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IPVS ABRAVES

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2022

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IPVS2022: Memories of resilience

It had all started in Dublin (June 2016), when Brazil got the right to organize the most important congress of the international pig veterinary community: the IPVS Congress. It was a great honor, but also a tremendous responsibility. As so, we needed to start immediately with the conference planning, in other words, bringing our dreams into the real world. It took around two years with the planning, and after the IPVS2018 China, the work got even harder each day: sponsorship contacts, putting together attractive scientific and social programs.

As the Chair of the IPVS2022 Congress, I should say that our event will be known as the congress of resilience: we overcome African Swine Fever by creating a Biossecurity committee, performing a nice risk assessment, and moving the host city to Rio de Janeiro. By then, we had to rearrange our plans to adapt for the new conference venue and establish a whole new structure within a few months. And we made it! Our congress was ready to go and beautifully organized to take place from June 2nd to 5th. However, unexpectedly, the whole world faced the COVID19 pandemic in the beginning of 2020. Given this scenario, the IPVS Brazil Local Organizing Committee, supported by the IPVS Board and the sponsors, decided to postpone our event to 2022. It was a tough, but a wise decision, aiming for the safety of all involved in our event (delegates, sponsors, and organizers).

After all that has been said, here we are to accomplish this great achievement: the IPVS2022! In this sense, we are pleased to deliver to the international pig veterinary community over 450 abstracts and almost 200 lectures, which are compiled in the IPVS2022 Proceedings. We wish to thank the Scientific Committee members for putting together this sensational scientific program, the speakers for sharing their knowledge and the reviewers for reading, scoring the abstracts, and assisting with the organization of the program. We would also like to thank the IPVS Board members, all sponsors for their support, which was crucial to preserve the LOC's soundness, and the suppliers. Therefore, our dream came true: Welcome to the IPVS2022! It is a great honor to have you all with us in Rio de Janeiro or attending online. Thank you very much for being here with us, and hope that you enjoy our conference, which was kindly prepared to meet the world's pig industry's demands.

Prof. Fernanda Almeida
IPVS2022 Chair

Preface

As mentioned by our president, "Resilience" or "Persistence" would represent very well the IPVS2022 by all aspects stated by her, but also considering the attempts to bring this greatevent to Brazil for the second time.

For this IPVS2022 the organizing committee tried to innovate and decided to offer six options ofpre-conference sessions: Antimicrobials, Agrobusiness, African Swine Fever, Mucosa ImmuneResponse and Vaccinology, Nutrition and Reproduction, that will be held in the morning andafternoon of the opening day. At the Opening Ceremony, we will have the Tom Alexander Memorial Lecture, by Dr. Robert Friendship, talking about "Simple things – the basic principles of swinehealth management". For the congress, we have chosen 12 subjects divided in 20 sessions and each of these sessions will have a keynote speaker to introduce the major topics. The final program consists of 122 oral presentations, chosen among more than 480 abstractsubmissions. This number of abstracts imposed a great task for the reviewers from all partsof the world. For all their support, we wish to thank the following reviewers for reading andscoring the abstracts:

Abelardo Silva Jr
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For the convenience of the readers the proceedings is consistent of only one volume organized in sections comprised by the I) Tom Alexander lecture, II) manuscripts from the speaker from the three pre-conference sessions, III) manuscripts from the keynote speakersfrom all sessions, and IV) all abstracts also divided by thematic area.

The Scientific Committee hope that the information contained in this book can be disseminated and shared to all interested professionals in up to date scientific results that certainly will contribute to the advancement of knowledge in different areas of swine production!

Prof Roberto Guedes
Chair of the Scientific Committee

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An event of such magnitude as the IPVS2020 Congress could not happen without the financial, professional and moral support of our sponsors.

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IPVS HISTORY

The specialization in Veterinary Medicine and the current increase in the importance of swine production have demanded the cooperation of Veterinary professionals from all over the world.This cooperation aimed the development of know-how to solve problems related to raising andreproducing this animal species.

The IPVS – International Pig Veterinary Society – was founded with this objective and with thepurpose to promote, every two years, a meeting with professionals from the swine productionchain to discuss the studies developed by the international scientific community.

In the last 50 years, this IPVS objective and purpose have been achieved. Since the first conferenceheld in Cambridge/England, in 1969, the scientific and technical community had shown its interest in this international forum, where all the problems related to pig production were presentedand debated. Thanks to the dedication and seriousness with which IPVS representatives havetreated the issues of this community´s interest, more than 40.000 people have already hadthe opportunity to participate in IPVS conferences, with the presentation of more than 13.000scientific papers. All the past editions of IPVS congresses are cited below.

HISTORICAL DATA

The interest in technical-scientific development is the main motivation to organize IPVS Congresses. Many of the debates have led to the development of procedures, which were incorporated into pig production systems, aiming to increase productivity. At each Conference, acomplete review of the new advances is presented in search of more efficient solutions to facepig production challenges.

Edition	Local	Date	Chair	Abstracts	Participants
1°	Cambridge, England	23 - 28/06/69	Dr. PD Storie-Pugh	123	500
2°	Hannover, Germany	23 - 26/05/72	Dr. W Schulze	179	900
3°	Lyon, France	12 - 14/06/74	Dr. J Tournut	187	854
4°	Ames, US	22 - 26/06/76	Dr. W Brandt	374	1250
5°	Zagreb, Yugoslavia	13 - 15/06/78	Dr. O Bohm	181	450
7°	Mexico City, Mexico	26 - 31/07/82	Dr. RR Necoechea	360	1250
8°	Ghent, Belgium	27 - 31/06/84	Dr. MB Pensaert	187	854
9°	Barcelona, Spain	15 - 18/07/86	Dr. JL Garcia-Ferrero	455	1026
10°	Rio de Janeiro, Brazil	14 - 17/08/88	Dr. L Roppa	368	1233
11°	Lausanne, Switzerland	01 - 05/07/90	Dr. H Keller	528	1718
12°	The Hague, the Netherlands	17 - 20/08/92	Dr. J Verheidjen	696	2004
13°	Bangkok, Thailand	26 - 30/06/94	Dr. S Laungtongkum	538	1621
14°	Bologna, Italy	07 - 10/07/96	Dr. E Seren	648	1614
15°	Birmingham, England	05 - 09/07/98	Dr. C Glossop	839	1800
16°	Melbourne, Australia	17 - 20/09/00	Dr. R Cutler	605	1614
17°	Ames, US	02 - 05/06/02	Dr. H Harris	688	1500
18°	Hamburg, Germany	27 - 01/07/04	Dr. H Bossow	872	2455
19°	Copenhagen, Denmark	16 - 19/07/06	Dr. B Nielsen	934	2486
20°	Durban, South Africa	22 - 26/26/08	Dr. DPB Evans	918	1900
21°	Vancouver, Canada	18 - 21/07/10	Dr. E Sanford	1149	2716
22°	Jeju, South Korea	10 - 13/06/12	Dr. WH Lee	–	3099
23°	Cancun, Mexico	08 - 11/06/14	Dr. A Stephano	978	2560
24°	Dublin, Ireland	07 - 10/06/16	Dr. P Kirwan	1100	3552
25°	Chongqing, China	11 - 14/06/18	Dr. Y Hanchun	903	5599

Abstracts

ANTIMICROBIALS

Antibiotic-resistant bacteria in environmental dust from pig farms

Nicoletta Formenti[1*]; Claudia Romeo[1]; Chiara Boifava[1]; Flavia Guarneri[1]; Federico Scali[1]; Matteo Tonni[1]; Antonio Maisano[1]; Patrizia Di Gennaro[2]; Giovanni Loris Alborali[1]
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Introduction

The colonisation of farm environments by pathogenic bacteria is a key factor in the persistence of diseases in pig production (1). Regular cleaning and disinfection should limit this spread, but in the case of antibiotic-resistant bacteria these protocols may fail and lead to persistent environmental contamination. Environmental analyses are thus fundamental to cope with the issue of antimicrobial resistance (AMR). Nowadays, thanks to the raising awareness about the risks posed by environmental contamination, the monitoring of surfaces is routinely carried out in hospitals (2). A similar attention to this issue should be payed in farms, especially to contamination by *Escherichia coli* producing extended-spectrum β-lactamases (ESBLs) and other β-lactamases (AmpCs), and by methicillin-resistant *Staphylococcus aureus* (MRSA), which are a recognized public health problem worldwide (4,5) and are common opportunistic pathogens in pig farms (6,7). In the present, preliminary study, the spread of ESBL/AmpC *E. coli* and MRSA in environmental dust within pig farms was investigated by analyzing samples collected before and after cleaning and disinfection procedures.

Materials and Methods

Sampling was carried out in 6 farms in Northeastern Italy from May to July 2021. Within each farm, three sampling sites were identified: the silo, the changing room, and the pig unit. Dust samples were collected by using wet sponges rubbed on a surface (100 cm^2) within each site. Overall, 67 sponges were collected by sampling each site in the "dirty" (N_{silo}=9; $N_{changing\ room}$=8; $N_{pig\ unit}$=9) and in the "clean" condition (N_{silo}=14; $N_{changing\ room}$=14; $N_{pig\ unit}$=13), before and after the routine cleaning and disinfection procedures. Each sample was microbiologically and molecularly analyzed for the identification of ESBL/AmpC *E. coli* and MRSA. The resistance profile of each isolate was assessed by MIC (Minimum Inhibitory Concentration) technique. The resulting data were analyzed through logistic regressions to identify the factors influencing bacteria presence (8).

Results

No *E. coli* ESBL/AmpC were found in any of the examined samples, while MRSA was detected in 41.8% (28/67) of them. MRSA prevalences observed in each sampling site before and after cleaning are listed in Table 1. Logistic regression revealed no significant difference in prevalences between sampling sites (p=0.70) or between dirty and clean condition (p=0.56). MIC analyses, performed on 25 *S. aureus*, showed that 100% were resistant to Cefoxitin, Clindamycin, Mupirocin, Tetracycline and Tiamulin, 88% to Quinupristin/dalfopristin, 68.0% to Erytromycin and 56.0% to Trimethoprim.

Table 1. MRSA prevalences (mean ± SE) in environmental dust sampled in pig farms before and after cleaning procedures.

SAMPLING SITE	P (%)	
	DIRTY	CLEAN
Silo	33.3 ± 16.7	35.7 ± 13.3
Changing room	50.0 ± 18.9	42.8 ± 13.7
Pig unit	55.5 ± 17.6	38.5 ± 14.0

Discussion and Conclusion

The lack of *E. coli* ESBL/AmpC in the analysed dust samples is a comforting result because of the associated potential health risks (6,9). On the contrary, the prevalence of MRSA found in the present study is rather high and independent from cleaning, confirming its high resistance against cleaning and disinfection procedures (1,5,10). Although the limited sample size might have hindered the detection of significant differences, the widespread presence of MRSA raises some concerns related to the potential risks for transmission among pigs and to workers, and the consequent occupational and public health issues, in addition to the potential economic implications (10). Moreover, these results suggest that new cleaning and disinfection protocols should be defined to control more effectively MRSA within pig farms. Overall, despite the limited sample size, these preliminary findings represent an interesting starting point for further studies aimed at elucidating AMR contamination in farm environments and at implementing new strategies for their control and eradication.

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Antibiotics use on nursery farms in Minas Gerais State - Brazil

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Introduction

The intensification of swine rearing systems has leveraged the occurrence of stressful conditions, which may cause immunosuppression. This scenario contributes to the emergence of potentially pathogenic microorganisms, increasing the animals' challenge. Therefore, the use of antibiotics is necessary for the treatment and prevention of diseases in pigs, especially in the nursery period, where they are more susceptible to bacterial diseases. Although antibiotics use is necessary, it is known that their excessive use in animal production is a global public health problem due to the development of resistance in human and veterinary medicine (1,2). The aim of this study was to list the use of antibiotics in piglets of full cycle farms in an area of Minas Gerais swine farming.

Materials and Methods

In 2021, 29 full-cycle commercial farms were visited in the municipality of Pará de Minas, state of Minas Gerais, southeastern Brazil. It was analyzed which antibiotics active principles were used in each farm and what was the frequency and duration of exposure to these drugs in days, in total and for each piglet growth phase. The different uses of antibiotics (as growth promotors, preventive or curative uses) and forms of administration (via water, feed or injectable) were jointly evaluated, and average results were analyzed per farm. Descriptive data analysis, Spearman correlation, principal components analysis and hierarchical clustering of main components were performed, identifying four clusters. Stepwise regression was performed and differences among groups were analyzed by ANOVA and Tukey's test. The significance level was $P<0.05$ for all analyses, which were performed with the R softwear (3).

Results

During the interviews, 28 antibiotics were mentioned in use on the farms, whose forms of administration were based on the different stages of piglet rearing. Of the 13 classes reported, bambemycin and streptogramin were used only in the grower and finisher phases, while hydroxyquinoline was used only in the farrowing and nursery facilities. The others were used in all phases of piglet rearing. Only three forms of administration of antibiotics were found in the herd: injectable, intramuscular and oral, via feed. The form of administration of antibiotics for piglets is mainly via feed. Of the 28 active ingredients used, only eight made up 77.3% of the total amount of ATM used on the farms: amoxicillin, tiamulin, oxytetracycline, florfenicol, lincomycin, tylosin, ciprofloxacin and norfloxacin. The number of active ingredients per farm was correlated with the number of sows (0.48, $p<0.05$) and with the total amount of ATM (0.49, $p<0.05$). In the nursery phase, 19 active principles were used, totaling 366,403.46 mg of antibiotics, or 28.04% of the total amount. Ten of them made up 94.26% of the amount used in this phase, especially Amoxicillin, followed by Neomycin, Colistin, Tiamulin and Ciprofloxacin.

Discussion and Conclusion

On average, there is an excessive use of antibiotics for piglets, without correct preventive or therapeutic justification and with no effect on productivity. In the farms studied in Pará de Minas, 21 of them use antibiotics preventively as a routine for suckling piglets in the farrowing room, with 62% of them using more than one active ingredient. These results are similar to those of Dutra et al. (2), when reporting that 72% of the studied farms used antibiotics preventively in the farrowing room and that half used more than one active ingredient. Only two forms antibiotics administration were found in the herd: injectable, intramuscular and oral, via feed. The form of administration of antibiotics for piglets is mainly via feed. These results portray a complex reality, since this use can change the population of microorganisms, such as bacteria that naturally inhabit the gastrointestinal tract, favoring the development of bacterial resistance. In the present study, all interviewees from the 29 farms declared that they used antibiotics in all stages of the piglets only for preventive purposes. The antibiotics most frequently cited by the farms in this study were Amoxicillin, Florfenicol, Colistin, Tiamulin, Tylosin and Lincomycin. The frequency of citations is comparable to that found by Dutra et al. (2). The results indicate the need to implement good practices and greater control in veterinary guidance, marketing and use of antibiotics for piglets on full cycle farms in Pará de Minas (MG).

Acknowledgments

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Antimicrobial susceptibility pattern to tildipirosin of *Actinobacillus pleuropneumoniae and Pasteurella multocida* serogroup A isolated in Brazil

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Introduction

Porcine Respiratory Disease Complex (PRDC) is the main cause of reduced performance of pigs and might be caused by several different types of pathogens (1). The involvement of *Actinobacillus pleuropneumoniae* (*App*) and *Pasteurella multocida* serogroup A (*PmA*) as primary and secondary agents of PRDC during the finishing phase, for instance, is often associated with bronchopneumonia, with or without involvement of the pleura (2). Some clinical strains of PmA, however, have been described as a primary agent of pneumonia or hemorrhagic septicemia in pigs (3). During clinical outbreaks of bacterial respiratory diseases, the prompt use of antibiotics is essential to control the infection; therefore, monitoring the antibiotic susceptibility of clinical strains of App and PmA is central to reduce outbreaks. In this work we present the sensitivity profile of Brazilian clinical strains of App and PmA to tildipirosin, a new semisynthetic macrolide molecule.

Materials and Methods

A total of 100 clinical strains of *A. pleuropneumoniae* (serotypes: 1, 5, 6, 7, 8, 14 and non-typeable) and 60 *P. multocida* serogroup A (all *toxA* gene negative and 9 of them *pfhA* gene positive) were isolated from porcine pneumonic lungs . The samples were collected from animals reared in eight different states of Brazil (RS, SC, PR, SP, MG, MS, MT and GO) between 2014 to 2018 for App, and from 2017 to 2021 for PmA. All clinical strains were recovered from pigs in growing and finishing phase (75 to 250 days old). The antimicrobial susceptibility to Tildipirosin was determined using the broth microdilution method in accordance with the recommendations by the Clinical and Laboratory Standards Institute (CLSI, 2018). Tildipirosin pure powder was supplied by Merck Sharp & Dhome (Germany).

Results

The antimicrobial susceptibility profile of the samples is shown in **Figure 1**. The Tildipirosin clinical breakpoint for App isolates is 16 µg/mL and the percentage of resistance to this macrolide was only 5 %. On the other hand, 26.7% of PmA strains were resistance to Tildipirosin. More than two-third of resistant isolates (68.7%) were recovered from fattening animals.

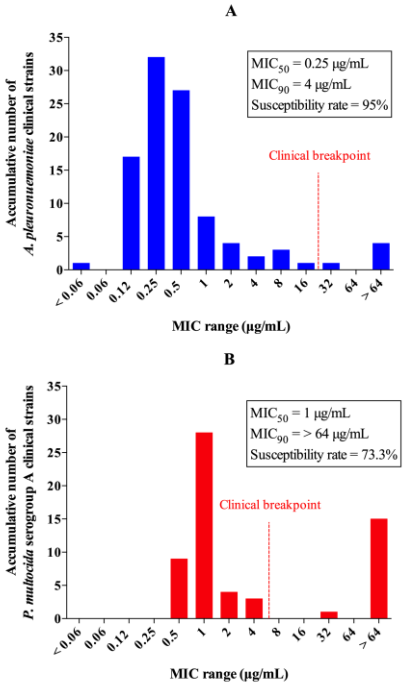

Figure 1. MIC range, MIC_{50}, MIC_{90} and percentage of susceptible App or PmA clinical strains recovered in Brazil to Tildipirosin.

Discussion and Conclusion

Tildipirosin was highly effective *in vitro* against most of the App clinical samples isolated in Brazil and, therefore, it is recommended for treatment of swine pleuropneumonia. MIC procedure must be used occasionally to set an accurate therapeutic dose as well as to monitor the evolution of App resistance to this novel macrolide under field conditions. Because of the high number of PmA strains resistant to Tildipirosin its use requires precaution and constant monitoring.

Acknowledgement

This work was financially supported by MSD Animal Health, AFK Imunotech and CEDISA.

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Belgian pigs "Raised Without Antibiotics"

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Introduction

Antibiotics are used to tackle infectious diseases caused by bacteria. However, there is an association between antibiotic use (ABU) and antibiotic resistance (ABR).(1) Reduction of antibiotic resistant bacterial isolates in pig production can be obtained through coaching, better herd management, improved biosecurity, and prudent or restricted ABU.(2,3) Raised Without Antibiotics (RWA) is a certification mark that is known in only a few countries, and it is unclear what the characteristics of RWA herds are, and which differences there are compared to conventional pig herds. Furthermore, the specific inclusion criteria for RWA production are not well specified in literature; and the implementation of RWA in a larger number of herds with varying management and housing conditions requires further investigation.(4,5)

The objectives of this study were to define the criteria of an RWA programme applicable to the Belgian pig industry; to guide farmers in the RWA programme and to assess if it was possible to achieve and maintain the RWA status for at least one year; and to determine the characteristics of farms that succeeded in the RWA programme.

Materials and Methods

Twenty-eight pig herds were visited three times for this study: 1) collecting data, 2) herd-specific coaching 2 months later, and 3) evaluation 7 months later. ABU was expressed as a treatment incidence namely the BD100 that is used in Belgium.

$$BD100 = \frac{amount\ of\ antibiotics\ administered\ (mg)}{DDDA_{bel} * kg\ animal\ 'at\ risk' * number\ of\ days\ 'at\ risk'} * LA_{bel} * 100$$

The BD100 was calculated for three periods. The first period was 14 months before the first herd visit (period A), the second period was the period between the first and third herd visit (period B), and the third period was the period one year after the last herd visit (period C). Resulting in a monitoring of ABU of 35 months per farm. Criteria for Belgian RWA production were defined, and the status of the herds ((non-)RWA) was verified for the three different periods. Antibiotic use, biosecurity (Biocheck.Gent), and herd characteristics of (non-)RWA herds were compared.

Results

RWA was defined as no antibiotics from birth until slaughter. Pigs requiring an individual treatment received a special ear-tag and were excluded from the RWA programme. The RWA-status of the herds varied over time, and the number of RWA vs. non-RWA herds was 10-18, 13-15, and 12-16, before intervention (period A), after coaching (period B), and after one year (period C), respectively.

There were no statistically significant differences in biosecurity status (Biocheck.Gent), but biosecurity improved significantly in the farms over the course of the study. RWA herds applied less vaccinations. RWA herds were smaller in terms of number of sows (median 200 sows, range 85 – 300) compared to non-RWA herds (median 350 sows, range 180 - 1250). The 4-week system was used significantly more in non-RWA herds, while the 3- and 5-week system were used most often in RWA herds. The weaning age was slightly higher (not significantly) on RWA farms (mean 24.9 days) compared to non-RWA farms (mean 23.9 days).

Conclusions and Discussion

This study showed it was possible for farmers to achieve and maintain the RWA status through herd-specific coaching related to prudent ABU and biosecurity. Characteristics of farms that succeeded were determined, and differences between RWA and non-RWA herds were elucidated. Further research is needed to show the feasibility of RWA on a larger scale, and to verify if there is reduced ABR on RWA farms.

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Comparative study between different formulations containing toltrazuril under field conditions

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Introduction

Cystoisosporiasis is caused by the infection of the parasite *Cystoisospora suis*. It is the most important disease caused by a protozoan in piglets (1). Toltrazuril is the pharmacological basis most used on pig farms to control the disease (2), and there are several commercial products based on this active principle available on the Brazilian market. Although the active principle concentration is usually the same among different products, formulation differences might influence the bioavailability of toltrazuril. Thus, this study aimed to analyze the effect of four formulations based on toltrazuril on pig performance.

Materials and Methods

The study was conducted in a Brazilian commercial pig farm *C. suis* positive. One hundred and fifty pigs aged 3 days old were randomly selected and divided into five groups (T1, T2, T3, T4, and Tc), 30 piglets each. T1 was treated with formulation 1 (Toltrazuril 5% - Baycox®, Elanco Animal Health), T2 with formulation 2 (Toltrazuril 5% - Company A), T3 with formulation 3 (Toltrazuril 5% - Company B), T4 with formulation 4 (Toltrazuril 5% - Company C), and Tc with placebo solution (control group). On the selection day, they were individually weighted and received the treatment (1mL of the product, oral), and they were weaned at 22 days old. Pigs were also weighted at 21 and 63 days old to measure weight gain. Parameters analyzed: average weight (AW) at 21 and 63 days old, average daily gain (ADG) on lactation, nursery, and the total period of the study (lactation + nursery), and pig weight gain relative to Tc at the end of the nursery. We used ANOVA for ADG on lactation and Mann-Whitney U test for AW and ADG on the nursery and total period for data analysis.

Results

AW: there were no differences between groups at any ages (Figure 1).

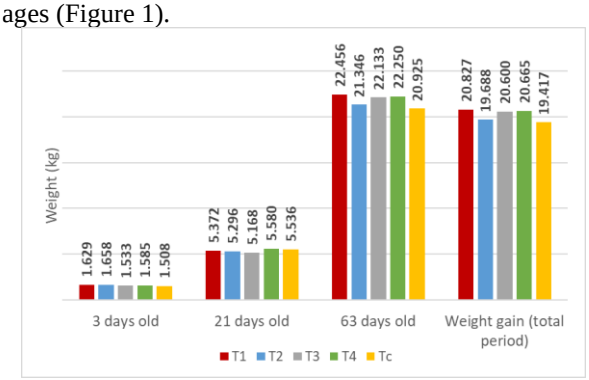

Figure 1. AW of pigs at 3, 21, and 63 days old and weight gain of the total period.

When we analyzed the average weight gain of the total period of the study, T1 gained 1.410 kg more than Tc, followed by T4 (1.248 kg), T3 (1.183 kg), and T2 (0.271 kg). ADG: there were no differences between groups on lactation or total period. The only difference was seen on the nursery - T1 (p=0.018) and T3 (p=0.039) had higher ADG than Tc.

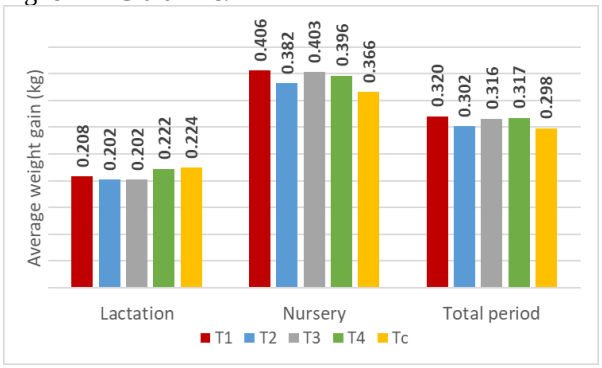

Figure 2. ADG of pigs during lactation, nursery, and the total period.

Discussion and Conclusion

The clinical signs of cystoisosporiasis usually occur up to 15 days old (2, 3). However, this pig farm had a delayed clinical sign, with diarrhea occurring around 20 days old. It led us to conclude that the damage on enterocytes caused by the infection had a major consequence not during lactation but on the nursery, affecting gut absorption and then impairing pig performance after weaning (2, 3). These results show the importance of measuring performance for longer periods. Also, although there were no differences on AW, the results can be extrapolated to a weight gain perspective, e.g., T1 showed the best weight gain relative to Tc, 1.410kg extra per pig. In a pig farm that sells 1000 pigs per week, it represents 1,410kg extra. It corresponds to 7,148 BRL (~1,355 USD) extra per week and 300,216 BRL (~56,947 USD) per year based on Brazilian hog price on February 2022, which was 5.07 BRL (0.96 USD). Thus, the control and treatment of cystoisosporiasis are critical to reduce the economic loss caused by the disease and improve pig farm productivity.

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Does the piglet fecal microbiome vary between individual, composite individual, and pooled DNA samples?: A pilot study

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Introduction

Processing of individual samples during large field-based microbiome studies is often resource-intensive. This study aimed to characterize and compare the piglet fecal microbiome obtained from samples processed using both individual and pooled workflows.

Materials and Methods

A total of 20 litters (N=10 contained piglets ~2 days of age, N=10 contained piglets ~20 days of age) reared in a single commercial swine facility were enrolled in the study. All piglets (N=258) from all litters were sampled individually by inserting a sterile cotton-tipped swab into the rectum. Additionally, 3 composite samples were collected from each litter by swabbing the pen floor with a cotton-tipped swab. Swabs were stored at -80°C and then processed using four different methods: 1) individual samples were processed for DNA extraction ("individual", N=258); 2) raw material from individual samples was pooled (4 pools/litter) and DNA was extracted ("fecal pools", N=80); 3) raw material from individual samples was processed for DNA extraction and then the DNA was pooled (4 pools/litter), ("DNA pools", N=80); and 4) composite floor samples (3/litter, N=60). All libraries (N=478 samples) were sequenced for microbiome analysis using the 16S rRNA V4 region. Amplicon sequence variants (ASVs) were identified using the DADA2 pipeline, and microbiome diversity and composition were compared between the four workflows using linear mixed effect models and permutational multivariate analysis of variance (PERMANOVA).

Results

As a set, the individual samples contained 865 ASVs that were not detected in any of the pooled samples, while the DNA and fecal pools contained 165 and 171 ASVs, respectively, that were not detected in any of the individual samples. However, these ASVs tended to be both low abundance and low prevalence. Alpha and beta diversity were not significantly different between fecal and DNA pools. Microbiome composition of composite floor samples was significantly different from individual samples, as well as fecal and DNA pools (PERMANOVA P= 0.002).

Discussion and Conclusion

Analyzing piglet fecal samples at the individual level provided the most comprehensive profile of the piglet microbiome. However, pooling samples captured most of the variability in individual microbiome composition and diversity, and thus should be considered for population-level piglet microbiome studies. Furthermore, the use of pooled raw feces generates very similar results to the use of pooled DNA and represents a significant cost savings. Finally, pen floor samples do not accurately represent the piglet fecal microbiome profile obtained from samples collected per rectum.

Acknowledgments

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Effect of ozone disinfection on transition pig farms

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Introduction

Biosecurity and cleanliness minimize the risk of introducing and spreading infectious diseases, it can endanger the health of the pigs. Taking care of disinfection, the animals are protected against infectious diseases. (1). On the other hand, the use of ozone in disinfection has been the focus of recent researches. It is rapidly converted to oxygen, making it less polluting and reducing drying wait times for the next batch of animals. (2) Therefore, the objective of this study was to determine whether on-farm ozone disinfection can match or be were effective than traditional disinfection with a disinfectant based on hydrogen peroxide.

Materials and Methods

Two transition pig farms in Castilla y León were used in this study. Two practically identical rooms were selected, "*room A*" and "*room B*". In "*room A*", a cleaning, drying and disinfection protocol was carried out with a conventional disinfectant used on each farm, and samples were taken for microbiological study after cleaning and disinfection. In "*room B*", a conventional cleaning was performed, but after drying, disinfection was performed with ozone, and in the drinkers and feeders sprayed with ozonized water. In the two of the farms, disinfection of feeders, drinkers and toys was performed with ozonated water.

Two ozone pumps and four ozonemeters were installed to quantify the amount of ozone present in a farm. Fans were also used to distribute the ozone evenly throughout the space. Once the desired ozone level was reached (1.5 ppm), the generators were turned off, and when the concentration reached a level that was harmless to humans (less than 0.1 ppm), sampling was performed. RODAC contact plates were used for sampling on regular surfaces (such as floors, walls and heating plates). For samples on irregular surfaces (such as feeders, drinkers, toys), microbiological sampling sponges with neutralizer were used. Samples were collected and processed according to Walia *et al.* (2017). (3)

Results and Discussion

The percentage of efficacy of each test was obtained according to those previously reports (4, 5), in such a way that a total of 6-$\log_{10}$ would correspond to a performance efficacy of 99,9999%; a 5-$\log_{10}$, 99,999%; a 4-$\log_{10}$, to 99,99% and so on.

Table 1. Percentage of efficacy of the conventional disinfection in comparison to ozonation.

Time	Place	UFC/mL	Logarithmic reduction [1]	Percentage of efficacy [2]	Place	UFC/mL	Logarithmic reduction [1]	Percentage of efficacy [2]
1	Feeders	$4{,}68{\cdot}10^{6}$	-	-	Walls	$5{,}25{\cdot}10^{7}$	-	-
2a		$9{,}7{\cdot}10^{4}$	1,684	90%		$3{,}7{\cdot}10^{6}$	2,144	99%
2b		$1{,}05{\cdot}10^{5}$	1,650	90%		$1{,}75{\cdot}10^{5}$	2,475	99%
1	Drinkers	$2{,}2{\cdot}10^{7}$	-	-	Toys	$6{,}6{\cdot}10^{7}$	-	-
2a		$1{,}8{\cdot}10^{5}$	2,086	99%		$8{,}4{\cdot}10^{6}$	0,900	--*
2b		$1{,}95{\cdot}10^{5}$	2,051	99%		$1{,}7{\cdot}10^{5}$	2,596	99%
1	Floor	$2{,}34{\cdot}10^{7}$	-	--	-Heating plates	$4{,}933{\cdot}10^{7}$	-	-
2a		$9{,}25{\cdot}10^{6}$	0,419	--*		$3{,}85{\cdot}10^{6}$	1,108	90%
2b		$5{,}35{\cdot}10^{5}$	1,657	90%		$2{,}76{\cdot}10^{5}$	2,254	99%

Samples were collected in two times: before (time 1) and after convectional disinfection (time 2a) and ozone disinfection. (time 2b). Logarithmic reduction compared to used control, no exposure.[1] *The percentage derived from the logarithmic reduction obtained is not considered sufficiently effective.

Ozone cleaning showed a similar activity a higher activity for regular surfaces that when using a conventional disinfection based on hydrogen peroxide (90% versus inactivity for floors; 99% versus 90% for heating plats) except for walls. (Table 1) for regular surfaces such as the heating plates. There was an improvement when on app this aspect by applying ozonated water to the most of irregular surfaces, ozonized water works better.

Ozonization is a disinfection method increasingly used in other fields, its role in farm disinfection can cause many problems if it is not carried out under optimal conditions. Factors such as a bad cleaning procedure and poor removal of organic matter can stimulated its ineffectiveness. The role of ozonated water is very important in this type of disinfection. The initial economic speending in of ozonation equipment can also be a factor. On the other hand, this good environmentally method of disinfection and the reduction of drying times for the next batch are circumstances to consider.

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High prevalence of antimicrobial resistance among intestinal isolates recovered from commercial pig farms in central Mexico

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Introduction

Antimicrobial resistance (AMR) is a global threat and one of the most important problems for public health (1). The use of antibiotic as a growth promoter during intense animal farming has been linked to the emergence and spread of AMR in bacteria (2). To identify the magnitude of this problem in Mexico, an AMR survey was carried out in commercial pig farms.

Materials and Methods

Samples were collected between September 2020 and January 2021 in six different States from Central Mexico. A total of 11 commercial pig farms were included in the study. At each farm, five fecal swabs were collected, immediately transported to the laboratory, and subjected to conventional microbiological methods targeting isolation of *Salmonella* sp, *Escherichia* sp., and *Pseudomonas* sp. All recovered isolates were subjected to AMR assays using the Disk-diffusion susceptibility testing against 22 different antibiotics at standardized concentrations (3,4). Results of prevalence between different bacterial species were analyzed by using the XLSTAT software.

Results

A total of 55 fecal isolates from healthy animals were recovered, *Escherichia* sp. (n = 44), *Salmonella* sp. (n = 6), and *Pseudomonas* sp. (n = 5). Overall, the study revealed a high prevalence of AMR among these intestinal isolates. Specifically, 50% to 100% of the bacterial isolates were resistant to 17/22 of the antibiotics tested. Only for a small number of antibiotics, Norfloxacin, Gentamicin, Ceftiofur, Ceftriaxone, and Amikacin, AMR was lover than 50%.

Discussion and Conclusion

The results of the present study revealed a high prevalence of AMR in intestinal bacterial isolates recovered from pig farms in central Mexico. These results are comparable with resistance patters observed in other countries; mainly Asian (*e.g.*, 5, 6). Importantly, based on these results, the repertoire of effective antimicrobial drugs available to treat bacterial infections caused by intestinal pathogens is very limited. Taken together, these results highlighted the current AMR emergency arising in commercial pig farms. Moreover, this study provided an initial framework to establish public health polices to reduce this important problem.

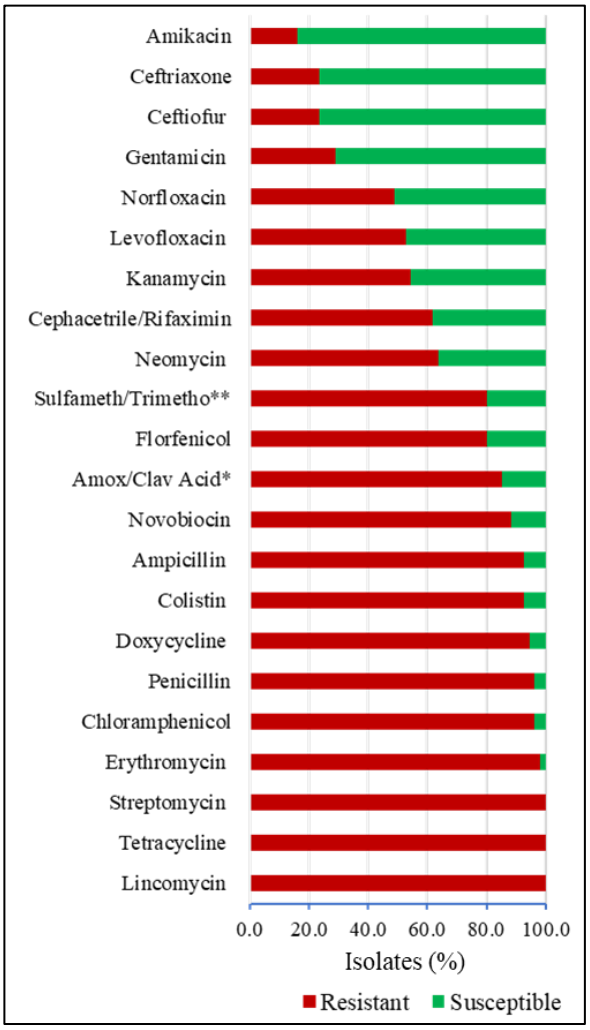

Figure 1. Prevalence of AMR among intestinal isolates recovered from commercial pig farms.

Acknowledgments

We thank managers and staff in commercial pig farms for facilitating the development of the study.

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In vitro antimicrobial activity of benzoic acid over *Brachyspira hyodysenteriae*

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Introduction

The increasingly control of antimicrobials in animal feed around the world as growth promoters has leaded to a run towards new products able to control and inhibit infections of various diseases (1), mainly nutraceuticals such as benzoic acid and essential oils (3). The swine dysentery (SD) is an enteric disease caused by some *Brachyspira* species, mainly *Brachyspira hyodysenteriae*, characterized by a mucohaemorragic diarrhea (2). The aim of this study was to evaluate the *in vitro* efficacy of Benzoic acid, 99.9% pure (VevoVitall®, DSM Nutritional Products), a commercial feed grade benzoic acid, against *B. hyodysenteriae*.

Materials and Methods

Nine Brazilians strains of *B. hyodysenteriae* isolated from clinical cases of swine dysentery from 2013 to 2019, and one reference strain were selected for this test. The strains were cultivated in Triptone Soy Agar (TSA) supplemented with 5% equine or goat blood, under anaerobic conditions for four days.

For evaluation of minimum inhibitory concentration (MIC), the cultivated strains were diluted on Brain Heart Infusion (BHI) supplemented with 10% fetal bovine serum at McFarland scale of 0.5. Benzoic acid was diluted in methanol at 250000 µg/ml and filtered at 0.22µm for sterilization. A serial dilution was performed, ranging from 10000 µg/ml to 4.88 µg/ml, including a methanol control and growth control, on 96 well plates and cultivated under agitation and anaerobic conditions for four days.

Results

MIC values are shown in table 1. Nine out of ten strains showed MIC values of 2500 µg/ml and a single strain had a MIC value of 1250 µg/ml. No influence was observed for methanol over the *B. hyodysenteriae* growth.

Discussion and Conclusion

Our results show *in vitro* antimicrobial activity efficacy of benzoic acid against *B. hyodysenteriae* is minimum of 2500 ppm.

There are few studies evaluating the antimicrobial effect of benzoic acid over *B. hyodysenteriae* (4), and the use of other methodologies make difficult the comparison of results. However, the present results are promising and a stimulus for future *in vivo* evaluations of antimicrobial activity of benzoic acid against *B. hyodysenteriae* in pigs.

Table 1. Minimum inhibitory concentration (MIC), in µg/ml, of benzoic acid for *B. hyodysenteriae*

STRAIN	Benzoic acid
B204	2.500
365/12	2.500
HK240	2.500
F28/19 – TP3	2.500
493/13	2.500
GB2	2.500
415/15	2.500
F26/18-1 (6)	2.500
F28/19-3650w	1.250
CP2	2.500

The antimicrobial activity of organic acids, such as benzoic acid, is associated with an acidification of gastrointestinal tract and, more directly, the diffusion through bacterial cell wall, lowering the cytoplasmatic pH leading to an activation of a Na^+/K^+ pump, depleting bacterial energy reserve, leading to bacterial death. Another consequence of cytoplasmatic acidification is denaturation of internal proteins, including bacterial DNA, compromising bacterial survival and replication (5). In addition of the direct effects over bacterial growth and gastrointestinal acidification, the modulation of microbial communities along the gastrointestinal tract can inhibit proliferation of pathogenic bacteria (6), such as *B. hyodysenteriae* and improve overall health.

These results improve the knowledge about the antimicrobial activity of nutraceuticals over *B. hyodysenteriae*. Future *in vivo* studies are necessary to confirm these *in vitro* results.

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DSM Nutritional Products

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Intramuscular vaccination against *Lawsonia intracellularis* as a tool to reduce antimicrobial consumption – a case study

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Introduction

Proliferative enteropathy in pigs, also known as ileitis, is caused by *Lawsonia intracelullaris*. This disease may lead to high antimicrobial use in affected herds (1), having an economic impact. Control of ileitis can be done by antimicrobials, vaccination and optimization of biosecurity and management practices. The aim of this case study was to reduce antimicrobial use by intramuscular vaccination against *L. intracellularis* in a farm with a history of acute ileitis, without an impact on ileitis-associated mortality.

Material and Methods

This case study was performed in a British indoor farrow-to-finish farm with a history of acute ileitis, which was historically controlled by medication with tylosin. A total of 74 batches were investigated between July 2020 and November 2021, allocated to three different control strategies: antibiotic treatment with tylosin for 7 days (T; 18 batches; Jul-Oct2020), antibiotic treatment with tylosin and vaccination with Porcilis®Lawsonia at weaning (T&V; 26 batches; Nov2020-Apr2021), and vaccination alone at weaning (V; 30 batches; May-Nov2021). Mortality was recorded at batch level. Antimicrobial Consumption (AMC; mg/kg) was registered using the eMB-pig system from UK. Average Daily Gain (ADG), Average Live Weight at slaughter (LW), and Days to reach slaughter weight (DtS) were recorded at batch level. The intervention cost was calculated per pig sold and per ton of pork sold. Data was statistically analyzed by SPSS software, using Chi-square test for mortality and ANOVA for ADG, LW and DtS. The batch was used as experimental unit.

Results

The mortality was significantly lower in the vaccinated group (0.57%) when compared with T (0.90%) and T&V (0.81%) groups (P<0.05) (Table 1). AMC was reduced gradually (T: 64.5mg/kg; T&V: 19.3-62.3mg/kg; V: 18.3-19.7mg/kg), as no clinical signs, nor ileitis-associated deaths were detected after vaccination. ADG was numerically (P=0.078) improved in the vaccinated groups (T&V: 885g/p/d; V: 845g/p/d) compared to treated pigs (T: 785g/p/d). Piglets treated and vaccinated were heavier at slaughter (116.4kg) when compared with T (107.9kg) and V (107.3kg) groups (P>0.05). Pig vaccinated reached slaughter weight slightly faster (V: 164.3d) when compared with T (165.7d) and T&V (166.8d) (P>0.05). The intervention cost was lower in vaccinated pigs when compared to treated pigs (Table 1).

Table 1. Overall results in pigs Treated (T), Treated & Vaccinated (T&V), and only Vaccinated (V).

	T	T&V	V	P-value
Mortality *(%)*	0.90^{B}	0.81^{B}	0.57^{A}	P=0.020
AMC *(mg/kg)*	64.5	19.3-62.3	18.3-19.7	N.A.
ADG *(g/p/d)*	785	885	845	P=0.078
LW *(kg)*	107.9	116.4	107.3	P>0.05
DtS *(days)*	165.7	166.8	164.3	P>0.05
Intervention cost				
£/pig	1.84	1.30-1.81	1.27-1.29	N.A.
£/ton pork	1.69	1.07-1.67	1.19-1.67	N.A.

^{A,B} Different superscripts between values from the same row represent statistical differences between groups. N.A. Not applicable

Conclusions and Discussion

Gradual removal of tylosin and replacement by intramuscular vaccination against *L. intracellularis* was demonstrated to be a successful strategy to control ileitis under the conditions of this specific herd. Despite the low level of ileitis-associated mortality seen in all three strategies, vaccination reduced mortality, even after major removal of macrolides. The combined strategy of Treatment & Vaccination provided the best growth performance. However, vaccination alone was demonstrated to be the most economic valuable strategy, being able to reduce the intervention cost per pig and per ton of pork sold, when compared to antimicrobial treatment.

Acknowledgments

Not applicable.

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Molecular characterization of LA-MRSA ST398 isolated of pigs and farm worker in the Rio de Janeiro state, Brazil

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Introduction

Antimicrobial agent use in livestock is a common practice to keep the animal health and to reduce production losses. However, multidrug-resistant bacterial strains are selected and spread in animals, humans, and environment (1). Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) is a pathogen isolated mainly from pigs and farm workers (2, 3). Although Brazil is one of the leaders in pork production and exportation (4), studies on the prevalence and characterization of MRSA in pigs and humans exposed to these animals are still scarce (5, 6). Therefore, we investigated the occurrence of LA-MRSA and transmission potential between pigs and workers in farms located in the Rio de Janeiro state.

