborn piglets by occurrence of prenatal stress (2). That could lead to weakening of the piglets immune system and therefore result in increased morbidity (4). The aim of the study was to examine if movement restriction of sow would affect piglets immune system by occurrence of prenatal stress.

Materials and Methods

The experiment was conducted at two farms that use individual and group housing system. Two research groups were established:

Free movement group (FM): Pregnant sows in this group were kept in group pens in a number of 10 animals in a pen and an area of 2.25 m^2 per animal. Sows in this group had a possibility to move freely. Before birth sows were moved to individual pens with a possibility of movement. Piglets stayed with sows up to 28th day of life.

Movement restriction group (MR): Pregnant sows in this group were kept in individual pens of an area of 1.3 m^2. Movement of these animals was limited only to the possibility of getting up and lying down. Before birth sows were moved to individual pens without possibility of movement. Piglets stayed with sows up to 28th day of life.

Blood samples were collected from piglets in all groups at 3th, 7th and 21th day of life.

Laboratory tests:

Cell proliferation assay was taken according to the method described by Tuchscherer et al (1998).

Neutrophil chemotaxis assay was performed according to the method described by Smith et al (1985).

Results

Lymphocytes proliferation in response to ConA was significantly lower in piglets from movement restriction group through all the experiment (up to 21st day). Lymphocytes proliferation in response to PHA was significantly lower in piglets from movement restriction group at first 3 days after birth. Lymphocytes proliferation in response to PWM was significantly lower in piglets from movement restriction group at first 7 days after birth.

Conclusions and Discussion

In presented study movement restriction of sow resulted in lowering of lymphocytes proliferation in piglets. This suggest that movement restriction of sows can lead to prenatal stress in piglets and through secretion of corticoid hormones, has negative effect on immune functions and results in weakening of the piglets immune system (2,5). Presented research shows that there is a direct link between sows welfare and piglets health condition.

Table 1. Lymphocytes proliferation in response to ConA, PHA and PWM in 3th,7th and 21st day of the experiment.

Parameter	day	FM (n=40)	MR (n=40)
lymphocytes proliferation (ConA)	3	1.21^{A}	1.09^{A}
	7	2.19^{a}	2.02^{a}
	21	3.28^{a}	3.07^{a}
lymphocytes proliferation (PHA)	3	1.6^{A}	1.41^{A}
	7	2.97	2.72
	21	3.24	3.07
lymphocytes proliferation (PWM)	3	1.45^{A}	1.28^{A}
	7	3.13^{a}	2.72^{a}
	21	3.75	3.42

Superscripts indicate: (A) highly significant differences ($p \leq 0.01$) and (a) statistically significant differences ($p \leq 0.05$) in columns.

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Physiological response of ear tagging in comparison with castration and tail docking

J Stark[a], M Ritzmann[a], N Übel[a], J Stadler, M Eddicks[a], S Zöls[a]
[a] *Clinic for Swine, Ludwig-Maximilians-University Munich, 85764 Oberschleissheim, Germany,* julia.grimm@lmu.de

Introduction

Piglets are subjected to several processing procedures early in life. Castration and tail docking have already been researched in previous studies (1,2,3). However, the effect of ear tagging on the welfare of the piglet is scarcely investigated. The objective of the present study was to compare the distress caused by ear tagging with the distress caused by castration and tail docking. In addition, the effect of analgesia with Meloxicam was examined.

Materials and Methods

In total 210 male piglets were randomized to equal numbers (n = 30) into one of seven groups: a control group which was only handled (H), an ear tagged group that received no analgesia (ET), an ear tagged group with analgesia (ETM), a castration group with no analgesia (C), a castration group with analgesia (CM), a tail docked group with no analgesia (TD) and a tail docked group with analgesia (TDM). The procedures were carried out on day three or four after farrowing. From all piglets five blood samples were taken: 30 min before the respective procedure (individual base value), as well as 30 min, 60 min, 4 h and 7 h after processing. Cortisol was measured as pain and stress parameter. Means as well as the AUCs (Area under the curve value) were analyzed and the effective sizes of the procedures were established.