Materials and Methods

Nasal swabs were collected from 250 pigs (16 farms) and 29 employees (9 farms) from pig farms located in the Rio de Janeiro state between 2014 and 2019. After bacterial identification by MALDI-TOF, isolates were subjected to the disk-diffusion method to 11 different antimicrobial agents. MRSA strains were detected by cefoxitin disk and PCR for *mecA* gene. Then, whole genome sequencing was performed for MRSA strains.

Results

Three pigs (1.2%) and one farm worker (3.5%) were MRSA nasal carriers. Of the *S. aureus* carriers, 20% (3/15) and 14.3% (1/7) of animals and humans, respectively, carried MRSA. All MRSA strains exhibited resistance to ciprofloxacin, clindamycin, erythromycin, penicillin G and tetracycline. One strain was resistant to sulfamethoxazole-trimetoprim. Methicillin resistance was not observed by cefoxitin disk in two *mec*A-positive strains. These two strains, recovered from one pig and one human in the same farm, had the same genotypic resistance profile. All strains were ST398-SCC*mec* V-t011 and carried resistance genes to several antimicrobial classes (Tab. 1). Two multidrug-resistant LA-MSSA ST398 were also recovered from one pig and one human.

Table 1. Resistance genes detected in LA-MRSA isolated from two pigs and one farm worker in the Rio de Janeiro state.

Isolate	Farm	Host	Resistance gene
SN51	E	Pig	*bla*Z[1], *mec*A[1], *erm*(C)[2], *lsa*(E)[2], *tet*(K)[3], *tet*(M)[3],*gyr*A[4], *fex*A[5], *dfr*G[6]
SN182	N	Pig	*bla*Z, *mec*A, *erm*(C), *lsa*(E), *tet*(K), *tet*(M), *grl*A[4], *fex*A, *dfr*G
HSN18	N	Human	*bla*Z, *mec*A, *erm*(C), *lsa*(E), *tet*(K), *tet*(M), *grl*A, *fex*A, *dfr*G

Resistance genes to beta-lactams[1], macrolides, lincosamides and streptogramins B[2], tetracyclines[3], fluoroquinolones[4], phenicols[5] e trimethoprim[6].

Discussion and conclusion

LA-MRSA ST398 nasal carriers were detected in both pigs and worker in farms located in the Rio de Janeiro state. The strains were resistant to several antimicrobial agents and shared most of the resistance genes. In one of the farms, strains isolated from pig and human exhibited identical phenotypic and genotypic characteristics, suggesting a zoonotic transmission. Molecular methods should be preferred whenever possible to identify MRSA, as the cefoxitin disk may not be effective as observed in our study. Therefore, more studies are necessary to know the LA-MRSA distribution in our country and the spread extension between pigs and farm workers.

Acknowledgments

CAPES, FAPERJ, PROPPI/UFF

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Preliminary in vitro activity of natural substances against bacterial pathogens in boar semen

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Introduction

The global impact of livestock industrialization has contributed significantly to one of most important human and animal health problem currently being fought: antimicrobial resistance. Immediate action must be taken to reduce the indiscriminate use of antibiotics and implement an integrated system that promotes a socially, economically, and ecologically production system(1). A semen extender plays an essential role in swine reproduction by reducing the bacterial load of sperm used for artificial insemination(2). Semen extenders typically contain antibiotic cocktails in their formulations. Unfortunately, antibiotic resistance created by the misuse of antibiotics in the human and veterinarian medicine setting has resulted in a loss of bacterial suppression efficacy(3). This study aimed to determine the effects of two substances of natural origin and protein character included in a formulation used to dilute boar ejaculate on sperm quality and the effects on lowering the initial bacterial load.

Materials and Methods

Tests were carried out in parallel with the semen of two 18-month-old Duroc males (A and B) to analyze the effect of two substances on cell vitality and antimicrobial activity coded as EL1 and EP1 in a short-term base formulation. The experimental design was based on two factors (substances) with three concentration levels (-1, 0, and +1). In addition, the differences between the two males were studied in terms of a decrease in bacterial load and sperm motility parameters. Sperm motility was followed daily for three days by computer-assisted sperm analysis (CASA). Aliquots of extended semen were used and analyzed for motility, progressive motility, acrosome, and microbiological CFU counts.

Results

The results obtained showed no differences in sperm quality among the males in the study. A negative correlation was observed between progressive motility and total bacterial counts at 0 h. The samples observed from both animals showed good quality in terms of motility (93%± 1.5) and progressive motility (86% ± 1.2) in the substance concentration groups of the experimental design.

Discussion and Conclusion

The groups with high concentration levels (+1) of the two substances showed lower mean total motility than the control positive control extender with antibiotic ($P < 0.001$) and negative control extender without antibiotic ($P < 0.05$). Based on these data, it was impossible to select a formulation with significant results showig higher performance based on motility results alone.

Further investigation was carried out by studying the effect of the concentrations of the formulations of the experimental design on the acrosome status. The results showed significant differences in acrosome integrity between the concentrations of the substances studied and the controls ($P < 0.001$). The worst performing formulations were those containing only EP1(+1) at high concentration, the formulation containing EP1(-1) at a lower concentration, and the mixture of EP1 (+1) and EL1 (+1) at higher concentration.

Sperm diluted with the EL1(+1)_EP1(0) encoded formulation showed a higher percentage of cells with intact acrosome (98%). On the other hand, the EL1(+1)_EP1(+1) and EL1(-1)_EP1(+1) groups provided a significant reduction in contamination. However, based on the data collected, the formulation that showed the best motility, viability, and decreased bacterial seed load was the experimental group EP1(0) _ EL1(+1).

A short-term semen extender formulation has been developed with an optimized combination of two substances of natural origin and protein character, which can be used as a replacement for antibiotics. The results showed that this formulation is highly biocompatible effective in reducing the biological load of males with an initial load not exceeding 10000 CFU/ml. It can be stated that the evidence provides a solid scientific basis for the development of an innovative antibiotic-free extender for swine artificial insemination.

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Real time sound based monitoring of Respiratory Health Status (ReHS) significantly reduces the overall antibiotic consumption in nursery facilities

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Introduction

Health monitoring systems are essential for detecting changes in disease status in a timely and effective manner (1). Of especial importance are those related to respiratory diseases of swine since we are dealing with very large populations and fast disease transmission processes. As a result, improving early detection of disease onset can have a tremendous impact on animal productivity as well as welfare (2). SoundTalks® (ST) is a cloud-based sensor technology that monitors 24/7 the sound emitted from pigs. Based on artificial intelligence, this technology processes the sound data collected at the farm and transforms it into a metric (ranging between 0 and 100) that represents the animals' respiratory health status (ReHS) as green (healthy, ReHSh), yellow (increased warning) and red status (immediate action) indicating respiratory problem (ReHSp). Despite the applicability of this technology, further research is needed to understand its functionality in a commercial farm regarding the early intervention in the face of an outbreak and specifically related to the total quantity of treatments. Therefore, the objectives of this study were to describe respiratory disease impact in a nursery facility monitored by ST and to evaluate the overall treatment consumption compared to the period prior to ST

Materials and Methods

The study was performed in a nursery facility as part of a 1300 sow farm in Northern Denmark (8 weaning rooms/site, 900 head spaces/room filled weekly with piglets of 4 weeks of age). All rooms were monitored for respiratory problems via sound by ST. For the purpose of the study, production variables such us batch number, mortality, as well as number and type of treatments (antibiotic vs antipyretic) were daily recorded and requested for the previous 12 months. Other health variables (i.e. ST daily average ReHS value) were recorded by ST and consolidated for the analysis. A Chi-square (non-parametric) test was used to study whether or not there was a statistically significant difference between the observed and the expected number of treatments before and after the monitoring period with ST.

Results

During the 6 months of the study period, between 2 and 3 batches per room were monitored by ST. Concurrent to the beginning of the sound monitoring the farm experienced an outbreak of PRRS. Results showed that, in all rooms, 9.9% (max=4.5%; min=19.4%) and 5.5% (max=6.7%; min=2.7%) of days the farm had respiratory alarms, as indicated by yellow or red warning signals, respectively. There was an evident room effect as the percentage of days with green, yellow or red ReHS significantly differed between rooms. Production performance of a total of 69, and 20 batches before and after ST monitoring were analyzed for the study. Fig 1 represent the total and percentage of individual treatments (antibiotic vs antipyretic) applied during the study period when Influenza (IAV) or other respiratory pathogens were diagnosed before and after T monitoring.

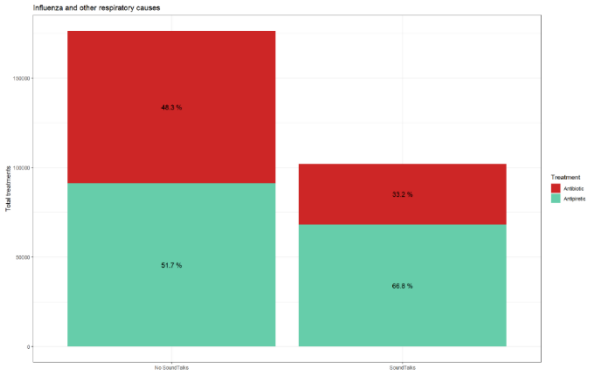

Fig 1. Number of total individual treatments (antibiotic vs antipyretic), as well as percentages, applied during the study period when Influenza (IAV) or other respiratory pathogens were diagnosed before (69 batches) and after (20 batches) the monitoring by SoundTalks®.

Results from the study shows that in these nursery rooms, the producer did not change the overall treatment consumption during IAV outbreaks after the implementation of ST monitoring, however there was a 38% antibiotic reduction and a 395% antipyretic increase when other respiratory pathogens were identified (p-value < 2.2e-16 Pearson's Chi-squared)

Conclusions and Discussion

Results from this study demonstrated the importance of continuous monitoring of nursery population to ensure the early detection/intervene in the face of outbreak. Under the conditions of this study, the implementation of SoundTalks® lead to a 38% reduction in the use of antibiotics with the exception of cases involving IAV as the primary pathogen. Despite the importance of these results, additional studies are needed to confirm these results under different health status and conditions.

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BACTERIAL DISEASES

A comparison between commercial *Mycoplasma hyopneumoniae* vaccines in swine in South Africa

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Introduction

Mycoplasma hyopneumoniae (*M hyopneumoniae*) is the etiologic agent of enzootic pneumonia(1). This agent causes a chronic, clinically mild infectious pneumonia of pigs characterized by a persistent dry cough with increased incidence of cranioventral pneumonia at slaughter. Postmortem lung lesion scoring systems are used to assess pneumonia associated with *M. hyopneumoniae* infection(3). Intradermal vaccination for *M. hyopneumoniae* using an intradermal device and a specially formulated intradermal vaccine were recently introduced inSouth Africa, where *M. hyopneumoniae* is endemic.

Materials and Methods

Four weekly batches of pigs were included in the study. Each batch of pigs was divided into 3 groups and each group was vaccinated with one of 3 commercial vaccines per the usual vaccination schedule of the farm. One group per batch receivedthe same vaccine that had been used on the farm prior to the trial via the intramuscular route (M+PAC); one group per batch received the intradermal vaccine using an intradermal device (Porcilis M Hyo ID Once)and one group per batch received a competitor vaccine via the intramuscular route. Lung lesion scoring was carried out in the abattoir and evaluated according to the Goodwin scoring system(4). Lung scores were compared using the Kruskal Wallis test.

Results

Results are presented in the order of groups (Porcilis M Hyo ID Once, Competitor Vaccine, M+PAC (Two injections). Statistical differences were observed in average lung scores at slaughter for week 1 (1.69 vs 2.45 vs 0.54, Kruskal Wallis P value = 0.031). No statistical differences were observed for week 2 (0.4 vs 0.37 vs 0.11), week 3 (0.05 vs 0 vs 0.02) or week 4 (1.73 vs 1.03 vs 1.47).

Conclusions

Selection of *M. hyopneumoniae* vaccines should be based on a combination of vaccine efficacy and labour. Two dose regimes have been documented to give greater protection but cause increased handling stress on the pigs(1). Farms should thus select the correct regime based on their *M. hyopneumoniae* situation and labour costs. In this farm, Porcilis M Hyo ID Once showed superior lung lesion reduction compared to a competitor vaccine (Respisure One). Equivalent performance was observed in Week 2, Week 3 and Week 4. Porcilis M Hyo ID Once may thus be a labour-saving tool delivering equivalent efficacy compared to some two dose competitor vaccines. In such a situation, there are also additional benefits compared to intramuscular vaccination, such as reduced stress on piglets(4) and the elimination of needles and the risk of needle breakage.

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A survey of Asian respiratory health in finishing pigs at slaughter age

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Introduction

Lung scoring at the slaughterhouse is a valuable tool for assessment of the respiratory health status of a large number of animals at a single visit, at relatively limited cost. Different from post-mortem investigation, it assesses lung health across the whole batch of animals. Moreover, a clear relation between lung lesions present at slaughterhouse and economic impact of respiratory disease has been reported (1), making lung scoring an attractive tool for decision making and effect monitoring of veterinary interventions.

To facilitate efficient and hygienic lung lesion scoring at slaughterhouses, Ceva provides a scoring methodology Ceva Lung program (CLP). A tablet-based software tool allowing for rapid recording of the results and their processing is a part of CLP.

Materials and Methods

In between January 2020 and December 2021, a total of 5960 batches of pigs (243,319 animals) were scored at time of slaughter, using CLP. Lung scorings were performed in Cambodia (5), China (170), Indonesia (27), Malaysia (18), Philippines (280), South Korea (189), Taiwan (60), Thailand (3902) and Vietnam (1310). Lungs were scored following the CLP method [2], with presence, type and extension of lung lesions described by:

- Enzootic pneumonia (EP)-like lesions following a modified Madec methodology.
- Cranio-ventral pleurisy, to describe EP-associated secondary pleurisy.
- Scarring, describing prevalence of fissures associated with older EP-like lesions.
- Dorsocaudal pleurisy, to describe *Actinobacillus pleuropneumoniae* (APP)-like lesions
- Actinobacillus pleuropneumoniae Index (APPI), using prevalence and grade of dorsocaudal pleurisy (scale 0-4).

Results

Results for the Asian region are presented in Table 1 and 2 using percentiles (P_{25} -median - P_{75}).

Table 1. EP-like lesions

	P_{25}	Median	P_{75}
Prevalence bronchopneumonia	39.1%	60%	80%
% lung surface with bronchopneumonia	2.2%	4.3%	7.8%
% Cranio-ventral pleurisy	0%	4%	13.3%
Scars	3.3%	18%	47.5%

Table 2. APP-like lesions

	P_{25}	Median	P_{75}
% Dorsocaudal pleurisy	2%	6.6%	15%
APPI index	0.05	0.19	0.41

Conclusions and Discussion

Results clearly indicate there is room for improving the respiratory health of finishing pigs in the sampled countries. Both lesions associated with *M.hyopneumoniae* and *A.pleuropneumoniae* have a high prevalence. While the data by country (not shown) suggests some differences between countries exist, these have to be interpreted with caution as farm selection was not randomized. Nevertheless, the distribution reported above could be a useful tool for interpretation of lung lesion scoring results, as well as for setting targets for farms aiming for improvement of their respiratory health.

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Active *Lawsonia intracellularis* Surveillance on Vaccinated and Non-vaccinated Canadian Farms

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Introduction

The extent to which *Lawsonia intracellularis* remains a common enteric pathogen on farms despite vaccine and antimicrobial control options has not been recently examined on Canadian farms (1). This study used fecal and oral fluid samples to examine the prevalence of *Lawsonia intracellularis* on finisher farms using different preventive and control strategies.

Materials and Methods

Forty Canadian finisher farms were enrolled in a prospective observational study from May to October, 2021. Inclusion criteria included ileitis vaccination status and willingness to allow on-farm surveillance and answer a health status survey.

Twenty-two farms used a commercial inactivated parenteral ileitis vaccine and 18 farms did not vaccinate for ileitis.Three pens/farm were selected with fixed spatial sampling and sampled at mid-finishing (15 to 17 woa) and late-finishing (20 to 22 woa). One oral fluid sample and one 5:1 pooled fecal sample were collected per pen for quantitative PCR analyses (Biovet Inc. Saint-Hyacinthe QC, Canada). Herd veterinarians completed a health survey for each farm.

Lawsonia prevalence between ileitis vaccinated and non-vaccinated was compared using a two proportion Z-test (Minitab 10.1.1 State College, PA. USA).

Results

At the farm level, 45% and 59% were positive to *Lawsonia intracellularis* by fecal and oral fluid samples respectively, as shown in Table 1. A total of 240 fecal samples (120 mid, 120 late-finisher) and 211 oral fluid (OF) samples (91 mid, 120 late-finisher) were analyzed.

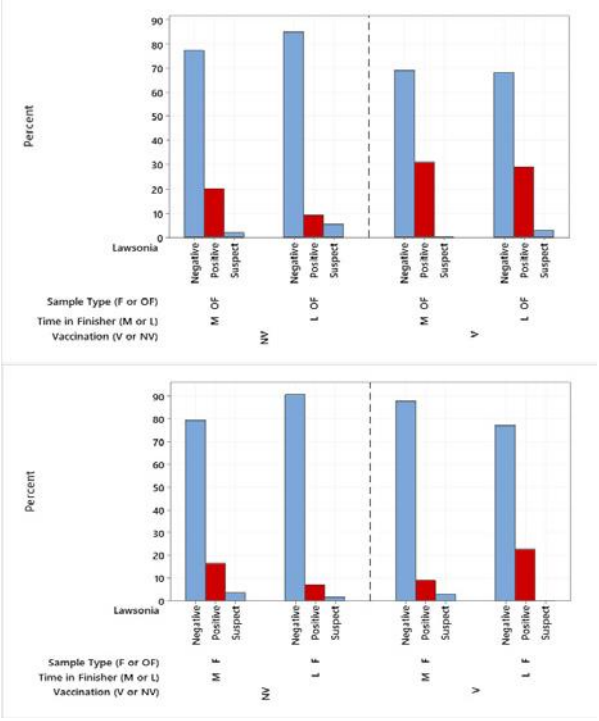
At the sample level, in **non-vaccinated herds**, 20% of fecal, 29% of OF **mid-finisher samples**, and 9.3%-fecal, 15%-OF **late-finisher samples** were Lawsonia-positive. The median Ct-values for positive samples were: **mid-finisher** 29.6 (feces), 29.1 (OF), and **late-finisher** 34.5 (feces), 33.2 (OF). Water/feed antimicrobial use was reported on 40% of non-vaccinated and 60% of vaccinated farms. Seven non-vaccinated farms reported finisher-diarrhea; 3 tested Lawsonia-positive. One vaccinated farm reported finisher-diarrhea and tested Lawsonia-positive. In **vaccinated herds**, 12% of fecal, 31% of OF **mid- finisher samples**, and 23% of fecal, 32% of OF **late-finisher samples** were Lawsonia-positive. The median Ct-values for positive samples were: **mid-finisher** 30.3 (feces), 30.2 (OF), and **late-finisher** 28.4 (feces), 28.2 (OF).

Within herds of the same vaccination status, there were no differences in fecal vs OF sample detection at mid or late-finishing. However, detection levels were different for **non-vaccinated vs vaccinated** herds in **late-finishing**: feces (9.3% vs 23%, P=0.054), OF (15% vs 32%, P=0.034), respectively.

Table 1. Farm level *Lawsonia intracellularis* prevalence by sample type and vaccination status

	Oral Fluid Samples	Fecal Samples
Non-Vaccinated Farms	28% (5/18)	28% (5/18)
Vaccinated Farms	59% (13/22)	45% (10/22)

Figure 1. *Lawsonia intracelullaris* prevalence by sample type, time in finisher and vaccination status



Discussion and Conclusion

OF are suitable for Lawsonia surveillance and a practical tool to assess control measures. Vaccinated farms had higher Lawsonia prevalence in late-finishing despite greater enteric-antimicrobial use than non-vaccinated farms. Vaccinated farms may have higher Lawsonia infection-pressure leading to both vaccination and increased antimicrobial use. Additionally, vaccine duration of immunity may be insufficient to control shedding in late-finishing. Further research of Lawsonia dynamics in **late-finishing** is recommended.

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An attempt to assess the usefulness of direct *Yersinia enterocolitica* DNA isolation from rectal swabs taken from fattening pigs

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Introduction

Pigs are the main reservoir of *Yersinia (Y.) enterocolitica*, and not sufficiently thermally processed pork is the main source of human infection (1). Species *Y. enterocolitica* has been divided into six biotypes (1A, 1B, 2, 3, 4, 5) and more than 70 serotypes (2). The most important virulence markers of *Y. enterocolitica* are genes: *ail*, which encodes the production of attachment-invasion locus (Ail) adhesin; *yst*, which encodes the production of enterotoxin Yst (*Yersinia* stable toxin) and *yadA*, which encodes the production of *Yersinia* adhesin A (YadA) (3). Rapid identification is one of the key requirements for the proper diagnosis and effective, targeted treatment. The aim of the study was to compare *Y. enterocolitica* DNA isolation directly from swabs with isolation after warm and cold enrichment.

Materials and Methods

One hundred and fifty rectal swabs were collected from 50 fattening pigs. Three swabs were taken from each animal. The first swab was pre-enriched in 9 ml of the ITC (irgasan, ticarcillin and potassium chlorate) medium (warm enrichment, 25°C, 48 h). The second swab was pre-enriched in 9 ml of the PSB (peptone, sorbitol and bile salts) medium (cold enrichment, 4°C, 3 weeks). Further analytical procedures were identical for both warm and cold enrichment were performed according to the methods described previously (4). The third swab was not pre-enriched, and DNA was isolated directly.

Genomic DNA was isolated using a Genomic Mini kit (A&A Biotechnology) according to the manufacturer's instructions. Triplex PCR involved the amplification of *ail* (365 bp), *ystA* (134 bp) and *ystB* (180 bp) gene fragments, and single PCR involved the amplification of *yadA* (849 bp) gene fragment. The applied reactions conditions included a preliminary denaturation step (95°C, 5 min), followed by 30 cycles of denaturation (94°C, 30 s), primers annealing (45°C, 30 s in triplex PCR; 60°C, 30 s in single PCR) and elongation (72°C, 1 min). The final chain synthesis was carried out at 72°C for 10 min. Randomly chosen amplicons were prepared for sequencing with the Clean-up kit (A&A Biotechnology) according to the manufacturer's procedure. The amplicons were sequenced by Genomed (Warsaw, Poland) to confirm reaction specificity.

Results

Forty-one *Y. enterocolitica* strains were isolated after warm enrichment, while 43 strains were isolated after cold enrichment. In 39 animals, *Y. enterocolitica* strains were isolated after both warm and cold enrichments. In 2 cases, *Y. enterocolitica* was isolated only after warm enrichment, and in 4 cases the pathogen was isolated only after cold enrichment. The molecular analyses of 84 *Y. enterocolitica* strains isolated during bacteriological examinations revealed that all strains harboured *ail*, *ystA* and *yadA* genes fragments. Only 9 samples where DNA was isolated directly from swabs were positive and harboured *ail*, *ystA* and *yadA* genes fragments. In all 9 cases, *Y. enterocolitica* strains were also isolated after both warm and cold enrichment, which suggests that *Y. enterocolitica* DNA was isolated from 9 animals with the use of three different methods. The results of molecular analyses are presented in detail in Table 1.

Table 1. Detection of *ail*, *ystA* and *yadA* gene fragments of *Y. enterocolitica*

DNA ISOLATION			
Direct	After warm enrichment	After cold enrichment	Positive samples (%)
+	+	+	9 (18)
-	+	+	30 (60)
-	+	-	2 (4)
-	-	+	4 (8)
-	-	-	5 (10)

Discussion and Conclusion

Bacteriological methods for the identification of *Y. enterocolitica* are laborious and time-consuming, especially when cold enrichment is conducted. PCR assays designed for the detection of *Y. enterocolitica* are rapid, highly specific and sensitive (5). This study demonstrated that *Y. enterocolitica* DNA can be isolated directly from swabs, but the isolation process is less efficient. This observation is in line with the study of Petsios et al. (5), who conclude that molecular methods should be combined with conventional culture-based techniques because isolation is a crucial step for complete characterization and subtyping.

Acknowledgments

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An experimental aerosol infection model for *Mycoplasma hyopneumoniae* in pigs

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Introduction

Mycoplasma hyopneumoniae (*M. hyopneumoniae*) isthe causative agent of enzootic pneumonia in pigs (1). The respiratory disease caused by *M. hyopneumoniae* affects swine production systems worldwide. *Mycoplasma hyopneumoniae* infections are usually associated with important financial losses to swine producers (2). Pig infection models provide excellent opportunities to study enzootic pneumonia, which has been experimentally reproduced by means of different inoculation methods (3). Aerosol is the least studied method described for experimental infections, although it mimics the natural route of *M. hyopneumoniae* infection. Therefore, the purpose of the present study was to develop and characterize an aerosol model to reproduce the infection and respiratory disease caused by *M. hyopneumoniae*.

Materials and methods

Twelve 6-week-old gilts were obtained from a *M. hyopneumoniae* and porcine reproductive and respiratory syndrome virus negative source and were housed at the Veterinary Isolation Facilities of the University of Minnesota, St. Paul, MN, USA. Gilts were randomly allocated to four experimental groups and individually exposed to *M. hyopneumoniae* infectious aerosols (Table 1). Aerosol exposure was performed using an isolator chamber and a combination of different doses, volumes, and times of exposure.

Table 1. Experimental groups.

Group	n	Titer	Volume	Exposure time
1	3	10^5 CCU/mL	10 mL	15-20 min/day
2	3	10^5 CCU/mL	20 mL	30-35 min/day
3	3	10^6 CCU/mL	10 mL	15-20 min/day
4	3	10^6 CCU/mL	10 mL	30-35 min/day

CCU= Color changing units.

All gilts were exposed to *M. hyopneumoniae* on two consecutive days. Starting at 7 days post exposure (dpe) and until the end of the study (28 dpe), cough was monitored daily, as previously described (4). Blood was collected at 0 and 28 dpe, whereas nasal (NS), laryngeal (LS) and deep tracheal secretions (DTS) were collected from each gilt at 0, 7, 14, 21 and 28 dpe. At necropsy, macroscopic lung lesions were scored, as previously described (5), and bronchial secretions (BS) and broncho alveolar lavage fluid (BALF) were obtained from each gilt. Seroconversion to *M. hyopneumoniae* was assessed by means of an indirect ELISA assay (IDEXX Laboratories, Inc.). Nasal, laryngeal, deep tracheal and bronchial secretions were tested for *M. hyopneumoniae* by qPCR, as described by Vilalta *et al.* (6). The local humoral immune response against *M. hyopneumoniae* (IgG and IgA) was evaluated in NS, DTS and BALF by modifying the abovementioned ELISA kit. All data collected was tabulated per gilt, treatment group and time point of examination.

Results

All gilts became infected with *M. hyopneumoniae* at a similar time post-exposure, irrespective of the different exposure characteristics. Infection with *M. hyopneumoniae* was confirmed by bacterial detection employing a qPCR from 7 dpe onwards. All BS collected at necropsy were positive for *M. hyopneumoniae.* Coughing was observed in experimental groups 2, 3 and 4, with onsets at 18, 12 and 10 dpe, respectively. Most gilts exhibited macroscopic lung lesions suggestive of mycoplasmal pneumonia at different extents. Circulating antibody responses to *M. hyopneumoniae* were detected only in gilts from experimental group 3. However, IgA and IgG against *M. hyopneumoniae* could be detected in NS, DTS, and BALF at different dpe in all experimental groups.

Discussion and conclusions

This preliminary study described the kinetics of infection and humoral immune response following an aerosol inoculation with *M. hyopneumoniae* in gilts, and further supports the utility of an aerosol model to replicate the mycoplasma-associated pneumonia. While only the exposure of gilts to high-dose aerosols consistently reproduced typical clinical signs and severe lung lesions, all gilts became infected at a similar time and developed similar mucosal and humoral immune responses.

Under the conditions of this investigation, the aerosol infection model was efficient in reproducing the disease caused by *M. hyopneumoniae* in gilts. The characterization of an aerosol infectious model may open a new range of possibilities to improve the study and understanding of this important swine bacterium.

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Antibiotic reduction in a farm with *Streptococcus Suis* diagnosis by vaccinating against VT2E

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Introduction

The present farm had a clinical history of *Streptococcus suis*, diagnosed by clinical signs (meningitis), necropsy and laboratory culture. This situation was managed with prophylactic antimicrobial therapy (amoxicillin) as a result of an antibiogram. Moreover, by using FTA-cards also the presence of VT2e ETEC was confirmed.
It has been widely proposed that co-infection with other pathogens can influence the severity of *S. suis*-associated diseases (1). Hence, in a context of the increasing global pressure to reduce antimicrobials due to drug resistance (2), the objective of this trial was to assess if antibiotics could be reduced on the farm by applying VT2e vaccination.

Materials and Methods

The piglets were divided into two groups: VEP (n=325, vaccinated with VEPURED® at 2 days of age) and AB (n=361, treated with 350mg/kg amoxicillin in feed after weaning). During the nursery period, mortality, antimicrobial treatments, average daily gain (ADG), and FCR were recorded. Weighing per pen was done at weaning, 32 days later, and at the end of nursery. An ANOVA was performed, with a posthoc Tukey in case of significance.

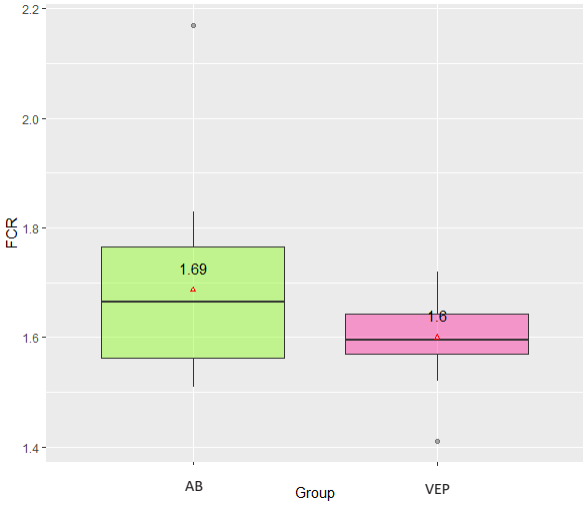
Results

Regarding productive data, no significant differences between VEP and AB groups were detected. (FCR 1,45 vs 1,41), P=0,57); ADG 332 vs 327, P=0.80) on the first period and the second period (FCR 1,69 vs 1,60), P=0,117); ADG 524 vs 511, P=0.71). There was no difference in mortality (5 animals in VEP vs 7 AB group). Overall medication and treatment costs were reduced in the VEP group (€1,2 vs. €1,91 in AB group), and the production cost per exit piglet at 36,6 kg was reduced by €1,32 in VEP group.

Discussion and Conclusion

Vaccination against VT2e permitted a reduction in antibiotics while reducing the cost per exit piglet. Clinical cases of edema disease and *S. suis* meningitis can be quite similar, and pathogens may interact in co-infections to enhance its pathogenicity. Therefore, further laboratory tests are strongly recommended in order to characterize the farm sanitary status. Moreover, when verotoxigenic *E. Coli* is present, it is important to consider vaccination against VT2e as an aid to control *S. suis* problems in nursery pigs.

Figure 1. Feed conversion rate on period 2.



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Antibody response induced by a multiserotype *Streptococcus suis* autogenous vaccine used in sows to measure the immunoglobulin passive transfer to piglets

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Introduction

Streptococcus suis is an important bacterial pathogen in swine production. It causes major economic burden to the industry, mainly in the post-weaning period. *S. suis* is classified in 29 serotypes with diverse geographical distribution (1). There is currently no commercial vaccine available in most countries. Autogenous vaccinesare thus the only preventive strategy used (1); however, field studies on the immunological response induced by these vaccines are scarce. Previous studies performed with North American autogenous vaccines showed that they increase antibody levels in sows when compared to placebo groups (2,3). Nevertheless, transfer of maternal immunity to piglets was either not improved (2) or did not last longer than 18 days of age (3). Since manufacturing procedures are different amongst companies, in this study we assessed a sow vaccination program with an autogenous vaccine containing five *S. suis* serotypes (2, 5, 7, 14 and 1/2) from a manufacturer never studied before. The response induced by the vaccine and transfer of maternal immunity to piglets (until 7 week-old) were analyzed on a commercial farm.

Materials and Methods

Blood was obtained from gilts pre-vaccination and after three doses of the autogenous vaccine in both vaccinated (n=28) and placebo (n=25) groups. After farrowing, piglets (2/litter, n=106) were tagged and followed for serology up to 7 weeks of age. ELISA test was done to measure and characterize the vaccine-induced antibody response in sows and the passive immunity in piglets. To measure antibody functionality (i.e. the capacity of antibodies to kill *S. suis*), an *in vitro* opsonophagocytosis assay (OPA) was used; this test is considered a correlation of protection in vaccine studies. Clinical evaluation, necropsy and bacteriology were done on piglets during the nursery barn period.

Results

Results targeting the response against serotypes 2, 5, 7, 14 and 1/2 showed that vaccinated sows present higher levels of antibodies against these serotypes than the placebo control group. Maternal antibody transfer to their litters was higher in piglets born from vaccinated sows compared to controls, which lasted until 3 weeks of age for *S. suis* serotypes 2, 7 and 14 (Figure 1).For serotype 5, passive antibody levels lasted until 5 weeks of age. This is the first time seeing this increase until 5 weeks of age (Figure 2).

Necropsy and bacteriology results showed that there was no *S. suis* outbreak at the time of the trial; however, a bacterial outbreak of *Erysipelothrix rhusiopathiae* was observed. Clinical signs observed were *S. suis*-like clinical signs; however, limited *S. suis* was identified through bacteriology.

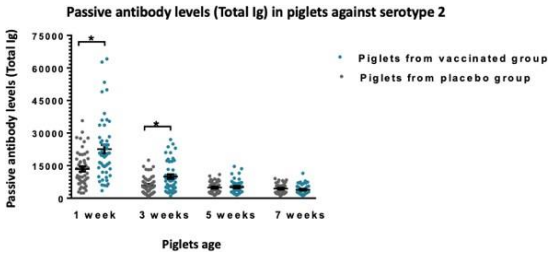

Figure 1: Passive antibody levels in piglets agaist serotype 2.

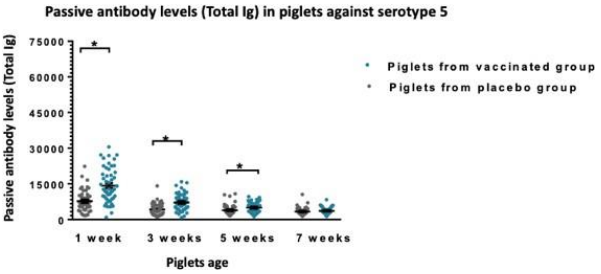

Figure 2: Passive antibody levels in piglets agaist serotype 5.

Disccusion and Conclusion

This autogenous vaccine program produced by a different company showed, for the first time, an increase of passive antibodies in piglets up to 3 and/or 5 weeks of age. This study proves that the use of a different manufacturing company may produce different results. Our data also prove the importance of quick and precise diagnostics in a herd, before and after application of an autogenous vaccine, to properly evaluate the efficacy andprotection induced by autogenous vaccines.

Therefore, more studies are required to fully characterize the clinical protective effect of this vaccine program during the complete nursery period.

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Assessment of Ep-like lesions in China under African swine fever epidemic environment during 2019-2021

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Introduction

First African swine fever (ASF) outbreak in China was officially announced in August 2018. Under ASF epidemic environment, Pig farms also face other important diseases challenge including Enzootic pneumonia (Ep), causing increased mortality, decreased growth rate and increased feed conversion ratio, etc. Lung lesion scoring at the slaughterhouse is a valuable tool for the assessment of the respiratory health status of pigs. The objective of this study is to survey the presence of Porcine Enzootic Pneumonia in China at slaughterhouses using Ceva Lung Program (CLP) which is an innovative approach for assessment of lung lesion scores.

Materials and Methods

Total 7,019 lungs from 187 batches were scored using Ceva Lung Program at slaughterhouse (Fig.1). Pneumonia was scored and calculated considering the proportion of each lobe of the lungs (1). The CLP was performed at difference slaughterhouses in China during 2019-2021. The mean values and quartiles were calculated for % of lungs with bronchopneumonia, % of affected lung surface out of all lungs and % scarring describing prevalence of fissures associated with older EP-like lesions.

Fig.1 Lung lesion scoring at the slaughterhouse

Results

Results of lung lesion scoring for China slaughter pigs were presented in Table 1 using percentiles (P25 – median - P75). The results of the lung lesion scoring indicated high prevalence of EP- like lesion in China under African swine fever epidemic environment. The median prevalence bronchopneumonia during 2019-2021 reached 51.60%, 92.78% and 52.14%, respectively. The EP-like lesion in 2020 was more severe than in 2019 and 2021, except for scaring.

Table 1 EP-like lesions

	Year	P25	Median	P75
Prevalence bronchopneumonia	2019	35.31%	51.60%	97.56%
	2020	40.42%	92.78%	100.00%
	2021	31.35%	52.14%	68.00%
Affected surface - all lungs	2019	1.19%	1.96%	2.95%
	2020	2.12%	16.92%	30.50%
	2021	1.62%	3.00%	6.73%
Scarring	2019	0%	10.00%	17.39%
	2020	0%	0%	15.83%
	2021	5.80%	10.00%	19.84%

Discussion and Conclusion

Under ASF prevention pressure, some pig farms ceased Mycoplasma hyopneumoniae (M.hyo) vaccination in china, causing serious enzootic pneumonia. According to previous study, 1% of affected surface out of all lungs decreases ADG by 3.74g and 0.25% FCR (2). This survey showed that Chinese pig farms had suffered huge losses due to the infection of M.hyo. Frequent use of antibiotics may be the cause of variation in the scarring ratio.

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Apparent ileal and total tract digestibility of amino acids and fecal microbiota of vaccinated, non-vaccinated clinically inconspicuous and non-vaccinated clinically conspicuous piglets under natural *Lawsonia intracellularis* infection

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Introduction

Lawsonia intracellularis (*L.i.*) is the cause of the porcine proliferative enteropathy (1). Although, levels of *L.i.* excretion correlate with the severity of ileal lesions (2) contact to the pathogen must not necessarily always lead to clinical disease (3). Clinically moderate *L.i.* infections adversely affect performance primarily caused by a lower feed intake, increased length of the small intestine and altered total N digestibility (4). This could have an impact on the nutrients that reach the hindgut which serve as substrate for the present bacteria. We hypothesize that this potential indirect effect of the pathogen in the hindgut could contribute to the development of a clinical manifestation of the disease. Therefore, the aim of the present study was to investigate amino acid (AA) digestibility and fecal microbiota composition of vaccinated or non-vaccinated clinically inconspicuous and conspicuous *L.i.* colonized piglets.

Materials and Methods

In three consecutive trials, 27 natural *L.i.*-infected pigs (19.0 ±1.50 kg body weight) of one farm were transferred to university and evenly divided into three groups at an age of 7 to 8 weeks (Vac = vaccinated via oral drenching on their 21st day of life (Enterisol®Ileitis, attenuated live vaccine (*L.i.*); Non-vac/cs- = non-vaccinated, without clinical findings and Non-vac/cs+ = non-vaccinated with clinical findings (healthy pigs with moderate to soft feces consistency)). Pigs were housed individually and fed a conventional diet *ad libitum* (CP: 176 g, CF: 23.5 g, EE: 33.6 g, ME: 13.8 MJ/kg diet) for 7 days to which 0.5% Chromium oxide was added. A bulk sample was formed from feces of the last five days, from which an aliquot was taken for further analyses. AA were determined (ion-exchange chromatography) to evaluate apparent total tract digestibility (ATTD). At the end of the experiment, pigs were dissected and digesta was taken for determination of AID of AA. Microbiota was analyzed in fecal samples (16S rRNA gene amplification within the hypervariable region V4, sequencing with Illumina MiSeq platform). Data visualization was done in R (version 4.1.2) with the R-package "phyloseq" (version 1.36.0). Total community structure was assessed by permutational multivariate analysis of variance (PERMANOVA) using Bray-Curtis distance (R-package "vegan", version 2.5.7). Differentially abundant OTUs (operational taxonomic units) between groups were identified with the help of the R package "DESeq2" (version 1.32.0), which uses tests based on the negative binomial distribution. Raw *p*-values were adjusted using the method of Benjamini and Hochberg. Additionally, a cutoff for the log2 fold change of +/-1 was set. Means of apparent digestibility of AA were first checked for normality by analyzing the model residuals with the Shapiro-Wilk normality test, before multiple and pairwise comparisons were conducted. Statements of statistical significance were based upon p-values < 0.05.

Results

PERMANOVA revealed significant differences in fecal sample's bacterial composition between the three groups (p = 0.001, R2=0.1432). Corresponding to the selected criterion, no significantly different OTU was found in Vac vs. Non-vac/cs-. However, 4 of 124 OTUs were identified as differentially abundant in Vac vs. Non-vac/cs+ and 6 OTUs in Non-vac/cs- vs. Non-vac/cs+. Only AID of cysteine was significantly different between the groups (p = 0.043). However, pronounced effects at clinical manifestation on ATTD were noted (see Table 1).

Table 1. Significantly different ATTD of AA (means)

AA	Vac	Non-vac/cs-	Non-vac/cs+	*p*-value
Alanine	79.3[a]	79.4[a]	75.8[b]	0.015
Arginine	91.8[a]	92.0[a]	90.1[b]	0.029
Aspartic acid	84.2[a]	84.9[a]	82.3[b]	0.043
Histidine	88.4[a]	88.0[ab]	86.3[b]	0.014
Isoleucine	81.9[a]	81.6[ab]	79.8[b]	0.031
Leucine	85.1[a]	85.1[a]	83.4[b]	0.027
Serine	88.0[ab]	88.9[a]	87.1[b]	0.230
Tyrosine	85.6[a]	84.9[ab]	83.2[b]	0.044

[a,b] Different superscripts indicate statistically significant differences at $p \leq 0.05$

Discussion and Conclusion

The pronounced differences of ATTD, but not of AID, of AA and of fecal microbiota composition in piglets with clinical signs, might result from different substrate availability with subsequent different bacterial fermentation characteristics and growth compared to clinically inconspicuous pigs. These different environmental conditions may in turn have promoted certain "non-beneficial" bacterial species in the hindgut.

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Case report: Prevention of proliferative intestinal adenomatosis using an oral live ileitis vaccine delivered with continuous membrane dosing pump

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Introduction

For the application of an oral vaccine it is important that the vaccine is applied long enough in the drinking water so that every pig gets access to the vaccine. The optimum time of vaccination by the drinking water, to get all the pigs to the drinker, is 6 hours (1). With traditional volume proportioners, this means the volume of stock solution should match with the volume of water which pigs are drinking. With the use of a continuous dosing system, with a fixed volume flow per hour, groups of pigs can be vaccinated without the required recording of drinking volume. The objective of this study is to evaluate the oral vaccination against ileitis using a continuous dosing pump.

Materials and methods

On a swine farm in the south of Germany ileitis due to infections *with Lawsonia intracellularis* (Li) was diagnosed. A continuous membrane dosing pump was installed (EMEC VCO 0501) to the pigs' drinking water system and set a dosing rate of 0.5 liter an hour. Different batches of pigs were vaccinated with 3 liters of stock solution, resulting in a fixed 6 hours dosing time for each batch of pigs, containing the required number of doses of an oral live ileitis vaccine (Enterisol Ileitis, Boehringer Ingelheim). To check the dosing accuracy, different water samples on different locations after the dosing point in the drinking water system (1.2 m, 16m, and at 22m at thenipple drinker) were taken with a 30-minute interval. Li concentration of water samples was measured using a qPCR (Acare, Germany) for Li. Performance of the finisher pigs was measured to evaluate the outcome of the vaccination intervention.

Results

It took about one hour after the start of dosing for the vaccine to arrive at the nipple drinkers. The water samples obtained from the nipple drinker and 16m after the dosing point were very homogenous (fig. 1). Samples from the nipple drinker showed a mean Log GE/ML of Li of 6.04 (95CI ±0.075; SD 0.13) for the duration of 6 hours. Shortly after the dosing point a higher variation in the vaccine concentration was noticed (SD 0.37)

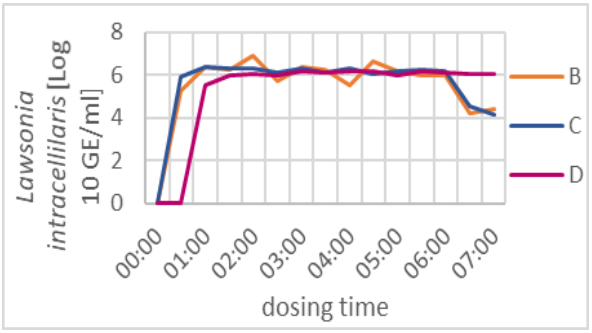

Figure 1 qPCR results of Li at various point of the drinking water system after the dosing point (B 1.2m; C 16m; D 22m/nipple drinker). Stock solution 7.94 LOG GE/ml of Li.

The overall performance of the pigs improved with an 74 grams increase of ADG, lower number of days to slaughter (-13 days) and lower mortality (-1%). The vaccine is known to reduce Salmonella antibodies due to the improvement of the intestinal microbiome (2). This was also confirmed in this farm by the significant drop in Salmonella positive animals for the vaccinated piglets when compared to the unvaccinated piglets (0% vs 42%; p<0.001).

Table 1 Technical performance of the finishing pigs

	Control	Vaccine	difference
ADG (gram)	781	855	+74
Mortality (%)	1.5%	0.5%	-1%
Finishing days	117	104	-13
Salmonella >OD10%)	42%	0%	-42%

Discussion/Conclusion

The use of a continuous dosing pump proved to be a very easy and reliable way to provide an oral vaccine to the pigs by the drinking water system. The amount of time spent by the farmer was less than 15 minutes per batch of pigs. Efficacy of this continuous dosing protocol was proven by the improvement of zootechnical parameters combined with a drop of Salmonella antibodies.

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Changes in the upper and lower respiratory microbiome during early *Mycoplasma hyopneumoniae* infection in pigs

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Introduction

Mycoplasma hyopneumoniae (*M. hyopneumoniae*) is a swine respiratory bacterium causing significant problems in the swine industry [1]. *M. hyopneumoniae* has been shown to facilitate further infections with other respiratory pathogens [1]. Recent studies have explored the composition of sections of the swine respiratory microbiome [2-4]. However, a thorough evaluation of the respiratory microbiome during the early stages of *M. hyopneumoniae* infection has not been performed. Therefore, the objective of this study was to assess the differences in the microbiome associated with the upper and lower respiratory tract during early *M. hyopneumoniae* infection in pigs.

Materials and methods

A natural *M. hyopneumoniae* transmission model was employed (approved by the University of Minnesota IACUC). Twenty-nine naive and one *M. hyopneumoniae* naturally infected gilt were allowed to commingle for 56 days and were humanely euthanized at the end of the study. Nasal, oropharyngeal, tonsillar, laryngeal, deep tracheal, and bronchial samples were collected from each gilt. DNA was extracted from the samples using PowerSoil Pro Kit (Qiagen). The detection of *M. hyopneumoniae* in the laryngeal, deep tracheal, and bronchial samples was evaluated through real time-PCR. Microbiome composition was ascertained after sequencing of the 16S rRNA gene V4 region. Reads were filtered and denoised using DADA2 pipeline in R 4.0.3. Microbiome analyses included the assessment of the contribution of *M. hyopneumoniae* status to the variance of beta diversity dissimilarity distances in PCoA plots and the evaluation of the association of *M. hyopneumoniae* status and the bacterial genera by sample type using ANCOM, correlation-based networks, and static discrete Bayesian networks.

Results and discussion

The total number of amplicon sequence variants (ASVs) ranged from 105 (bronchial swabs) to 1531 (tracheal fluids). *Streptococcus*, *Moraxella*, *Clostridium sensu stricto*, *Rothia*, and *Actinobacillus* were among the five most abundant genera. These results agree with previous studies on healthy pigs [2, 4] but disagree with the findings observed in pigs with respiratory disease [2, 3]. Oropharyngeal swabs, tonsillar swabs, laryngeal swabs, and tracheal fluids constituted a cluster different from nasal swabs and bronchial swabs (Figure 1). Interestingly, ASVs classified as *Blautia massillensis*, *F0058*, *Porphyromonas*, and *Neisseriaceae* were more abundant in the upper respiratory tract in *M. hyopneumoniae*-positive pigs and the probability of being positive for *M. hyopneumoniae* increased when *Megasphaera* and *Filobacterium* were present in the bronchi, suggesting that *Megasphaera* and *Filobacterium* may potentially increase the susceptibility to *M. hyopneumoniae* infection.

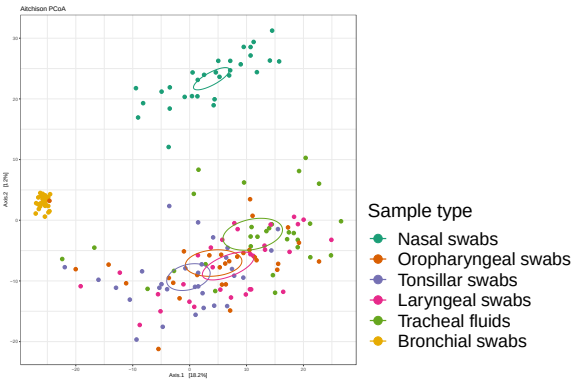

Figure 1. Plot of samples distributed according to their microbiome composition and colored by sample type.

Conclusions

Results from this study showed the association of *M. hyopneumoniae* with bacteria in the upper and lower respiratory tract during early infection, suggesting that *M. hyopneumoniae* can alter the microbiome at several sites of the respiratory tract. Importantly, these interactions may change over time as *M. hyopneumoniae* has been shown to persist in infected pigs for up to 8 months.

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Combined stress and *S. suis* serotype 2 oral challenge to attempt a disease model

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Introduction

Streptococcus suis (*S. suis*) disease in pigs is an endemic and zoonotic problem, with serotype 2 (SS2) being important worldwide (1, 2). The disease is mainly associated with respiratory and tonsil colonization (2), but gastrointestinal infection and intestinal lining translocation have also been reported (3). Outbreaks and disease are often associated with co-infections, with stress factors identified as potential triggers (2). The natural infection process is still not fully understood, and a repeatable model that mimics the first steps of the disease is lacking. Furthermore, the interaction between disease susceptibility and stress related to feeding pattern post-weaning is unknown. It was hypothesized that competitive stress and an abrupt feed intake change could influence the disease. The present objective was to evaluate a SS2 oral challenge in response to feeding stress.

Materials and Methods

A total of 45 pigs (5.85 ± 0.35 kg BW) were weaned and distributed across 15 pens at 3 piglets per pen. Three treatments were administered: 1) unchallenged control group with stress (NC); 2) SS2 challenged without stress (NOstress); and 3) a positive challenged control group with stress (PC); see Figure 1 for the model set up. The feed stressor consisted of a feed allowance set to 75% maintenance (MEm at 200 kcal/kg BW^0.6) for day 0, 1, and 2 with leftovers removed at 8:00am next day and feeder refilling only at 1300h. From day 3 until day 7, feed allowance was set to 110% of maintenance with feeder refilling at 1300h daily, followed by ad libitum access from day 8 until day 14 post-weaning. All piglets were remixed within treatment on day 4 before *S. suis* challenge., which consisted of 3 ml oral gavage of 8.7 log virulent SS2/ml to 2 pigs/pen. Hence, 1 pig/pen was left as sentinel. Tonsillar swabs were collected for SS2 qPCR to assess prevalence and load (log10). On day 14, all piglets were euthanized, and swabs form joints, cardiac valves, and spleen were plated on Columbia blood agar at 37°C in serial dilutions to assess tissue colonization. Growth performance and feed intake (FI) were monitored.

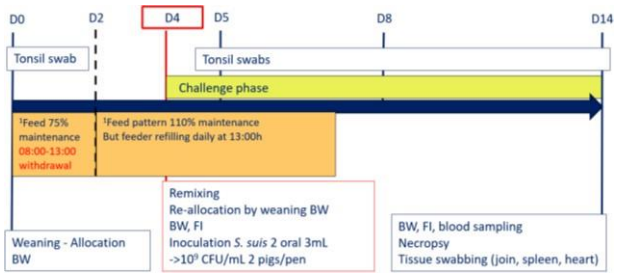

Figure 1. Scheme of measurements and activities.

Data was analyzed (SAS Inst. Inc., Cary, NC) with treatment as fixed effects and BW block as a random effect (PROC MIXED). The experimental unit used was the pen.

Results

For the pre-challenge phase, ADG from weaning to day 4 tended to be higher (P = 0.062) for NOstress pigs than the NC or PC. Similarly, BW at day 4 was higher in NOstress than NC (P < 0.05). For the post-challenge phase, the SS2 challenge model failed to cause streptococcal disease or affect performance. The SS2 prevalence at weaning was 20-47%. After challenge, day 5 and 8 post-weaning prevalence (67-100%) was high in general and significantly higher in challenged treatments (P>0.05). The prevalence by day 14 was about 80% in all treatments and not different between treatments. Pigs from NC did not show significantly different tonsillar loads to those from NOstress and PC challenged treatments (P > 0.05). Tissue sampling and swabbing at time of euthanasia demonstrated no tissue colonization by inoculated SS2.

Table 1. Means for performance and SS2 prevalence.

Treatment	NC	NOstress	PC	Pre-challenge
Pre-challenge				
BW weaning	5.83	5.88	5.89	
BW day 4 (challenge)	6.09^{b}	6.42^{a}	6.19^{ab}	
ADG 0-4	66.3^{y}	136^{x}	75.7^{y}	
ADFI 0-4	93.1	108	99.6	
Post-challenge				
BW day 14	8.94	9.23	8.89	
ADG 4-14	285	281	254	
ADFI 4-14	303	322	278	
ME Intake vs. Requirements[1], %	134^{xy}	140^{x}	124^{y}	
***S. suis* SS2 prevalence**				
Weaning, %	20	47	27	
Day 5, %	73^{a}	100^{b}	100^{b}	
Day 8, %	67^{a}	100^{b}	100^{b}	
Day 14, %	80	80	79	

[1]MEm at 200 kcal/kg BW^0.6. Superscripts ($^{a-b}$) indicate statistically difference at P<0.05 or a tendency ($^{x-y}$) at P < 0.10.

Discussion and Conclusion

Feeding stress resulted with reduced performance before infection which was mostly compensated by day14 post-weaning. The SS2 challenge did not influence growth performance and only modified temporary the SS2 prevalence measured in tonsils. In conclusion, the current challenge model failed to induce disease and colonize internal tissues of pigs under a *S. suis* serotype 2 oral challenge with or without feeding stress.

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Comparison of pathogenicity and immune response to *Mycoplasma hyopneumoniae* infectionin naïve pigs and super-infection in *M. hyorhinis* infected pigs

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Introduction

Mycoplasma (M.) hyopneumoniae is a major cause of chronic respiratory disease (Enzootic Pneumonia, EP) and is an important primary pathogen in the porcine respiratory disease complex (PRDC). It is widespread in pig farms and occurs in most pig farms around the world. The economic loss is significant and primarily related to decreased production performance. *M. hyorhinis* most commonly causes polyserositis, such as pleuritis, pericarditis, and peritonitis or arthritis and septicemia in nursery-age pigs (1,2). *M. hyorhinis* is considered a commensal bacterium in swine (1). In this study, we compare the pathogenicity and immune response of *M. hyopneumoniae* infection in naïve pigs and by super-infection in *M. hyorhinis* infected pigs. The *M. hyopneumoniae* field isolate was used for this study (provided by Careside Korea Inc., Gyeonggi-do, Republic of Korea).

Materials and Methods

Two weeks old pigs were used for the experiment. Animals were treated antibiotics under controlled conditions for 4 weeks to avoid any unexpected bacterial problems. *M. hyorhinis* infected pigs were selected by polymerase chain reaction (PCR). We assigned three pigs as *M. hyopneumoniae (Mhp)* and *M. hyorhinis (Mhr)* super-infection group. Six pigs without *M. hyorhinis* were assigned as *Mhp natural* infection group. Pigs were inoculated 10^9 CCU/ml of *M. hyopneumoniae* using a inhalation device. For clinical evaluation, pigs were monitored daily such as respiratory signs, pyrexia, arthritis, and lethargy and scored for each sign (2). Blood samples were collected at 4wpi (weeks post inoculation), 6wpi, and 8wpi for Enyzme-linked immunosorbent assay (ELISA). Pigs were necropsied at 4wpi, 6wpi, and 8wpi. Lungs were scored pneumonia lesions described by Straw (1986) and histopathology by H&E staining as described in Calsamiglia *et al*. (2000).

Results

Both groups mainly showed respiratory signs until the end of the experiment. *Mhp* and *Mhr* super-infection group showed higher mean clinical sign scores than *Mhp* natural infection group at 3wpi, 6wpi, 7wpi, and 8wpi and significant difference between two groups was confirmed at 3wpi ($p<0.05$). The mean values of *M. hyopneumoniae* ELISA S/P ratio is shown in Table 1. *Mhp* and *Mhr* super-infection group's *M. hyopneumoniae* ELISA S/P ratio was higher than *Mhp* natural infection group. Significant differences in the mean S/P values between two groups were confirmed– at 4wpi and 6wpi ($p<0.05$). *Mhp* and *Mhr* super-infection group elicited a 100% positive response by *M. hyopneumoniae* ELISA antibody test. Conversely, *Mhp* natural infection group showed 67%, 75%, and 100% in each 4, 6, and 8wpi, respectively. Pneumonia was observed in all pigs. Pneumonia score ranged from 17.3 ± 15.6 (*Mhp* and *Mhr* super-infection) and 20.5 ± 10.8 (*Mhp* infection), without any statistically significant differences. Both groups were histopathologically diagnosed with Grade 4 showing perivascular and peribronchiolar lymphoplasmacytic hyperplasia, type II pneumocyte hyperplasia, alveolar spaces with edema fluid, neutrophils, macrophages, and plasma cells with perivascular and peribronchial lymphoid nodules.

Table 1. Mean and SD of *M. hyopneumoniae* ELISA S/P ratio.

Group	S/P ratio of *M. hyopneumoniae* mean±SD (n[1])			
	0wpi	4wpi	6wpi	8wpi
Mhp natural infection	0.08 ±0.03 (6)	0.48 ±0.16 (6)	0.66 ±0.24 (4)	0.74 ±0.18 (2)
Mhp and *Mhr* super-infection	0.2 ±0.05 (3)	1.36 ±0.16 (3)	1.68 ±0.28 (2)	1.64 ±0 (1)
*p**	-	<0.0001	0.0094	-

[1]Number of pigs **p*-value for student t-test. Results are statistically significant if $p<0.05$

Discussion and Conclusion

There were no significant pathological differences between *Mhp* and *Mhr* super-infection and *Mhp* natural infection. However, there was a tendency that *Mhp* and *Mhr* super-infection group's *M. hyopneumoniae* ELISA S/P results were higher than *Mhp* natural infection group. These data support that *Mhr* either cross react and/or stimulate *M. hyopneumoniae* immune response and can be used as comparative data in conventional farm where the *M. hyorhinis* is commensal bacterium.

Acknowledgments

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Correlating diagnostic metrics and performance in *Lawsonia intracellularis* challenged pigs

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Introduction

Porcine proliferative enteropathy or ileitis,caused by *Lawsonia intracellularis*, is endemic to swine herds worldwide (1,2). Antemortem diagnostic assays of fecal polymerase chain reaction (PCR) assays and serum antibody assays are widely utilized to diagnose disease, however the relationship between these diagnostic assays and performance metrics is notfully characterized (3,4). Thus, this study aimed to determine how *L. intracellularis* diagnostic metrics (fecal shedding, antibody levels) correlate to production metrics in both vaccinated and unvaccinated pigs.

Materials and Methods

Thirty-six barrows, individually confirmed negative for *L. intracellularis*, were selected and assigned to the following treatment groups (n=12/trt):

1) nonvaccinated, *L. intracellularis* negative (NC); 2) nonvaccinated, *L intracellularis* challenged (PC); and 3) *L. intracellularis* challenged, vaccinated with Enterisol® Ileitis (Boehringer Ingelheim Animal Health, Duluth, GA) via oral drench at 1-week postweaning (VAC). At 11 weeks of age, on days post inoculation (dpi) 0 PC and VAC pigs were inoculated with *L. intracellularis* via gastric gavage (2.7×10^8 organisms/mL). Individual feed disappearance and body weight were recorded weekly. Additionally, fecal and blood samples were collected weekly to evaluate fecal shedding by qPCR and serum antibody levels by commercial ELISA. At dpi 21, pigs were euthanized and scored for gross and microscopic lesions characteristic of *L. intracellularis* infection. To investigate the relationship between diagnostic parameters and performance metrics, correlation coefficients were calculated in the PC and VAC groups.

Results

As expected, ileal gross lesion length was negatively correlated with overall ADG (r =-0.616; P=0.033) and positively correlated with overall feed conversion ratio (FCR; r =0.613; P=0.034) for PC pigs, but did not correlate with overall growth in VAC pigs. Fecal shedding at dpi 14 did correlate with overall FCRin PC pigs (r=0.587; P=0.045; Table 1); every 1 $\log_{10}$ genomic copy increase in fecal shedding was associated with a 0.17 unit increase in FCR. Fecal shedding did not negatively correlate with FCR in VAC pigs at any timepoint ($P \geq 0.262$). No other significant correlations were found among fecal shedding values at other timepoints and overall production performance metrics. Serum antibody concentrations did not correlate with overall ADG, ADFI, or FCR in either PC or VAC pigs.

Table 1. Correlation coefficients amongst *Lawsonia* fecal shedding level and production performance

		Days post inoculation		
		7	14	21
PC	ADG[1]	0.210	-0.413	0.025
	FCR[2]	0.084	0.587*	0.210
VAC	ADG[1]	0.181	0.261	0.530
	FCR[2]	-0.065	-0.352	0.181

[1]Average daily weight gain from days post inoculation (dpi) 0-19; [2]feed conversion ratio from dpi 0-19; *statistically significant (P<0.05).

Discussion & Conclusions

Taken together, under the conditions of this study, this research indicates that correlations of fecal shedding values to production performance metrics vary depending on the stage of disease the sample is collected. In the field it is not possible to know the exact stage of infection, thus making the direct correlation to production loss more difficult. Rather, positive results mostly confirm the presence of disease, which itself leads to performance loss (1,2,4). A reduction in performance (ADG and FCR) was found in pigs that shed as low as 100 organisms/gram of feces at 7 dpi. We also found that correlation metrics were different among vaccinated and non-vaccinated animals. While higher shedding levels were correlated with worsened FCR in non-vaccinated pigs, this was not found in vaccinated pigs. Thus, fecal PCR results should be interpreted differently in pigs depending on their vaccination status. Finally, *L. intracellularis* antibody concentrations were not indicative of performance metrics during *L. intracellularis* challenge, particularly in pigs that had been vaccinated.

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Declined *Lawsonia intracellularis* in feces by phytogenic feed additive supplementation in fattening pigs in different herds system

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Introduction

Lawsonia intracellularis is a gram-negative bacterium, a causative agent, of worldwide spread pig enteric disease, the porcine proliferative enteropathy (PPE). (1). The decreased susceptibility of *L. intracellularis* to antibiotics is observed (2) as well as novel a plant-based solutions to control the PPE (3). The aim of our study was to investigate effects of plant-based feed additive (PBFA) on *L. intracellularis* presence in pig feces that originated from 2 herds that differ in temperature regulating systems.

Materials and Methods

The trial was performed on *L. intracellularis* naturally infected pigs on one a conventional open-housing system farm and one evaporative cooling-housing system farm. On each farm, 40 fattening pigs (12-week-old) were randomly allocated in to two groups including control group (n=20) and treatment group (n=20). The pigs in each group were fed with a conventional diet (Control) and the same diet supplemented with 2 kg/ton of commercial PBFA, PATENTE HERBA® PLUS, PATENT CO. DOO, Serbia), for 14 days (Treatment). The feces samples were collected and were scored on day 0, 7 and 14 after supplementation and were determined by quantitative real time PCR.
The number of DNA copies of *L. intracellularis* were logged transformed, after testing the assumption of normal distribution using skewness, kurtosis, and Shapiro–Wilk normality test. The effect of the phytogenic feed additive on herd and on the number of DNA copies of *L. intracellularis* were analyzed by using general linear model (GLM). Values with $P < 0.05$ were regarded as statistically significant.

Results

The number of DNA copies of *L. intracellularis* are presented in Table 1. In control groups of all herds, the number of DNA of *L. intracellularis* did not differ significantly throughout the study. In herd A, the number of DNA copies of *L. intracellularis* in the Treatment group at 14. day of PBFA supplementation (1.0 ± 2.7 copies/µL) was lower than at the day 0 (0.8 ± 2.2 copies/µL) and at day 7 (0.8 ± 2.2 copies/µL) but statistical difference was not observed.
In herd B, the number of DNA copies of *L. intracellularis* in the Treatment group at day 14 (0.8 copies/µL), after supplementation of PBFA, was statistically significantly lower than at day 0 (5.5 copies/µL; P = 0.023) and at day 7 (1.4 copies/µL; P = 0.131).

Discussion and Conclusion

The plant-based feed additive can reduce the number of *L. intracellularis* after 7 days of supplementation in fattening pigs in both, an evaporative cooling system and an open-housing system. Therefore, phytogenic feed additive may be applied to control *L. intracellularis* in commercial swine farms when the usage of antibiotic was limited.

Table 1. DNA copy number of *Lawsonia intracelluralis* in a pooled samples of fattening pig feces in the Control (n=30) and Treatment groups (n=30) in each day of supplementation in each herd

Herd	Group	day of supplementation					
		0		7		14	
Herd A	Control	2.0	±	3.7	±	2.9	±
		2.2		1.7		2.2	
	Treatment	4.2	±	0.8	±	1.0	±
		1.9		2.2		2.7	
Herd B	Control	2.6	±	5.0	±	2.3	±
		1.9		2.2		1.9	
	Treatment	5.5	±	1.4	±	0.8	±
		1.7[a]		2.7[b]		1.9[b]	

[a, b] Different superscript letters within group indicate significant differences $P < 0.05$.

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Detection dynamics of *Mycoplasma hyopneumoniae* under controlled aerosol exposure forgilt acclimatization

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Introduction

Mycoplasma hyopneumoniae (*Mhp*), the causative agent of enzootic pneumonia in swine [1-2], continues to cause significant economic and health losses in the industry worldwide [3]. Gilt acclimation methods for *Mhp* control, such as vaccination [4], a "seeder" model [5], or controlled exposure [6], are employed with the intent to minimize dam-to-piglet transmission and to establish Day 0 for elimination strategies [7]. Controlled exposure of gilts via the aerosolization of *Mhp* herd-specific lung homogenate [8] has been attempted at an increasing rate in the industry to induce infection under field conditions [6]. However, limited information regarding the validation of such administration technique to obtain a reliable and uniform exposure is available. Therefore, the objective of this study was to evaluate the spatial and temporal detection of *Mhp* under controlled aerosol exposure conditions using various clinical and environmental samples.

Materials and methods

Spatial and temporal detection dynamics of *Mhp* were evaluated by conducting a longitudinal prospective study. A total of five rooms (A-E) from three *Mhp* negative gilt developing units (GDUs) were selected. The GDU sites were managed in an all-in/all-out fashion and housed a similar population size. For each room, a *Mhp* herd-specific lung homogenate was procured based on a diagnostic criterion (8) and was aerosolized from a single location within each room using a hurricane fogger. At 0,7 and 14 days post-exposure (dpe), environmental and air particle deposition (APD) samples were strategically collected at different locations (up to 9.8 meters) from the origin of exposure (i.e., hurricane fogger). Tracheal secretions were collected from randomly selected four- to seven-week-old gilts (n=30/room) at 0, 7, 14, and 28 dpe. Sampled gilts (n=5/pen) were housed in pens throughout the room to assess *Mhp* detection dynamics. Samples were stored at -20ºC and later processed individually for DNA extraction and tested for *Mhp* using a real-time PCR. Samples with a ≤39 Ct value were considered *Mhp* positive. Data wasdescriptively analyzed.

Results

Lung homogenate samples were *Mhp* positive with Ct values ranging from 27.2-30.3. Prior to exposure, all samples were *Mhp* negative by PCR. At 0 dpe, *Mhp* was detected in environmental and ADP samples up to 5.8 meters from the hurricane fogger, in which the relative bacterial load numerically decreased as distance increased. For three rooms (A, B, and E), giltsremained *Mhp* negative until approx. 14 dpe, in which10% of pigs housed in neighboring pens located within 4.6 meters of the exposure origin became positive. In comparison, *Mhp* was detected in 20 and 26% of gilts in rooms C and D, respectively at 7 dpe. At 28 dpe, 20-57% of gilts became *Mhp* positive in rooms C-E, in which a high proportion (80-100%) of infected gilts per pen was observed in pens located within 5.8 meters from the exposure origin.

Conclusions

Overall, *Mhp* detection dynamics appeared to be spatially associated, however, inconsistent results were observed across the five rooms. It is important tomention that *Mhp* strain differences among the GDU sites could not be ruled out as a contributing variable. Additional research is warranted to confirm findings and to provide further insight towards the optimization of gilt acclimation procedures via aerosol exposure.

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Distinguishing viable from non-viable *Mycoplasma hyopneumoniae* by PCR

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Introduction

An accurate diagnosis is a critical component for assessing the success of disease control interventions. Due to its intrinsic difficulty for isolation, microbiologic culture is not routinely performed to identify *M. hyopneumoniae* in clinical specimens (1). Thus, the detection of *M. hyopneumoniae* in clinical samples is usually determined by PCR targeting bacterial DNA. However, DNA molecules are known to be very stable in the environment and resistant to degradation, including resistance to pathogen inactivation. Hence, there is no direct relationship between viability (or potential infectivity) of bacteria and their detection by PCR, which can lead to diagnostic uncertainty and interpretation issues. Therefore, the objective of this study was to develop a PCR assay for the detection of viable *M. hyopneumoniae*.

Materials and Methods

Validation of the new assay followed the guidelines developed by the Laboratory Technology Committee of the American Association of veterinary Laboratory Diagnosticians (2) and encompassed analytical and diagnostic performance and repeatability, using reference strains and clinical specimens commonly collected for routine diagnostics.

Side-by-side comparisons were performed to evaluate the detection of viable *M. hyopneumoniae* and genetic material after bacterial inactivation using various methods.

To determine the analytical sensitivity of the assay, a standard curve spanning several orders of magnitude was prepared from serial 10-fold dilutions of a quantified targeted synthetic oligonucleotide. The limit of detection (LOD) was calculated based on the mean of the Ct value at the lowest copy number wherein 100% of the replicates were positive. The LOD was established based on the results of replicates in independent runs. To define the analytical specificity of the assay, a panel comprised of several *M. hyopneumoniae* reference and field strains, other *Mycoplasma* species, and bacterial and viral species commonly present in the respiratory tract of pigs was assembled. Repeatability was measured testing replicates of various concentrations of the target in separate runs. In order to determine the assay's selectivity and diagnostic characteristics, different clinical specimen matrices, including secretions from the upper and lower swine respiratory tract were collected and tested. The above-mentioned clinical specimens were obtained from both from experimentally infected and naive pigs.

The detection of viable *M. hyopneumoniae* disappearance was evaluated after culture inactivation by various methods.

Mycoplasma hyopneumoniae bacterial culture was maintained at 37ºC, in the incubator, and at 25°C (room temperature). Then, genetic material was extracted prior to and post-inactivation at 1, 2, 6 and 12 hours, and at 1, 2, 3, 5, 10 and 20 days. All samples were analyzed by means of a commercially available real time PCR and the viability PCR assay. Moreover, the decay dynamics of viable *M. hyopneumoniae* was compared to the standard detection and was evaluated by culture inactivation immediately prior to and 1, 2, 3, 4, 5, 10, 15, 20, 25 and 30 minutes post-inactivation.

Results

The analytical LOD was 1 genome copy number/µL (8 genome copy numbers/reaction). The assay met all the analytical specificity requirements, as all tested *M. hyopneumoniae* strains were detected and the genetic material of other pathogens was not amplified. PCR amplification was obtained in clinical specimens from all tested swine matrices. As for repeatability, there was very low intra and inter-assay variation, as all SDs were <1.0.

Mycoplasma hyopneumoniae DNA detection by the real time PCR (current PCR assay) was consistent over time, irrespective of the viability status and up until 20 days post-inactivation (the last time it was measured). In contrast, viable *M. hyopneumoniae* was no longer detected either at 25 minutes or immediately after, depending on the method used for inactivation. For the cultures maintained at 37 and 25°C, viable *M. hyopneumoniae* was detected up to the last time point in which it was evaluated (at 20 days).

Discussion and Conclusions

This study describes, for the first time, the development of a quantitative and rapid method for *M. hyopneumoniae* viability assessment *in vitro* and in clinical specimens in pigs.

This assay can aid to circumvent the current inability to differentiate viable from non-viable bacterial cells. Lack of viability differentiation has been a key limitingfactor for the diagnosis of *M. hyopnuemoniae*, especially when assessing the efficacy of control interventions in the field.

Important scenarios in which this assay can be applied may include the evaluation of the effects that antibiotic treatments have on the clearance of *M. hyopneumoniae* from the respiratory tract of pigs, and the detection or pathogen genetic material in the tail end of disease eradication programs.

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Effect of two immunoglobulin treatments to control clinical digestive problems and improvethe viability of suckling piglets.

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Introduction

In Mexico, mortality in lactating piglets ranges between 3% and 30%, of which diarrhea contributes 15%. Currently, one of the challenges facing the swine industry is to develop alternative ways to control diarrhea during lactation. Egg yolk antibodies (IgY) have been of great interest as an alternative to the use of antibiotics (1). This practice could decrease the use of antibiotics and improve mortality rates in suckling piglets associated with diarrheal problems.

Objetive

To evaluate the effect of two treatments with immunoglobulins of different origin on productive parameters in an outbreak of diarrhea in suckling piglets.

Material and Methods

The work was carried out in a commercial farm, located in Tamaulipas, Mexico. Digestive clinical signs were observed in lactating piglets during the first 3 days of life, reducing their viability during thisphase. Samples of intestinal contents were taken for qPCR tests (PED, GET, Rotavirus and Porcine Deltacoronavirus) in addition to bacteriological isolation for *E. coli*. The experimental phase was carried out in two groups, each with 110 Large White x Landrace sows. Both groups were placed in complete maternity units. Group 1 group was treated with colostrum with immunoglobulins obtained from spray drying (Product A) and group 2 was treated with immunoglobulins of avian origin specific against E.coli, Rotavirus and TGE (Product B). A single dose of 2mL of each product was administered per newborn piglet during the first 6 hours of life. A statistical test (T-Student Test) was used to compare the average mortalities and weights of both treatments.

Results and Discussion

The qPCR results for TGE, Rotavirus and porcine Deltacoronavirus were negative and Escherichia coli was isolated and was sensitive to colistin sulfate and Fosfomycin [data not shown]. Overall mortality decreased considerably in the group treated with Product B with a difference of 5 percentage units ($P<0.05$). In addition, weaned piglet weights had a considerable difference of 730g and a weight gain of 670g with Product A ($P<0.05$) [See Table 1]. Oral administration of specific IgY antibodies has been reported to be highly effective against intestinal pathogens that cause diarrhea in suckling piglets: *Escherichia coli* enterotoxigenic (ETEC): K88, K99, 987P and F18.; *Salmonella spp*, Porcine Rotavirus, Transmissible Gastroenteritis (TGE) and Porcine Epidemic Diarrhea Virus (PEDV) (2)

Table 1	Groupup 1 / Product A	Group 2 / Product B
Gilts	30	33
Sows	80	77
Total piglets at birth	1332	1306
Average total piglets at birth	12.11	11.87
Born alive piglets	1267	1250
Average born alive piglets	11.52	11.36
Average birth weight	1.7	1.64
% Mortality	6%[a]	1%[b]
Piglets weaned	1197	1237
Average piglets weaned	10.88[a]	11.25[a]
Weaning weight	8346.8	7718.3
Average weaning weight	6.97[a]	6.24[b]
Weight gain Kg	6188.1	5673.2
Average Weight gain Kg	5.27[a]	4.60[b]

Different literals indicate statistical difference P<0.05

Marquardt et. al. 2005 (3) reported the protective effects of IgY against ETEC K88 fimbriae in neonates. Pigs treated with IgY (soluble fraction) from immunized hens showed no clinical signs of diarrhea 24 and 48h after treatment and improved their weight gain. In this study, the group treated with product B with specific IgY, the suckling piglets did not show clinical signs of diarrhea 24 and 48h after treatment, their overall mortality was low and their weights remained stable. In contrast to the previous, piglets treated with product A, there was severe diarrhea during 24h and 48h with overall mortality of 6%. Pigswith diarrhea died within the first 48h of infection, however pigs that were not observed to be affected byclinical infection improved considerably in weight. Inthis case, Chu et., al. 2006 (4) have reported control scenarios in 3-day-old suckling piglets against ETEC
- F18 and ETEC K88, with results that decreased mortality in clinical challenges, as was the case in this work.

Conclusion

Oral administration of IgY offers a prophylactic and therapeutic approach to control clinical diarrhea associated with E. coli. Product A improved weaning weights, but did not protect the piglets as a whole, while product B reduced mortality with efficient weaning weights. This work shows an option to control diarrhea problems in neonatal piglets as well as having an alternative to improve piglet development.

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Effect of vaccination with Porcilis Ileitis on productive parameters in fattening in a commercial farm.

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Introduction

Ileitis or Proliferative Enteropathy is caused by an obligate intracellular bacterium, *Lawsonia intracellularis*, and manifests clinically in several ways. The objective of this study was to evaluate the impact of vaccination with Porcilis Ileitis (containing inactivated *L. intracellularis* bacteria in XSOLVE adjuvant) on the technical and economic results in the fattening stage in a commercial farm, without restricting the use of antibiotics.

Materials and methods

The trial was conducted on a farm with 2100 sows located in the region of Antioquia (Colombia) with a history of Ileitis, diagnosed by Elisa (Svanovir® L. intracellularis/Ileitis-Ab) and clinical signs associated with intestinal hemorrhagic syndrome at the end of the fattening. For this study, 4085 piglets were selected at the beginning of the fattening phase with an average age of 75.3 days of life, which were distributed in two treatments, 1942 piglets vaccinated with Porcilis Ileitis (Treatment 1) with 25 replicates and 2143 unvaccinated piglets (Treatment 2) with 30 replicates. The Porcilis Ileitis group was vaccinated IM at 21 days of age (weaning), while the control group was not vaccinated. The environmental and management conditions were the same for both groups. The 1942 piglets vaccinated with Porcilis Ileitis were distributed in 25 groups or experimental units and the 2143 non-vaccinated piglets were distributed in 30 groups or experimental units. The total of each treatment was weighed at the beginning of the evaluation (75.3 days of life +/- 0.81 days), then it was weighed randomly, selecting 20% at 103 and 133 days of life, age at which the harvest was made or sale of pigs to the market begins. All pigs had ad libitum access to feed and water throughout the trial. Diets were formulated to be identical in all treatments, the % Crude Protein (PC) of the diets was 15.50%, 15.02 and 16.50 for the grower, fattening and finishing feeds, respectively. The fattening feed was medicated with 200 ppm tiamulin, 600 ppm chlortetracycline, 82.5 ppm methylene disalicylate bacitracin and 80 ppm halquinol. Average daily gain (ADG), feed conversion ratio (FCR), feed consumption (FC) and mortality % (M) was evaluated at 133 days of life. Initial conditions (weight and age), individual weight on day 133, and feed conversion were analyzed by analysis of variance, the animal growth (GDP) was analyzed using a mixed linear regression model, feed consumption was analyzed using multiple linear regression model mortality was analyzed using the relative risk analysis methodology. The level of significance used was 90%, that is, the results whose test yielded a P value less than 0.1 are reported as significant. All the analysis was performed in R software (1).

Results

The group vaccinated with Porcilis Ileitis obtained better results in the productive parameters compared to the non-vaccinated pigs (summary in table 1).

Table 1. Evaluated parameters

Parameter	Vaccinated group	Control group
Final Age	132,4	133,6
Final weight (kg)	100,57	98,80
ADG (grs)	1109[a]	1078
FCR	1,96	2,06
FC (kg)	124,72[a]	128,10
Mortality (%)	0,41	0,65

a: statistically significant difference

The pigs vaccinated with Porcilis Ileitis treatment presented an average weight of 1.2 kg higher than the animals of the control treatment, this difference was statistically significant (P=0.0251). Likewise, the average daily gain (+31 grs) throughout fattening was significantly higher in the vaccinated animals (P = 0.0932). The difference in feed consumption per animal was 3.37 kg lower in pigs vaccinated with Porcilis Ileitis, this difference is equivalent to a daily consumption lower by 30.7 grams per animal, this difference was not statistically significant (P=0.1569); however, it is recommended to consider its practical importance given the low P value and the apparently large magnitude of the difference and its practical importance in the cost of feeding pigs. The feed conversion ratio throughout in the evaluated period was lower in the animals vaccinated with Porcilis Ileitis treatment. The % mortality was lower in the group vaccinated with Porcilis Ileitis Vs the unvaccinated group, (0.412% Vs 0.653%), additionally and considering mortality due to intestinal hemorrhagic syndrome, the difference was also better in the vaccinated group Vs the unvaccinated group (0.103% vs. 0.280%).

Conclusion and Discussion

In this study, it was observed that that the animals vaccinated with Porcilis Ileitis achieved a better performance in the productive parameters during the fattening period. These results may be associated with the improvement of the intestinal integrity of the piglets vaccinated with Porcilis Ileitis, which allows a better utilization of the nutrients included in the diet even with the administration of antibiotics in the feed.

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Effects of acute-phase proteins after *Salmonella* Typhimurium challenge in piglets submitted to a *Salmonella* bacterin vaccination.

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Introduction

Salmonella Typhimurium recently became the most common serovar isolated from pigs in Europe, the United States, and Brazil. Its prevention is necessary since the colonization in the intestinal tissue leads to intense production losses. Acute-phase protein responses (APPs) are useful tools to evaluate the efficacy of the vaccine and to monitor the intensity of the infection injuries (1). This study aimed to compare the APPs in piglets submitted to an inactivated *S.* Typhimurium vaccine commercially available - followed by an experimental challenge with *S.* Typhimurium.

Materials and Methods

Twenty piglets were divided into two groups (n=10), as follows: (G1) were immunized subcutaneously with two doses (21-day interval) of an inactivated vaccine against swine pneumoenteritis, containing strains of *Salmonella* Choleraesuis, *Pasteurella multocida*, and *Salmonella* Typhimurium; (G2) were not immunized. All animals were challenged at 65 days of age (D0) with 5 mL of culture medium containing 10^8 CCU/mL of *S.* Typhimurium. Collection of blood samples and rectal swabs was performed before the vaccination (D42 and D21), and before the inoculation (D0) and after that, the samples were collected on D3, D6, and D9 to determine the serum concentration of total proteins and APP by SDS-PAGE assay. Furthermore, *Salmonella* excretion in feces was checked by microbiological isolation. Immediately before each sampling, the piglets will be submitted to physical examination to evaluate rectal temperature and feces consistency.

Results

Serum concentration of α1-acid-glycoprotein, ceruloplasmin and α1-antitrypsin was lower in G1 than G2, evidencing the vaccine efficacy due to stimulation of the immune system. Furthermore, the concentration of immunoglobulin A was higher in G2 than during the whole experiment, presenting statistical differences on D3. Additionally, transferrin was higher in G1 than G2, which may indicate a less intense inflammatory process after challenge. Additionally, albumin was higher in G1 at D9 and monocytes were higher in G1 at D3 and D6, which had a less severe clinical presentation of the infection.

Table 2. Mean of imunnoglobulin A (mg/dL) and transferrin (mg/dL) results on SDS-PAGE by serum samples.

	G1	G2
Immunoglobulin A (mg/dL)		
D-42	95,8 ± 25,2 [a]	134 ± 31,6 [a]
D-21	90,3 ± 18,1 [a]	107 ± 13,2 [a]
D0	65,5 ± 26,5 [a]	59,1 ± 11,8 [a]
D3	49,8 ± 19,6 [a]	83,3 ± 25,8 [b]
D6	56,9 ± 15,5 [a]	65,8 ± 15,1 [a]
D9	50,9 ± 11,7 [a]	66,8 ± 17,7 [a]
	G1	G2
Transferrin (mg/dL)		
D-42	358 ± 62,7 [a]	321 ± 33,1 [a]
D-21	371 ± 27,5 [a]	331 ± 27,6 [a]
D0	449 ± 53,1 [a]	459 ± 28,9 [a]
D3	468 ± 23,7 [a]	431 ± 23,5 [a]
D6	513 ± 28,1 [a]	480 ± 38,1 [a]
D9	576 ± 31,1 [a]	512 ± 46,5 [b]

[1]Superscripts indicate statistically significant differences within the main effect ($p \leq 0.05$).

Discussion and Conclusion

Increasing the concentration of the evaluated APPs in the serum may be related to the intensity of the immune response caused by the infection by the pathogen and/or the effect of vaccination (2).

Transferrin protein profiles between D0 and D9 were higher in animals vaccinated with the pneumoenteritis vaccine, than in the other group, which may indicate a lower intensity of the inflammatory process caused by the inoculum of *S.* Typhimurium.

Higher levels of immunoglobulin A in animals that have not received the vaccination (G2), may indicate the establishment of infection after inoculation since S. Typhimurium colonizes intestinal mucosa (3).

The inactivated vaccine was capable of decreasing the shedding and colonization of *S.* Typhimurium (4) and stimulated the innate immune system to an inflammatory response conferred by vaccine protection, minimizing the effects of infection by *S.* Typhimurium in piglets.

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Efficacy of an Oral Vaccine Against Monophasic *Salmonella* I 4,[5],12:i:-

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Introduction

The monophasic *Salmonella* I 4,[5],12:i:- serovar has emerged as the predominant *Salmonella* serovar isolated from clinical cases in the U.S. swine population, and is also prevalent worldwide (1,2). Infection can cause clinical disease in pigs as well as pose a public health threat as a human foodborne pathogen (1,3). This study had the objective of evaluating a commercial vaccine, Enterisol Salmonella T/C®, in conferring protection against monophasic *S.* I 4,[5],12:i:- as measured by clinical signs, enteric lesions and average daily weight gain.

Materials and Methods

A randomized, blinded, controlled study was conducted evaluating pigs in three different treatment groups. Each treatment group consisted of 20 pigs, blocked for the effects of litter, sex and weight. The treatment groups were: 1) non-vaccinated non-challenged (NVNC); 2) non-vaccinated challenged (NVC); 3) vaccinated with Enterisol Salmonella T/C® and challenged (EVC). Animals were challenged four weeks post vaccination at approximately 7 weeks of age with a dose of $2x10^9$ *S.* I 4,[5],12:i:- strain SX 240. This is a multidrug resistant strain of *S.* I 4,[5],12:i:- associated with a human foodborne outbreak from pork and that also causes clinical disease in pigs (3,4,5). Pigs were monitored for clinical signs, macroscopic enteric lesions and evaluated for weight gain for a period of 14 days following challenge.

Results

Following challenge, NVC animals had a significant increase in the incidence of diarrhea (59/254), compared to NVNC pigs (0/280) and EVC pigs (12/280) ($p<0.05$). This represented a significant increase of both the duration of diarrhea as well as the number of pigs with diarrhea (Table 1). One pig in the NVC group died two days following challenge and had intestinal lesions compatible with enterocolitis due to salmonellosis. At 14 days post challenge, four pigs in the NVC group had intestinal lesions while no pigs had lesions in the NVNC and EVC groups ($p<0.05$, Table 1). Average daily weight gain (ADG) was significantly decreased in the NVC group (0.543 kg) compared to the NVNC group (0.768 kg) and the EVC group (0.697 kg) ($p<0.05$, Table 1). The difference in ADG equated to vaccinates being 2.15 kg heavier than non-vaccinates over the challenge period.

Table 1. Post challenge evaluations of clinical signs, weight gain and gross lesions measured at necropsy

	NVNC	NVC	EVC
Number of pigs with diarrhea	0% (0/20)[a]	84% (16/19)[b*]	30% (6/20)[c]
Average duration of diarrhea (days)	0[a]	3.58[b]	1[c]
Number of pigs with intestinal gross lesions	0% (0/20)	22% (4/18)[*]	0% (0/20)
Average daily weight gain (kg)	0.768[a]	0.543[b]	0.697[a]

a,b,c Different superscript letters within a row indicate statistical significance ($p<0.05$). NVNC= non-vaccinated non-challenged; NVC = non-vaccinated challenged; EVC = vaccinated and challenged. *One pig died prior to challenge (not evaluated for diarrhea) andone pig died prior to study end point (only lesions measured in study end point reported).

Discussion and Conclusion

Enterisol Salmonella T/C® is a vaccine that contains a live Choleraesuis vaccine strain and a live Typhimurium vaccine strain, the latter is a serovar of the same serogroup (B) as *S.* I 4,[5],12:i:-. The results suggest that the Enterisol Salmonella T/C® vaccine conferred protection to *S.* I 4,[5],12:i:- under the conditions of this study, as measured by clinical signs, intestinal lesions and improved average daily weight gain.