Results

At 7 h after the experimental treatment, cortisol concentrations returned to base values in all groups. ET evoked a more elevated cortisol response than H piglets at 30 min ($p < 0.001$) and 60 min ($p = 0.001$). ET seemed to be less stressful than C at 30 min ($p = 0.001$) but did not differ significantly at the other sample times. In comparison to TD, ET did not result in a significant difference when comparing the means; taking both intensity and duration of the procedure in account (AUC), a significant effect could be shown (p of AUC = 0.019). Analgesia (ETM) resulted in lower cortisol levels than ET at 30 min ($p = 0.001$) and 60 min ($p = 0.003$) post-procedure. Castration (C) provoked the highest stress response of all procedures; a significant analgesic effect (CM) could be shown only at 60 min post-procedure ($p < 0.001$). TD resulted in significant higher cortisol levels than H piglets only at 30 min ($p < 0.001$); analgesia (TDM) reduced the cortisol response at 30 min ($p = 0.003$).

Conclusions and Discussion

Castration seemed to produce the greatest distress of the three investigated procedures. Ear tagging followed next and effectuated a greater cortisol response than tail docking, which can be explained by the different degree of sensitivity of the ear and the tail (4). Analgesia was especially effective with ear tagging. We concluded that the distress caused by ear tagging piglets is substantial and needs to be further researched.

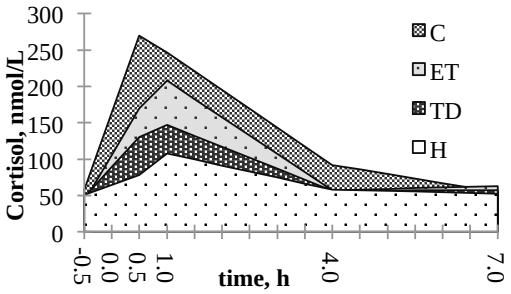

Figure 1. The course of cortisol concentration (nmol/L) over time (h). Treatments: control handling (H; n = 30); ear tagging without pain medication (ET; n = 30); tail docking without pain medication (TD; n = 30); and castration without pain medication (C; n = 30).

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Evaluation of cortisol levels of sows during piglet castration – comparison of castration with and without isoflurane anesthesia

D Hoeltig[1], C Schwennen[1], M Piechotta[2], N Kolbaum[1], K-H Waldmann[1]
[1]*Clinic for Swine and Small Ruminants, Forensic Medicine and Ambulatory Services,*
[2]*Clinic for cattle; both University of Veterinary Medicine Hannover, Foundation, Germany,* doris.hoeltig@tiho-hannover.de

Introduction

Although it is evidenced that it is combined with pain and stress (1) surgical castration without anaesthesia is the most common practice on commercial pig farms. There are many studies examining the stress of castration for the directly involved piglet (2, 3), but no studies regarding a possible influence of the castration procedure and the piglets' vocalisation on the stress level of other pigs housed in the same stable. Never the less, in animal welfare discussions it is often assumed that plenty of shrieking from the piglets leads to higher stress levels of their mother and therefor might also influence the welfare of sows as well as the piglets performance (4). The aim of this study was to compare cortisol levels of sows whose litters had been castrated with and without isoflurane anaesthesia, as anaesthetised piglets show a significant lower level of vocalisation.

Materials and Methods

On three different commercial swine herds (Farm A and B: 200 sows; farm C: 540 sows) sows where divided into two groups per castration day. The piglets of sows of the first group were castrated without anaesthesia, piglets of sows of the second group where castrated under isoflurane anaesthesia using the PIGNAP Pro® automated anaesthetic device (Agrosystems GmbH, Switzerland) with two fixation cups (5Vol.% isoflurane in 30% oxygen; flow rate: 2L / min). Cortisol levels in saliva of the sows were measured 1 hour before castration as well as one 1 hour after castration of their piglets (Salivette®, Co. Sarstedt, Germany; Cortisol free in Saliva ELISA®, Co. Demeditec Diagnostics GmbH, Germany) and a behaviour score system based upon vocalisation, movements and enragement of the sows was surveyed. Over all 84 sows, 28 (14 isoflurane / 14 conventional) sows per farm, were monitored. The observed groups consisted of gilts as well as older sows.