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Experimental model: reproduction of erysipelas in pigs

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Introduction

The bacterium *Erysipelothrix rhusiopathiae* (ER) is endemic in swine farms. In immunosuppression situations, infection by this agent causes swine erysipelas, a systemic disease that induces significant economic damage to producers and industries, because of reproductive losses, carcass condemnation, animal deaths, drug costs and losses in zootechnical indices. The use of vaccines is one of the main tools of controlling the disease. The objective of this work was to standardize an experimental model for reproduction of erysipelas in pigs, an important method to evaluate the efficacy in future vaccine and medicines development, besides strain pathogenicity tests.

Materials and Methods

Twenty three specific pathogen-free pigs, negative for ER by PCR (2) and for anti-ER antibodies by Elisa (CIVTEST® SUIS SE/MR, HIPRA), were divided into: T1) 8 pigs challenged at 70 days of age with 10^8 Colony Forming Units (CFU) of ER per pig; T2) 8 challenged at 70 days with 10^7 UFC; T3) 1 pig inoculated with Feist broth at 70 days (negative control); T4) 6 challenged at 90 days with 10^7 UFC. The challenge was performed via dorsal intradermal injection, after shaving, with 0.1mL of cultured strain BRMSA 0558, from Embrapa's Collection of Microorganisms of Interest for Swine and Poultry (CMISEA). The pigs were then evaluated daily for survival, rectal temperature, diameter of skin erythema at the inoculation site and presence of disseminated lesions, for a period of 4 days for those challenged at 90 days of age and 7 days for those challenged at 70 days. Afterwards, they were euthanized by electrocution followed by bleeding and then necropsied for macroscopic evaluation and samples collection for laboratory tests aiming the isolation and characterization of ER (1, 2, 3) and for histopathology analysis and ER detection by immunohistochemistry (IHQ). Animals that were suffering were immediately euthanized.

Results

There was no natural death of any pig. The unchallenged pig (T3) did not show any alteration. It was possible to observe clinical signs and lesions in more than 80% of the challenged pigs. The skin lesions started with erythema at the inoculation site 1 day post inoculation (dpi); increase in lesion diameter by 2 dpi; disseminated multifocal lesions between 3 and 6 dpi and at 7 dpi the lesions became less evident.

In challenged pigs there was reisolation of the ER BRMSA 0558 strain from the skin culture (100%), feces (95.5%), blood and liver (9%) and from the spleen (4.5%). All pigs had histopathological skin lesions compatible with ER infection, which ranged from areas with mild to moderate inflammatory infiltrate in the dermis and subcutaneous tissue, hyperemia of the dermis and subcutaneous tissue, foci of hemorrhage, fibrin thrombi, and areas of necrosis in the dermis and epidermis. In some challenged pigs, discrete alterations compatible with septicemia were observed in the spleens and livers, such as mild to moderate infiltration of neutrophils in the red pulp and mild leukocytosis in the sinusoids, respectively. The IHC for ER of the skin samples was positive in 70% of the challenged pigs and the most evident staining was observed in the samples collected before 7 dpi, results are presented in Table 1.

Table 1. Percentage of pigs with clinical signs, lesions and positive laboratory assay for ER

Treatment	T1	T2	T3	T4
Number of Animals	8	8	1	6
Elevated T°C* (%)	87,5	87,5	0	83,3
Focal skin lesion (%)**	87,5	87,5	0	100
Disseminated skin lesion (%)	75	87,5	0	83,3
Skin - ER isolation (%)	100	100	0	100
Skin - ER compatible histopathology (%)	100	100	0	100
Skin - positive IHQ (%)	75	37,5	0	100

*Temperature above 40.5 on at least one day post challenge;
**At the inoculum injection site;

Discussion and Conclusion

The proposed experimental model allowed the reproduction of the disease in pigs without corticosteroid treatment, at different challenge ages tested and in the two concentrations of inoculum studied,being able to be used for tests of vaccine protection, medicines efficacy and strain pathogenicity.

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Field data of use of Porcilis Lawsonia IM and ID vaccination on a Dutch closed sow herd

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Introduction

Ileitis is an old disease in pigs but the Lawsonia intracellularis bacterium is still causing significant economic damage, with often no obvious clinical symptoms on the farms. Several controlling interventions are available for the veterinarian and farmer, like antibiotics, feed additives and a live oral vaccine. Recently in Europe a new killed vaccine against Lawsonia intracellularis was introduced (1). The case study describes the technical performance before and after the usage of Porcilis Lawsonia under Dutch field conditions on a closed Dutch pig farm.

Material and Methods

The 220 closed sow herd with 1600 finishing places in The Netherlands had a history for years of an oral Lawsonia vaccine administered via the water at the start of finishing phase to control the Ileitis in the finishers. By time, the farmer still needed tylosin for aweek to diminish further clinical symptoms due to acute losses to Ileitis.

In November 2019, the farmer started at 12 weeks of age with Porcilis Lawsonia © (PL) (MSD Animal Health) by intramuscular injection, and going back till the 3 weeks of age vaccination. The farmer switched to Porcilis Lawsonia ID with the IDAL device at 3 weeks, by dissolving the dry lyophilized powder of 50dose Porcilis Lawsonia in the 50 dose bottle Porcilis PCV ID.

The monthly technical results like ADG, FCR, and mortality before – after were primary parameters used for evaluation, and antibiotic use, defined by DDD (3). The observed period was one year before the first Porcilis Lawsonia vaccinated pigs were slaughtered versus one year after start. Statistical analysis was done by 2-sample t-Tests, with Minitab. Due to seasonality influences, the same corresponding months were compared before after.

Results

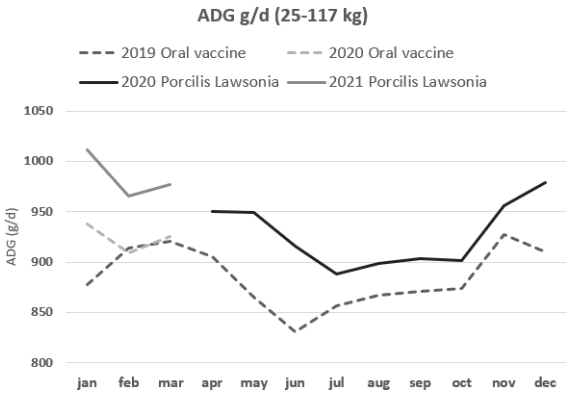
Since the start of Porcilis Lawsonia vaccinated pigs were slaughtered, on a whole year base before- after the ADG improved by +51 gr/day (p=0.002), FCR by – 0.08 (p=0.035) and mortality by 0.4% (p=0.16) (table and graph 1). Also, the antibiotic usage was lowered by 90 %: 12,9 DDD (2019) to 1,2 DDD (2020).

Table 1: technical results whole year Oral vaccine protocol vs whole year PL vaccinated pigs slaughtered

	Oral vaccin	Porcilis Lawsonia	difference
ADG[1]	890	941	+51*
FCR[1]	2,77	2,69	-0,08*
mortality % [2]	2,2	1,8	-0,4

[1] april'19-mar'20 vs april'20-mar'21 *p<0,05
[2] jan'19-jan'20- vs feb'20-mar'21

Graph 1: ADG figures per month from January 2019 till March 2021, for different vaccination protocols



The first Porcilis Lawsonia IM vaccinated pigs were slaughtered mid-march 2020. Comparing the corresponding months of the oral drinking water period vs IM period (April '19 – Aug'19 vs April'20 -Aug'20) the ADG were 865 vs 920 gr/day (p<0,05); FCR 2,75 vs 2,68 (p>0,05) and mortality 2,3 vs 1,9 %(p>0,05). Comparing the corresponding months of the oral drinking water period vs ID period the ADG were908 vs 956 gr/day (p<0,05); FCR 2,79 vs 2,70 (p>0,05) and mortality 2,1 vs 1.7 % (p>0,05).

Discussion and conclusion

This case report shows the results of the successful implementation of the new killed IM and ID Lawsonia vaccine under field conditions a whole year around. The results are in line with other side by side results (3). The gross margin for this farm is estimated on + €3,70 vs the old vaccine protocol, based on the improved technical parameters (this is excluding antibiotics).

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Genetic identification of Shiga toxin 2e produced by different Shiga toxin-producing *Escherichia coli* strains in Korea

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Introduction

Porcine Edema disease (ED) is caused by Shiga toxin-producing *Escherichia coli* (STEC) strains which produce Shiga-toxin 2e (Stx2e) and may possess F18 fimbrial unit. Following intestinal colonization of STEC, Stx2e enters the systemic circulation and causes vascular necrosis of arterioles in the brain and gastrointestinal tract. The clinical severity of ED varies depending on the farm, as some affected farms show typical clinical signs, whereas others show subclinical symptoms. Although STEC strains producing typical ED clinical signs are known to express identical Stx2e regardless of serotypes (1), the genomic characteristics of Stx2e by different clinical manifestations are not yet identified. In this study, Korean STEC strains were isolated from pigs with classical or subclinical ED, and the full Stx2e nucleotide sequence of each strain was analyzed to investigate if the genomic characteristics of Stx2e are responsible for the different severities of ED.

Materials and Methods

A total of 57 diarrheic samples of 40 to 100 days-old weaning piglets were collected from 9 conventional pig farms in different regions of South Korea; 11 were from piglets showing the typical ED clinical signs and 46 were from unthrifty piglets. The samples were individually streaked on a MacConkey agar, incubated overnight, and five susceptible colonies in each plate were tested of 13 virulence factors (toxins, LT/STa/STb/Stx2e/EAST1; adhesins, F4/F5/F6/F18/F41/paa/AIDA-I/EAE) by polymerase chain reaction. The virulence gene combination was determined, and the entire Stx2e domain of STEC strains was amplified by using gene-specific primers. The individual amplicons were sequenced by the Sanger's method, and the amino acid sequence identities were estimated in BioEdit 7.2.5.

Results

The detection rate of Stx2e gene was 37% (21/57), and STEC strains were grouped into 3 groups (Table 1): **1)** STEC without fimbriae (12/21), **2)** STEC producing Stx2e and F18 (6/21), **3)** STEC producing enterotoxins, Stx2e and F18 (3/21). While the STEC strains isolated from pigs with typical ED clinical signs all belonged to Group 2, the strains isolated from pigs with subclinical ED belonged to Group 1 or 3. Interestingly, all STEC strains shared 100% of amino acid sequence identity of Stx2e regardless of groups and virulence factor combinations (Table 2).

Table1. Virulence factor combination of Korean Shiga toxin-producing *Escherichia coli* strains

Group	Toxins	Adhesins	Clinical manifestations
1 (n=13)	Stx2e Stx2e:STb	- -	SED
2 (n=6)	Stx2e Stx2e	F18:AIDA F18:AIDA:paa	ED
3 (n=2)	Stx2e:LT:STa Stx2e:STa:STb :EAST1	F18 F18:EAE:paa	SED

ED, clinical edema disease; SED, subclinical edema disease

Table2. Pairwise amino acid sequence comparison between Shiga-toxin 2e domain of Korean Shiga toxin-producing *Escherichia coli* strains

	Sequence identity (%) of Stx2e domain between STEC groups		
	Group 1	Group 2	Group 3
Group 1	ID	100.0	100.0
Group 2		ID	100.0
Group 3			ID

Stx2e, Shiga toxin 2e; STEC, Shiga toxin-producing *Escherichia coli*; ID, identical

Conclusions and Discussion

Porcine ED has been widespread in the swine industry, presenting diverse clinical manifestations depending on the affected pig farm. In Korea, Stx2e was frequently (37%) detected from weaning piglets, and all the STECs causing typical ED clinical signs (sudden death/palpebral edema/ neurological signs) presented only Stx2e without enterotoxins and F18 and AIDA for adhesins, while the strains causing subclinical ED (diarrheic/wasting) showed various virulence gene profiles. To our knowledge, the severity of ED does not correlate to the genomic characteristics of Stx2e since all STEC strains possess identical Stx2e amino acid sequences. The establishment of risk assessment criteria for the clinical severity of ED by farm levels is necessary for prevention of acute ED outbreaks, and the classification system for STEC strains seem to be a potential index for the risk assessment.

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Global mass screening on sub-optimal farms reveals a high risk of Oedema Disease

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Introduction

Oedema Disease (OD) is a major problem in pig production worldwide, causing mortality and impaired productivity. Two main presentations of this disease have been described, the clinical form, with typical symptoms such as swelling of the eyelids, and acute outbreaks with high mortality; or the subclinical form, without apparent clinical signs but with performance parameters severely affected[1]. The aim of this study was to evaluate the presence of the gene coding for verotoxin on farms where OD was suspected in a selection of countries worldwide.

Materials and Methods

For the analytical procedure, oral fluid samples were obtained from 3 to 5 different pens on 1304 farms, from animals that were 6 to 16 weeks old. These oral fluids were then transferred to FTA cards and samples were sent to HIPRA DIAGNOS (Spain) for performance of a qPCR analysis targeting the gene coding for the verotoxin (Vt2e)[2]. A farm was considered positive when at least one of the samples was positive.

Results

The mean prevalence of positive farms on a global basis was reported to be 62% positive (809 out of 1304 farms). When assessing the prevalence by region, we observed a high prevalence in all of them, led by LATAM countries (72% positive; 178 out of 247 farms), followed by Europe + Canada (60% positive; 608 out of 1014 farms) and Asia (59% positive; 261 out of 443 farms). When analyzing the data by country, excluding countries with a very limited number of samples (fewer than 5 samples), the top 5 countries with the highest prevalence were Brazil (85% out of 84 farms), Ireland (79% out of 38 farms), Poland (76% out of 38 farms), Mexico (76% out of 79 farms) and Argentina (76% out of 41 farms). On the other hand, the lowest prevalence was observed in Peru (14% out of 7 farms), Russia (33% out of 104 farms), Italy (36% out of 72 farms), Portugal(45% out of 40 farms) and Colombia (46% out of 35 farms). See figure 1.

Conclusions and Discussion

The results obtained are similar to those contained in a previously published systematic literature review, in which the Vt2e gene prevalence was estimated to be between 30 and 70% depending on the country and health status (reported as healthy or unhealthy)[3]. These data may be particularly challenging in European countries, which have now drastically reduced their reliance on antimicrobial use in the nursery, often relying on therapeutic high levels of zinc oxide[4], and which need an alternative strategy in place by the deadline in 2022.

Figure 1.Percentage positivity in the different countries. Number in brackets indicates the farms tested.

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Identification and elimination of Swine Dysentery from a herd in a large production system

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Introduction

Swine Dysentery (SD) is classically caused by *Brachyspira hyodysentery* or other emerging brachyspira such as *Brachyspira var. hampsonii*. In the severe form it causes frank blood and mucus in diarrhea pigs 10 weeks or older[1]. Reports of SD in the United States and Canada have increased in the last decade.[1,2,3] The long term costs and biosecurity threat of swine dysentery to large production systems makes eradication a viable option.[2,3,4] Long term control of SD has led to the evolution of multidrug resistant brachyspria.[1] Elimination programs using medication, sanitation with white wash, strict biosecurity, and rodent control are highly successful.[1,4,5] This case report details the identification and elimination of SD in a herd within a large production system.

Materials and Methods

The first indication of SD were production staff reports of higher than normal loose stools in a multisource pig flow. These quickly escalated to mucohemorrhagic stools at this site. Fecal samples were positive by PCR for *nox* and *tlyA* hemolysin genes were in this finishing sites[1]. As no clinical signs were noted in the 8 sow farms supplying pigs to the multisourced pig flow, a focused fecal collection was scheduled. Eight sow farms were tested focusing on sows with loose stool and young parity sows. 30 samples were collected and pooled by 5 for anaerobic culture.

One 35000 head four barn site in a low pig dense was diagnosed as SD positive via fecal PCR as described above. One 2500 head breed to wean sow farm was identified infected visa testing.

Because of the potential spread and estimated economic impact of $10 to $12 (USD)[2,3,4] per finishing pig, the following interventions were considered: pig flow segregation, sow herd depopulation and medical elimination were evaluated. Segregation would significantly slow the wean to finish barn and site fill time because of the large numbers of pigs required. Depopulation cost and pig flow impacts were negative considerations. Although Medical eliminationrequires extensive cleaning coordinated during medication, the disruption to pig production and flow was deemed comparatively minimal. A medical elimination was designed using cleaning with lime wash and Denagard® (tiamulin hydrogen fumarate)[1] in a high/low modality [200g/ton (220ppm) for 2 weeks followed by 35g/ton (38 ppm) for 2 weeks] to the entire breeding herd. Extensive cleaning and medication continued for 10 weeks in the lactation barns as sows were weaned. Personnel movements during cleaning and medication, reduction in inventory, medication logistics, cleaning logistics and lime wash were planned and implemented.

Results

After the detailed medication and cleaning program was completed, known SD negative gilts were entered and comingled with resident sows. No loose stools were noted or collected in “sentinel” gilts post entry. Two weeks after entry and at two-week intervals for a total of 4 collections (30 fecal samples per collection in pools of 5) were randomly collected in late gestation. PCR and anaerobic culture were performed, and no samples were detected with *B. hyodysenteriae* or *B.var hampsonii*. Ongoing clinical and diagnostic investigations have not identified either agent after seven months post elimination.

Conclusions and Discussion

Medical elimination costs were estimated at $101,750 (USD) for the elimination plan. An SD infected pig flow cost estimated on a 28 pig/sow/year basis is $700,000 (USD) per annum. The return was estimated to be 1.75 months. With swift identification, evaluation of options and implementation of a solution,this large integrated pig production system has limited and eliminate a costly disease within the production system. This case report exempifies active surveillance and swift action of the veterinary and production staff to mimimized this costly disease.

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Identification of extracellular vesicles from type strain and contemporary clinical isolate of *Mycoplasma hyopneumoniae*

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Introduction

In the last decade, researches in extracellular vesicles (EVs) from cells of the three domains of life has become an explosion of interest (1). Gram-negatives EVs are referred to as outer membrane vesicles (OMVs) because of LPS-containing outer membrane. It has been identified a lack of studies in Gram-positive compared to Gram-negative bacteria, due its lower yield and release performance of EVs (2). In absence of an outer membrane, Mollicutes class is phylogenetically related to a Gram-positive ancestor (Clostridium spp). These microorganisms are wall-less with a cholesterol-rich membrane, and due to the lack of a cell wall physical barrier, the EVs production is expected (3, 4, 5). In this work, we showed for the first time EVs from *Mycoplasma hyopneumoniae* (*Mhy*) type strain and at a clinical isolate under stress cultivation conditions.

Materials and Methods (Clinical strains)

Strains and culture

Mycoplasma strains used in this study were stype strain J (NC_007295.1) and a clinical isolate (SAMN11634267). Briefly, cells were harvested by differential centrifugation (dC) washes and supernatant harvest at 500 x g for 20min (4°C) followed by 3.000 x g for 20min (4 °C). Then, supernatants were filtered in 0.45 µm PVDF membranes. The filtrates were centrifuged again at 11.000 x g for 20 min (4 °C). The pellets obtained after the last centrifugation were fixed as described posteriorly. The ultracentrifugations were carried at 100,000 x g and at 250.000 x g per 3h at 4°C (SW 70 Ti rotor). Fractions of 1 mL were collected from the top of the tube to recover the fractions containing EVs. Each fraction was centrifuged again at 11.000 x g for 20 min (4 °C) to constitute the pellets.

Transmission electron microscopy (TEM)

All pellets obtained in dGU were fixed, postfixed, dehydrated and infiltrated with epoxy resin for ultramicrotome cutting and the grids were counterstained, according to usual techniques in electron microscopy. The grids were observed under a TEM (Zeiss Transmission Electron Microscope, EM 109).

Results

It was possible to detect EVs of size ranged from 200 to 500 nm in both isolates from both growth conditions.

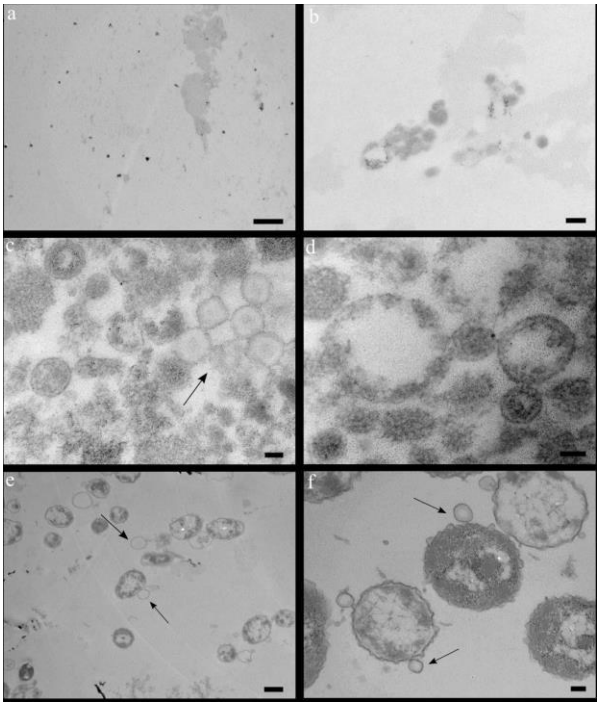

Figure 1. A, B: control of EV purification technique, 10 and 100 mL Friis medium non-inoculated, after early purification steps. Bar 500. C: 100 mL Friis medium inoculated with type strain and clinical isolate under normal growth condition. Bar 500 nm. E, F: 10 mL Friis medium inoculated with type strain under oxidative stress, separated only by differential centrifugation (dC). Bar 100 nm.

Discussion and Conclusion

Early purification steps were enough to remove large particles, and constituents of the medium from both experiments, suggesting that large EVs from eukaryotes were not determinant for sample differentiation. The filtration process based on differential centrifugation, allows larger vesicles to be selected. Especially for clinical isolate the production of EVs can contribute to microbial survival or competition. This study demonstrated for the first time that *Mhy* type strain and a clinical isolate have the ability of producer EVs.

Acknowledgments

Núcleo de Microscopia Eletrônica e Microanálise

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Immunoglobulin status and bacterial tonsillar load in *S. suis* naturally affected piglets

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Introduction

Streptococcus suis (*S. suis*) disease in pigs is endemic and zoonotic while still has gaps knowledge remain. Most common *S. suis* serotypes (SS) causing streptococcal disease in Europe are SS2 and SS9 (1, 2). Passive maternal immunity may protect progeny, but results are controversial (1). Clearance of *S. suis* maternal antibodies occurs about 18 days of life, temporarily exposing vulnerable piglets after weaning (3). Furthermore, information about antibody status during natural infections is scarce. Based on clinical signs and outbreak diagnoses, we studied piglets suspected of *S. suis* disease and compared these with randomly selected healthy pen-mates (control) for tonsillar load of total *S. suis*, SS2 (and/or SS1/2) and SS9, and total Ig, IgM and IgG2 levels reactive to three *S. suis* isolates.

Materials and Methods

A total of 56 piglets were sampled (blood and tonsillar swab) across three main outbreaks, comprising 28 control and 28 piglets with suspected *S. suis* infection, including 20 with severe neurological signs. Diagnosis was not done case-by-case, but outbreaks and deaths were confirmed to be caused by *S. suis*. Three isolates were collected from meninges and heart valves from infected pigs as two SS2 and one SS19. These were used to develop an ELISA to quantify reactive total Ig, IgM and IgG2 in sera (3). DNA from tonsillar swabs were analyzed by qPCR for total bacteria, total *S. suis*, and SS2 (and/or 1/2) and SS9. Regression models (Proc Mixed) were performed to study the effect of severe disease in total Ig, IgG2 and IgM reactive to *S. suis* isolates (log2 transformed). The model was: Y = Intercept + beta*Sickness + beta*Time + beta*Sickness*Time + $E_{REP\ (repeated\ measures\ pig)}$ + $E_{RA\ (random\ statement\ =\ isolate)}$ + $E_{R\ (residuals)}$. Proc GLIMMIX with normal (most variables) or binomial (prevalence) distribution were used to compare diseased and control pigs. Relative abundance analysis as total *S. suis* in total bacteria or serotype groups in total *S. suis* were compared with PROC NPAR1WAY and Wilcoxon two-sample test. All analyses were conducted using SAS v9.4 (SAS Inst. Inc., Cary, NC).

Results

Prevalence of SS2 (and/or SS1/2) in sick pigs tended to be increased (72%; P = 0.083), and for severely sick pigs, were significantly increased (81%; P = 0.039) compared to controls (44%). In addition, SS9 load was significantly higher in severely sick pigs relative to controls (P = 0.008). As relative abundance, sick pigs significantly lowered (P = 0.03) total *S. suis* compared to controls. Otherwise, relative abundance of SS2 (and/or SS1/2) tended to increase in severely sick pigs (P = 0.086). Total Ig significantly increased with time (P = 0.017) but did not increase with sickness (P = 0.101). IgM significantly increased with time (P < 0.001) and with sickness severity (P = 0.025), but an interaction between time and sickness indicated a lower slope for severely sick pigs (P = 0.038). IgG2 were not affected by time but were higher in control than in severely sick pigs (P = 0.027).

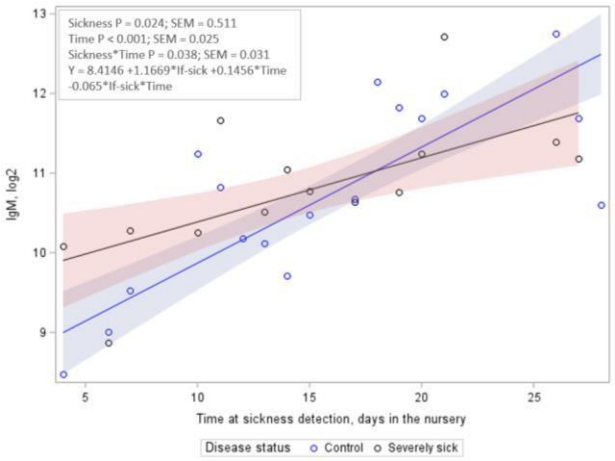

Figure 1. IgM levels[1] in pigs severely sick (n = 20), or control pen-mates (n = 28) at different times post-weaning. [1]Light red or blue areas indicates 95% confidence interval. Points indicate means for "n" cases on a same age for three isolates.

Discussion and Conclusion

The higher levels of IgM in severely sick piglets and their lower slope over time post weaning, suggests these pigs had an earlier contact with *S. suis* than healthy pen-mates. However, lower levels of IgG2 in severely sick piglets suggests they were more susceptible. Increased prevalence of SS2 (and/or 1/2) suggest that most cases were caused by the isolated SS2. In conclusion, meningitis in naturally infected piglets was associated with higher and earlier *S. suis* reactive IgM, lower IgG2, increased SS2 (and/or 1/2) prevalence and relative abundance, and increased SS9 in tonsils, but reduced relative abundance of total *S. suis*.

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Impact on the incidence of virulence factors of enterotoxigenic *Escherichia coli* in pigs after restrictions in the use of antimicrobials in the United States

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Introduction

Enterotoxigenic *Escherichia coli* (ETEC) is a commensal enteric bacterium, but it also has the ability to express virulence factors (i.e. fimbriae and enterotoxins) causing severe diarrhea in pigs, humans and other mammals. The main pathotypes associated with colibacillosis in pigs are: ETEC (neonatal diarrhea); EPEC and ETEC (post-weaning diarrhea) and STEC (edema disease) (1). Polymerase chain reaction (PCR) has been applied to identify virulence genes and to characterize its virulence factors. In addition, *E. Coli*is a prominent pathogen in the scenario of antimicrobial resistance (AMR). Factors such as the acquisition and horizontal transfer of resistance genes and the increase of multidrug-resistant strains have raised public health concerns (2). As a result of the AMR warning, several changes in the use of antimicrobials have been made, pointing to rational use and banning of growthpromoters in animal production. In the US, the FDA's publication of #GFI 213 (in January 2017), restricted the veterinary use of important antimicrobial bases in order to minimize the risks in public health scenario (3). Therefore, the aim of this study was to compare the virulence factor profile of *Escherichia coli* samplesfrom piglets before and after the establishment of #GFI213 to comprehend its impact in the swine industry.

Materials and Methods

For this study, a database containing 3,158 *Escherichia coli* isolates from piglets with enteric symptoms were analyzed. Those samples were received at the Veterinary Diagnostic Laboratory (VDL), University of Minnesota between 2013 to 2020. Initially, bacteriological isolation was performed, and then a multiplex PCR was done to characterize the following virulence factors: fimbriae (F18, F4, F41, F5, F6), AIDA, paa, eae and the toxins EAST-1, LT, Sta, Stb and Stx2e.

Results

The main differences observed in the panel of virulence factors during the period of analysis were an increase in the occurrence of F18+ strains and reduction of F4+, after 2017. In addition, the F18+ genotype showed an increase in the expression of Stx2e toxin, with 27.3% of the F18+ samples expressing the gene of this toxin during 2013-2017 and 40.0% in the period of 2017-2020. Also, the percentage of F18+ strains producing the four toxins concurrently has increased, going from 0.8% during 2013-2017 and achieving 24.0% in the period between 2017-2020.

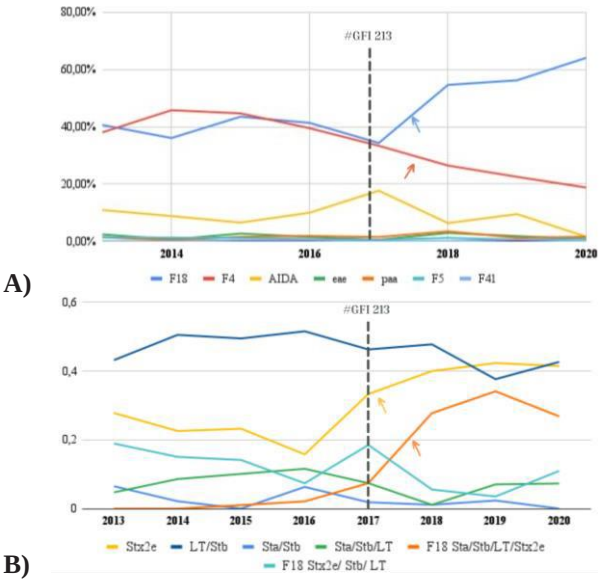

Figure: A) Occurrence of adhesion-related genes of *E. coli* (2013-2020) **B)** Genotype of *E. coli* F18+ associated with toxin-related genes (2013-2020).

Discussion and Conclusion

The present study describes important changes in the incidence of virulence-related genes after regulatory changes in the use of antimicrobial in the swine industry. Identification of a high occurrence of F18+ genotype producing multiple toxins, most importantly including Stx2e, was a significant finding. The existence of Stx2e-producing strains of ETEC is challenging for the swine industry, both related to the possibility of concomitant development of clinical signs of diarrhea and edema disease in those piglets (1), and because of the identification of multidrug-resistant strains of this genotype (4). The fact that the F18 gene and others virulence factors are located in the plasmid raises the possibility of horizontal transfer of these genes between strains (4). Finally, a more comprehensive analysis such as genome sequencing canbe explored to identify the genomic changes on ETEC
E. coli and its impact on the virulence and AMR profile in pigs.

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Incidence of *Salmonella enterica* serovars: an evaluation of cases

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Introduction

The presence of *Salmonella* in swine production systems has been a worldwide concern, both for reasons related to public health and for differences resulting from the presence of this microorganism in the products (Spricigo et al., 2008). This agent causes salmonellosis, one of the most important enteric diseases in swine (Kim and Isaacson, 2017). *Salmonella* serovar Typhmurium has been prevalent in Brazil (Guerra -Filho et al., 2016; Dos Santos Bersot et al, 2019), followed by serovar Choleraesuis (Meneguzzi et al., 2017).

To understand the epidemiology of this disease, the phenotypic investigation of strains present in different areas can provide important information. In Brazil, there are few modified data for the characterization of clinical isolates of *Salmonella* (Meneguzzi et al., 201). Therefore, the objective of this study was to evaluate the presence of less recurrent *S. enterica* serovars in Brazil in samples evaluated by the Microvet Diagnostic Laboratory.

Material and Methods

Samples of pigs with suspected clinical signs of infection by *S. enterica*, from different regions of the Brazil, were evaluated by the MV Diagnostic Laboratory.

Isolation of the strains of interest was performed from a diverse samples: intestine, liver, lymph node, lung and feces. Bacteriological examination was performed to select colonies with suggestive morphology of *S. enterica*. After identification, a serotyping test was performed to determine the serovars in the samples. After confirmation, colonies of *S. enterica* were submitted to MALDI-TOF to identify the protein profile of the strains and finally, the samples were submitted to next generation sequencing (NSG). Porechop and Minimap2 softwares were used for sequence trimming and mapping, respectively with reference genomes. Raw sequencing reads of the isolates were used for serotype prediction by Seqsero2.

Results and Discussion

The incidence of atypical Salmonella's serovars from different regions of the Brazil evaluated in the present study is presented in Figure 1. The results showed that the higher frequencie of *Salmonella* spp. was Bredeney (40%) followed by Panama (20%), Infantis and London (15%), and Derby (10%).

The occurrence of the serovars *Salmonella* spp. according to the swine's age and organs by type of serovar are available in Table 1. The lymph nodes was the major reservoirs of *Salmoenlla* Bredeney and Infantis.

The *Salmonella* serovars analyzed in the present work were observed more frequently between 50 and 99 days, except serovar London, which was present only in the first days of the animal's life (Table 2).

In Brazil, several studies show that the prevalence of these atypical Salmonella serovars are those reported in the present work (Weiis et al., 2022; Kich et al., 2011).

The serovar Bredeney was one of the most frequently found in mesentetic lymph nodes in pigs from farms in southern Brazil (Weiss et al., 2022), which corroborates with the present work. The London serovar was also observed in the termination phase, which was not observed in the present work (Weiss et al., 2002). Kich et al (2011) also observed a higher prevalence of atypical *Salmonella* Panama and Derby serovars in pig mesenteric lymph nodes in the slaughterhouse installation phase.

Conclusions

The Bredeney serovar had the highest incidence in mesenteric lymph nodes in swine from farms in southern Brazil based on the cases analyzed. Animals between 50 and 99 days had the highest rate of infection by *Salmonella* Bredeney, Derby, Infantis, and Panama, while the serovar Londos was observed in animals between 0 and 49 days.

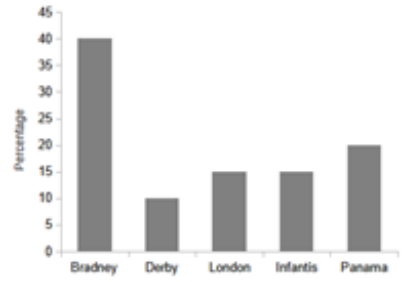

Figure 1: Incidence percentage of atypical *S. enterica* serovars, from different regions of the Brazil.

Table 1: Frequency of *S. enterica* serovars according to serovars and origin of the isolate samples.

Serovars	Isolation				
	Intestine	Liver	Lynph node	Lung	Feces
Bredeney		1	4	2	1
Derby			2		
London	2		1		
Infantis			3		
Panama	1	1	1	1	

Table 2: Frequency of *Salmonella* spp. strains along the pig production.

Serovars	Age (days)			
	0 - 49	50 - 99	100 - 150	Uninformed
Bredeney		5	3	
Derby		2		
London	3			
Infantis		1		2
Panama		1	3	

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Index of pneumonia, microbiological identification and microscopic lesions from pig's lungs with macroscopic lesions at slaughter age

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Introduction

Several bacterial and viral pathogens can be involved in the porcine respiratory complex diseases (PRCD) and the knowledge of the distribution of those agents are crucial for the successful implementation of control programs, and the reduction of direct and indirect losses as such the necessity of antibiotic treatments, increase of mortality, weight lost and carcass convictions (1). Therefore, the objective of this study was to determine which pathogens are involved in the lungs with macroscopic lesions at slaughter age in three samples, and the impact of those pathogens in the index of pneumonia (IP) and the correlated microscopic lesions.

Materials and Methods

At the slaughterhouse, 589 lungs from three collections days, were inspected for macroscopic lesions and the index of pneumonia it was determined. From those, 162 lungs were collected for microscopic evaluation, microbiological analyses and PCR analysis.

Results

The index of pneumonia is a tool widely used for the determination of the general wealth of the lung and helps to establish a guideline for the actions in the pig farming, were indexes higher than 0.9 is strongly associate with pneumonia (2). On this study, the higher IP found was 0.64 with some animal scored in the category 2 to 5 (Table 1).

Table 1: Macroscopy evaluation and index of pneumonia (IP) from pig's lung at slaughter age.

Evaluated parameter	Sample 1[1]	Sample 2	Sample 3
Number of pigs	195	186	208
Lungs with consolidation	99	87	85
% consolidation area	50.7	47.0	40.8
General IP	0.51	0.,47	0,64
IP category 0[2]	96	99	123
IP category 1	99	87	60
IP category 2	0	0	11
IP category 3	0	0	6
IP category 4	0	0	7
IP category 5	0	0	1
% of pulmonary adherence	0	2,7	1,44

[1]Samples collected on 05/05/2018; 09/17/2018 and 06/25/2019. [2]IP 0 = 0% of hepatization; IP 1 = 0,1-11% of hepatization; IP 2 = 11,1-21% of hepatization; IP 3 = 21,1-31% of hepatization; IP 4 = 31,1-41% of hepatization and IP 5 = 41,1-51% of hepatization.

Whereas in 20.9% of lungs were identified a single infection, in 76.5% a coinfection of two to four different agents was identified (Table 2). *M. hyopneumoniae*, *P. multocida* type A and PCV-2 was the most frequen agents detected both in single infections and i coinfection.

Table 2: Isolations and microbiological characterizatio of the agents found in the pig's lung with macroscopi lesions at slaughter age.

Microbiological agent	Single infectior	Co-infections				
		Mhyo	PCV-2	*P. multocida*	Mhyo + PCV2	PCV-2 + GPS
APP	-	-	-	-	-	-
AAA	-	1	10	-	8	1
E. coli	-	4	-	-	1	-
GPS	1	1	1	1	4	-
Klebsiela sp.	-	-	-	-	-	-
M. haemolytica	-	-	4	-	1	-
Mhyo	3	-	-	-	-	-
P. multocida	10	7	8	-	26	-
PCV-2	16	20	-	-	-	-
S. suis	-	4	3	1	7	-
T. pyogenes	-	-	-	-	1	-
Negative	4	-	-	-	-	-

*coinfection of four agents are not shown. *APP* - *Actinobacillu pleuropneumoniae*; *A. suis* - *Actinobacillus suis*; *E. coli* - *Escherichi* coli; GPS - *Glaesserella parasuis*; *M. haemolytica* - *Mannheimi haemolytica*; *M. hyo* - *Mycoplasma hyopneumoniae*; *P. multocida* *pasteurella multocida*; PCV-2 – *porcine circovirus 2*; S. suis *Streptococcus suis*; *T. pyogenes* - *Trueperella pyogenes*.

Discussion and Conclusion

The higher IP was 0.64, and this sample shows som animals with IP categorization of 2, 3 and 4, as well som animals with pulmonary adherence. Even though we di not have IP values that could be classified as acceptable we found microscopic lesions in all the samples teste and in nearly all samples at least one microbiologica agent involved. Therefore, the slaughter evaluations ar an important tool that can be used to get mor information and correlated with the historic on life fron those pigs. The type of microbiota found in the literatur can be very diverse, *P. multocida* (3) and *M hyopneumoniae* (4) are common agents to be found in th lungs. All that information can be used in the decision making of procedures changing and adjustment of th vaccination's protocols.

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Influence of parity and reproductive stage on the prevalence of *Mycoplasma hyopneumoniae* in breeding animals

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Introduction

Mycoplasma hyopneumoniae (*M. hyopneumoniae*) isthe primary agent of enzootic pneumonia, a chronic respiratory disease in pigs that causes significant economic losses (1). Infection occurs often early in life as dam-to-piglet transmission plays an important role (2). Previous studies investigated the (sero)prevalence of *M. hyopneumoniae* in breeding animals and the presence of the pathogen in replacement gilts (3,4). The objective of this study was to investigate the prevalence of *M. hyopneumoniae* in gilts and sows in different stages of the reproductive cycle.

Materials and Methods

On ten commercial Belgian farrow-to-finish farms a cross-sectional sampling was performed in the breeding animals. All animals were group housed from at least four weeks of gestation onwards till one week before farrowing, gilts were vaccinated on nine farms against *M. hyopneumoniae* prior to or in the quarantine unit and sows were not vaccinated.

Tracheobronchial swabs (TBS) and blood (serum) were taken from 80 animals on each farm (n=800). At four different time points in the reproductive cycle i.e. 30-40 and 75-85 days of gestation, 3-5 days post farrowing and 1-3 days post weaning, ten sows and ten gilts (if possible) were sampled.

TBS were analyzed for the presence of *M. hyopneumoniae* DNA with a nested PCR (5). Serum samples were analyzed for the presence of *M. hyopneumoniae*-specific antibodies using a commercial ELISA (*M. hyo* Ab test, IDEXX Laboratories Inc., Westbrook, ME, USA).

A generalized linear mixed model with farm as random factor was used to test the associations of *M. hyopneumoniae* prevalence with time point in the reproductive cycle and parity. IBM SPSS statistics 26.0 was used.

Results

M. hyopneumoniae DNA was detected in 26.5% of the gilts (91/344) and in 10.7% of the sows (49/456). At farm level *M. hyopneumoniae* prevalence ranged between 0-62.5% and 0-37.5% for gilts and sows, respectively (Fig. 1A). Significantly more gilts were *M. hyopneumoniae* positive than 2-4th parity sows (P=0.024) and >4th parity sows (P=0.019). At 30-40 days of gestation significantly more breeding animals were positive as compared to 75-85 days of gestation (P=0.035), 3-5 days post farrowing (P=0.016) and 1-3 days post weaning (P=0.02) (Fig. 1B).

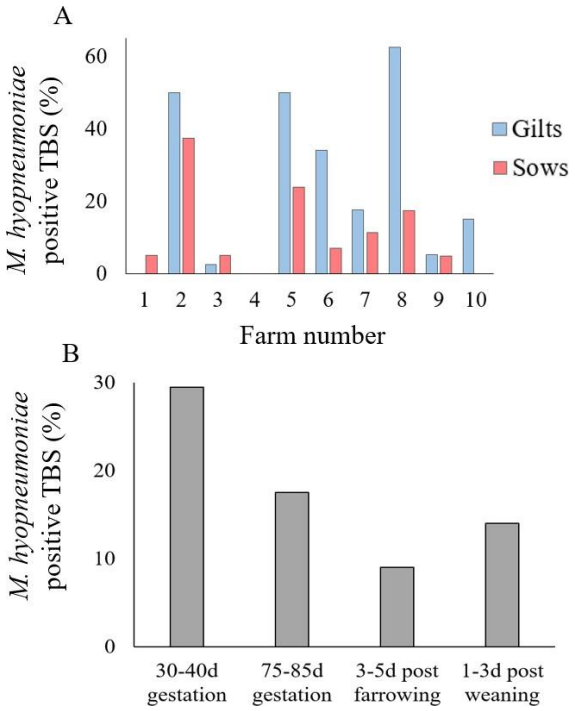

Figure 1: Percentage of (A) gilts and sows on each farm, and (B) breeding animals at the different time points, infected with *M. hyopneumoniae*. d: days

At farm level the seroprevalence ranged between 65.0-100% and 19.7-95.5% for gilts and sows, respectively. Gilts were significantly more often seropositive than sows (P=0.027). No significant difference was observed in seroprevalence between the different time points.

Discussion and Conclusion

The percentage of *M. hyopneumoniae* infected breeding animals varies a lot between farms (3,6). Due to vaccination of the gilts and circulation of the pathogen on most farms, the seroprevalence is high and the variation less than the percentage of *M. hyopneumoniae* infected animals. Despite vaccination, gilts were more often *M. hyopneumoniae* infected than sows and most positive animals were found in the first half of gestation. This emphasizes the importance of proper quarantine and acclimation practices for gilts before introducing them to the sow herd.

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Large scale *Lawsonia intracellularis* antibody prevalence survey of swine production herds in China

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Introduction

Lawsonia intracellularis (Li) is the causative agent of proliferative ileitis in pigs. The disease is known to have a global spread (1). However, reports of prevalence of this disease in China are scarce, despite the high production of pork in this country. A report from 2014 investigating the prevalence of Li farms located in North, Central and South China estimated the prevalence of Li infections to be 77% (2).
However, this was a relatively small sample size of 14 farms in total. Objective of this study is to increasethe knowledge of the presence of Li infections in swine production locations in China.

Materials and methods

Different farms located in various regions of Chinawere sampled for the screening of LI antibodies.
Serum was collected in 2020 from different age groups (finishing pigs and breeding sows). At bleeding the age of the animals was noted. Both finishing pigs and sows were sampled. Lawsonia antibodies were measured with an ELISA (An Wu Biotechnology, China) following the kits instructions.

Results

In total 43 farms were investigated for the prevalenceof Li. In total 1378 blood samples were analyzed of which 794 were collected from finisher pigs (various age groups), 125 from gilt developers and 459 from breeding sows. All 43 farms were positive for antibodies against Li. From the finishing pigs, in total31% of the samples were positive. Gilt developers had slightly higher amounts of positive animals whencompared to breeding sows (89% vs 76%). Focusing on the spread among the different age groups of slaughter pigs, a clear rise in prevalence is seen with increasing age of the pigs (Table 1 and Figure 1).