Results

There were no significant differences in the cortisol levels of sows whose piglets were castrated with (iso) or without (con) anaesthesia (p: 0.385). Regarding all monitored sows the saliva levels of cortisol decreased in 16 sows (iso, mean: -2.98 ng/ml) and 19 sows (con, mean: -5.16 ng/ml) between to two measurements. Also no differences in the behaviour of the sows of the different groups during the castration of their litters (p: 0.385) occurred. The results were repeatable when the data were appraised on herd level. The p-values for cortisol levels were 0.575 (farm A), 0.646 (farm B) and 0.362 (farm C) and for behaviour 0.077 (farm A), 0.404 (farm B), 0.645 (farm C). As for the fact that the stress of the sows might be more influenced by the different management strategies of the castration day than by the castration method the data were also evaluated comparing behaviour and cortisol levels between the different herds. Here too, no differences could be shown. The p-values for cortisol levels were 0.295 (farm A vs. B), 0.678 (farm A vs. C), and 0.111 (farm B vs. C) and for behaviour 0.351 (farm A vs. B), 0.359 (farm A vs. C) and 0.990 (farm B vs. C).

Conclusion and Discussion

Due to the results it seems that regarding a mixed population of sows of different ages the castration method and the amount of vocalisation of their piglets seems not to have a significant impact on the sow's welfare. This might be based upon the fact that the sows get used to the daily farm routine as well as the castration procedure. The dropping of the cortisol levels in a relatively high number of sows also might lead to the hypothesis that a short interval of separation from the piglets seems to be tolerated quite well or even result in some stress relaxation of the sows. The results might differ when monitored only in gilts, as they experience the castration of their litters for the first time and might therefor be more influenced of their piglets shrieking.

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Performance of piglets after castration with or without isoflurane anaesthesia

C Schwennen[1], D Hoeltig[1], N Kolbaum[1], K-H Waldmann[1]
[1]*Clinic for Swine and Small Ruminants, Forensic Medicine and Ambulatory Services, University of Veterinary Medicine Hannover, Foundation, Germany,* doris.hoeltig@tiho-hannover.de

Introduction

Surgical castration without anaesthesia is the most common practice on commercial pig farms, although it is evidenced that castration is accompanied by pain and stress (1, 3). Isoflurane anaesthesia is offered as an alternative and has already been used in countries like Switzerland and on organic farms in Germany. It permits a safe, rapid anaesthetic induction and maintenance, as well as brief and smooth recovery (2). As previous experimental studies showed that there might be some differences in wound healing and development of piglets castrated with and without isoflurane anaesthesia (4), the objective of this study was to evaluate if these differences are repeatable on farm level.

Materials and Methods

The study took place in three commercial swine farms in Germany (Farm A and B: 200 sows; farm C: 540 sows). The castration was performed at the average age of 3 to 6 days. The piglets were given a NSAID treatment directly before castration. All farms used the PIGNAP Pro® automated anaesthetic device (Agrosystems GmbH, Switzerland) with two fixation cups (5Vol.% isoflurane in 30% oxygen; flow rate: 2L / min). The litters were divided into two groups per farm per day. Piglets of one group were castrated conventionally (con) and piglets of the other group under anaesthesia (iso). The separation time from the sow, rectal temperature directly before and after castration, suckling behaviour after castration and the course of wound healing and growth were compared between the two groups. Therefore 256 (204 iso / 52 con) litters were observed for separation time and the rectal temperatures of 527 piglets (264 iso / 263 con) piglets were noted. The time until the first suckling, duration of the suckling, as well as time until the second suckling was measured. The assessment of wound healing and growth took place on day 10 after castration and 28th day of life. Wound healing of 2023 (965 iso / 1058 con) piglets on day 10 and 1334 (636 iso / 698 con) on the 28th day of life was rated using a score system (7). At last the weight of 525 (268 iso / 257con) piglets on day 10 and 420 (202 iso / 218 con) on the 28th day of life was noted.

Results

Castration under anaesthesia takes significantly more time compared to the conventional method (8min iso / 5min con; p< 0.0001). There was no significant difference between the groups in terms of suckling behaviour (mean of time until first suckling: 29min for both groups, p=0.664; duration of first suckling: 10min iso / 7min con, p=0.063; time up to second suckling: 35min iso / 34.5min con, p=0.875). The conventionally castrated piglets showed a significantly higher increase of rectal temperature as the anesthetised animals (+0.0129˚C iso / +0.2989˚C con, p=0.029). The assessment of wound healing on the 10th day after castration as well as on the 28th day of life showed no significant difference between both groups (day 10: p=0.386; 28th day of life: p=0.718). A significant difference in weight of the piglets was detected on day 10 after castration (4.45kg iso / 4.63kg con, p = 0.0360), albeit this difference could no longer be seen on the 28th day of life (7.53kg iso / 7.73kg con, p=0.1567).

Conclusion and Discussion

The significantly lower increase of rectal temperature in anesthetized animals can be explained with lower stress levels compared to those of conventional castrated piglets. Regarding the performance of the piglets after castration, it can be said that the piglets experience neither advantages nor disadvantages in their suckling behaviour and their further performance if the castration is performed under anaesthesia. The extended separation time is a result due to the 70 seconds lasting induction time of anaesthesia. This delay could be used for the implementation of other management measures (for example vaccination) or be shortened by using an anaesthetic device with more than two fixation cups.

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Evaluation about welfare parameters in fattening pig farms

F Ronco[1], G Martano[1], M Tarantola[2]
[1] *ASL TO3, Pinerolo, Italy,* [2] *Department of Animal Production, Epidemiology and Ecology, Turin University,*
beppemartano@virgilio.it

Introduction

The international research developed in the last decades in the field of applied ethology has demonstrated clearly that the respect of animal welfare is applicable to different types of farming and can lead, as well as proper management of farm animal populations, even in a quantitative and quality of the productions.
Animal welfare in livestock is strongly influenced by environmental parameters and management that are heavily dependent on man, you should then be able to identify the needs of the latter in order to keep under control and be able to change the harmful and stressful situations, that may affect their health and therefore the yield and quality of food products derived from it.
The aim of this study is to evaluate welfare parameters in 26 fattening pig farms and to study correlation with lameness.

Materials and Methods

We collected data from 26 fattening pig farms located in Piemonte, North West Italy. The herds size ranged from 35 to 3700, for a total of 34.000 pigs.
In order of collected data it has been used a questionnaire divided in different sections regarding master data of the breeding, evaluation of welfare parameters, Global Score (tool developed by Candotti et al, 2007, based on observation of abnormal behaviors and injury.), feeding and sanitary treatments, evaluation of lameness and mortality. For the compilation of the Global Score and assessment of lameness were observed fifty pigs in each farms, presents in 3-4 box depending on the size and relative concentration of the same pigs in herds. Observation of the various behaviors and the search for any injury lasted at least 30 minutes. The Global Score range from 0 (worst) to 8,907 (top). In this study the worst welfare herds was 2,64 and the best farms was 7,574.

Results

Based on the rating of the Global Score farms were divided into 3 groups (group 0: score < 4; group 1: score >4 and <6; group 2 : score > 6). The data were first analyzed with analysis of variance (ANOVA) followed by Bonferroni test. The statistical analysis of the data showed a statistically significant difference between group 2 (global score > 6) and group 0 (global score < 4) with respect to the parameter of lameness. It was founded a correlation between Global Score and lameness; in fact to decrease the rating of the Global Score increases cases of lameness. It is displayed in Figure 1.

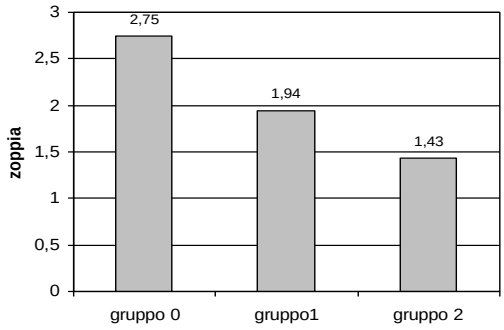

Figure 1.