Table 1 *Lawsonia intracellularis antibody Elisa results over the different ages*

	Start	Grow	Finish	Gilts	Sows
Age (days)	84-94	120	150-175	230	>365
Pos (%)	6%	25%	34%	89%	76%
S/P (± 95% CI)	0.09 (0.04)	0.36 (0.11)	0.54 (0.06)	1.31 (0.12)	1.25 (0.09)

At the start of finishing (84-90 days of age) infections are quite low with 6% prevalence. This is increasing with age to 25% for growers (120 days of age) and to 34% for finishers (150-175 days of age).

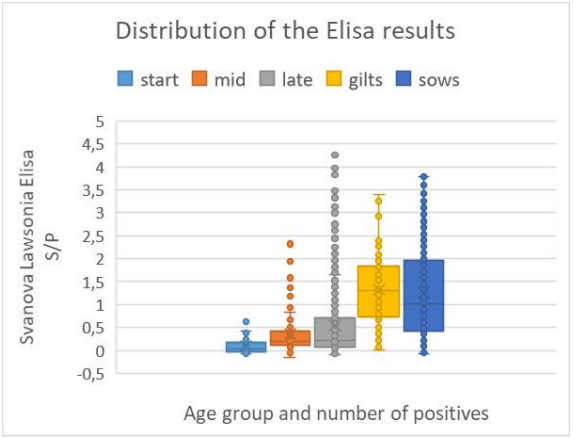

Figure 1 *Distribution of the Lawsonia intracellularis antibody Elisa results of different ages of finishing pigs in China. Respective ages: start, mid early 120 days, mid late 150-170 days, end 230 days.*

Discussion

In all 43 farms positive samples were found, suggesting a high prevalence of Li in Chinese production systems. The increase of prevalence with an increase of age suggests a slow spread of the infection within a herd. Considering 3-4 weeks of seroconversion after first infection, most of the infections take place during the duration of the starter and grower phase of slaughter pigs. This is in line with other reports in the literature (1).

Conclusion

Prevalence of infection with Li is widespread in China. This will most probably affect finisher pig performance, equally to other parts of the world. The onset of seroconversion after start of finishing, leaves room to implement a vaccination program in either the suckling or the post weaning phase of finisher pigs.

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Lessons learned during a *Mycoplasma hyopneumoniae* (Mhp) infection in a naïve sow herd

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Background

An acute Mhp infection was investigated in a naïve 4200 sow breed-to-wean site in the Midwest. The dynamics of Mhp infections are becoming better known by practitioners as improved diagnostic methods are utilized in determining infectious state of animals. Due to the improved diagnostic methods, a common management practice is to perform herd closure when pathogen elimination is desired. This site went through a typical herd closure process with a successful elimination of Mhp. Several economically important lessons became apparent.

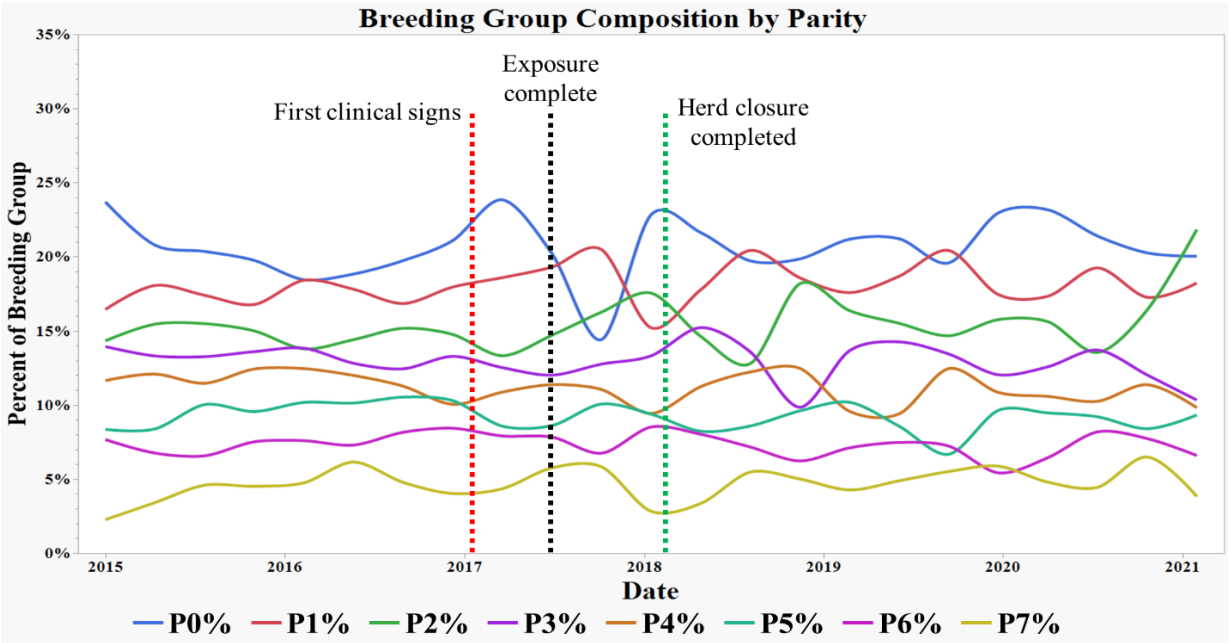
Methods

During week 3, 2017 the clinical sign of coughing in sows started in farrowing rooms with nursing piglets that were 12 to 18 days of age. Positive diagnosis of Mhp was confirmed week 4, 2017 by PCR on laryngeal swabs and ELISA antibody tests. Natural exposure was selected to infect the rest of the animals within the site, including the developing females in the gilt development unit (GDU). Exposure was complete week 24, 2017 when over 90% of the animals were positive to PCR by laryngeal swab and or ELISA antibody tests. The immune management time period started next which is called herd closure. The completion of the 36-week herd closure was week 8, 2018 when the first negative piglets were born. Production records of 6years were analyzed for comparing the three time periods – naïve (up to week 3, 2017), infection (week 4,2017 till week 8, 2018), and post-infection (week 9, 2018 till week 5, 2021.

Results

The following lessons learned comparing production records of the three times periods; naïve (107 weeks), the infection period (56 weeks), and post-infection period (157 weeks). In this study, all animals were enrolled at service (all females bred within one week became the breeding group) for charting and analysis. A significant increase in sow mortality (4.16% 8.33%, and 3.89%) and pre-weaning mortality (10.45%, 12.38%, and 12.06%) occurred during the naïve, acute infection, and post-infection respectively. Further production losses included: Weight decrease (kg) in weaned weight/sow/year (166.3, 158.3, and 164.2) and pigs weaned/mated female/year (29.43, 28.35, and 28.28) in naïve, during acute infection and post-infection phases respectively. The reduced farrowing rate during the herd closure meant fewer pigs at weaning, which resulted in the most severe economic loss. The disease cost of Mhp presented with a higher cost of $6.82 for every sow bred per week during the infection with a total cost per week of $681.72 due mostly to a higher preweaning mortality. A $45 US per weaned piglet was utilized to calculate opportunity costs. Additional costs are sow mortality and feed cost from "open" sows (any reason) post-service. Because the parity structure of each breeding group impacts a herd's overall performance there was concern herd closure contributed to the economic losses. Figure 1 illustrates the "ripple" effect that occurred in parity 0 and continued in each subsequent parity. It was determined that in the long-term, herd closure did not contribute additional economic losses.

Figure 1



Minimal inhibitory concentrations of enrofloxacin, tiamulin and valnemulin against 10 contemporary field strains of *Mycoplasma hyopneumoniae*

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Introduction

Mycoplasma hyopneumoniae (Mhy) is a member of the class Moliculites. It is known as the agent responsible for Swine Enzootic Pneumonia (SEP), the bacteria does not have a cell wall, making it resistant to antimicrobials that directly affect this synthesis, in addition to having a limited metabolism and a slow and fastidious growth, causing great difficulties for *in vitro* cultivation. Vaccination is not fully effective in preventing the pathology that will colonize the respiratory tract, so treatment with antimicrobials is necessary. The objective of this study was to determine the minimum inhibitory concentration (MIC) of most used antimicrobials at pig farming against 10 clinical isolates of *Mhy* recently isolated in Minas Gerais, Brazil.

Materials and Methods (Clinical strains)

Ten clinical isolates were obtained from collectionsfrom lungs with macroscopic lesions (2018-2019) (1,2). Genetic profile was determined for genes involved with mechanisms of virulence and the reference strain J was used to control the performance of test (Embrapa) (1) All isolates had a count between 10^3 and 10^5 CCU/mL, assessed using the microbroth dilution method (3). Dilutions of the antibiotics were performed, ranging from 0.0001 to 64 mg/L. The microbroth dilution test was performed using a 96-well plate. Previously, 100 µL of the culture was added to the plate and incubated for at least 1 h. All plates had a positive control (culture without antibiotic) and negative control (modified Friis medium). After 1 h of incubation, 100 µL of the antibiotic at each concentration (0.0001–64 mg/L) was added to the wells. The plates were then sealed and incubated at 37 °C for 15 days. The plates were monitored daily for any color change in the wells.

Results

Table 1.MIC results for 10 clinical isolates of *Mhy*

	Range µg/ml	MIC90
Enrofloxacin	0.0001-0.015	0.03 µg/ml
Tiamulin	0.0001-0.125	0.125µg/ml
Valnemulin	0.0001-0.001	0.001µg/ml

The MIC of the isolates was higher for tiamulin, whichreached the MIC90 (0.125 µg/ml) *vs.* enrofloxacin thatreached the MIC90 of 0.03 µg/ml. Valnemulin had alower MIC90 (0.0001 µg/ml). The J strain (type strain)reached 0.003 µg/ml and 0.25 µg/ml for valnemulin,tiamulin and enrofloxacin respectively.

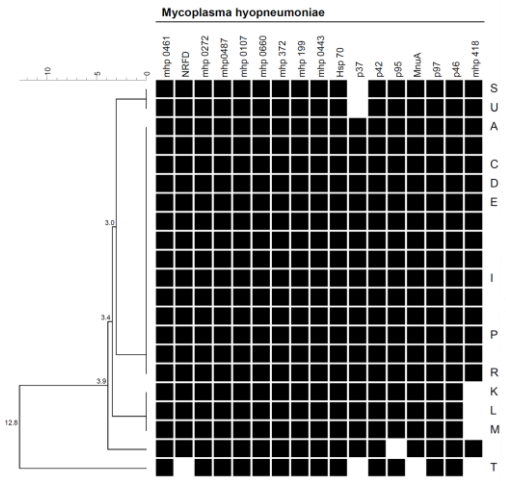

Figure 1. Dendogram based on 17 pathological-related genes. All 10clinical isolates are highlighted. KLM are type strain (Bionumerics®)

Discussion and Conclusion

The MIC for broth dilution is defined as the lowest antibiotic concentration that inhibits growth when growth is observed in the antibiotic-free growth control well (3). However specific clinical breakpoints or Epidemiological cut-off (ECOFF) for *mycoplasmas* makes it difficult to evaluate for *Mhy* (*vivo vs. in vitro*). Therefore, MIC results are often compared to clinical breakpoints determined for other bacteria (3). Some authors have suggested the correlaction PK/PD/MIC Integration can be a robust tool (4). MICs for pleuromultilins (tiamulin and valnemulin) were lower, suggesting a greater effect *in vitro.* However, the quinolone (enrofloxacin) was also shown to be effective, and in this specific case is indicated only as the last choice because of importance in human health.

Our results indicate that the use of 2 pleuromutilins should be considerer and microbroth dilution method continues to be an important tool to monitoring about emergence of resistent *Mhy* strains in the field.

Acknowledgments

We would like to thanks Federal University of Viçosa, Permanent Commission on Intellectual Property and Brazilian Agricultural Research Corporation for all support.

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Molecular characterization by serotyping PCR and LS-PCR of *Glaesserella (Haemophilus) parasuis* strains isolated from clinical cases in Peru

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Introduction

Molecular serotyping and LS-PCR are valuable and practical tools for *Glaeserella parasuis* characterization (1, 2). Early in 2020, and due to the forced post-pandemic closure, several swine production systems in Peru reported clinical cases of *G. parasuis*, and a total of sixty-eight qPCR-positive microbiological isolates of clinical cases were obtained over two years. The objective of this study was to identify the circulating serotypes, and evaluate the presence of predictor *vtaA* virulent genes of the *G. parasuis* isolates from clinical cases in Peru. This study represents the first molecular diagnostic and serotyping report of virulent *G. parasuis* in Peru.

Materials and Methods

Pericardium, thoracic and synovial fluid, pleura, fibrin, lymph nodes, and lung specimens were collected at necropsy from suspicious animals of *G. parasuis*-associated disease from commercial swine farms in Peru. Primary *G. parasuis* isolation was performed on sterile blood agar plates with a *Staphylococcus aureus* nurse streak, and incubated at 37 °C for 48 hours. Subsequently, suspected *G. parasuis* colonies were purified by subculture on sterile trypticase soy + NAD agar plates at 37°C for 24-48 hours. The identification of purified isolates was performed using biochemical tests followed by subsequent molecular characterization. Bacterial DNA extraction was carried out using a Real PCR RNA/DNA Magnetic Bead Kit (IDEXX Laboratories, USA). Molecular diagnostic confirmation was made by amplification of *inf*B gene by qPCR (3). For molecular serotyping, a multiplex PCR (mPCR) based on the variation within the capsule loci of the 15 serovars of *G. parasuis* was used (1). Additionally, a LS-PCR based on the leader sequences of the *vtaA* genes was performed to evaluate virulence (2). Both the molecular serotyping and LS-PCR were read in an electrophoresis gel agar.

Results

Sixty-eight isolates *G. parasuis* qPCR positives were obtained (Figure 1A), the most common was serotype 7 (n=55), following by serotype 1 (n=3), serotype 4 (n=2), serotype 13 (n=1), serotype 5-12 (n=1) and six isolates were nontypeable (Figure 1B). All isolates had the *vtaA* gene virulent predictor of *G. parasuis*

Discussion and Conclusion

The clinical cases associated with serotype 7 of *G. parasuis* denote attention since it was previously considered non-virulent (4). The present work reports the first molecular detection and identification of serotypes 1, 4, 7, 5-12, 13, all virulent and associated with clinical cases in Peru, and highlights the detection of more than 55 virulent isolates positive for serotype 7. There is no cross-protection between *G. parasuis* do serotypes nor even isolates from the same serotype. Therefore, the identification of several *G. parasuis* virulent serotypes (serotypes 1, 4, 5-12, 13, and 7) circulating in Peru should generate special attention and reinforce the importance of timely and accurate diagnosis and subsequent implementation of specific control measures.

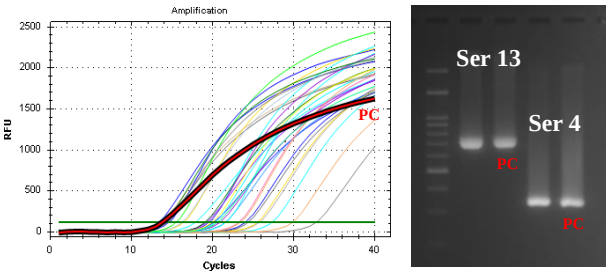

Figure 1. Results *G. parasuis*: (A) qPCR; (B) identification of serotypes 4 and 13. PC: Positive Control

Acknowledgments

Dr. Luis Gimenez-Lirola for his great support and kind recommendations.

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Molecular identification of *Streptococcus suis* serotypes 3, 7 and 2-1/2 isolated from Peruvian swine farms

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Introduction

The COVID-19 pandemic caused a decrease on the demand of pork in Peru, and led to the overcrowding of animals commercial farms. As a consequence, there was an increase in clinical cases with a range of signs, including fever, pneumonia, arthritis, polyserositis, cyanosis, prostration and tremors, compatible with bacterial infection of unknown origin. Due to the high frequency and severity of clinical signs, and high prevalence of disease in young animals (5-11 weeks of age), *Streptococcus suis* was included for differential diagnosis among other swine pathogens. Due to the absence of *S. suis* serotype identification in Peru, samples identified as *S. suis* by qPCR were also analyzed by multiplex PCR to detect 33 serotypes of *S. suis* (1)

Materials and methods

Pigs from different farms were necropsied, and sterile samples from lungs, meninges, pericardium, peritoneum, joint capsule and pleural swabs were collected for microbiological analysis. Samples were cultured on Blood Agar and incubated for 24 to 48 hours at 37°C under microaerophilic conditions (3-5% CO_2). After incubation, small, translucent and alpha hemolytic colonies were observed, which corresponded to gram-positive, catalase-negative cocci. Suspicious colonies compatible with *Streptococcus suis* were submitted for molecular identification. DNA was extracted from bacterial colonies suspended in 1 ml of PBS using the MagMAX CORE Nucleic Acid Purification kit (ThermoFisher Scientific, USA). A SYBR green-based qPCR, targeting the capsular polysaccharide synthesis (cps) gene cluster (1), identified all the samples as *S. suis*. After *S. suis* confirmation, serotyping was performed by multiplex gel-based PCR, and a total of 33 serotypes were analyzed (1).

Results

Colonies compatible with *Streptococcus suis* were isolated from lungs, brain and meninges. In total, 10 isolates were submitted for molecular identification of *Streptococcus suis.* All 10 isolates were confirmed, where 1 isolated was classified as serotype 2-1/2, 3 as serotype 3, 4 isolates as serotype 7 and 2 isolates as non-typeable.

Discussion and conclusions

The serotype identification of *S. suis* isolates is crucial due to their zoonotic potential and veterinary relevance. It is known that serotypes 1, 2, 4, 5, 14, 16, and 24 have been involved in more than 700 clinical cases in humans, whereas serotype 2 have been widely detected

Discussion and conclusions

The serotype identification of *S. suis* isolates is crucial due to their zoonotic potential and veterinary relevance. It is known that serotypes 1, 2, 4, 5, 14, 16, and 24 have been involved in more than 700 clinical cases in humans, whereas serotype 2 have been widely detected in diseased pigs and currently, it is the unique serotype that is included in a commercial swine vaccine. Serotypes 9, 1 and 14 have been often isolated from clinical cases in Europe, while serotypes 2, 3, 1/2, 4, 7 and 8 are prevalent in Canada (2). However, in South America: In Argentina and Brazil serotype 2 have been isolated from systemic infection, pneumonia, and healthy pigs (3). Moreover *S. suis* serotypes ½, 3, 5, 6, 7, 8, 9, 10, 11 and 14 have been identified in Brazil (4; 5, 6), while cases of purulent meningitis in humans have been attributed to serotype 2 in Chile (7, 8). Despite its relevance, no foregoing studies have reported any *S. suis* serotypes in Peru. Hence, this preliminary study represents the first identification of *Streptococcus suis* serotypes 3, 7 and 2-1/2 in clinically affected pigs from Peruvian farms. Further studies including larger sampling for bacterial isolation and characterization, including the identification of molecular markers of virulence are needed to classify the non-typeable isolates and identify the presence of other posible relevant *S. suis* serotypes.

Acknowledgments

Dr. Luis Gimenez-Lirola for his great support and kind recommendations.

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Molecular Typing and Phylogenetic Analysis of *Glaesserella parasuis* Isolated in Spain, and similarity with other Brazilian *G. parasuis* clinical strains

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Introduction

Glaeserella parasuis (*Gp*) is a gram-negative organism that causes a systemic infection (Glässer's disease, GD), characterized by polyarthritis, polyserositis and meningitis, leading to significant economic losses to the pig intensive industry (1). GD has been reported in many countries, with a different geographical distribution of serovars (SVs). A high number of clinical isolates belonging to SVs 2 and 5 has been previously reported in Spain (2).

Gp possesses iron acquisition routes mediated by a surface receptor which binds specifically porcine transferrin, that is composed of transferrin binding protein A (TbpA) and TbpB. Both proteins have become potential candidates for the development of vaccines against the pathogens, such as *Gp*, bearing this iron acquisition system.

The aim of this study was to type a high number *Gp* isolates collected in Spain in 2018-2021 to better understand the phylogeny of *Gp* by comparing these Spanish with other Brazilian isolates. For this purpose, the analysis of TbpB sequences was performed, with the hope of getting better protective vaccine candidates against GD.

Materials and Methods

Gp were recovered from samples collected from different pig farms located in Spain, and its identification was performed by a *TbpA* gene PCR (3). Then, a multiplex PCR was carried out to know the SV to which each isolate was connected.

In addition, the amplification of *TbpB* gene was performed by other PCR (4). Amplicons were purified, the DNA concentration was measured, and samples were later sequenced by the Sanger method, using an intermediate primer (5).

After sequencing, samples were edited with the Chromas™ software, and the contigs were performed with the Bioedit™ software. Alignment and phylogenetic analysis of sequences were carried out using Clustal W and Mega7™.

Results and Discussion

SV 5 was the most prevalent followed by SVs 10, 2, 4, 1, 3, 7, 8 and 12; non-typable isolates were not found (Table 1).

Sequencing of TbpB of 59 *Gp* allowed to establish eight clusters (Figure 1). **Clade 1** was the highest (16 *Gp*) group, followed by **5** (14 *Gp*), **2** (11 *Gp*), **4** (10 *Gp*), and **3, 6, 7 and 8** (each 2 *Gp*). **Clades 1 and 2** were the most heterogeneous because six of nine SVs appeared in each; however, clade 4 was highly homogeneous because all Gp belonged to SV 5.

Table 1. Serovars of *Gp* according to the isolation source.

Sero-var	Source of isolation (number and percentage)			Total
	Respiratory	Systemic	Unknown	
1	3 (42.9)	3 (42.9)	1 (14.2)	7 (10.3 %)
2	7 (77.8)	2 (22.2)	-	9 (13.2 %)
3	3 (75.0)	1 (25.0)	-	4 (5.9 %)
4	7 (77.8)	2 (22.2)	-	9 (13.2 %)
5	15 (75.0)	3 (15.0)	2 (10.0)	20 (29.4 %)
7	4 (80.0)	-	1 (20.0)	5 (7.4 %)
8	1 (100.0)	-	-	1 (1.5 %)
10	8 (66.7)	3 (25.0)	1 (8.3)	12 (17.6 %)
12	1 (100.0)	-	-	1 (1.5 %)
Total	49 (72.1)	14 (20.6)	5 (7.3)	68

Figure 1

Comparing with Brazilian *Gp* isolates (1), **clades 1 and 3** sequences were clearly different, but they were very similar in the phylogenetic tree obtained by us, because Hps.174.SV7 and App.h397.SV1 (representing **clades 1 and 3**, respectively) sequences appeared one close to the other, with a 98.5 % similarity.

Based on TbpB sequences of our Spanish *Gp* isolates, new information is gotten about this protein as a useful target for resulting in effective vaccines against GD.

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Multi-farm long-term investigation of *Mycoplasma hyopneumoniae* detection in processing fluids

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Introduction

Mycoplasma hyopneumoniae, the primary etiologic agent of porcine enzootic pneumonia, is considered to be restricted to the respiratory system, with very occasional detection in extra-respiratory tissues reported in experimental infection studies (1). However, recent *in vitro* studies have shown internalization of the bacterium into epithelial cells and paracellular migration (2). In addition, the detection of *M. hyopneumoniae* in processing fluids (PF) was recently described, both in a subclinically-infected farm (3) and in the context of a Porcine Respiratory Disease Complex farm outbreak (4). Although valuable, these reports included a limited number of samples collected during a reduced timeframe, in very particular farm conditions. Thus, the aim of this study was to further evaluate the PCR-detection of *M. hyopneumoniae* in PF in farms with various health status and management conditions, by regularly testing for an entire year.

Materials and Methods

Ten farms from three different production systems were enrolled in the study. Five farms were *M. hyopneumoniae*-negative at enrollment and five were positive. Processing fluid collection was performed by farm personnel and consisted in obtaining the testicles from litters born to gilts, to second parity sows, and to third parity and above sows. Processing fluids were collected in individual bags on a monthly basis for an entire year, for a total of thirty PF/month/farm. Processing fluid samples were frozen at the farm until shipment to the laboratory. At arrival at the laboratory, samples were thawed at room temperature and PF were collected and aliquoted. Samples were then submitted to the Veterinary Diagnostic Laboratory at the University of Minnesota for *M. hyopneumoniae* rt-PCR testing by pools of five, maintaining the same parity and month within every pool. More than 600 aggregated samples, representing near 3,000 individual litters were tested. Results were statistically analyzed by constructing a mixed effects logistic regression model. Additionally, a selection of positive pools from different farms were individually tested, and the resulting samples were submitted for *M. hyopneumoniae* DNA sequencing.

Results

Based on PCR detection in PF throughout time, three farm profiles or scenarios were identified: farms that were constantly negative; farms with variable detection; and farms that were constantly positive over the entire sampling period. *Mycoplasma hyopneumoniae* farm status (negative or positive for the pathogen) was significantly associated with the detection of the bacterium in PF. The proportion of positive samples among parity groups and by month of the year was similar, hence, no association with bacterial detection in PF was observed for these two variables. The obtained six partial and one complete DNA sequences from three different farms were identical and showed 99.8-100% homology to *M. hyopneumoniae* J strain.

Discussion and Conclusions

The *M. hyopneumoniae* status of the farm appeared to be one key factor for *M. hyopneumoniae* detection in PF. The origin of the genetic material is uncertain at this point of the investigation and further studies would be needed to determine if environmental contamination can be ruled out.

Pre-clinical cross-contamination of PF samples was not investigated as part of this study. However, the percentage homology of *M. hyopneumoniae* of the sequenced PF with strains used in commercial vaccine products warrants further investigation.

Results from this study emphasize the fact that, for PCR interpretation, the context of the diagnostic investigation, such as reported clinical signs or pathologic findings, is key, and is worthy to remember that many variables, such as the collection procedure and handling of samples or the DNA/RNA persistence or degradation in the environment can dramatically affect PCR results. Thus, a meticulous sampling methodology, aiming at minimizing both cross- and environmental contamination of the samples, should be implemented and monitored for all the procedures involving nucleic acid detection by PCR.

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Mycoplasma hyopneumoniae break in a naïve gestation barn

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Introduction

Mycoplasma hyopneumoniae is a common endemic pathogen around the world. But increasingly many farms are negative, especially breeding genetic farms. This report describes the clinical signs of an incursion into a *M. hyopneumoniae,* free farm and the course of the outbreak, investigation methods and treatment and control measures instigated.

Materials and Methods

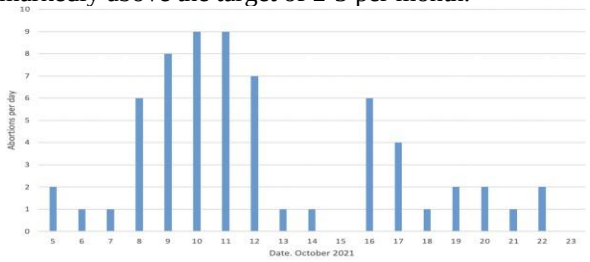
The farm is set up as three units, each three week batching with 240 sows in each batching unit. Therefore, the farm is approximately a 5500 sow unit. With a weaning expectation of 2880 pigs each week, producing a total of 14,000 tonnes of pork each year.
The farm is a specific pathogen free farm including *M. hyopneumoniae.* However, the farm has a low level of Porcine Reproductive and Respiratory Syndrome virus on the farm and is undergoing an elimination programme. The farm is ASF free.

Results

In October (autumn) there was a sudden increase in abortions, coughing, loss of appetite, and the death of 5 parity 1 sows. None of the affected pigs demonstrated any appreciable pyrexia. There was a significant rise in the number of stillborn pigs with no rise in mummifications.

Abortions

In October 70 abortions were recorded which is markedly above the target of 2-3 per month.



The farm uses breeding production boards and they clearly illustrated the clinical problem. The example below illustrates the abortion issue in unit 3, the red blocks represent the same weeks of production on the farm. Abortions are recorded as the small number lower right corner.

The abortions started from day 30 of gestation to term.

Total born and stillbirths

The farm previous to October recorded a normal 7% stillbirth rate. During October the stillbirth rate rose to 24% with many litters having complete stillborn litters of 14 plus, which is very distressing to the stockpeople. The mummification rate stayed at 0.5%. There was no impact on total born but the born alive dropped significantly.

Investigation

Initially the clinical signs pointed towards a PRRS break and PRRS virus was found in 2 out of 5 aborted fetuses'. But, aborted and sick sows were PCR negative with moderate or low antibody levels. Subsequent analysis demonstrated no rising titres,
In addition, the type of early abortions are not typical of a PRRS abortion storm. In previous abortion storms associated with PRRS, coughing has not been seen but severe coughing can be seen in finishing pigs breaking with PRRS.
Swine Influenza was considered as part of the differential diagnosis although the lack of pyrexia and the high stillborn rate was considered unusual. There was coughing but no sneezing throughout the farm. However, PCR nasal swabs were negative of SIV (H_1N_1, H_1N_12009, H_2N_3 and H_1N_2) and antibodies, were inconsistent, some negative and did not demonstrate a rising titre.
Mycoplasma hyopneumoniae was considered despitethe fact the farm demonstrated negative status in quarter3 tests a months earlier.
Nasal swabs were PCR positive in all clinically affected sows with Ct values of 26 to 30. Initial antibodies were inconclusive in 5 out of 20 animals. The following testing was 20 out 20 antibody positive
The farm was checked for ASF and was negative.

Treatment and control

Whole herd *M. hyopneumoniae* vaccination single shot was performed.
All gestation and lactation sows were given Tiamulin in-feed antibiotics with aspirin, despite the lack of pyrexia.

Outcome

The coughing and abortions stopped 6 weeks after the break of disease with no reoccurrence.
There has been a reduction in litter sizes associated with mating's from the on-farm boar stud in semen collected during this October period but this is recovering.

Discussion and Conclusion

A naive *Mycoplasma hyopneumoniae* pig farm became positive in October. The clinical signs were typical of a *M. hyopneumoniae* outbreak with spreading coughing around the farm. This case is interesting as it reports the impact on niave breeding stock resulting in abortions, stillborn piglets and some fertility impact in boars.

Further reading:

Maes, D.; Sibila, M. and Pieters, M. (2020). Mycoplasmas in swine. ISBN 978-94-66379-79-62

Mycoplasma hyopneumoniae P102 gene detection using a digital PCR assay

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Introduction

Mycoplasma hyopneumoniae, the primary pathogen of enzootic pneumonia in pigs, is highly prevalent in swine producing areas (1). At present, the amount of *Mycoplasma hyopneumoniae* DNA in clinical samples is quantified indirectly using quantitative PCR (qPCR) and a standard curve with reference material (2). However, direct quantification of target nucleic acids without the need for a standard curve can be obtained with digital PCR (dPCR). Next to increased interlaboratory repeatability, dPCR also provides a higher quantitative accuracy and precision compared to qPCR. Therefore, we established a dPCR assay using the primers and probe designed by Marois et al. (2010) (3) targeting the P102 gene of *M. hyopneumoniae*.

Materials and Methods

An experimental challenge study with *M. hyopneumoniae* was conducted during which 150 clinical broncho-alveolar lavage (BAL) samples were collected at 3 different time points. All samples were analyzed both with a qPCR assay using a standard curve, as described by others (4,5,6,7), and with our newly developed dPCR assay. Also, the limit of blank (LOB) and the theoretical limit of detection (LOD) of the dPCR assay were determined. Therefore, 63 non-template controls containing 10 ng/µL salmon sperm DNA were measured. Next, the LOB and theoretical LOD were calculated.

Results

According to Marois et al. (2010) (3), the LOD for qPCR was 1.3 equivalent organisms/µL. A tenfold dilution series of *M. hyopneumoniae* DNA was used to convert the threshold values to the number of organisms (4,5,6,7), and values below the last dilution (1.50 x 10^1/µL, which corresponds with 1.18 log organisms/µL) were considered as negative. The LOB (95 %) of the dPCR assay was 3 positive partitions per well, providing a theoretical LOD (95 %) of 0.47 organisms/µL. The qPCR and dPCR results for the clinical samples are shown in Figure 1.

Discussion and Conclusion

First, we have shown that the primers and probe used in the P102 targeted qPCR assay work also in a dPCR assay. Interestingly, the dPCR LOD (95 %) was 3 times lower compared to the LOD of the qPCR assay.

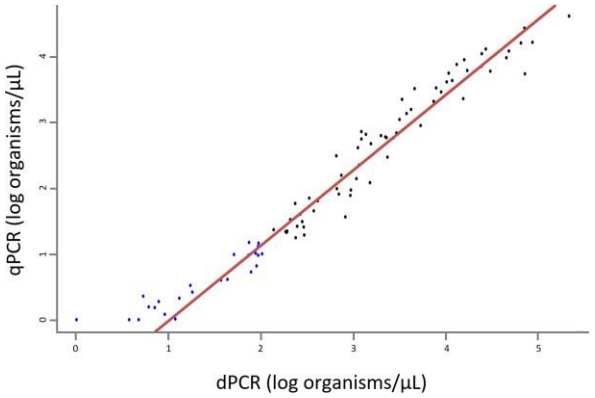

Figure 1. qPCR and dPCR results of the 150 clinical BAL samples.

Samples with a concentration below 1.18 log copies are considered negative based on qPCR (blue). Only values with a concentration higher than 1.18 log copies were used to calculate the regression line (red). The slope of the regression line, intercept and R^2 value is 1.14, -1.16, 0.93 respectively.

According to the qPCR assay, 87 clinical samples had a concentration lower than 1.18 log copies (concentration of the last dilution), meaning that these samples would be considered negative. However, the *M. hyopneumoniae* DNA concentration of all samples was also determined using dPCR, and 23 out of the 87 samples classified as negative based on the qPCR assay, were positive according to the dPCR assay. These 23 samples had an average concentration of 43.35 organisms/µL on dPCR.

Due to its increased sensitivity, high precision and accuracy, dPCR is a promising quantitative tool to measure *M. hyopneumoniae* DNA in clinical samples, especially for studies in which small differences in DNA concentration need to be measured accurately. Also, a standard curve is not needed when using dPCR, which increases interlaboratory repeatability and allows to compare the results from different studies more easily.

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Mycoplasma hyorhinis detection in dams and piglets during lactation

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Introduction

Mycoplasma hyorhinis (*M. hyorhinis*) is considered a commensal bacterium that inhabits the swine upper respiratory tract (1). However, *M. hyorhinis* can cause, in some instances, polyserositis and polyarthritis in nursery-age piglets through mechanisms of invasionthat still remain unknown (1). *Mycoplasma hyorhinis* can cause disease even in farms where other swine mycoplasma species have been eradicated (2).Detection of *M. hyorhinis* in dams and piglets prior to weaning has been reported using different sample types (3,4). Nevertheless, knowledge regarding colonization and distribution of this important swine bacterium in modern pig production systems is still lacking. In the present study, we aimed to evaluate the dynamics of detection of *M. hyorhinis* in dams and piglets during thelactation period at different time points using various sample types.

Materials and Methods

The study was conducted in a commercial 5,000 sow farm. Twenty-four dams in a weekly farrowing group were randomly selected and enrolled in the study. From each dam, two piglets were randomly selected at birth and were ear tagged. Additionally, from each dam, different samples were collected, including one vaginal swab (prior to farrowing), and three oropharyngeal swabs collected at three specific time points: prior to farrowing, at mid lactation and at weaning. Piglets were sampled during the lactation period. A total of six samples were collected from each piglet. Nasal and tonsillar secretions were both collected from each piglet at three different sampling events: birth, mid-lactation, and weaning. All samples collected during the study were processed for DNA extraction and were tested by a real-time PCR, employing custom primers and probe for *M. hyorhinis*, as previously described (5). Detection of *M. hyorhinis* between time points, and sample types at weaning stage were analyzed using a McNemar's Chi-squared test with continuity correction.

Results

Mycoplasma hyorhinis genetic material was not detected in vaginal swabs from dams tested in this study (data not shown). In oropharyngeal swabs, 8 % of the dams (2/24) were positive prior to farrowing, while 0% were positive at mid lactation (0/24), and 4% (1/23) resulted positive at weaning (data not shown). It is important to note that *M. hyorhinis* was detected only in parity one dams. Two percent (1/48) of piglets were positive for *M. hyorhinis* in nasal swabs at birth, 4.35% (2/46) were positive at mid-lactation and 44% (20/45) were positive at weaning. Tonsillar swabs were negative in all piglets at birth, whereas *M. hyorhinis* was detected in 2% (1/46) of the samples at mid-lactation and 53% (24/45) at weaning. Detection of *M. hyorhinis* increased significantly at weaning (p<0.001), and tonsillar swabs showed similar sensitivity to detect the bacterium DNA when compared to nasal swabs (p=0.288)

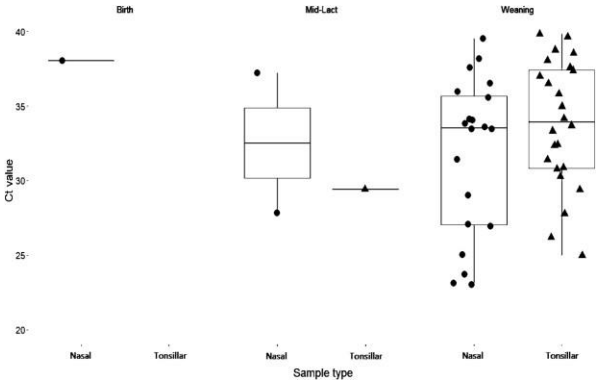

Figure 1. *Mycoplasma hyorhinis* detection by rt-PCR in piglet nasal (●) and tonsillar swabs (▲) at different time points.

Discussion and Conclusions

Detection of *M. hyorhinis* in dams using oropharyngeal swabs was inconsistent overtime, and only observed in first parity dams, whereas all vaginal swabs collected prior to farrowing were negative. The lack of detection of *M. hyorhinis* in vaginal swabs prior to farrowing potentially suggests that piglets are not colonized in the birth canal. However, the fact that piglets detected positive to the bacterium at birth and mid-lactation were born to first litter dams, suggests that young parity sows may play an essential role in early colonization of *M. hyorhinis*.

Detection of *M. hyorhinis* in the nasal and tonsillar swabs of piglets increased slowly until the weaning age, as previously reported (3,4). Detection patterns in piglets at the weaning stage were similar regardless of the sample type used.

Despite efforts to assess colonization and circulation patterns of *M. hyorhinis* in swine herds, knowledge gaps are still present and need to be addressed to characterize the infection, pathogenesis, and circulation of this swine bacterium, which will allow the development of new disease control strategies.

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Oral vaccination mitigates weight and feed efficiency reductions during *Lawsonia intracellularis* experimental challenge

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Introduction

Lawsonia intracellularis and its associated disease is endemic to swine herds worldwide, and is a significant cause of reduced growth and feed efficiency in growing pigs (1,2). Currently, vaccination is the most effective and cost-efficient strategy to mitigate performance losses, however the extent of this mitigation, particularly regarding feed efficiency, is not fully understood (3). Thus, the objective of this study was to characterize the impact of vaccination on growth performance during an experimental *L. intracellularis* challenge.

Materials and Methods

For this study, 36 barrows were selected, individually confirmed negative for *L. intracellularis*, and assigned to treatment groups as follows (n=12/trt): 1) nonvaccinated, *L. intracellularis* negative (NC); 2) nonvaccinated, *L intracellularis* challenged (PC); and 3) *L. intracellularis* challenged, vaccinated with Enterisol® Ileitis (Boehringer Ingelheim Animal Health, Duluth, GA) via oral drench at 1-week postweaning (VAC). On days post inoculation (dpi) 0 (7 weeks post-weaning), PC and VAC pigs were inoculated with *L. intracellularis*. Individual feed disappearance and body weight (BW) were recorded for each pig on dpi 0, 7, 14, and 19. At dpi 21, pigs were euthanized and scored for gross lesions characteristic of *L. intracellularis* infection.

Results

Loose, formless stools were first observed in 4 of the PC pigs at dpi 7, and continued for the remainder of the experiment. Overall, 100% of PC pigs demonstrated clinical signs consistent with enteric disease, whereas clinical signs were observed in 75% of VAC pigs. For overall performance (dpi 0-19; **Table 1**), ADG was 41% lesser in PC ($P < 0.001$) pigscompared with NC pigs, while ADG was 26% greater in VAC pigs compared with PC pigs ($P < 0.001$). Similarly, ADFI was 24% lesser in PC pigs ($P < 0.001$)compared with NC pigs, and was greater in VAC pigscompared with PC pigs (14%, $P = 0.032$). Overall FCRwas 25% greater in PC pigs compared with NC pigs ($P< 0.001$). Overall FCR was 18% lesser in VAC pigs compared with PC pigs ($P = 0.003$). At necropsy, average lesion length in the ileum was greater in PC pigs (118 cm) compared with NC (0.0 cm, $P = 0.007$)and VAC (3.0 cm, $P = 0.045$) pigs, confirming the challenge model.

Table 1. Growth performance of non-infected pigs (NC), *Lawsonia intracellularis* inoculated pigs (PC), and vaccinated *Lawsonia intracellularis* inoculated pigs (VAC) from days post inoculation (dpi) 0-19

	Treatment				*P*-value
	NC	PC	VAC	SEM	
dpi 0-7					
ADG	1.30[a]	0.87[b]	0.93[b]	0.049	<0.001
ADFI	2.41[a]	1.75[b]	1.99[b]	0.083	<0.001
FCR	1.89	2.03	2.15	0.104	0.193
dpi 8-14					
ADG	1.22[a]	0.83[b]	1.10[a]	0.070	0.001
ADFI	2.72[a]	2.31[b]	2.40[b]	0.095	0.011
FCR	2.25[a]	3.43[b]	2.26[a]	0.342	0.029
dpi 15-19					
ADG	1.31[a]	0.45[b]	1.03[a]	0.115	<0.001
ADFI	3.01[a]	2.03[b]	2.81[b]	0.169	0.001
FCR	2.12[a]	2.82[b]	2.31[a]	0.103	<0.001
dpi 0-19					
ADG	1.27[a]	0.75[c]	1.02[b]	0.044	<0.001
ADFI	2.68[a]	2.03[c]	2.35[b]	0.086	<0.001
FCR	2.13[a]	2.82[b]	2.30[a]	0.103	<0.001

[a,b,c]Means with differing superscripts differ significantly at $P < 0.05$

Discussion and Conclusions

Few studies have quantified the impact of vaccination on growth performance and feed efficiency during controlled experimental challenge conditions. These data characterize the negative impact *L. intracellularis* has on growth performance of grow-finish pigs. Further, these data highlight the importance of vaccination, as vaccination mitigated performance losses and reduced ileal lesion severity compared with non-vaccinated pigs.

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Patterns of *Salmonella* spp presentation in Mexican swine farms

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Introduction

Porcine salmonellosis, caused by thegenus *Salmonella* spp, produces different clinical presentations. Salmonellosis is a significant disease for both animal and public health worldwide (1). In addition, Salmonella is recognized as the principal etiological agent of diseases transmitted through food and water (2). In Mexico, the General Directorate of Epidemiology has published that around 70,000 cases may occur per year (3).
This study aims to assess the association between the presence of *Salmonella* spp in different swine systems in Mexico and their geographic and demographic characteristics.

Material and methods

A directed cross-sectional study was carried out in 18 pig producing systems, representing 50 production sites distributed over two different regions of Mexico: north, and south. A total of 1181 stool samples were processed between August 2020 and April 2021 for presence of *Salmonella* spp, *S.* Choleraesuis and *S.* Typhimurium by real time PCR.Pigs from 5 age groups (6, 10, and 14, 18, and 22 weeks of age) were sampled. Per group four samples were collected and pooled by group. The Odds Ratio (OR) test was calculated to compare *Salmonella* prevalence by age (≤ 10 weeks of age vs. ≥ 14 weeks of age) and by region (north vs. south), applying a significance level of $p = 0.05$. The program Minitab 19 (Minitab, USA) was used to calculate the OR.

Results

From the 1181 samples tested, 33% (348/1181) tested positive for Salmonella.Analyzing age as a risk factor for the detection of *Salmonella* (table #1), it was found that pigs 10-week-old or younger had a significantly higher risk of being positive to *Salmonella* spp (OR = 1.44, $p = 0.004$) and *S.* Typhimurium (OR = 1.75, $p = 0.007$) than those of 14-weeks-old or older., In contradiction, the prevalence of *S.* Choleraesuis tended to be higher for the older pigs (OR = 0.54, $p = 0.089$) . In the caseof geographic region as a risk factor for the detectionof Salmonella, it was found that pigs from the northern region of Mexico have a greater risk of being positive to *Salmonella* spp (OR = 2.74, $p < 0.0001$), *S.* Typhimurium (OR = 1.15, $p = 0.698$), and *S.* Choleraesuis (OR = 1.02, $p = 0.936$) than those from the south.