Conclusions and Discussion

The assessment of animal welfare is one of the components of swine breeding in close contact with the component health, nutrition and management of the same. Modern farming techniques lead increasingly to considere the resolution of business problems in multi-view, of which the welfare part is primary. The use of a card with the assesment of the Global Score, practical and fast compilation, making it more easily measurable animal welfare, highlighting how the welfare state affects the production and health parameters of swine fattening herds.

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P.714

The welfare of pigs during transportation to slaughter house in Nigeria.

JO Abiola
Department of Veterinary Medicine, University of Ibadan, Ibadan. Dnk12_day@yahoo.com

Introduction

Scientists agree that transport is generally an exceptionally stressful episode in the life of an animal. (6) It involves changes to the animal's whole environment. It may be handled and mixed with unfamiliar animals, subjected to changes in temperature and air movement, possibly hurt or injured and restricted in space, feed and water.

Transportation is completely unnatural for animals. Forms of suffering caused by transport include hunger, thirst, discomfort, pain, frustration, fear, disease and distress. Suffering increases directly with the length of journey endured. The issue of whether live long distance transport of animals, only to be slaughtered at the journey's end, is justified at all when they could be slaughtered on the farm, or at one of the nearest abattoirs, deserves much more attention.

According to (FAWC) (4) one of the five freedom of animal during transportation is Freedom from discomfort by providing an appropriate environment, including shelter and a comfortable resting area.

For pigs, the transportation process is a combination of unfamiliar or novel experiences that could be perceived as stressful. If the pig is not able to cope with these sequential and additive stressors, increased losses through elevated mortality, morbidity and decreased product quality were observed during transport and lairage at the slaughter houses. Transport losses are multi-factorial and involve people, pig, facility design, management, transportation, processing plant, and environmental factors (1, 2, 3, 8). In pigs, long distance transport exhausts the animals due to excessively long feed withdrawal times, while the physical and psychological stress tends to produce Dried Firm and Dark (DFD) meat. Transport is also particularly stressful for pigs because they can suffer motion sickness. (7) The main problems associated with transport and meat quality are the Porcine Stress Syndrome (which results in live weight loss in 4-6% and mortality in 0.1- 0.4%), injuries, bruises, skin damage, abnormal colour, DFD meat or 'Pale, Soft, Exudative' (PSE) meat, and contamination by Salmonella. (5)

Animal welfare during transportation of pigs to slaughter houses must be of utmost importance to provide a comfortable environment for the pigs.

Materials and Method

Visit to the abattoirs revealed that pigs were usually crammed into small vehicle, tied with ropes, pigs struggle to get air and are usually given no food or water for the entire journey (often hundreds of kilometers). They suffer from temperature extremes and are forced to inhale ammonia fumes and diesel exhaust from the transporting vehicles. Many of the animals were presented to the abattoir very weak, lame or death may even occur depending on the time of the day the animals were transported.

Discussion and

Factors affecting the welfare of animals before, during and after transport must therefore be taken into consideration. By identifying potentially additive factors within the loading, transporting and marketing stages that may be potential stressors to the pig, we may attempt to reduce stressors placed upon the pig and subsequently reduce transport losses.

Good animal welfare during transport and slaughter must therefore be monitored to minimize the losses incurred during transportation. The following may therefore be considered:

- training and supervision of employees or handlers about the welfare of the pigs.
- well designed Vehicle utilizing behavioral principles of the animals
- good Vehicle maintenance
- Vehicle must have sufficient capacity for the number of pigs being handled

One of the most important factors which determines if a pig is fit for transport is the condition of the pig that is loaded onto the truck. The welfare of pigs during transport can be easily monitored with numerical scoring to prevent abuse. Legislation about animal welfare during transport may equally help in reducing the stress observed on the animals during transportation.

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