Table 1. Risk factors for positivity to *Salmonella*. Levels by factor: Age (≤ 10 weeks of age vs. ≥ 14 weeks of age) and Region (north vs. south).

Risk Factor	OR	95% CI	*p*
Age			
***Salmonella* spp**	1.44	1.13-1.85	0.004
***S.* Choleraesuis**	0.54	0.26-1.10	0.089
***S.* Typhimurium**	1.75	1.16-2.64	0.007
Region			
***Salmonella* spp**	2.74	2.07-3.61	0.000
***S.* Choleraesuis**	1.15	0.58-2.28	0.698
***S.* Typhimurium**	1.02	0.67-1.54	0.936

Discussion & Conclusion

This data shows that salmonella infections are quite common in different stages of production. The prevalence of Salmonella serotypes is different between ages of pigs, but also different prevalence patterns are observed between the various serotypes. The higher prevalence of Salmonella detected in the northern region could be due to regional environmental cooling practices that greatly increase the barns humidity.
Salmonella infection in pigs is multifactorial, and forits control, different strategies, including feed interventions, biosecurity, and control of other concurrent diseases must be considered, throughout all the ages of pig production
Among the different control strategies, vaccination against Salmonella should be considered to control the disease's clinical presentation and reduce the bacteria's excretion from subclinically infectedanimals.

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Performance improvements in growing pigs by vaccination with Enterisol® SC54

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Introduction

Salmonella infection in pigs is manifested by different clinical presentations such as enteric, septicemic -both acute and chronic-, as well as respiratory and cutaneous symptoms.[1] This agent is an intracellular facultative anaerobic bacillus capable of surviving in various conditions for long periods.[2] Salmonellosis continues to be a risk for public health and food safety due to the contamination of pig products.[3]

The objective of this study was to evaluate the reduction of mortality and the improvement in weight gain at a site with clinical problems of salmonellosis by applying a live oral vaccine against infections with *Salmonella choleraesuis* (SC) vaccine under field conditions.

Material and methods

The study was conducted on a 1400-sow farm located in Puebla, Mexico. A total of 101 groups of pigs weaned during the period January 2019 and August 2021 were compared in terms of percentage of mortality and weight (kg) when moving to the finishing area at approximately ten weeks of age. A total of 29,088 pigs corresponded to Group A (unvaccinated), while 21,924 pigs were in Group B (vaccinated). Group B pigs were orally immunized at four days of age with 2 ml of Enterisol® SC54. The population of this farm is positive for PRRSV, PCV2, *Mycoplasma hyopneumoniae*, *Lawsonia intracellularis*, and *Salmonella* spp.

The percentage of mortality between treatments was compared using the X^2 test, while the t-student test was applied to compare weights; α=0.05. Graphs and statistical tests were performed with the Minitab 20.0 program (Minitab, USA).

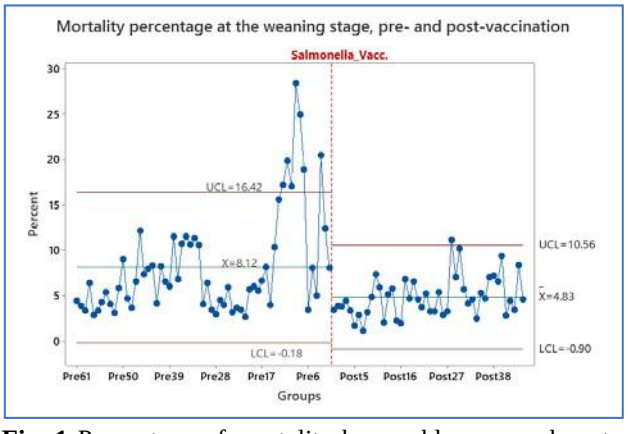

Fig. 1 Percentage of mortality by weekly pre- and post-vaccination groups.

Results

The percentages of weekly mortality by groups pre- and post-vaccination with Enterisol® SC54 are displayed in figure #1. Mortality was reduced by 40.7%, dropping from 8.1% (2354/29088) in the unvaccinated pigs to 4.8% (1062/21924) in the vaccinated pigs (P<0.001).

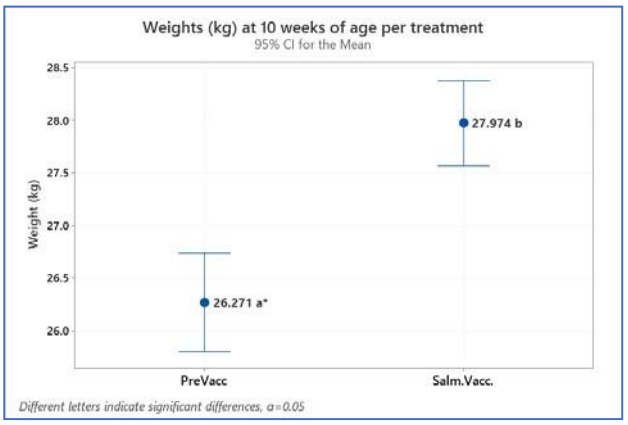

Fig. 2 Weights (kg) per treatment, at ten weeks of age.

Figure #2 shows weights at ten weeks of age. Pigs from the vaccinated group were 1.70 kg heavier than the unvaccinated group (27.97 vs. 26.27 kg; P<0.001).

Conclusions

The results obtained from this study demonstrated the productive and economic advantages of using live oral vaccination of Enterisol® SC54. The improvement of the parameters measured in the nursery pigs could improve the animal's subsequent growing phases as well as a reduced risk of contamination with SC. Further data from the finishing stage will be collected and reported once the corresponding farm reports are obtained. This study highlights the potential of reducing mortality togetherwith an increase of body weight at ten weeks of age due tothe intervention with an SC vaccine.

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Persistence of *Mycoplasma hyopneumoniae* following controlled aerosol exposure

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Introduction

Mycoplasma hyopneumoniae is the causative agent of enzootic pneumoniae in pigs and one of the primary agents involved in porcine respiratory disease complex (1). Herd closure and medication programs are commonly used for eradication of *M. hyopneumoniae* (2) and are based on breeding herd population exposure and the duration of pathogen persistence post-infection. Exposure of all replacement gilts and breeding females to *M. hyopneumoniae* prior to the beginning of the eradication program establishes Day 0 (2). The expected duration of *M. hyopneumoniae* persistence, which may last longer than 214 days, defines the end of herd closure (3). Eradication program success is determined after 240 days post-exposure (2,3). Aerosolization of lung tissue containing *M. hyopneumoniae* has been rapidly adopted in the North American swine industry to achieve population exposure (4), and preliminary data has indicated PCR detection of individuals beyond 240 days post-exposure. Therefore, the objective of this study was to determine the duration of *M. hyopneumoniae* detection in sows and replacement gilts following aerosolized lung homogenate exposure, as part of a *M. hyopneumoniae* and porcine reproductive and respiratory syndrome virus (PRRSv) herd closure and elimination program.

Materials and Methods

A 3,000-head commercial sow herd with an off-site gilt development unit (GDU) undergoing a dual *M. hyopneumoniae* and PRRSv herd closure was identified for the study. Sows were stratified by parity and gilts by age group. Sows (n=70) and gilts (n=70) were randomly selected and tagged for longitudinal sample collection following purposeful aerosol exposure (4). Sample size estimates assumed a 90% diagnostic sensitivity, 100% diagnostic specificity, 5% prevalence, 95% population sensitivity, appropriate population sizes, and allowed at least four extra individuals in event of fall-out.

All gilts were sourced from a *M. hyopneumoniae* negative multiplier, confirmed by diagnostics and prior exposure of the sow herd was assumed based on historical diagnostics and clinical signs. Deep tracheal catheter samples (5) were collected from study animals at 30, 60 120, 180, and 240 days post-herd closure. Samples were individually processed for DNA extraction and the extracted genetic material was tested for *M. hyopneumoniae* using a species-specific real-time PCR at the University of Minnesota Veterinary Diagnostic Laboratory. Samples with a cycle threshold (Ct) value < 40 were considered positive.

Results

Mycoplasma hyopneumoniae detection by real-time PCR is shown in Table 1. A greater proportion of gilts exposed at the off-site GDU were detected positive for *M. hyopneumoniae* at each sampling event, with lower average Ct values compared to sows exposed at the sow farm.

Table 1. Deep tracheal catheter detection by *M. hyopneumoniae* real-time PCR by exposure location.

Days post-exposure	DNA detection[1] # positive/# tested (%)		
	Exposure location		
	Combined*	Off-site GDU	Sow farm
30	80/139 (58)	67/69 (97)	13/70 (19)
60	71/140 (51)	66/70 (94)	5/70 (7)
120	70/134 (52)	60/67 (90)	10/67 (15)
180	25/129 (19)	19/64 (30)	6/65 (9)
240	2/126 (2)	2/63 (3)	0/63 (0)

[1]Real-time PCR. Samples with Ct values < 40 were consider positive. *Combined date for GDU and sow farm.

Discussion and Conclusions

Under the conditions of this study, gilts were responsible for the *M. hyopneumoniae* PCR positive results at the end of the herd closure. As anticipated, based on previously published data (3), a sharp decline of PCR positives for *M. hyopneumoniae* was observed at the end of the herd closure. There was no indication that the two *M. hyopneumoniae* PCR positive gilts at 240 days into the herd closure were false positive. Thus, the decision was made to move forward with additional treatments and added time prior to naïve gilt introduction (herd opening).

Assaying for *M. hyopneumoniae* genetic material using deep tracheal catheter samples allowed for extended detection at least until 240 days post-exposure in this study. This data suggested that a diagnostic sampling plan based on specimens collected from the lower respiratory tract is critical for accurate diagnostics, especially late in the herd closure timeline. These results justify additional research on duration of bacterial shedding post- exposure under field conditions.

Acknowledgments

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Preliminary surveillance for a sudden death in breeding pigs caused by *Clostridium novyi* type B in large herds in Thailand

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Introduction

C.novyi is one of the underestimated pathogens leading to sudden death in sows. To achieve an accurate diagnosis, it is necessary to perform a necropsy and to collect samples after death, no later than 12 hours in accordance with rapid post-mortem decomposition **(**2,3**)**.

Nowadays, this cause of sow mortality is less known in Thailand due to a lack of accurate information about the prevalence of *C. novyi*. Hence, the objective of this study was to confirm the suspicion of *C. novyi* type B in breeder pigs on several farms in Thailand.

Materials and Methods

Five commercial farms were selected, averaging >2,000-sow farrow to finish pigs, based on history of sow sudden death located in western and northern areas in Thailand during 2020-2021. All farms operated on an all-in all-out basis and had good on-farm biosecurity.

Clinical signs of suspected pigs: The deaths of ≥7-10 breeder pigs per week had been recorded. Amoxicillin and penicillin G were used in the feed stuff during pregnancy for treatment. However, medication was not effective in alleviating the disease.

Necropsy examination: A suspected case of a 39-week-old pregnant gilt **(**P0**)** and 2–3-year-old pregnant and lactating sows (P3-P6) were observed. Mild jaundice, panting, convulsion (Figure 1) and death the next morning were noted. Necropsy was promptly performed following the death of breeding pigs.

Figure 1. (L and R) Pregnant gilt displayed mild jaundice and increased panting.

Tissue sample and molecular assay: 10g of liver sample from the necropsied pigs were aseptically collected. DNA extraction process and the real-time PCR procedure for amplification of the portion of virulence genes of α-toxin of *C. novyi* as followed by HIPRAdiagnos, Thailand. A positive result indicates the presence of *C. novyi* Type B in the sample.

Results

The overall prevalence of *C.novyi* type B in five swine farms was proved by real-time PCR diagnostic assay manifesting 60% **(**3/5**)**, whereas *C. novyi* in 3 out of 9 samples from 5 farms was undetectable **(** 33%**)** . Macroscopic lesions were present in the liver and the spleen (Figure 2). The surface of the liver with swelling and pale necrotic areas was scrutinized. Splenomegaly was noted. Moreover, no abnormalities were found in several organs such as brain, intestines and respiratory organs.

Figure 2. Gross lesions of *C. novyi* infection in pregnant sow. (A) Jaundice and increase breathing.
(B) Hepatic necrosis and pale **(**C**)** Splenomegaly

Conclusions and Discussion

The current preliminary study described the presence of *Clostridium novyi* (*C. novyi*) type B isolated from breeder pigs with sudden death manifesting 60%. These novel findings encouraged us to continue longitudinal surveillance the *C. novyi* type B in Thailand in the future. Furthermore, it is clearly accepted that the existence of *C. novyi* type B is an underlying problem pathogen across several herds and is likely an important cause of sow mortality.

This field study may provide key data for swine field surveillance in Thai piggeries which in turn would have an impact on vaccine selection for controlling thedisease. Additionally, HIPRA Thailand is the only pharmaceutical company for animal health that has an establishing laboratory guideline and applicable sample kit for proving the *C. novyi* type B in suspected in breeder pigs.

Acknowledgments

The authors would like to express their gratitude to the farm owners and the sales team for their assistance.

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Prevalence and severity of Enzootic pneumonia and pleuropneumonia in Czech pig farms based on lung lesion scoring in 2021.

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Introduction

Monitoring of respiratory disease by lung scoring is beneficial to assess the farm health status. Ceva Lung Program (CLP) was confirmed as a valuable tool to establish the prevalence and severity of Enzootic Pneumonia (EP) and pleuropneumonia (1). The aim of this study is to evaluate the level of EP and *Actinobacillus pleuropneumoniae* (A.p)-like lesionson Czech pig farms in 2021 compared to the previous years.

Material and Method

The survey was conducted on conventional pig farms belonging to 12 swine producers. A total of 1190 lungs in 20 batches (30-100 lungs/batch) of slaughtered pigs were scored using the CLP method. Percentage of lungs with Bronchopneumonia lesions (BP), the extension of the lesions out of sick lungs were recorded and scored. Dorsocaudal pleurisy (DP) suggestive for previous pleuropneumonia was scored to describe A.p-like lesions. Data were compared to the year 2019.

Results

The prevalence of 28% of BP was found (Q1=9.67; Q3=62), compared to 16.67% in 2019. The area of affected surface of lung parenchyma in pneumonic lungs reached 3.85% (Q1=2.53; Q3=6.23). As for pleuropneumonia – 12% (Q1=6; Q3=17) of lungs were affected by A.p-like lesions, compared to 25.69% previously in 2019). The APPI index was 0.3 (Q1=0.2; Q3=0.37) in comparison to 0.59 in 2019. All values are expressed as median and quartiles 1 and 3.

Discussion

EP-like lesions have an increasing tendency compared to previous period. That indicates that Enzootic pneumonia probably affected again previously well controlled or repopulated farms. Lesions characteristic for A.p infections were less prevalent than previous years. This may be due to improved control of the infection or due to lower N° of scorings because of Covid restrictions. Both types of respiratory diseases however deserve high attention to be controlled. Efficient preventive measures shall be implemented and maintained in the Czech farms.

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Prevalence of enteropathogenic-virulence determinants in commercial pig farms from Mexico

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Introduction

The presence of bacteria harboring enteropathogenic-virulence determinants (EVD) in the intestinal tract can be associated to high mortality, poor animal performance, and significant economic losses in swine production systems (1). Moreover, these bacteria represent a serious threat to public health worldwide (2). Unfortunately, our knowledge about these microbial threats is limited. To solve this problem, a molecular survey to estimate the prevalence of EVD in pig commercial farms from Mexico has been initiated. The aim of the study was to identify the frequency of five well-stablished EVD. Herein, the initial results are presented and discussed.

Materials and Methods

Fecal samples were collected during the year 2020 from commercial pig farms located in central Mexico. Samples were sent to the Laboratory of Molecular Microbiology, Autonomous University of Queretaro, for microbial analysis. These samples (n = 57) were collected aseptically from apparently healthy animals, immediately transported to the laboratory, and then, subjected to DNA extraction, using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, USA). DNA was amplified by PCR to detect the presence of five virulence genetic determinants, *hlyF*, *iroN*, *iss*, *iutA*, and *ompT*, linked to enteropathogenic bacteria. PCR primers and protocols are detailed elsewhere (3). The presence of 3 or more EVD suggest an intestinal colonization by pathogenic enterobacteria (3).

Results

The present study revealed a high prevalence of EVD in fecal samples from apparently healthy commercial pig farms. Specifically, 17% and 49% of the fecal samples, possessed between 3 and 5 virulence genes, respectively (**Figure 1A)**. Also, it was identified that the most prevalent EVD, among these samples, was *iutA*,followed by *ompT*, *iroN*, *hlyF*, and *iss* (**Figure 1B).**

Discussion and Conclusion

Findings of the present study revealed a high frequency of virulence genes in fecal samples from commercial pig farms from Mexico. These results are comparable with reports obtained from healthy pigs sampled in China and Spain (4,5). Together, these results suggest that the intestinal tract of pigs from commercial farms could be frequently colonized by bacterial pathogens such as *E. coli*, *Yersinia* and *Salmonella*, the main reservoirs of these five EVD (6).

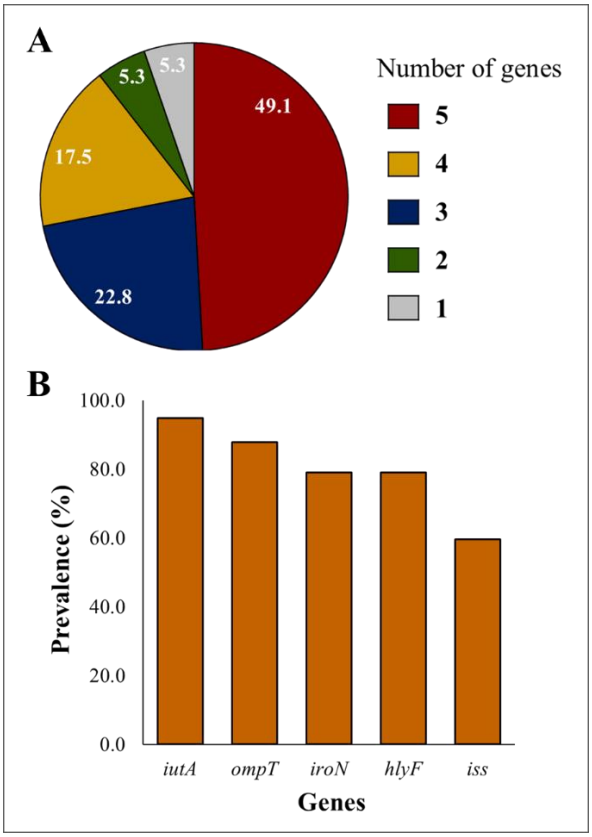

Figure 1. Prevalence of enteropathogenic-virulence determinants (A) and recovery frequency (B) in commercial pig farms from Mexico.

Acknowledgments

We thank managers and staff in commercial pig farms for facilitating the progress of the present study.

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Prevalence of Verotoxin-producing *Escherichia coli* (VTEC) on Korean swine farms

Introduction

Verotoxin-producing *Escherichia coli* (VTEC) causes swine oedema disease by producing verotoxin 2e (VT2e), which induces degenerative angiopathy in susceptible pigs. It is well established that Swine Oedema Disease (SOD) has a huge negative impact on the productivity of the swine industry. Sudden deaths, neurological symptoms, and retarded growth followed by large economic losses can be caused by SOD. However, up until now, the prevalence of VTEC on Korean swine farms has not been well studied due to the limited sensitivity of traditional diagnostic methods.The objective of the present study was to investigate the prevalence of VTEC with a new method using oral fluid samples and FTA (Flinders Technology Associated) cards.

Materials and Methods

From November 2019 to April 2021, a total of 959 oral fluid samples were collected from 196 farms distributed in all provinces of South Korea. The screened farms were selected randomly, without knowing the VTEC infection status of the farms and without SOD being suspected in the majority of cases. *Verocheck*, a commercial test developed by HIPRA, was used (1). The process consisted of collecting oral fluids, transferring them to FTA cards, and evaluating the presence of VT2e genes by real-time PCR assay. Positive cut-off was established at 38.5 CT, and other semi-quantitative values were established (see Table 1).

Table 1. Designation according to Ct value by real-time PCR assay targeted to *Verotoxin 2e* genes

CT value	<30	30-35	35-38.5	>38.5
Designation	Positive+++	Positive++	Positive+	Negative

Regarding the analysis and interpretation of the data, the laboratory results were further correlated with farm information such as herd size and sampling age. Logistic and linear regressions were performed in both cases.

Results

Overall, 132 farms out of 196 were positive for VTEC (see table 2).

Table 2. Nationwide positivity of *VTEC* at farm level in South Korea

	Positive	Negative	Total
No. of farms	132	64	196
Proportion	67.3%	32.7%	100%

There was a significant correlation (P<0.001, r=0.26) with sampling age (see figure 1). The mean percentage positivity was significantly higher in samples from animals older than 12 weeks (77.4%[a]) than animals 9-12 weeks old (60.0% [b]), 6-9 weeks old (47.1% [c]) and younger than 6 weeks (33.9%[d]).

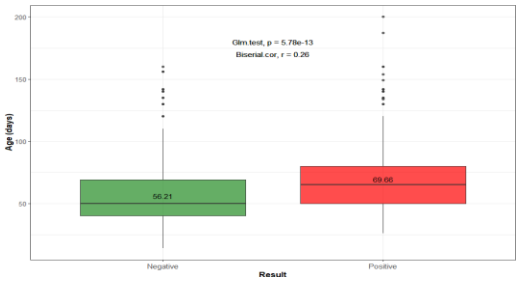

Figure 1. Correlation between VTEC positivity and sampling age.

When positivity was analyzed according to herd size, a negative correlation was observed (P=0.042), the herds with fewer than 600 sows having a higher rate of positivity (see figure 2) and the semi-quantitative positivity increasing as herd size was reduced (see figure 3).

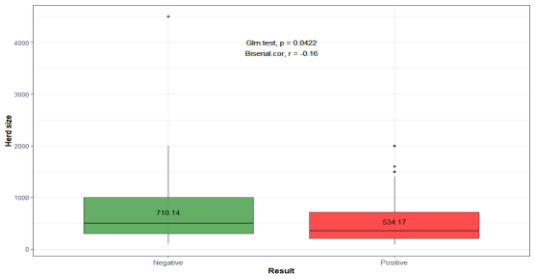

Figure 2. Correlation between VTEC positivity and herd size.

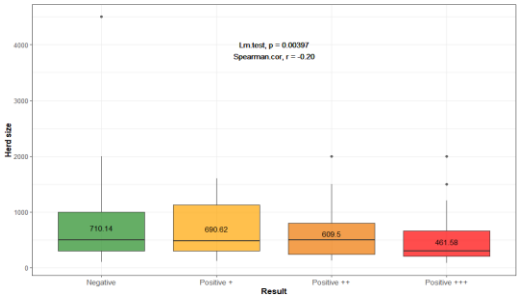

Figure 3. Correlation between herd size and semi-quantitative positivity.

Discussion and Conclusion

The results of the current study indicate that VTEC is present nationwide in South Korea, with a high prevalence at farm level. Moreover, herd size and age of the animals significantly affects positivity.

Acknowledgments

This study was financed by HIPRA.
The authors would like to acknowledge all the vets and farmers who supported them in obtaining the samples.

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Prevention in the control of chronic leptospirosis infections, reducing antibiotic use and improving production parameters

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Introduction

Leptospirosis is one of the major swine reproductive diseases globally. In recent years, the frequency of clinical presentation has increased, inpart due to group housing in gestation for sows' Bratislava is the most prevalent Leptospira serovarin Spain., generally causing chronic herd infections, with reproductive clinical symptoms mostly affecting farrowing rates and increasing litter scatter. Control requires a significant use of antibiotics. The aim of this study is to assess whether vaccination can reduce antibiotic use and improve reproductive parameters in large herds with chronic problems caused by this disease

Materials and Methods

The study was carried out under field conditions, in a farm with 3500 sows. The farm had a chronic reproductive failure problem (low farrowing rate, increased abortion rate and litter scatter). The problem was diagnosed using the microscope agglutination test (MAT, Neiker Tecnalia), in problem sow serum samples (20 sows, 80% with titers >= 1/100 Bratislava serovar). Different reproductive parameters were monitored: total born (TB), mummified (M), ultrasound fertility (F), farrowing rate (FR), litter scatter (LS), as well as antibiotic use, from January to October 2019.In July a multivalent vaccine against Leptospira was introduced (Porcilis Ery+Parvo+Lepto®). Weekly data were used for analysis (n=42)
2 periods were studied, (P)n=29 before vaccinationand with antibiotics in feed (oxytetracycline), and after vaccination (V) n= 13

Results

During P, antibiotic was used twice a month due to reproductive problems. During V no antibiotics were used.
Ultrasound fertility (V) 93.37% vs 92.9% (P) p=0,837; FR 91.14% (V) vs 89,17% (P) $p<0.05$, average mummified fetuses per sow 0.146 (V) vs 0.58 (P) $p<0.001$; LS<9 piglets, 6.70% (V) vs 14.57% (P) $p<0.05$. In multiparous sows, TB18.84 (V) v 18.94 (P) $p>0.05$, in primiparous 15.99 (V) vs 15.69 (P) p=0.049, primiparous sows returning to estrus 3.45% (V) vs 7.95% (P) statistically significant ($p>0.05$)

Discussion and Conclusion

In this field study we have observed that preventive control in chronic leptospirosis situations, significantly reduces antibiotic use as well as improving reproduction parameters over what was achieved with antibiotic control. There were statistically significant differences in farrowing rates, mummified fetuses per litter, litter scatter and even fertility and total live born in primiparous sows with their first Leptospira infection.

Rapid detection of *Actinobacillus pleuropneumoniae* from clinical tissues using recombinase polymerase amplification (RPA)

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Introduction

Actinobacillus pleuropneumoniae (APP) is the causative agent of pleuropneumonia and is responsible for substantial economic losses worldwide (1,2). APP outbreaks can occur when infected pigs are introduced into a naïve herd.

Although APP infections respond well to antibiotics, their overuse has resulted in the spread of antimicrobial resistance genes that may limit treatment efficacy (3–5). Therefore, the continued surveillance and rapid diagnosis of APP is paramount in reducing the use of antibiotics and limiting economic losses.

The gold standard for APP diagnosis is the use of the polymerase chain reaction (PCR) which is costly and time-consuming. Therefore, there is the need for a rapid, sensitive, and specific method of detecting APP infections, without the transportation of samples, e.g., on-farm at the point of care.

Recombinase polymerase amplification (RPA) is an isothermal diagnostic technique that amplifies DNA at 37°C. RPA reagents come in a lyophilized format enabling cold-chain free transportation, ideal for use in a point-of-care diagnostic. Here we use the APP species-specific *apxIVA* gene to formulate an APP-RPA assay and assess its clinical performance in a range of clinically relevant tissue types.

Materials and Methods

APP-RPA *apxIVA* primers and probes were screened using APP serovar 8 reference strain 405.

129 clinical samples, including 100 lung tissues, 15 tonsil scrapings, six oral fluid, four tonsil, three heart and one synovial fluid sample were collected from farms in England, Portugal, Austria, and the Netherlands. Commercial kits were used for DNA extraction as previously described (6).

RPA was performed with TwistAmp Liquid Basic kits (TwistDx, UK) as per the manufacturer's instructions, supplemented with 2 U/µL of Exonuclease III (NEB, UK) in a fluorimeter at 37°C for 20 minutes. APP quantification of clinical samples was done using a previously described APP-qPCR assay (7).

Results

The APP RPA assay had a limit of detection of 80 copies/µL (Figure 1), with all 19 APP serovars being detected at 100 copies/µL.

The APP-RPA assay had an overall sensitivity of 77.33%, with 100% specificity using the 129 clinical samples obtained from various farms throughout Europe.

The majority of false-negative APP-RPA results were in samples with low APP copy numbers.

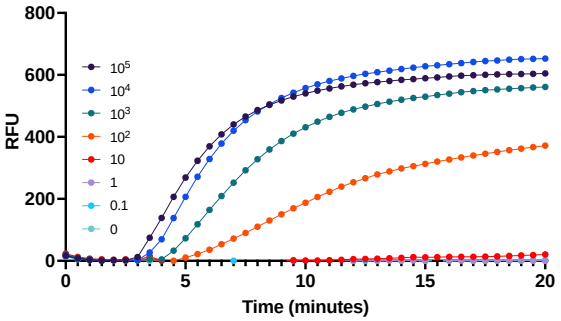

Figure 1. APP-RPA limit of detection. APP-RPA limit of detection performed in triplicate serial dilutions of strain 405 in copies/µL reaction volume.

Therefore, APP-positive samples were grouped into low (<200 copies), medium (201-2000 copies) and high (<2000 copies) bacterial load as determined by qPCR (Table 1).

Table 1. APP-RPA results of 129 clinical samples

Bacterial load	Number of samples (*n*)	Sensitivity (%)
High	24	95.83
Medium	26	96.15
Low	25	40.00
Negative	54	N/A
Total	**129**	**77.33**

Discussion and Conclusion

The APP-RPA assay resulted in rapid (<20 minutes) amplification of clinical samples, achieving high sensitivity in instances where the bacterial load was medium or high. The poor sensitivity of clinical samples with low bacterial load suggests that this APP-RPA assay will not be suitable for the detection of sub-clinically infected animals. However, it would be suitable for the rapid detection of APP outbreaks in naive herds.

Acknowledgements

We thank CEVA Animal Health Ltd for providing clinical samples.

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Rapid, efficient, and safe FTA card-based serotyping of *Actinobacillus pleuropneumoniae* by multiplex-PCR

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Introduction

Actinobacillus pleuropneumoniae (APP) is the causative agent of porcine pleuropneumonia which has a high economic burden worldwide. There are currently 19 known serovars of APP with some considered more virulent than others. Therefore, seroprevalence is an important consideration for surveillance and implementing appropriate vaccination strategies.

Molecular serotyping of APP relies on amplification of serovar-specific capsule synthesis (*cps*) genes (1). Previously, we have described two multiplex polymerase chain reaction assays (APP-mPCR) for the definitive serotyping of all 19 serovars of APP (2). However, mPCR serotyping requires the transportation of potentially infectious and hazardous samples to centralized laboratories, with the use of specialist couriers being expensive and discouraging farms from engaging in routine testing programs.

Whatman FTA cards are chemically coated filter paper, which lyse bacteria on contact and entrap nucleic acids (3). We sought to implement FTA cards in our APP-mPCR serotyping assays, to facilitate the transportation of tissue samples by national and international shipping methods.

Materials and Methods

Twenty-two lung samples were collected from farms within England, Northern Ireland, and Portugal. The tissue surface was disinfected with 70% ethanol prior to an aseptic incision being made in visibly lesion areas, where possible. A QIAamp DNA Mini kit (Qiagen, UK) was used to extract genomic DNA from infected lungs (4).

Tissue imprints were made onto FTA cards (Whatman, UK), via pressing the excised tissue onto the FTA card sample area until visibly saturated and air-dried for at least 2 hours. The processing of FTA cards involved taking a 3-mm biopsy punch of the card, transferring the punch to a 1.5 mL tube, and washing three times in 1 mL of distilled water prior to immediate transfer to the PCR reaction. Extracted gDNA and FTA cards were compared in APP-mPCR assays, using published primer sets and cycling conditions (2).

Results

The sensitivity of our rapid three-water-wash FTA card protocol was equivalent to that achieved with the recommended FTA protocol which requires multiple washes and drying steps (3).

A direct comparison between lung tissue spiked with APP showed that gDNA extraction results in a 100-fold higher sensitivity than FTA-mPCR. mPCRs were carried out on 22 clinical lung samples to enable comparison of FTA-PCR and genomic extraction, representative examples are shown in Figure 1. Identical mPCR results were found with FTA-mPCR and mPCR performed on genomic extractions (Figure 1).

Cultivation of bacterial preparations of APP placed on FTA cards was unsuccessful, indicative of complete inactivation of the bacterium after application to the surface of the card. Additionally, bacteria isolated on FTA cards remained detectable by FTA-mPCR for over 6 months at 37°C.

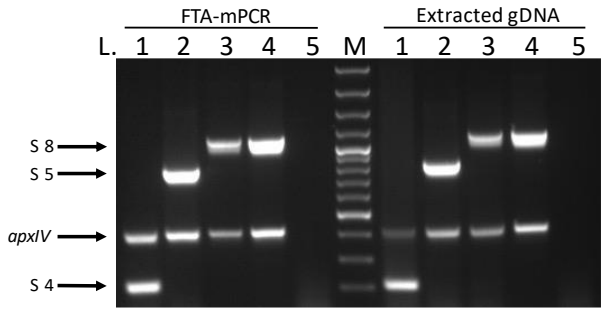

Figure 1. Lung samples processed with FTA-card compared to gDNA extraction.
mPCR results from five lung samples that were positive for APP serovar 4 (L1), serovar 5 (L2), serovar 8 (L3 and L4), and APP-negative (L5).

Discussion and Conclusion

The transportation of animal products poses a significant biosecurity risk, a source of reluctance for many farms and laboratories. Our data suggest that the use of FTA-card sampling of lung lesions is a safe, stable, and rapid sampling method for APP serotyping.

Although a loss of sensitivity is seen compared to performing a full genomic extraction on the tissue, our results show the bacterial burden in infected animals is sufficient to show a 100% agreement between the two extraction methods. Therefore, careful evaluation is required when implementing FTA-card PCR detection of other pathogens, to ensure no loss of diagnostic sensitivity is observed.

These results validate the use of lung imprints on FTA cards for the rapid, safe, and efficient diagnosis of APP by APP-mPCR. We hope this method will increase the use of APP serotyping in remote areas where resources are scarce.

Acknowledgements

CEVA Animal Health Ltd for providing lungs.

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Resistance profiles of Salmonella spp against antibiotics for veterinary use in Mexico

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Introduction

Salmonella is a genus of Gram-negative bacteria and is among the most important foodborne pathogens and tenththird leading cause of human death among diarrhea diseases worldwide, with *Salmonella typhimurium* being the leading serotype of *Salmonella enterica* subsp. *enterica* implicated in foodborne diseases worldwide[1]. The increasing incidence of resistant bacteria in humans, animals, and the environment is a major globalconcern and is being monitored more widely[2]. In theMexican swine industry, the use of antibiotics to control infectious bacterial diseases, both respiratory and enteric, is carried out routinely; however, in many instances, no sensitivity and resistance tests are executed. The objectiveof this study was to determine the resistance profile of *Salmonella* spp against antibiotics of veterinary use in Mexico.

Material and methods

From August 2020 to March 2021, 1130 rectal swabs (feces) samples were collected from farms located in different Mexican regions. The 235 by culture obtained *Salmonella* isolates or, were selected to execute antibiogram testing for 16 antibiotics. The BD BBL™ Sensi-Disc™ antimicrobial susceptibility test was used, according to the manufacturer's instructions and interpretation. Antibiotic inhibition zones for low, intermediate, and high sensitivity were defined as low sensitivity 11-15 mm; intermediate sensitivity 16-22 mm; high sensitivity 23 mm or more.

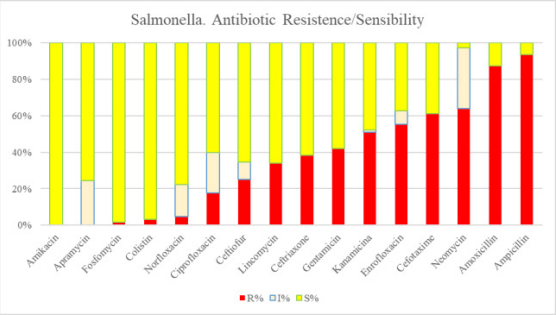

Fig. 1 Proportion of samples resulting in resistance (red), intermediate sensitivity (light yellow), or sensitivity (yellow) to the tested antibiotics.

Results

Figure #1 shows the proportion of samples (out of in total of 235 *Salmonella* isolates) resulting in resistance,intermediate sensitivity, or sensitive to the tested antibiotics. Of those 235 isolates, 94% were resistant to ampicillin and 87% to amoxicillin; on the other hand, over75% of the isolates resulted sensible to amikacin (100%), fosfomycin (98%), colistin (97%), norfloxacin (78%), and apramycin (76%).

Discussion & Conclusions

This study shows the (multi) resistance of Mexican pig feces obtained *Salmonella* spp isolates to various antibiotics for veterinary use. The tested antibiotics are the primary antibiotics used in the Mexican swine industry parenterally or in the feed. According to information obtained from the Infarvet 2020-2021, enrofloxacin (quinolones), amoxicillin, lincomycin/spectinomycin, and penicillin are the most commonly consumed antibiotics during the 2020-2021 period.

Antibiotics are necessary for the treatment of animal diseases. However, it is also evident that theindiscriminate use of antibiotics can result in (multi) resistant bacteria. This is the reason to implement complementary, responsible, and targeted antimicrobial strategies in swine production to improve the animals' health and wellbeing. Reduction of antibiotic use in pig production can lead to a reduction of antimicrobial resistance[3]. Vaccines against swine salmonellosis provideprotective immunity against the main *Salmonella* serotypes affecting pigs and could be an alternative to prevent clinical disease due to infection with Salmonella without the negative consequences of the careless use of antibiotics.

This study proves the high antibiotic resistance of several Salmonella isolates to several classes of veterinary antibiotics. Better education/training is needed to improve the prudent use of antibiotics in the Mexican swine industry and increase the use of non-antibiotic disease control tools like vaccines.

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Saliva sampling as an alternative method besides pooled faeces samples for measuring qPCR Lawsonia levels

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Introduction

Lawsonia intracellularis bacterium is still causing significant economic damage in pigs with pooled faeces sample tested by qPCR as an ordinary measure of the disease level. Saliva sampling method is recently getting more attentions due to its advantage as being more user-friendly by veterinarians.
The aim of this study is to investigate whether a saliva sampling can be adapted as an alternative method in the field using statistical correlation analysis compared to the pooled faeces sampling.

Material and Methods

In a Dutch finishing farm samples were taken at different time points from 12 different compartments. At sampling point, in different pens a pooled faeces sample was collected from different fresh faeces present in that pen with a small spoon in a small container and stirred for making a homogenous sample. At the same time in that same pen, a saliva sample was collected by a chewing rope offered to the same pigs (1).
In total 195 times both individual samples of faeces and saliva were tested by qPCR Lawsonia in the BactoReal Lawsonia kit of Ingentix at the CDS in Boxmeer, The Netherlands. The samples were also pooled by 3 to represent the measurements per compartment side. Statistics calculations were conducted using Spearman's correlation and inter-rater reliability by Cohen's kappa (on Lawsonia status with 38.5 as the cut-off of Saliva sampling and 0 or not 0 as the cut-off of faeces sampling).

Results

A significantly strong correlation on individual samples (r=-0.804, p<0.001) was detected between both sampling methods using the Spearman's correlation. The Kappa value was 0.49 with p<0.001 to show the concordance of both measurements (110/195 being positive by both sampling methods; table 1). Also between the pooled samples a strong correlation (r=-0.818, p<0.001) was shown in the Spearman's correlation.

Table 1. Calculation of the Interrater reliability (individual samples)

		Faeces		Total
		POS	NEG	
Saliva	POS	110	28	138
	NEG	16	41	57
Total		126	69	195

Figure 1. Comparison of LI qPCR BactoReal Ingenetix Saliva rope samples (Ct) vs faeces (log copies/ µl) (individual samples)

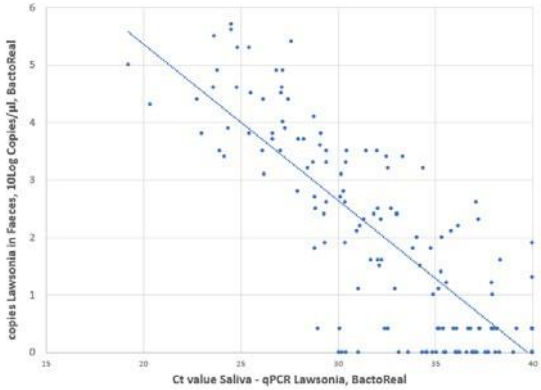
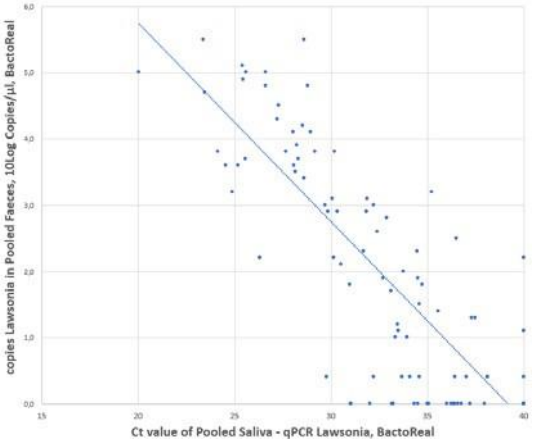


Figure 2. Comparison of LI qPCR BactoReal Ingenetix pooled Saliva rope samples (Ct) vs Pooled faeces (log copies/ µl)



Discussion and conclusion

This study presents a strong correlation between both sampling methods for qPCR Lawsonia. It indicates that saliva sample is a reliable alternative sampling method for practical use in the field. Lawsonia is not excreted by the saliva; but it corresponds with the pen contamination. Saliva sampling outperforms faeces sampling mainly because that with a rope more pigs are sampled compared to some fresh faeces of that same pen from unknown sources. In addition, with rope sampling a veterinarian can collect a sample without entering the pen and when necessary, conduct additional PCR testing on the same sample on for instance PRRS, Flu or mycoplasma. Last but not least, compared to individual sampling, for practical reasons the use of pooled samples can be recommended as very similar strong correlation was shown using our data.

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Salmonella enterica sv Bredeney: A recent case report in Southern Brazil

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Introduction

The identification of *Salmonella enterica* sv Bredeney in Brazil was reported between 1985 and 2009. It is associated with food outbreaks and animals at slaughter, mainly in the Southern region of the country (1,2,3). Considering the importance of correct diagnosis of *Salmoenella* sp. in swine to outline treatment and prevention strategies, the objective of this work was to identify the serovars isolated from swine in Southern region of Brazil.

Materials and Methods

Organs were collected from an 84-day-old porcine and referred to the MV Diagnostic Laboratory for evaluation. The isolates of *Salmonella* sp were previously identified by mass spectrometry using MALDI BioTyper with log values (score) > 2.0. Biochemical and serological tests were performed on the sample to identify the serovars Choleraesuis and Typhimurium, serovars with the highest occurrence in Brazil, but without positive results.Therefore, 5h ofNGS sequencing was performed by MinION from Oxford Nanopore Technologies (ONT) with SQK- RBK004 kit. Real-time basecalling was performed by MinIT and Guppy v3.0.4 software and sequences with quality scores >7 were evaluated by EPI2ME software by WIMP classification. Porechop and Minimap2 software were used for sequence trimming and mapping, respectively. Raw sequencing reads of the isolate was used for serotype prediction.

Results

The sequencing run ranked 18,555 sequences with ≥ quality (Q7) and 16,081 were ranked in the EPI2ME identification software. The average sequence quality score was 11.03 with a total yield of 97.9Mb, about 20x the size of the *Salmonella* genome (4.8Mb) and the average size of the sequences was 5.275bp. Of the 16.081 sequences, 14.309 were identified as *Salmonella enterica* and 1.949 sequences were identified as serovar Bredeney str. CFSAN001080. Sequence mapping analysis of the isolate with the reference genome of *Salmonella* serovar Bredeney NCTC 6026 resulted in an alignment of 86.7% (15.555/17.934 *reads*) and the coverage rate was 15% (Figure 1). Using raw reads workflow SeqSero2 accurately predicted the serovar Bredeney, recognizing the somatic and flagelars antigens (antigenic formulae 4,12:l,v:1,7).

Discussion and Conclusion

The results obtained from Salmonella serotyping are in agreement with those reported in (4). The authors the authors correlated different Salmonella genome coverage to accuracy of serovar's prediction.

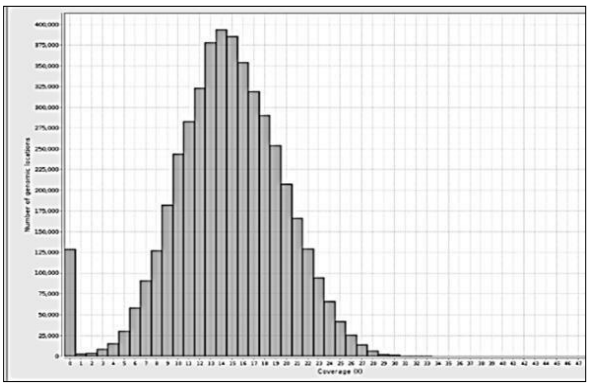

Figure 1. Coverage histogram of MinION readsmapped with reference genome of Salmonella sorovar Bredeney obtained by Qualimap software.

Accordingly, this report shows the presence of *Salmonella enterica* sv Bredeney from a mesenteric lymph node sample of swine in a farm in Southern Brazil. This results was corroborated by NGS sequencing with mapping of 86.7% of the sequences of the isolate with the genome of serovar Bredeney and from SeqSero2's result.

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Salmonella Typhimurium reduction to below the bacteriological detection level in a farrow-to-finish herd using a live attenuated *Salmonella* Typhimurium vaccine

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Introduction

Salmonella Typhimurium (ST) is an important zoonotic pathogen in swine, that can cause clinical disease (1). This case report describes a farrow-to-finish swine herd, producing its own replacement gilts. The farm experienced clinical outbreaks of salmonellosis caused by ST regularly since 2002, occurring in weaned piglets, growers, finishers and gilts, leading to high antimicrobial (Colistin) use. Aim of this study was to see whether next to reduction of clinical signs and thus reduction of antimicrobial usage, it was possible to reduce ST to below the bacteriological detection level, using vaccination of sows and piglets with Salmoporc a live attenuated ST vaccine according to SPC, in combination with standard hygienic measurements.

Materials and Methods

Monitoring of Salmonella on farm was done every month and later every two months, using in total 20 pooled fecal, sock and dust samples per farm visit in the period after implementation of the vaccination from September 2016 to November 2019. Follow up samplings were performed in October 2020 andOctober 2021)

Results

After starting vaccination in August 2016, within the first ten months the results showed a rapid decline in clinical symptoms, the antimicrobial usage and the number of Salmonella-positive samples from 50% to 0%. During the winters of 2017/18 and 2018/19 the number of positive samples increased due to management factors. In July 2019. Only two samples from a corridor were positive and in the samplings in November 2019, October 2020 and October 2021 all samplings were completely negative (Figure 1).

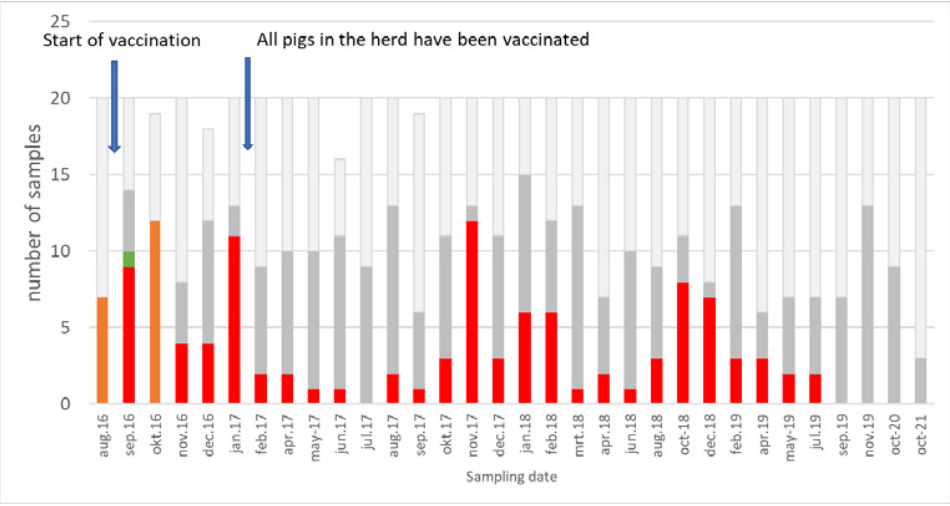

Figure 1. Number of samples taken per sampling date, differiented by bacteriological testing result (orange: *Salmonella* Typhimurium (ST), no differentiation field/vaccine strain, red: ST field strain, green: Salmonella enterica serogroup C, dark grey: Salmoporc®vaccine strain, light grey: negative

Discussion and Conclusion

Eradication of *Salmonella* Typhimurium from pig farms is a difficult goal (2). As Salmonella is present in the whole production chain and infection already takes place early in piglets' lives (3), the best results can be archived in a combined vaccination approach of sows and piglets. This case can be seen as a proof of principle that vaccination with Salmoporc in addition to improved management factors can be a tool to reduce ST contamination within a herd to below the bacteriological detection level. Also clinical signs and antimicrobial use were no longer present or needed. Vaccination during an extended period of time can be a valuable additional tool, like in poultry industry, to reduce the number of herds supplying *Salmonella* Typhimurium infected swine to slaughterhouses and ultimately reduce the number of salmonellosis cases in humans.

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Screening of *Glaesserella parasuis* on farms with polyserositis in LATAM

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Introduction

Glaesserella parasuis is a major pathogen affecting the pig industry, causing meningitis, polyserositis, polyarthritis and bacterial pneumonia; an infection known as Glässer's Disease (GD) (1). Antimicrobials have been commonly used to treat this bacterial disease in farm animals, but the emergence of antimicrobial resistance, a serious threat for public health, necessitates the implementation of alternatives for disease control (2). Vaccines are the preferred alternative for control of the infection, as they have been shown to be useful in the prevention of this disease (1). The objective of this study was to evaluate the presence of *G. parasuis* on farms in LATAM countries with relevant symptoms.

Materials and Methods

For the analytical procedure, samples were obtained from animals 4 to 8 weeks old with symptoms consistent with GD (polyserositis, polyarthritis meningitis and bacterial pneumonia). The animals were necropsied, sampled with a sterile swab, the contents were transferred to FTA cards and the cards were sent to HIPRA DIAGNOS (Brazil, Peru and Mexico) for performance of a qPCR analysis of *G. parasuis* (3). A farm was considered positive when at least one of the samples was positive. These samples came from farms (N=296) in different LATAM countries (Mexico, Colombia, Argentina, Brazil, Costa Rica, Peru, Guatemala, Ecuador, Chile and Paraguay) where 1 to 4 animals were sampled per farm.

Results

The mean prevalence of positive farms on a global basis was reported to be 84% (250 out of 296 farms). When analyzing the data by country, excluding countries with a very limited number of samples (fewer than 5 samples), the top countries with the highest prevalence were Mexico (95% out of 89 farms), Brazil (83% out of 41 farms), Argentina (79% out of 52 farms) and Colombia (67% out of 54 farms). Two other countries stand out, with fewer samples but with a high prevalence, Costa Rica (100% out of 20 farms) and Peru (79% out of 19 farms). See figure 1.

Conclusions and Discussion

The results obtained show a high presence of *G.parasuis* on farms with polyserositis in LATAM countries, and are in line with previously published scientific literature in which it was demonstrated that *G.parasuis* is one of the main agents involved in polyserositis cases (4,5).

As *G.Parasuis* can be controlled with the use of vaccines (1), these results indicate that it is possible to reduce the use of antibiotics for the prevention or control of polyserositis cases in LATAM. Hence, it is essentialto perform a correct diagnosis in order to be able toassess the best treatment or preventive method otherthan the non-rational use of antibiotics.

Figure 1. Percentage positivity in the different LATAM countries. Number in brackets indicates the farms tested.

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Serological monitoring of *Mycoplasma hyopneumoniae* vaccination uptake using a new ELISA kit.

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Introduction

Mycoplasma hyopneumoniae (Mhyo) is the primary pathogen of Enzootic pneumonia in pigs (1). Humoral response to the infection can be measured by different available types of ELISA (2). The aim of this study was to assess the potency of different Mhyo vaccines using serology and propose the optimal sample testing for semi-quantitative evaluation.

Material and Methods

In total, 250 samples from pigs vaccinated with Hyogen® (H), (Ceva) or vaccines A and B were analyzed using the ID Screen® *Mycoplasma hyopneumoniae* Competition ELISA (cELISA) test (IDvet).

To highlight post vaccination monitoring and immune response, several serial dilutions of positive vaccinated sample were tested. Standardized values were calculated and the percentage of positive samples for each dilution was compared among differently vaccinated groups of pigs. Positivity at dilutions 1:1 and1:5 (out of positives in dilution 1:1) were evaluated to better objectivate seroconversion.

Results

With only two dilutions per sample, it is possible to assess a semiquantitative interpretation. The first dilution (1:1) measures the overall immune response in a population, and the second dilution (1:5) determines the strength of the immune response. Analysis with the Mhyo cELISA allows to measure the seroconversion with pigs' samples vaccinated with vaccine H (79% and54% positivity in dilutions 1:1 and 1:5). Such strong seroconversion is not observed with other commercial vaccines (45%, 32% in dilution 1:1 and 16%, 0% in dilution 1:5 for vaccines A and B).

Discussion and Conclusion

Based on available results analyzing blood serum samples in dilutions 1:1 and 1:5 can be proposed. This allows not only to define the prevalence of true positiveresponders but also to estimate the magnitude of the humoral immune response. In this study Hyogen® provided stronger serological response than other testedvaccine. The IDvet Mhyo cELISA can be potentially used for this vaccine uptake monitoring.

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Serology by Flow Cytometry: a smart strategy to assess *Lawsonia intracellularis* circulation in pig farms

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Introduction

Lawsonia intracellularis is the causative agent of Porcine Proliferative Enteropathy, an important worldwide distributed enteric disease of swine (1). Infected animals can be diagnosed by detecting *L. intracellularis* on fecal samples by qPCR and by serological assays such as ELISA, IPMA and Flow Cytometry (FC) that are used to assess the contact of pigs with *L. intracellularis*. We recently described the use of FC to detect anti-*L. intracellularis* IgG in pigs (2). Here, we evaluated the concomitant use of qPCR and FC for the detection of farms positive for *Lawsonia intracellularis*.

Materials and Methods

We evaluated the sensitivity of qPCR and FC to detect the presence of *Lawsonia intracellularis* in conventional farms. A total of 17 farms with frequent episodes of diarrhea during the growth and finishing phase were selected. From these farms, 820 serum and feces samples were transversally and concomitantly collected from pigs with different ages (65 up to 210 days). At least twenty animals per farm or age were included. Faeces samples were diluted to 10% in PBS and used for total genomic DNA extraction (MagaZorb DNA Mini-Prep Kit, Promega, USA). The molecular detection and quantification of *L. intracellularis* was performed by qPCR as previously described (3). Serum samples were used to detect *L. intracellularis* IgG by Flow Cytometry as described previously by our group (2).

Results

All farms (n=17) had at least one animal shedding *L. intracellularis* on faeces, which was detected by qPCR. In parallel, serum samples from the same animals were analyzed by FC and all farms had positive animals. As illustrated in Figure 1, the level of positivity at the different moments evaluated varied considerably according to the sample and diagnostic method used. Although the qPCR technique is specific and able to detect a low number of *L. intracellularis* in the faeces, we noticed that the percentage of pigs with shedding *L. intracellularis* in the faeces (*n*=254, 30.97%) was consistently lower than the number of pigs with specific *L. intracellularis* IgG (*n*=617, 75.24%). Although approximately two thirds of the animals were not shedding *L. intracellularis*, the FC results showed that most of them were infected at some point during the growth and finishing phase; and possibly at the time of sampling they had already naturally controlled the infection.

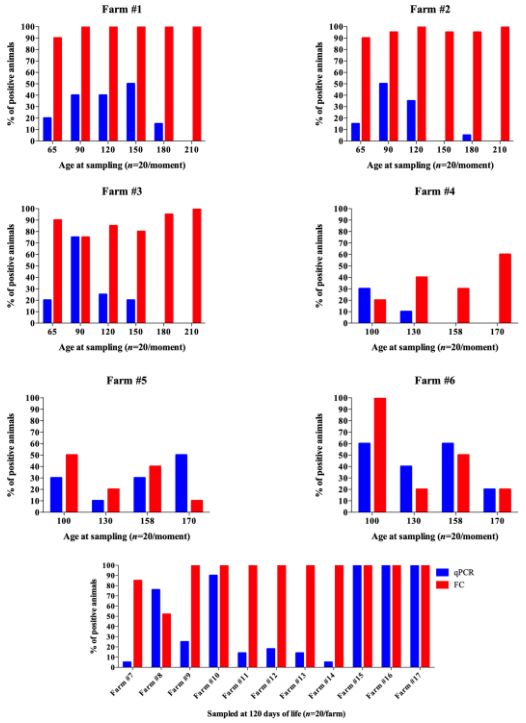

Figure 1. Concomitant detection of *L. intracellularis* by qPCR or specific *L. intracellularis* IgG by Flow Cytometry.

Discussion and Conclusion

Molecular or serological diagnosis of *L. intracellularis* is important for detecting pigs with subclinical infection. Here we demonstrated that FC is a suitable technique for monitoring herds for *L. intracellularis*. Strategically, FC serology can be used primarily to assess the dynamics of infection on the farm. After identifying the time when pigs become positive for the presence of IgG against *L. intracellularis*, fecal samples can be collected (~14 days before the first IgG peak) and qPCR used to understand the infection burden and estimate the economic losses resulting from the infection process.

Acknowledgments

This work was financially supported by MSD.

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Sock sampling detection of *Escherichia coli* F4/F18 in 30 pig farms using antibiotic medicated feed for post-weaning diarrhea: how to develop a new preventive strategy?

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Introduction

Post-weaning diarrhea (PWD) due to *Escherichia coli (E. coli)* causes mortality and reduces performance in pig farms. PWD is due to *enterotoxigenic E. coli* (ETEC) with fimbriae F4 or F18. Sources of ETEC are usually found in the environment of the piglets: mammary gland of the sow, farrowing pens, nursery pens, piglets with ETEC diarrhea or subclinical carriers (1). Despite a significant decrease, nursery period is the production phase with the highest antibiotic usage and mainly for digestive disorders. Medicated feed was the second pharmaceutical form used in post-weaning with 48% of usage in 2019 (2).

In France, regulation on antibiotic medicinal products used for prophylaxis has changed in January 2022 (3). Several alternative strategies exist to prevent PWD without antibiotics in particular *E. coli* F4/F18 vaccination (1). In order to prevent PWD and to promote a prudent use of antibiotics, the diagnosis of ETEC presence in farms and the assessment of risk factors are determinant.

The detection of *E. coli* F4 and F18 with sock samples showed acceptable performance and repeatability for detecting groups of ETEC-positive diarrheic nursery pigs. Furthermore, socks detect contamination of the environment due to previous excretion of the group of pigs (4,5). Thus, under medicated feed containing antibiotic, sock samples could detect excretion of piglets under treatment and after treatment (6).

The objective of this study was to detect *E. coli* F4/F18 with sock samples and assess risk factors for PWD in farms using medicated feed during post-weaning in order to consider alternative preventive solutions forthe future.

Materials and Methods

From November to December 2021, 30 pig farms using medicated feed containing antibiotic to treat against PWD were selected in order to develop an alternative prevention strategy for future batches after January 2022. In each farm, two post-weaning pens were sampled with 2 pairs of socks (ColiBoots™ diagnostic tool, Elanco) 7 days after the end of treatment. It was performed between 4 to 10 weeks of piglet's age to assess the status of the farms for *E. coli* F4/F18 and risk factors for PWD through an audit tool.

The 2 pairs of sock samples were sent and pooled at the laboratory in order to perform a qPCR for *E. coli* F4/F18. The detection limits in CFU per gram faeces

Results

Above the qPCR breakpoint, 13% of the farms were positive for F4, 27% of the farms were positive for F18 and 37% of the farms were positive for F4 and F18 (figure 1).

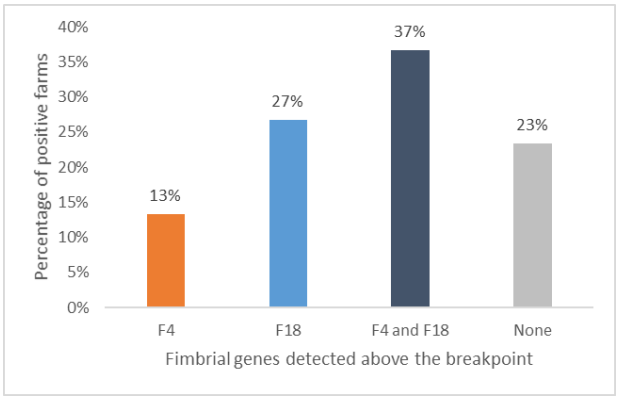

Figure 1: Sock samples results for detection of *E. coli* F4/F18 by qPCR (n=30 farms)

The prevalence of *E. coli* F4 and/or F18 in post-weaning pens was 77%. Despite the use of medicated feed, 57% of farmers declared to have episodic or frequent diarrhea during post-weaning over the year. PWD due to *ETEC* with fimbriae F4 or F18 isa multifactorial disease. The presence of ETEC strains is a determining factor and contributing factors (temperature, ventilation, feed, water, ...) play a role in the development of clinical manifestation (1).

Discussion and Conclusion

This study confirms a high prevalence of *E. coli* F4 and/or F18 in post-weaning pens. Sock sampling is a suitable tool to determine *E. coli* F4/F18 prevalence.

With uncontrolled contributing factors and the end of prophylaxis with medicated feed, these positive farms could be at risk to develop clinical manifestation of PWD. It's time to focus on risks factors and consider alternative preventive solutions.

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Swine Erysipelas risk on non-vaccinated farms in China

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Introduction

Swine Erysipelas (SE) is an economically significant disease capable of affecting all stages of production. In sows, SE can cause abortions at any moment of gestation, but also acute death, skin lesions, fever, or lameness (1). The protective role of specific antibodies against SE enhanced through vaccination, is the key to controlling infectious reproductive problems in sows (2). Recently SE has been categorized as a potential disease to cause outbreaks in China (3). Serological assays such as ELISA have been used to detect antibodies against *Erysipelothrix rhusiopathiae* in pigs. The contact with the bacterium or after vaccination develops a humoral immune response (4).

The objective of this study was to evaluate whether *E. rhusiopathiae* is present or not on Chinese farms that are not vaccinating against it, as a prospective causative agent of reproductive disorders, evaluating through the immune status of the animals if the bacterium is circulating on the farms.

Material & Methods

A total of 2681 sows were included in the study (parity 0 to 7) from 68 different farms located in 13 provinces from North and South China. Farm size varied between 564 to 35,000 sows. None of these farms include the immunization against *Erysipelothrix rhusiopathiae* in their vaccination schedule. Blood samples were collected from January 2020 to December 2020.

Serum samples were tested using a commercialized ELISA kit (CIVTEST® SUIS SE/MR; Cut off-IRPC: 40). The ability of this kit to detect anti-SE antibodies without bias has been previously reported (5).

Results

From the total number of animals, 22.7% were SE positive (IRPC >40). Sows of parity 1 (29%) and parity ≥2 (24%) had a higher positivity rate than parity 0 (15%), with these differences being statistically different ($p<0.05$, Mann-Whitney U test).

The number of farms within each range of positive animals was: 3 (0% positivity), 12 (2%-9% positivity), 12 (10-19% positivity), 24 (20-36% positivity), 5 (37-54%) and 12 (55-70% positivity) (Table 1).

Table 1. Number of farms within each range of positive animals

Range of positivity	Nº of farms
0%	3
2-9%	12
10-19%	12
20-36%	24
37-54%	5
55-70%	12

Discussion & Conclusion

Based on these results, *E. rhusiopathiae* is circulating in 95.5% (65/68) of the nonvaccinated Chinese farms, as a different percentage of animals have developed an immune response against the bacterium. So, SE is a bacterial disease that could be causing different reproductive disorders on Chinese swine farms that could be going unnoticed. Furthermore, the positivity in sows of parity ≥1 is higher than in parity 0, which indicates that the prevalence in the production units is higher.

As prevention of SE is best accomplished by immunization programs (1) the inclusion of vaccines including antigens of *E. rhusiopathiae* , which elicits a strong immune response, must be used to prevent reproductive disorders.

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The effect of two different vaccine strategies against *Actinobacillus pleuropneumoniae* on lung lesions

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Introduction

Actinobacillus pleuropneumoniae (A.p.) is one of the main respiratory pathogens causing great damage to the swine industry (1). One of the tools to monitor the challenge and effectiveness of control programs is lung lesions investigation at the slaughterhouse (2). The aim of this study was to compare two vaccine protocols against A.p. through the presence of A.p.-like lesions by the Ceva Lung Program.

Materials and Methods

The study was carried out in 3 finishing farms in different geographical locations, inside the same production system, and with history of A.p. issues. Two vaccines to control A.p. according to same protocol were evaluated. Each pig received 2 doses of 2 mL, 7 and 10 weeks of age. From October 2018 to April 2019 approximately 1700 animals per month were vaccinated with a commercial vaccine B (G2 group). From April to August 2019, there was a transition period (TP), with animals vaccinated with either of the two vaccines. From August 2019 until April 2021, the same number of animals per month, as in G2, were vaccinated with Coglapix® (Ceva, France – G1 group). The piglets in the three farms had the same sanitary, genetic, and nutritional conditions and were destined for the same slaughterhouse. Lung lesions were evaluated using Ceva Lung Program, a tool described in previous studies (3). From the three farms a total of 429 lungs G1, 563 lungs of G2, and 192 lungs in TP were inspected.

Results

The evolution CLP, lung lesion investigations data are shown in Figure 1.

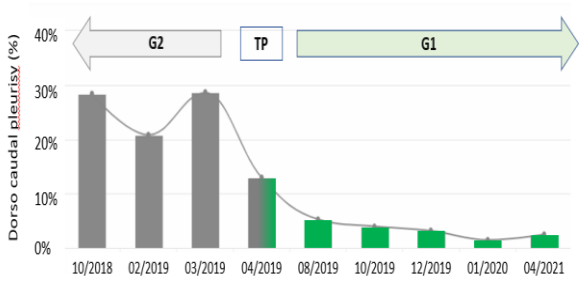

Figure 1: A.p.-like lung lesions (percentage of dorsocaudal pleurisy) over time.

A.p.-like lesions values are shown in table 1. Animals from G1 group had significantly lower prevalence of dorsal caudal pleurisy than animals vaccinated with vaccine B (3.17% vs 25.89%) p <0.05. In addition, the A.p. pleurisy (severity) index was lower in animals from G1 group (0.10 Vs 0.74).

Table 1. Comparison of lung lesion values between groups G1 and G2. Different superscripts indicate a significant difference (p<0.05).

Protocol	Dorsocaudal pleurisy	A.p.p.-Index
G1	3.17%[a]	0.1
G2	25.89%[b]	0.74

Discussion and Conclusion

Special scoring systems, like CLP on lung lesions, are used to generate numeric data from clinical and pathological observations. These data are evaluated with suitable statistical methods. In this study it was possible to use CLP as a tool to monitor health challenges, vaccination programs and indicate the best health measures to be adopted. It was possible to observe two important reduction moments of A.p.-like lesions: the first reduction occurred in the transition phase of the protocols between the months of April and August 2019. And the second significant reduction, from August 2019 over time following total change of A.p.-vaccine (G1) to Coglapix® compared to G2.
Coglapix® has presented itself as a useful alternative to reduce the challenges caused by A.p., as observed in other studies (4,5). Assertive tools in the control of this agent contribute to greater herd health and, consequently, greater profitability for the producer.

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THE PRESENCE OF *E. COLI* WITH A VT2E GENE RELATING TO OEDEMA DISEASE IN PIGS FROM INDUSTRIAL FARMS IN VIETNAM

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Introduction

Oedema disease is an important cause of mortality and hence economic losses in the pig industry. It is caused by Verotoxigenic *E. coli* (VTEC) due to the production of the Verotoxin 2e (VT2e). The disease has been thought to be highly prevalent in backyard farms in Vietnam[1], however, little information is available in the literature. Also, there are no reports on the pressure of this pathogen on the industrial farms that pig production in Vietnam is moving towards. The aim of this study was to determine the prevalence of VTEC in pig herds in Vietnam.

Materials and Methods

Farrow-to-finish farms (n=39) with a herd size of at least 1,000 weaners and productive impairment acknowledged by the veterinary herd manager were recruited and studied from 10/2020 to 04/2021. The presence of VT2e was assessed in oral fluid samples collected from pigs at different ages (6- 8, 11-13 and 15-17 weeks of age) by qPCR[2]. A total of 9 samples per farm (3 samples per age group) was collected. Each oral sample was taken from one pen with 30 pigs.

Results

Table 1. The true prevalence of farms with *E.coli* Vt2e at 95% confidence level.

qPCR results	Nº of sampled farms	True Prevalence (%)
Negative	10	25.64 (13.04-42.13)
Positive	29	**74.36 (57.78-86.96)**
Total farms	39	100

Table 2. The prevalence of positive samples with *E.coli* Vt2e by age groups.

	Age			**Total**
PCR results	6 – 8 weeks	11 – 13 weeks	15 – 17 weeks	
Total samples	118	111	109	338
Negative	74	69	70	213
Positive	44	42	39	125
Positive rate (%)	37.29	37.84	35.78	36.98
95% confidence level	28.56-46.67	28.80-47.54	26.83-45.53	31.82-42.37

The results show that VT2e was detected on 74.36% of the farms (see Table 1). The positive oral fluid samples within farms ranged from 10-100%, 20-40% being the most prevalent. The presence of VT2e was homogeneously distributed among the different ages, ranging from 37.84 to 35.78% (see table 2). Among the positive farms, 17/29 had a prevalence of over 40%. There were no statistically significant differences in positivity between age groups.

Conclusions and Discussion

The findings highlight a high prevalence of VT2e and risk of oedema disease on industrial pig farms that might have been neglected until now. The clinical problems of the disease might be hidden due to the availability of antibiotic or Zinc oxide strategies which are commonly used by pig producers. However, with Vietnam's progressive reduction of antibiotic and ZnO use, there is definitely a risk of the disease appearing. This finding is especially relevant in a context of antibiotic reduction in farming, and it demonstrates the need for alternatives to control this disease.

Acknowledgements

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The vaginal microbiome from vulval discharge in postpartum sows

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Introduction

In the first few days after farrowing, sows excrete some discharges from the vulva that can be either normal or abnormal. The vulval discharge may originate from the uterus, vagina, vulva, or the urinary tract. Bacteria identified using bacterial culture technique from pus exudates included *Escherichia coli* (33.3%), *Staphylococcus sp.* (17.5%), *α-hemolytic Streptococcus sp.* (14.3%), and *β-hemolytic Streptococcus sp.* (9.5%) (Tummaruk et al., 2010). These bacteria can compromise physiological function of the sow uterus and vagina, cause inflammation in the walls of the vagina and can also lead to endometritis. The advent of next-generation sequencing (NGS), which use the 16s ribosomal RNA region of the bacterial genome to identify bacteria has enable more accurate characterization of human and animal microbiome (Farahani et al., 2020; Wang et al., 2020). However, the vaginal microbiome study in pig is limited. The aim of the present study was to determine and characterize bacteria from the abnormal vaginal secretions in postpartum sow in tropical environment by using NGS.

Materials and Methods

Pus exudate (2 mL) from 10 Landrace × Yorkshire postpartum sows that had vaginal discharge syndrome was collected from a commercial swine herd in Thailand. The bacterial 16S rRNA gene (rDNA) sequence universal primers were used to amplify 16S rRNA sequence from the isolated bacteria. Microbiome analyses was performed by using 16S rRNA metagenomic Sequencing. The saturation of microbial richness of all samples was 55,802 sequencing depths.

Results

The result was obtained from 845,405 high quality reads of 16S rRNA. The observed abundance of ASVs, bacterial abundance (Chao1) and bacterial diversity (Shannon) from the vulval discharge samples are presented in Table 1. High variability of bacterial abundance was observed among samples. Taxonomic profile of bacteria detected from vulval discharge in postpartum sow is illustrated in Figure 1. Bacteria identified from pus exudates in postpartum sows by NGS included *Fusobacterium* (25.0%), *Peptostaphylococcus sp*. (9.0%), *Bacteroides* (8.0%), *Streptococcus sp.* (7.0%), *Proteus* (6.0%), *Porphyromonas* (4.0%), *Parvimonas* (4.0%), *Enterocuccus* (3.0%), *Staphylococcus* (3.0%), *Alloprevotella* (3.0%), *Erysipelatoclostridium* (2.0%), *Escherichia-Shigella* (2.0%), *Actinobacillus* (2.0%), *Prevotella* (2.0%), *Veillonella* (2.0%) and miscellaneous (18.0%). However, the type of bacteria identified from the vaginal microbiome differs among individual sow. For instance, *Staphylococcus* was identified in 23.0% of the vaginal bacteria in one sow, while *Proteus* was found in 57.0% of the vaginal bacteria in another sow.

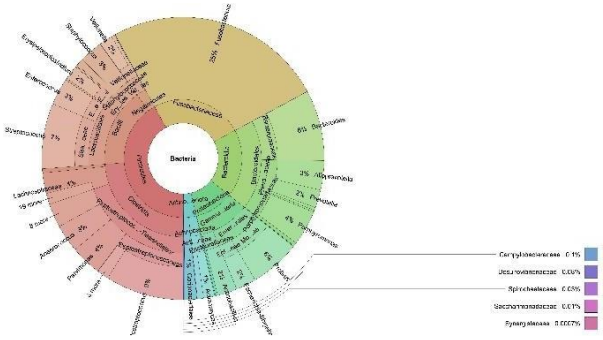

Figure 1 Taxonomic profile of bacteria detected from vulval discharge in postpartum sow.

Table 1 Alpha diversity measure of the vaginal microbiome in postpartum sows

Sample	Observe ASVs	Chao1	Shannon
1 – 7	24 – 83	24 – 83	1.6 – 2.8
8 – 10	96 – 129	96 – 130	3.1 – 3.4

Conclusions and Discussion

The present study demonstrated the efficacy of using NGS to identify the vaginal microbiome in pus exudate obtained from postpartum sows under tropical environment. Both the type and the proportion of bacteria differ from earlier studies using conventional bacterial culture technique (Tummaruk et al., 2010). This new technology allows veterinary practitioners to have a more accurate bacterial identification.

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Use of organic acids to reduce *Salmonella* Typhimurium colonization in pigs

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Introduction

Salmonella spp. can be present at all stages of pig production, but the finishing phase has been identified as the most frequently involved in the infection of swine herds. Its prevention is necessary since the colonization in the intestinal tissue leads to carcass contamination, presenting a risk for public health. The use of acidifiers as an alternative for the prevention and control of *Salmonella* can improve zootechnical performance, facilitating the digestion process and reducing the number of pathogenic microorganisms in the intestine (1). This study aimed to evaluate the effectiveness of using an organic acidifier to control *Salmonella* Typhimurium in 65-day-old pigs by detecting the pathogen excretion during the finishing phase and in organs after slaughter.

Materials and Methods

For this purpose, two experimental groups consisting of 12 piglets each were used. For 10 days (D-5 to D5) one group was treated with a liquid organic acidifier (Axeed® Liquid) in drinking water, while the other did not receive any type of treatment. Five days after the start of treatment (D0), the animals were orally inoculated with 10^6 CFU of *S.* Typhimurium. Every three days (D3, D6, D9, and D12), three piglets from G1 and G2 were euthanized. Samples from lungs, liver, spleen, mesenteric lymph nodes, ileum, cecum, and ileocolic lymph nodes were collected for the microbiological isolation and molecular detection of loads *Salmonella* gene *hilA* by qPCR (2).

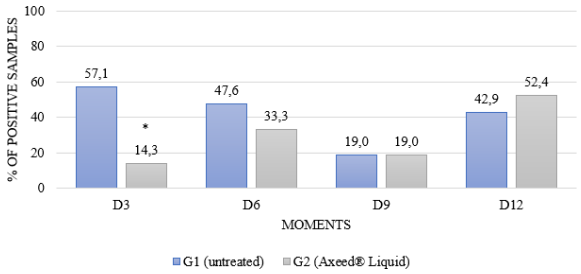
Results

The untreated group had 91.7% of positivity in cecum samples in qPCR, while the treated group had 50%, which characterizes a reduction of 45.5% in the total cecum samples positive throughout the experimental period. All values resulting in qPCR by gene *hilA* are shown in Table 1. Additionally, regarding the other samples, it was noted that the treated group presented a reduction of 11.8% in the quantified bacterial load compared to the control.

Table 1. Absolute number and percentage of cecum samples positive for *S.* Typhimurium, by qPCR, in G1 and G2.

	G1		G2	
Timepoints	+/total	%	+/total	%
D3	3/3	100	1/3	33.3
D6	3/3	100	1/3	33.3
D9	3/3	100	2/3	66.7
D12	2/3	66.7	2/3	66.7
Total	11/12	91.7[a]	6/12	50.0[b]

*Values followed by the same letter in the column do not differ by the chi-square test or Fisher's exact test (p>0.05)



Percentage (%) of organ samples positive for *Salmonella Typhimurium* in the microbiological isolation in G1 and G2. * p<0.05.

Discussion and Conclusion

The longer supply of acidified water with a mixture of acids (lactic, formic, propionic, and acetic) at a concentration of 0.035% during the fattening period (6-7 weeks of treatment) showed a reduction in the number of seropositive animals when compared to the control, as well as a decrease in the excretion of the bacteria in the feces of the treated group (3).

Our findings have shown an elevated percentage of positive cecum samples by qPCR (91.7%) in the untreated group. This high bacterial load in the cecum can be attributed to re-infection or recent infection, as during periods of stress, carrier pigs may experience a recrudescence of infection, and *Salmonella* counts in their intestines and feces may increase (4).

Beyond that, the presence of piglets with high *Salmonella* load in the cecum influenced the proportion of carcasses contaminated by the bacteria on the same day, a fact observed by a correlation, between carcass contamination and *Salmonella* load in the cecum of the even pig (4)

The results indicate that acidifying the drinking water of piglets at strategic moments and at times of great stress remains an option to be considered in the control of *Salmonella* in swine herds, as it may reduce intestinal colonization and bacterial excretion. Thus, organic acids could be strategically used to replace antimicrobials to reduce the occurrence of *Salmonella* in piglets.

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Use of quantitative PCR to diagnose *Clostridium perfringens* type A in neonatalpiglets.

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Introduction:
C. perfringens type A (CpA) is a normal component of the swine intestinal microbiota (1). CpA is also considered by some authors as a cause of enteric disease in neonatal and, occasionally, weaned pigs (2). Nowadays, the main challenge to face is that CpA is the most ubiquitous toxinotype in the intestine of neonatalpiglets[1], in fact its role in the neonatal diarrhoea disease is controversial. It has been suggested that when some strains of CpA multiply beyond control, probably 10^8-10^9 CFU per gram of intestinal content, they produce toxins that induce enteric disease(3). The aim of this study was to evaluate the potential of PCR based testing, targeting alpha, beta, and epsilon toxins, to establish a correlation between the bacteria load and the development of diarrhoea inneonatal piglets.

Materials and Methods:
A total of 674 piglets, from 218 farms in 14 European countries were included and analysed in the present study. Samples were taken from piglets suffering from neonatal diarrhoea (Group A, n=531) and healthy piglets as a control group(Group B, n=143). Diarrhoea and faeces samples were fixed in FTA® ELUTE cards (Whatman) and sent to DIAGNOS Laboratory (Amer, Spain). A multiplex quantitative polymerase chain reaction (PCR) test was performed to detect genes encoding alpha, beta, and epsilon toxins of *C.perfringens*. Statistical logistic regression was performed.

Results:
CpA was isolated from 97% of the total amount of samples. No significant differences were seen regarding the average positivity rate of CpA comparing healthy and diarrhoeic piglets *(p- value > 0,005)*. It is noteworthy that the presenceof piglets with diarrhoea was significantly higherwhen the Ct value of the sample was lower than 32 *compared* with negative or samples with a higher Ct value than 32 (*p-value < 0.005*) (Fig.1).

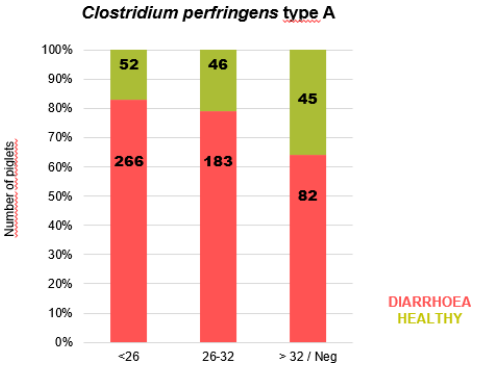

Figure 1. Neonatal diarrhoea correlation with the bacteria load (Ct-value).

Discussion and Conclusion:
Despite CpA being considered a ubiquitous pathogen and an early colonizer of the intestinal tract, the present study reinforces previous research that describes the possible involvement of the intestinal dysbiosis as an important risk factor. These results suggest that the quantification of CpA could be a valuable diagnostic technique to diagnose infections and thus, supports the importance of piglet's microbiota during the first days of age to prevent neonatal diarrhoea disease. The use of a quantitative PCR, as seen in this study, could be an improvement for CpA diagnosis.

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Using APXIV to adjust vaccination programs in two Chinese swine farms

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Introduction

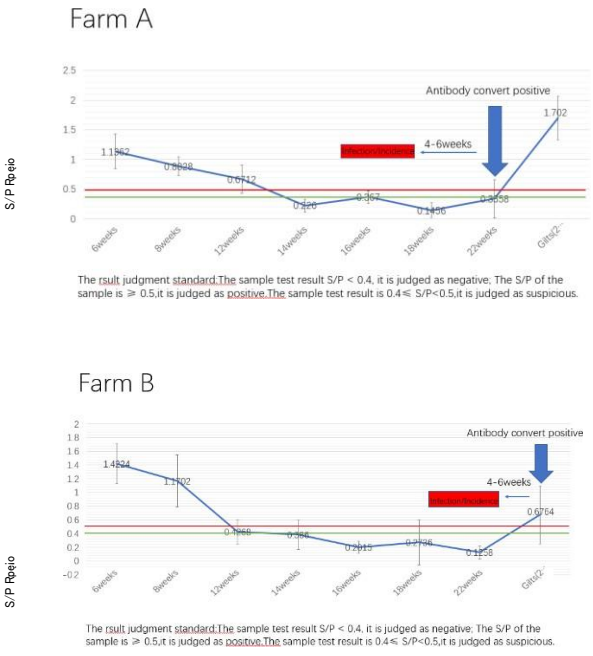
Porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae* (APP) is capable of affecting swine of all ages. It commonly causes clinical problems from 12-24 weeks of age and the economic impacts are higher if older pigs are affected[1]. The APP organism can secrete 4 kinds of toxins, APX I, APX II, APX III and APX IV[2]. APX IV can only be secreted in vivo. APX IV antibody testing can be used to guide ideal vaccination timing.

Serological testing was conducted in two farms in Yunnan, China with mortality attributed to APP in growing pigs at 15% of a batch or higher from 12-24 weeks of age. The initial vaccination timing was at 8 and 12 weeks of age.

Materials and methods

Serum was collected from swine at 6, 8, 12, 14, 16, 18 and 22 weeks of age and gilts (about 210 days old) (n=5 in each age group). Samples were tested with APX IV (IDEXX) Elisa test.

Results



Conclusion

The maternal derived antibody (MDA) level of APP in the two pig farms was high, suggesting that sow herds continued to serve as a reservoir of APP. MDA levels were also high at the time of vaccination. High levels of MDA at vaccination were demonstrated to interfere with efficacy in studies of other pathogens[3]. The vaccination program of both farms was adjusted to first dose at 10 weeks and second dose at 14 weeks, subsequently mortality in both grow finish herds improved to <5% from 12-24 weeks of age. A possible strategy to optimize APP vaccination is to use APX IV serology to understand the epidemiology and MDA status of a farm.

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Vaccinated pigs against *Lawsonia intracellularis* (li) presented better productive results at slaughterhouse

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Introduction

Intestinal disease is an important cause of production losses in slaughterhouses. Focusing on enteric diseases of swine, as ileitis (caused by Li), impacts may be related to condemnations of white viscera for example (1). It is important to consider other possible losses related to intestinal integrity damage, such as impact on casing (used to sausage production) or even to the carcass. The goal of this study was to evaluate and compare carcasses, white viscera condemnations and casings from animals not vaccinated (NVAC) and vaccinated against Li (VAC) at slaughterhouse.

Materials and Methods

The study was carried out in a slaughterhouse located in southern Brazil with animals from 6 different farms nearby: 3 farms with NVAC and 3 with VAC animals (Porcilis®Ileitis, one 2 ml dose at 28 days of age). Evaluations and comparations were performed on animals of the same sex and slaughter age (average of 108 days). Carcass data (n= 1248 NVAC, 1746 VAC) were collected from the slaughterhouse weighing database and information on intestinal condemnations was obtained from the Federal Inspection Service. A total of 430 intestines were collected from each group to assess the casing obtention yield, by calculating the total amount of casings obtained after intestinal cleaning and processing. Mann-Whitney test was used to statistical analysis and p values<0,05 were considered to indicate statistical significance.

Results

There were significantly (p<0,05) better criteria related to carcass in VAC animals, compared to NVAC. Carcasses information means and SD are shown inTable 1. In this study, white viscera condemnations did not differ (p>0,05) between the groups (VAC, 3%,SD±1.73 vs NVAC, 3.33%, SD±0.58). Although there was also no statistical difference (p>0,05) related to theYield of Casing Obtention, there was a numerical improvement in the amount of casing (m/pig) obtained from VAC animals. Casing obtention yield means and SD are shown in Table 2. (21.04, SD±0.11 vs 18.71, SD±0.26).

Discussion and Conclusion

There was a correlation between slaughterhouse results and vaccination against Li in the animals. These benefits are possibly related to improved intestinal health, consequently protecting the intestinal structure and improving its function.

Table 1. Carcasses information of VAC and NVAC animals

	NVAC	VAC
Carcass Weight*	98.04 KG SD±11.11	100.32 KG SD±9.85
Bacon Thickness*	13.95cm SD±3.28	13.52cm SD±3.29
Percentage of Lean Meat*	59.65% SD±2,02	60.07% SD±1.86

* Statistically significant differences ($p \leq 0.05$)

Table 2. Casing Obtention Yield of VAC and NVAC animals

Casing Obtention Yield*	NVAC	VAC
Mean*	18.71 meters of casing per intestine	21.04 meters of casing per intestine
SD	±0.26	±0.11

* Statistically significant differences ($p \leq 0.05$).

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Virulence differences between *Mycoplasma hyopneumoniae* Brazilian strains at experimentally infected swines

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Introduction

Mycoplasma hyopneumoniae (*Mhy*) is the primary pathogen of enzootic pneumonia (EP), a chronic respiratory disease in pigs. Infections occur worldwide and cause major economic losses to the pig industry (1). However, a better understanding of virulence between strains could further approaches to prevention and treatment of animals. The objective of this work was to evaluate the virulence between two strains previously isolated from Minas Gerais, Brazil. We carried experimental challenge in a swine model under experimental conditions to evaluate the virulence differences from recent Brazilian strains.

Materials and Methods

Characterization strains UFV01 and UFV02

The isolation from clinical samples occurred (2). Whole genome sequencing was made by Illumina platform and the data were analyzed using bioinformatic. Whole genome sequences were previously deposited in GenBank as UFV01 (SAMN11634266) and UFV02 (SAMN11634267).

Experimental infection

Twenty piglets (13,750 + 1.33 kg) with 35 days of age negative for *Mhy* were used. Two test groups (n=8) each, and a negative group (n=4) were used. One test group was inoculated with the UFV01 strain, the second with the UFV02 strain, and the negative control (NC) was inoculated with Friis medium. The animals were challenged intratracheally with $1x10^7$ CCU per each strain. The ELISA test was performed on days 0, 7, 14, 21, 28, and 35-days post inoculation (DPI), using the Mhy Ab kit (IDEXX, USA).

At DPI 35 the animals were euthanized. The macroscopic lesions were scored according to the methodology described by (3). The quantification of the microscopic lung lesions was evaluated for the presence of BALT hyperplasia, pleuritis and bronchopneumonia.

Results

Figure .1- Percentage of macroscopic lesions.

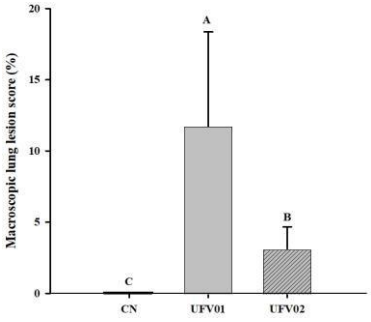

Figure. 1 - Bar on a scale from 0% (no lesions) to 100% (whole lung affected).*Different letters indicate significant difference detected in the Tukey test ($p<0.05$), (4).

The group NC showed no lesions, the group UFV01 showed a higher % of macroscopic lesions 11.75% (SD±6.60) when compared to group UFV02 3.125% (SD±1.55).

Figure. 2- Bar graph quantitation of microscopic lung lesions.

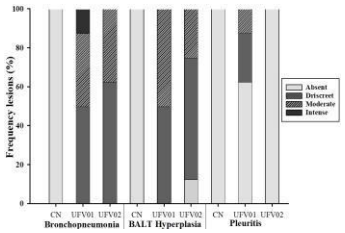

Figure. 02 -Describes the quantified microscopic lesions (bronchopneumonia, BALT hyperplasia and pleuritis).

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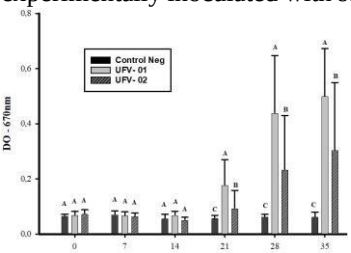
The group NC did not show lesions. The group UFV01 presented a frequency of 50% of discreet bronchopneumonia lesions (4/8), 37.5% moderate (3/8) and 12.5% (1/8) intense lesions. In BALT analyses, 50% presented moderate lesions (4/8) and 50% intense lesions (4/8). The group UFV02 presented 62.5% of discreet bronchopneumonia lesions (5/8) and 37.5% of moderate lesions (3/8). BALT lesions have a frequency of 62.5% discreet bronchopneumonia lesions (5/8), 25% moderate (2/8). One (1/8) animal showed no lesion in group UFV02. Only the group UFV01 showed cases of pleuritis: 25% moderate (2/8) and 12.5% intense (1/8).

Figure. 3.-Seroconversion dynamics of pigs experimentally inoculated with strain UFV01 e UFV02.



*Different letters indicate significant difference detected in the Tukey test ($p<0.05$). (4).

The NC animals remained negative throughout the study. All animals in the groups inoculated with the strains seroconverted. However, animals inoculated with the UFV01 strain reached higher IgG antibody levels when compared to UFV02. Significant statistical differences were detected on days 21, 28, and 35 DPI.

Discussion and Conclusion

The UFV01 and UFV02 strains were able to cause the disease with an experimental challenge of 35 DPI. However, the UFV01 strain proved to be more pathogenic, inducing more capacity to produce micro and macroscopic lesions and production of IgG antibodies than UFV02 strains. More studies are needed about genetic difference to understand the virulence between strains.

Acknowledgments

Agroceres, FAPEMIG, CNPq, Pigpork and CAPES.

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Yearly evaluation of lung lesions in slaughter pigs in Europe

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Introduction

Scoring of lung lesions in the slaughter pigs belongs to major diagnostic methods to evaluate the presence, incidence and severity of respiratory diseases in commercial pigs (1). Lesions suggestive for previous M.hyo or A.p. infections and their scoring were described before. The aim of this survey was to collect the results of lung scoring performed in most of swine producing European countries in 2021.

Materials and methods

Ceva Lung Program scoring methodology was implemented to score lung lesions at the slaughterhouse. The results were collected from 20 European countries in the 12 months period fromDecember 2020 till the end of November 2021. The mean values and quartiles were calculated for % of lungs with bronchopneumonia (%BP), % of affected lung parenchyma out of sick lungs (% parenchyma), % of dorso-caudal pleurisy (%DP) and APP index (APPI).

Results

The total number of scored lungs was 381748 from 4470 reports with the average of 85 lungs per batch. The median value of %BP was 24.% (30.6% in 2020) with the Q1=11% and Q3 46%. The median of affected parenchyma was 1.75% (2.07% in 2020).

Table 1. EP-like lesions

	% of lungs with EP-like lesions	% of affected lung parenchyma
Median 2021	24%	1.75%
Median 2020	30.6%	2.07%

For % DP the median, was 3% (4.1% in 2020). ForAPPI the corresponding value was 0.07 (0.11 in 2020).

Table 2. APP-like lesions

	Percent of Dorsocaudal Pleurisy	APPI Index
Median 2021	3%	0.07
Median 2020	4.1%	0.11

Discussion and Conclusions

The results of this survey conducted in European countries in 2021 revealed lower values compared to previous years. Three factors are apparently behind this reduction: 1) the general continuous improvements in the control of those two infections (reported from several countries); 2) a significant amount of CLP results from high health status Dutch farms came into the database; 3) farms with better control of respiratory disease began to perform CLP more frequently.

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CLINICAL CASES

A high mortality of H1N1pdm vaccinated sows related with H1N2 subtype

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Introduction

Influenza A virus (IAV) causes an acute respiratory disease in swine which imposes substantial economic losses in pig production, especially when paired with additional respiratory pathogens (1). In Brazil, since the emergence of 2009 H1N1 pandemic virus (pdm09) several outbreaks associated with H1N1, H1N2 and H3N2 viruses have been described in pig herds (2). To minimize the impacts of IAV in pig farms, the use of vaccines has been applied preventively (3). However, the great antigenic and genetic diversity of IAVs has limited the vaccine efficacy or cross-protective immunity against heterologous homosubtypic or heterosubtypic IAVs (4). Also, a vaccine-associated enhanced respiratory disease (VAERD) in pigs that received a whole inactivated virus (WIV) vaccine and were challenged with a heterologous H1 virus has been reported (5,6). The aim of this study was to report an influenza outbreak with a high mortality rate in vaccinated sows.

Materials and Methods

This case report refers to a breeding herd of 1,800 sows, located in Paraná state, routinely vaccinated with H1N1pdm inactivated virus, presented a mortality surge of 40 deaths in 8 days (28 gilts and 12 sows). A morbidity rate of 90% and a mortality rate of 2.2% were associated with clinical signs of fever, lethargy, nasal discharge, cough, and respiratory distress.

Necropsy was performed in 3 sows and tissue samples were collected for laboratory analysis. Analyzes were conducted for bacterial isolation on blood Agar plates and incubated at 37°C for 24-48 hours in microaerobic atmosphere, qPCR for *Actinobacillus pleuropneumoniae* using NewGene APPAmp kit (Simbios Biotecnologia), RT-q-PCR for IAV (7) and multiplex RT-PCR assays for IAV subtyping (8). For histopathologic examination, formalin-fixed lung tissue samples were routinely processed and stained with hematoxylin and eosin and immunohistochemistry for IAV using Universal LSAB™+/HRP Kit (Dako).

Results

Gross lesions were characterized by interlobular edema, dark red firm multilobular to coalescing lung lesions and congestion of the lung and trachea. Histological lesions consisted of moderate diffuse neutrophilic bronchopneumonia with necrotizing bronchiolitis, congestion and moderate edema. There was moderate diffuse lymphoplasmacytic tracheitis. No other significant lesions were observed in other tissues. On immunohistochemistry, all lungs were positive for IAV staining. Lung samples showed no bacterial growth, and they were negative for *A. pleuropneumoniae*. All lung samples were positive for IAV, and the virus subtype was characterized as H1N2 by multiplex RT-PCR.

Discussion and Conclusion

This study describes an influenza outbreak with a high mortality rate of sows previously vaccinated with an H1N1pdm virus and infected with an H1N2 virus. Influenza vaccines licensed in Brazil are a commercial monovalent H1N1pdm09 WIV and an autogenous WIV. Currently, IAV is endemic in Brazilian pig herds and genetically diverse virus subtypes circulate in the herds (9). A higher occurrence of H1N1pdm from 2012 to 2015, H3N2 in 2017, and H1hu in 2017 to 2019 has been shown (10), demonstrating a variability of IAV subtypes over time. Similar to this case, several studiesdescribed VAERD in pigs vaccinated with a WIV that is antigenically mismatched with the infecting virus (6).In these studies, severe inflammation and pneumonia were observed (5).

Although the HI test or phylogenetic analysis were not performed in our study, we can suggest the occurrence of VAERD based on the clinical signs, gross/histopathologic lesions, and mortality intensity. Regardless, the increasing genetic and antigenic diversity of H1 IAVs circulating in swine added to the use of WIV vaccines creates optimal conditions for vaccine-virus mismatch and potential VAERD in the swine population. In conclusion, it is recommended to determine the IAV that is circulating in a specific herd before the utilization of commercial inactivated IAV vaccines.

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Assessment of an Aujeszky's Disease control strategy on a highly prevalent pig farm based on systematic vaccination with an inactivated gE-negative marker vaccine

Introduction

Aujeszky's Disease (AD) is endemic in Argentina, where 19.1% of farms and 8.9% of sows are infected (1). As there are no national AD vaccines in the country, the Official Veterinary Service (Senasa) decided to import an inactivated vaccine (IV), to minimize the risks associated with adventitious viruses involved in the use of modified live vaccines (MLV). The inactivated gE-negative marker vaccine is commonly used in combination with a MLV (AUSKIPRA® GN) (2,3). Nowadays, Argentina is the only country that carries out a control strategy plan using only the inactivated AUSKIPRA® BK (HIPRA, personal communication) as a vaccination tool.

The objective of the study was to describe the results of a control programme on a farrow-to-finish farm with high initial AD prevalence based on systematic vaccination with an IV, detection and elimination of seropositive pigs, replacement of sows with vaccinated gE-negative gilts and introduction of artificial insemination.

Materials and Methods

The study was performed on a commercial farrow-to-finish farm with 278 sows, located in General López, province of Santa Fe, Argentina. The initial AD prevalence was 33% in sows, 25% in boars, and 50% in gilts. The implemented control strategy started in July 2017 and was based on: vaccination of sows (twice, every 21 days, and before farrowing) and piglets (at weaning amd 21 days after); purchase of ELISA gE-negative gilts from Officially AD-free farms vaccinated twice, at 120 and 141 days of age; culling of gE-positive females, culling of all boars and introduction of artificial insemination, serological tests to monitor all ELISA gE-negative sows and gilts and 30 finishing pigs every 6 to 9 months. Also, some basic biosecurity improvements were implemented.

Results

The monitoring dates, the number of sows and gilts positive and negative for the AD virus (ADV), the total number tested and the percentage of positives are presented in Table 1. Viral circulation was found in finishing pigs only at the first monitoring.

Discussion and Conclusion

Four months after the beginning of the control strategy, vaccinating twice (and 3 times, in some cases), was not enough to avoid transmission among breeding females. Also, there were some gilts that seroconverted when they entered the farm, even with 3 doses, although the majority remained negative. The viral circulation continued in the finishing sector, similar to what has been described previously (2) , indicating that the pigs became infected as piglets. Ten months after implementation of the control strategy, the situation was more favourable, with no new infections in sows or finishing. Unlike what happened on the Chinese farm described in (2), no gilt was positive. This may be due to the fact that in our study the replacement was carried out by introducing ELISA negative gilts. Then, 20 and 48 months after the beginning of the interventions, no seropositive animal was detected.

MLV are generally acknowledged to be more efficacious than IV (4,5). However, based on the results, we can observe that not only MLV but also IV can be a good method to eradicate ADV. Vaccination and additional management measures implemented quickly and together can allow the eradication of AD on a farm and this situation can be sustained over time.

The control strategy presented here based on vaccination and culling of seropositive animals with certain improvements in biosecurity was suitable for reducing the incidence and the prevalence to levels compatible with virus eradication.

Table 1: monitoring dates, number of sows and gilts positive (+) and negative (-) for ADV, total tested and percentage positive. *: 3 gilts entered the farm in 17-08-10 and 1 in 17-09-27.

MD	Sows				Gilts		
	+	-	n	%	+	-	n
17-11-04	38	71	109	34.9	*4	72	76
18-05-15	0	182	182	0.0	0	80	80
19-02-27	0	176	176	0.0	0	75	75
21-06-25	0	186	186	0.0	0	14	14

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Benefits observed in the field after using an oral vaccination against *Lawsonia intracellularis*

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Introduction

Porcine Proliferative Enteropathy (PPE) caused by *Lawsonia intracellularis* (L.i.) is an enteric disease of pigs affecting most of the farms worlwide[1]. Most of pigs are infected subclinically, but the economic impact is estimated between 1.3€ and 18.5€ per affected pig[2,3] Nowadays the meat industry and its costumers ask for a reduction of antibiotic use in animals. Oral vaccination against Ileitis can be considered as an effective prophylactic tool to control clinical and subclinical PPE. The aim of this study was to evaluate the efficacy of an oral vaccination against L.i. on antibiotic use reduction and performance improvement in a Spanish pig farm.

Materials and Methods

This study was conducted in a multi-site farm with 2,000 sows located in the north-east of Spain. Although no clinical signs consistent with Li were observed, infection with L.i. was confirmed by ELISA (L.i IgG). A total of 20,160 fattening pigs were included in the study (10,080 non-vaccinated and 10,080 vaccinated with the oral live vaccine Enterisol® Ileitis Boehringer Ingelheim Vetmedica GmbH). The first 14 batches served as control (2019), and the 14 subsequent batches were vaccinated (2020). The pigs were orally vaccinated one week after weaning via drinking water in the nursery unit. An EMEC continuous dosing pump was used to guarantee that the appropriate dose was administered. Pigs were raised under the same conditions. Production data were recorded throughout the fattening period.

For these study average batch production data have been compared: initial body weight (kg), feed consumption (kg), total medication cost (€), final live weight (Kg), days on feed, total live weight gained (kg), feed conversion rate (FCR), days to slaughter, mortality rate (%), average daily gain (ADG, kg/d). ADG and FCR were standardized previously to the statistical analysis. An ANOVA test was used to analyze those parameters following a normal distribution and the Mann Whitney for non-normal distributed data. Data was analyzed using Software R (The R foundation).

Results

The results are summarized in Table 1.

For the vaccinated group a significant reduction of the total medication cost was observed in comparison with the non-vaccinated group (39.39%; p = 0.0001). (Figure 1)

In addition, statistically significant differences were also observed for the mortality rate (3.61% vs 4.10%; p=0.024), (Figure2).

Table 1: Comparative efficacy of Enterisol Ileitis® at fattening.

	Mean		
Vaccination	**No**	**Yes**	P value
Initial weight (kg)	11.16	12.97	**p=0.003**
Total feed consumption (kg)	229.6	224.3	p=0.16
Medication cost/pig (€)	1.32	0.8	**p=0.0001**
Final weight (kg)	114.3	113.2	p=0.35
Mortality rate (%)	4.1	3.61	**p=0.024**
Days on feed	125.1	121.6	p=0.16
ADG (g/day)	824	824.8	p=0.87
FCR	2.33	2.33	p=0.14
Days to slaughter	27.64	20.64	**p=0.0001**

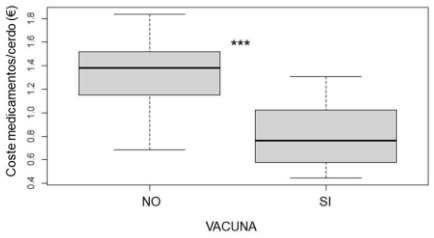

Figure 1: Boxplot graph showing the total medication cost in control and vaccinated batches

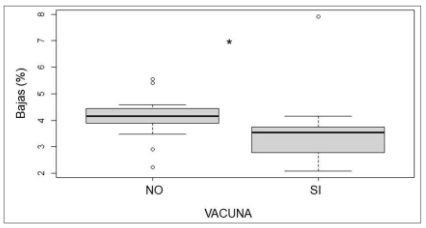

Figure 2: Boxplot graph showing the mortality rate in control and vaccinated batches

There were no significant differences in ADG nor feed conversion rate between both groups.

Conclusions and Discussion

In this field experiment, it was observed that to control ileitis, total medication cost use can be replaced by vaccination. Zootechnical parameters like ADG and FCR remained the same. Additional for the vaccinated group, the mortality was decreased. It can be concluded that oral vaccination against Li is an alternative for antibiotic treatment to control ileitis, even improving the herds performance when mortality is also taken in account.

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3. Hardge, T. Et al (2005) Proc APVS P 114

Declined *Lawsonia intracellularis* in feces by phytogenic feed additive supplementation in fattening pigs in different herds system

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Introduction

Lawsonia intracellularis is a gram-negative bacterium, a causative agent, of worldwide spread pig enteric disease, the porcine proliferative enteropathy (PPE). (1). The decreased susceptibility of *L. intracellularis* to antibiotics is observed (2) as well as novel a plant-based solutions to control the PPE (3). The aim of our study was to investigate effects of plant-based feed additive (PBFA) on *L. intracellularis* presence in pig feces that originated from 2 herds that differ in temperature regulating systems.

Materials and Methods

The trial was performed on *L. intracellularis* naturally infected pigs on one a conventional open-housing system farm and one evaporative cooling-housing system farm. On each farm, 40 fattening pigs (12-week-old) were randomly allocated in to two groups including control group (n=20) and treatment group (n=20). The pigs in each group were fed with a conventional diet (Control) and the same diet supplemented with 2 kg/ton of commercial PBFA, PATENTE HERBA® PLUS, PATENT CO. DOO, Serbia), for 14 days (Treatment). The feces samples were collected and were scored on day 0, 7 and 14 after supplementation and were determined by quantitative real time PCR.

The number of DNA copies of *L. intracellularis* were logged transformed, after testing the assumption of normal distribution using skewness, kurtosis, and Shapiro–Wilk normality test. The effect of the phytogenic feed additive on herd and on the number of DNA copies of *L. intracellularis* were analyzed by using general linear model (GLM). Values with $P < 0.05$ were regarded as statistically significant.

Results

The number of DNA copies of *L. intracellularis* are presented in Table 1. In control groups of all herds, the number of DNA of *L. intracellularis* did not differ significantly throughout the study. In herd A, the number of DNA copies of *L. intracellularis* in the Treatment group at 14. day of PBFA supplementation (1.0 ± 2.7 copies/µL) was lower than at the day 0 (0.8 ± 2.2 copies/µL) and at day 7 (0.8 ± 2.2 copies/µL) but statistical difference was not observed.

In herd B, the number of DNA copies of *L. intracellularis* in the Treatment group at day 14 (0.8 copies/µL), after supplementation of PBFA, was statistically significantly lower than at day 0 (5.5 copies/µL; P = 0.023) and at day 7 (1.4 copies/µL; P = 0.131).

Discussion and Conclusion

The plant-based feed additive can reduce the number of *L. intracellularis* after 7 days of supplementation in fattening pigs in both, an evaporative cooling system and an open-housing system. Therefore, phytogenic feed additive may be applied to control *L. intracellularis* in commercial swine farms when the usage of antibiotic was limited.

Table 1. DNA copy number of *Lawsonia intracelluralis* in a pooled samples of fattening pig feces in the Control (n=30) and Treatment groups (n=30) in each day of supplementation in each herd

Herd	Group	day of supplementation		
		0	7	14
Herd A	Control	2.0 ± 2.2	3.7 ± 1.7	2.9 ± 2.2
	Treatment	4.2 ± 1.9	0.8 ± 2.2	1.0 ± 2.7
Herd B	Control	2.6 ± 1.9	5.0 ± 2.2	2.3 ± 1.9
	Treatment	5.5 ± 1.7[a]	1.4 ± 2.7[b]	0.8 ± 1.9[b]

[a, b] Different superscript letters within group indicate significant differences $P < 0.05$.

Acknowledgments

The present study was financially afforded by Faculty of Veterinary Science, Chulalongkorn University and Amcovet. Co., Ltd. (RES_63_139_31_ 018).

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Cystoisospora suis resistant to toltrazuril on a commercial farm in the Czech Republic

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Objective

In the European Union, control of *Cystoisospora suis* is commonly achieved with a single oral administration of toltrazuril during the prepatent period (day 3-5 of life of piglets). Constant treatment regimens with toltrazuril have been applied for controlling porcine cystoisosporosis for almost 25 years. Despite this long-term use, cases of *Cystoisospora suis* resistance in pigs are very rare.

Materials and Methods

Cystoisosporosis outbreak was reported in the Czech Republic in a 2000 sows commercial farrow-to-finish Danbred production system with SPF status. Mortality of the piglets in the farrowing unit before the outbreak ranged from 10 to 12%.

Coccidiosis outbreak started at the beginning of summer 2020, approximately 3 months after the purchase of gilts from a new Danish gilts supplier. Piglets from the 8th day of age until weaning developed yellowish to grayish diarrhea. Approximately 50% of the piglets were affected despite treatment with the recommended dose of toltrazuril (20mg/kg body weight). Small piglets became dehydrated, and had lower weight gains. Mortality of piglets increased almost twice, losses in the most affected batches were more than 27%. The number of small non-standard piglets dramatically increased too.

Laboratory examination confirmed massive infection of *Cystoisospora suis*. Oocysts were detected in the fecal samples from piglets at the age of 6 days and older. Other laboratory tests did not reveal infection caused by another specific pathogen. Quality of feed was also analyzed, again with negative results.

Evaluation of the administered amount of toltrazuril on the farm level revealed no under-dosing. Application of 30 mg/kg body weight of toltrazuril and application of diclazuril in the same dose did not have any effect on the clinical signs and therefore we suspected a loss of efficacy. Due to the high losses that continued, we decided to start treatment with sulfonamides. The oral application of sulfonamides via medicated water did not work, the piglets did not drink it probably due to its bitter taste. For this reason we started with an injectable application of trimethoprim+sulfonamide in dose 15 mg/kg body weight, repeatedly administered at day 5, 6 and 10 of life of piglets. Other responses included disinfection of the environment with Kilcox Extra (destruction of oocysts) and culling of the most affected piglets not responding to treatment.

Results

There was a significant reduction of mortality almost immediately after the start of this injectable sulfonamide treatment (Table 1). The effect onset was very rapid, while almost 20% piglets without treatment died, only 11,7% of piglets died in the first treated batch. Diarrhea never completely disappeared, but feces were pasty and no liquid. Currently, a maximum of 10% of piglets have diarrhea and they are almost always piglets 1 week before weaning. These piglets are able to manage diarrhea and dehydration much better. The health situation and mortality have never returned to pre-outbreak levels, but losses of around 20% and above no longer occur. The situation in the herd remains stable with this long-term and consistent treatment.

Table 1. Mortality of the suckling piglets before and after starting treatment with injectable sulfonamides, March 2020-March 2021

Month	Mortality (%)	Comment
March	10,8	
April	10,7	
May	14,9	onset of infection
June	16,8	outbreak
July	22,2	high dose of toltrazuril
August	23,6	peroral sulfonamides
September	19,6	peroral sulfonamides
October	19,6	injectable sulfonamide
November	13,0	injectable sulfonamide
December	14,8	injectable sulfonamide
January	14,8	injectable sulfonamide
February	12,5	injectable sulfonamide
March	13,3	injectable sulfonamide

Conclusion

The effect on parasite development and clinical improvement was achieved only by repeatedly administered injectable sulfonamides. This treatment can be considered as an alternative in cases of the toltrazuril resistance, however, it is labor-intensive and very difficult for routine treatment. Better results could be achieved if we administered injectable sulfonamides repeatedly for 6 days in a daily interval, but it would be practically impossible under the conditions of a high production farm.

Reference

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Field evaluation of the efficacy of a commercial vaccine against *Glaesserella parasuis*

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Introduction

Glaesserella parasuis is an important pathogen affecting the pig industry, causing meningitis, polyserositis, polyarthritis, and bacterial pneumonia; an infection known as Glässer's Disease (1). Antimicrobials have been commonly used to treat this bacterial disease in farm animals, but the emergence of antimicrobial resistance, a serious threat to public health, necessitates the implementation of alternatives for disease control (2). Vaccines are the preferred alternative for control of the infection, as they have been shown to be useful in the prevention of this disease (1). The objective of the present study was to determine the effect that a commercial vaccine against *G. parauis* had on mortality during a disease outbreak.

Materials and Methods

The trial was performed from January to June 2020 in an 8,500 multiple site production farm, negative for PRRS, located in Villaflores (Chiapas, Mexico). The average weaning age was 21 days. The farm had a diagnosis of Glässer´s Disease and it was decided to start a vaccination programme in piglets (two shots at 1 and 3 weeks of age) with HIPRASUIS® GLÄSSER (HIPRA) to control the clinical signs and mortality that appeared from the 5th week of age. The comparative study was done by implementing vaccination and comparing the period before and after its introduction. For this, 6 consecutive batches (from farrow to finish) were assessed, 3 batches vaccinated and 3 batches without vaccination (n=6,213 and n=6,327). Differences in the main productive parameters between groups were tested by T-test and Wilcoxon test. Mortality was assessed by a Test of Proportion. All the statistical analyses were performed using R software.

Results

A significant reduction in mortality (from 4.78% to 3.22%; *P*<0.001) was observed between periods when comparing batches without vaccination and batches with vaccination (Figure 1).

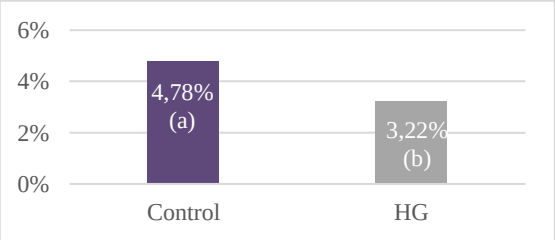

Figure 1. Mortality. Prop. Test. (*P*-value<0.001)

No differences were observed in the mean productive parameters. However, a statistical trend was observed in the ADWG of the post-weaning phase and numerical differences in the fattening phase, with higher homogeneity in the vaccinated animals (see Figures 2 and 3).

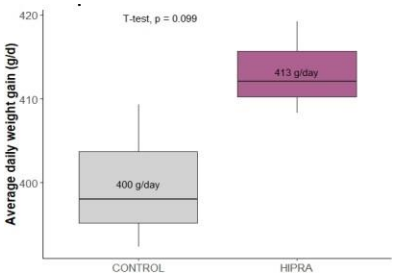

Figure 2. Comparison of the ADWG in the post weaning unit.

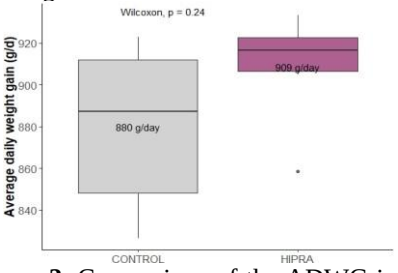

Figure 3. Comparison of the ADWG in the fattening unit.

Although the improvements in the productive parameters observed were not statistically significant, an important reduction in the cost of slaughtered pigs was observed (Table 1).

Table 1. Productive cost of slaughtered animals (vaccinated and control group).

	Control	HIPRASUIS GLASSER®
Entry weight	26.39 kg	27.07 kg
Exit weight (standardized 163 days)	104.4 kg	106.61 kg
Total feed cost	383,195 €	412,555 €
Total vaccine cost	-	4,428.90 €
Cost of slaughtered pig	106.94 €	105.26 €
Cost per kg	1.05 €	1.03 €

Conclusions

On this farm, HIPRASUIS® GLASSER significantly reduced the mortality, caused numerical improvements in the ADWG and reduced the cost of pig production.

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Field experience of antibiotic reduction while improving productive parameters with Oedema Disease vaccination

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Introduction

Oedema disease (OD) is a major challenge for pig production, particularly in the weaning phase, causing high mortality rates and impairing performance. Historically, OD has been controlled by management, disinfection, use of zinc oxide, antimicrobials and probiotics; with variable outcomes. Vaccination of pigs against VT2e (verotoxin) has been proven to be a highly effective alternative (1). Hence, the aim of this study was to evaluate whether the use of antibiotics could be reduced by administering a VT2e vaccine on a farm with a clinical diagnosis of OD.

Materials and Methods

The trial was performed on a 1500 sow farm in Minas Gerais (Brazil), where the animals were receiving antibiotics for the control of OD. The animals were monitored to compare the standard farm treatment: Group 1, n=2,818 (6 batches), receiving 10 mg colistin + 7.5 mg fosfomycin; Group 2, n=1,272 (3 batches), receiving VT2e vaccination (VEPURED®) + 5 mg colistin + 3.4 mg florfenicol. Farm productive data were recorded and compared through an ANOVA. The analysis of mortality data was performed using a Test of Proportions.

Results

Regarding productive data, no significant differences were observed between groups in entry and final weights, however significant improvements were observed in Group 2 regarding Average Daily Gain (ADG) and Feed Conversion Ratio (FCR) (see table 1). Moreover, global mortality was significantly reduced in Group 2 (see figure 1). Finally, overall treatment costs were reduced in Group 2 (8.3 R$ Group 1 vs. 5.3R$ Group 2), and the production cost per exit piglet was reduced by 3.45 R$ in Group 2 (249.12 R$ Group 1 vs. 245.67 R$ Group 2).

Discussion and Conclusion

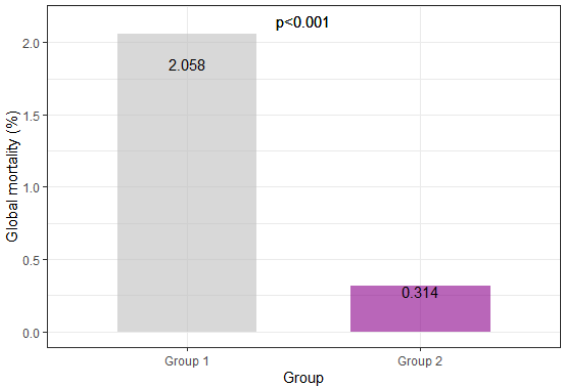
Treatment with VT2e vaccination allowed a reduction in the use of antibiotics whilst increasing productivity and reducing treatment costs. These results are in line with previous data reported in Europe, where a reduction in antibiotic use was reported after the use of a VT2e vaccine. However, on previous occasions the change from antibiotics to vaccination did not affect mortality or productive parameters; whilst in the present case the results were improved. These improvements may be explained by the high antimicrobial resistance reported for *Escherichia Coli* (3), which probably prevented full control of the disease with antimicrobial treatment.

Antibiotics are an important tool for tackling infectious disease on pig farms; however extensive research indicates that their frequent mis/over-use maycontribute to the development of antibiotic resistance; and the WHO has declared that this issue should be addressed (4). In this context, the use of VT2e vaccinesis shown to be a highly effective alternative to antibiotics in the management of OD.

Table 1. Productive parameters.

	Group 1	Group 2	SD	P value
Entry weight (kg)	1.55	1.82	1.99	0.461
Final weight (kg)	21.06	22.10	2.38	0.586
ADG (kg/day)	0.35[b]	0.37[a]	0.03	0.004
FCR	1.61[b]	1.36[a]	0.16	0.022

Figure 1. Global mortality (%).



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Field experience of ZnO withdrawal and antibiotic reduction by using a probiotic and vaccinating against VT2E

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Introduction

In a context of the increasing global pressure to reduce ZnO and antimicrobials due to the public health and environmental reasons (1), there is a need for farms to find treatments to replace them. The objective of this trial was to assess if ZnO and antibiotics could be reduced by alternative methods in a farm with a clinical diagnostic of oedema disease.

Materials and Methods

The trial was performed in an 1800 sows farm from Paraguay, with severe mortality problems associated with a multidrug-resistant verotoxigenic *E.Coli* and *Streptococcus suis* in the nursery period. Animals were followed during a 6 months demedicalization process (three 2-month periods) being: (1) n=5.395, 23 mg of colistin + 12 mg of neomycin + 3200 ppm of ZnO (initial farm treatment); (2) n=5.342, same treatment + VT2e vaccination (VEPURED®) + probiotic; (3) n=5.408, 10 mg of amoxicillin + VEPURED® + probiotic. Farm data was tested through an ANOVA and a posthoc Tukey test.

Results

Both periods with vaccine and probiotic had a significantly better gain:feed ratio (1,686(b); 1,636(a); 1,638(a) for periods 1, 2 and 3, respectively; p-value=0,002). However only numerical differences were obtained for daily average (0,472; 0,486; 0,490 for periods 1, 2 and 3; p-value=NS). Total mortality was reduced with the vaccine and probiotic (4,77%(b), 1,89%(a); 1,67%(a); for periods 1, 2 and 3; p-value<0,001), basically attributed to a decrease in mortality with farm diagnostic of OD (3,78%(b), 0,11%(a); 0,06%(a); for periods 1, 2 and 3; p-value<0,001). Finally, regarding treatment costs, the cheapest approach for the farmer was the one used in period 3 (1,52€/piglet), compared to groups 1 (1,94€/piglet) and 2 (3,1€/piglet).

Discussion and Conclusion

The treatment of VT2e vaccination and probiotic permitted the withdrawal of ZnO and a relevant reduction of antibiotics (eliminating the highest priority critically important antibiotics) while increasing productivity and reducing the treatment costs.

Figure 1. Gain:feed ratio.

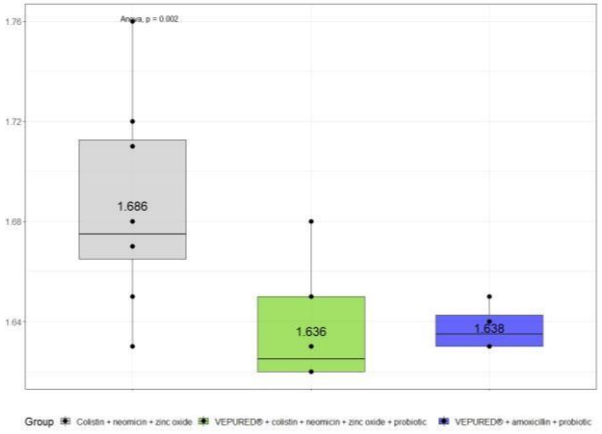
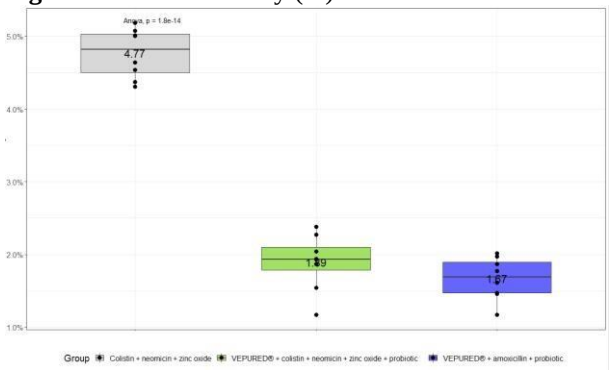


Figure 2. Global mortality (%).



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Improvement of growth performance in nursery house at sow farmafter PCV2 and MH vaccine change

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Introduction

Porcine circovirus Type-2 (PCV2) and *Mycoplasma hyopneumoniae* (MH) are prevalent diseases in South Korea and most swine farms use vaccines for the control. But not all vaccines show same efficacy, and economic losses due to PCV2 and MH may be easily overlooked in a vaccinated farm. For this reason, it is very important to evaluate vaccine efficacy in terms of economic value. However, differences of efficacy among PCV2 and MH vaccines are not well described in South Korea, especially in nursery houses. The objective of this study is to compare the efficacy of two commercial vaccine programs against PCV2 and MH with respect to growth performance during nursery period on a sow farm.

Materials and methods

The field observation was conducted on a 1,200-sow, PRRS negative farm in South Korea, in a before and after study model. For about one year, pigs were vaccinated against PCV2 and MH with a RTU (ready-to-use) commercial vaccine: DS CircoMyco Pigvac (Vac A) at 15 days of age and weaned around 21 days of age. At 70 days of age, pigs were transferred from nursery to the grower/finisher. Despite vaccination, pigs demonstrated wasting and respiratory symptoms at 7 weeks of age and PCV2 was detected in serum by RT PCR at 7 and 10 weeks of age in Feb/2021 (~4 log10 PCV2 genome copies/ml). In May/2021, vaccination protocol was changed, and piglets started to be vaccinated with 2 mL of FLEXcombo® – Ingelvac CircoFLEX® and Ingelvac MycoFLEX® freshly mixed (Vac B) at 15 days of age. To compare the vaccine efficacy, number of dead pigs every week and average body weight (BW) at 70 days of age were analyzed. This nursery house was managed by the same employee during the evaluation period and there was no change in management practices except for the PCV2 and MH vaccines.

Result

A total of 24,752 pigs were monitored for this trial. Improvement in growth performance was observed during the nursery period after switching from Vac A to Vac B. The number of dead pigs decreased dramatically (Figure 1) and average BW at 70 days of age increased gradually (Figure 2). The results demonstrate that the vaccine change had a positive impact on nursery house. Furthermore, in terms of growth performance, PCV2 and MH vaccine selection is very important on the sow farm though they only raise pigs around 70 days of ages.

Figure 1. Number of dead pigs by week. Vac B was introduced from batch 18.

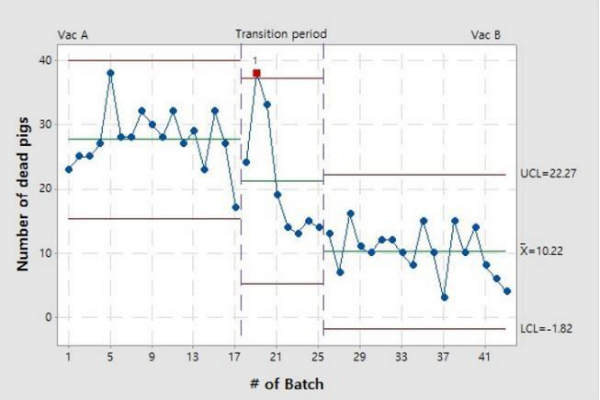
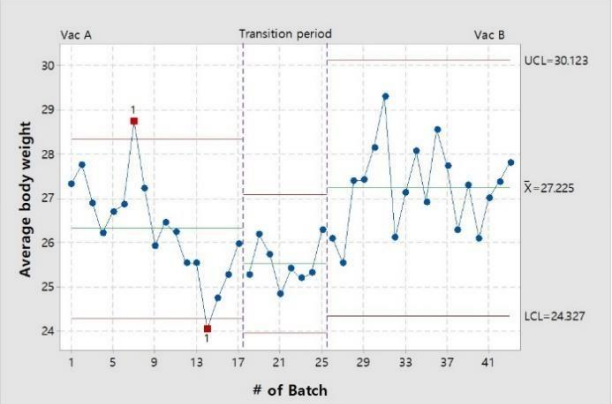


Figure 2. Average BW of pigs around 70 days of age. Vac B was introduced from batch 18.



Discussion

The results from this study indicate that the selection of PCV2 and MH vaccine can impact pig mortality and growth performance during the nursery period on the sow farm. As there was no change in farm management, except for PCV2 and MH vaccine, these improvements are due to the vaccine change. Further studies monitoring mortality rate in finishing houses should be done to evaluate the impact of PCV2 and MH vaccine change in this stage of production.

Reference

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In-field evaluation of early-piglet vaccination in piglets from vaccinated and non-vaccinated sows

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Introduction

Glaesserella parasuis (G. parasuis) is the aetiological agent of Glässer's Disease (GD), pathologically characterized by serofibrinous to fibrino-purulent polyserositis, arthritis and meningitis (1). The colonization of the upper respiratory tract of piglets occurs soon after birth through contact with the sow (2). One key to controlling GD is immunization by vaccination, early-piglet immunization (off-label)being a common field procedure. However, this carries the risk that its safety has not been evaluated inregistration trials and its efficacy can be limited by interference from colostrum antibodies. The aim of this study was to assess the safety and efficacy of early-piglet vaccination in field conditions.

Materials and Methods

The study was carried out on a swine farm in southern Brazil using a total of 33,045 piglets divided into 3 groups with 25 replicates each. Group 1 (control) (*n*=11,136), piglets and their mothers were not vaccinated. Group 2 (*n*=10,789), only piglets were vaccinated at 3 days of age and at weaning. Group 3 (*n*=11,135), piglets were vaccinated at 3 days of age and at weaning, and their dams were vaccinated at 70and 90 days of gestation. The same vaccine was applied in groups 2 and 3: HIPRASUIS® GLÄSSER (HIPRA). Safety (temperature and local reactions), and efficacy parameters (mortality and weights at the end of lactation, nursery and fattening) were collected.Statistical analysis was performed by T-test and Wilcoxon test. Mortality was assessed by a Test of Proportion.

Results

No significant differences were observed in temperature and local reactions (data not shown).
The mortality rate for G1, G2 and G3 was 8.63%, 8.59% and 6.11%, respectively (Figure 1). Piglets in G3 had a significantly lower mortality rate (*p-value*<0.001). Average Daily Gain (ADG) was improved in thenursery phase in G2, whilst G3 showed significant improvements in the lactation and nursery phasesn (Table 1).

Conclusions and Discussion

Maternal antibodies induced by vaccination and transferred to piglets by colostrum ingestion are an important source of protection in the first weeks of life,as was observed in G3.

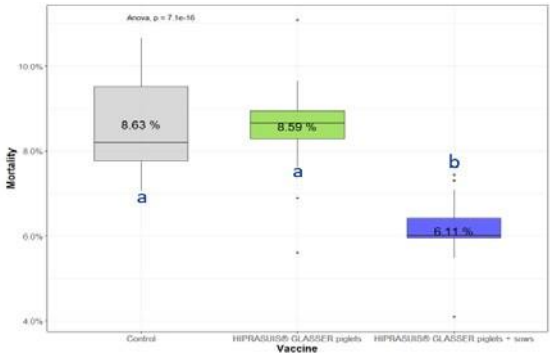

Figure 1: Mortality rate by group.

Table 1: ADG by group between production phases.

	Lactation	Nursery	Fattening	All
G1, g	205[a]	399[a]	973[a]	686[a]
G2, g	206[a]	425[b]	975[a]	695[b]
G3, g	214[b]	449[c]	975[a]	702[c]

[a,b,c]Means within a column with different superscripts differ. (*p-value* <0.05)

However, maternally-derived antibodies, protective against GD, decrease during lactation (2) and piglet vaccination is necessary. It was previously reported that early-piglet vaccination effects could be limited by maternally derived antibodies (3). In this study, under field conditions, it was observed that G3, compared to the other two groups (G1 and G2), had a lower mortality rate and a higher average daily gain in the lactation and post-weaning phases, with no apparent interference between maternal immunity and vaccination efficacy. Therefore, it can be concluded that there were no safety contraindications to early-piglet vaccination, and it was effective in piglets from both vaccinated and non-vaccinated sows.

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Investigation and analysis of Porcine epidemic diarrhea cases and evaluation of different immunization strategies in the large-scale swine farming system

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Introduction

Porcine epidemic diarrhea (PED) is a contagious intestinal disease caused by porcine epidemic diarrhea virus (PEDV) characterized by vomiting, diarrhea, anorexia, and dehydration, which have caused huge economic losses around the world (1,2). However, it is very hard to find completely valid approaches to control the transmission of PEDV. At present, vaccine immunity remains the most effective method (3). To better control the spread of PED and evaluate the validity of different immunization strategies, 240 PED outbreak cases from 577 swine breeding farms were collected and analyzed. The objective of the present study was to analyze the epidemic regularity of PEDV and evaluate two kinds of different immunization strategies in controlling PED.

Materials and Methods

The commercial live attenuated vaccine: Porcine Transmissible Gastroenteritis and Porcine Epidemic Diarrhoea Vaccine, Live, Strain WH-1R + Strain AJ1102-R, Wuhan Keqian.

The commercial inactivated vaccine: Porcine Transmissible Gastroenteritis and Porcine Epidemic Diarrhoea Vaccine, Inactivated, Strain WH-1 + Strain AJ1102, Wuhan Keqian.

The highly virulent live vaccine: field strain, isolated by Swine Research Institute of New Hope Group.

The location of swine farms from the large-scale swine farming system distributed throughout almost the whole Chinese mainland. The number of sows in those swine farms was from 1000 to 3000.

All swine in the large-scale swine farming system were vaccinated with two kinds of strategies. The one immune commercial live attenuated vaccine and inactivated vaccine. The other one immune highly virulent live vaccine by oral immunization and the commercial inactivated vaccine.

The PED status of all swine farms was collected every month since January 2021.

The statistical analysis was performed using two-tailed t-tests in Graph Pad Prism 7.0 (GraphPad Software Inc., USA) (4). The significant difference was defined as $*p<0.05$, and the various degrees of significant difference were designated as $**p<0.01$, respectively.

Results

The results showed that the main reasons which lead to the outbreak of PED were the introduction of gilts (36.9%) and delaying piglets from the normal production flow (17.5%). The prevalence rate of PEDV in the hot season (May to October) was obviously higher than that of in the cold season (January to April, November to December) (Fig.1). Results of different vaccine immunity cases showed that immunization with the high virulent live vaccine (2020Ta strain) and the commercial inactivated vaccine can significantly decrease the frequency rate of swine breeding farms (5.9%), duration time of PED cases (1.70 weeks) (Fig.2), and the week batches of dead piglets (0.48 weeks weaned piglets) (Fig.3), compared with immunization with commercial attenuated vaccines and inactivated vaccine of PED.

Discussion and Conclusion

Therefore, immunization with highly virulent live vaccine and inactivated vaccine of PED is more effective in the prevention and controlling the outbreak of PED in the large-scale swine farming system.

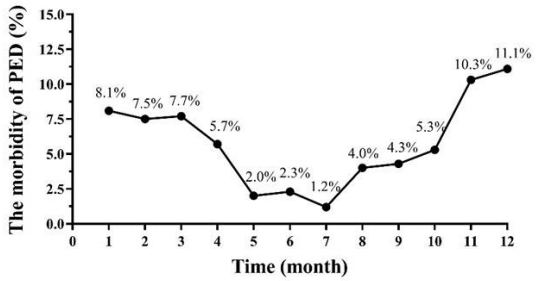

Figure 1. The seasonal prevalence rates of PEDV.

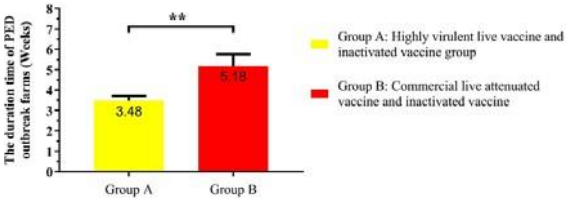

Figure 2. The duration time of PED outbreak farms with different immunization strategies

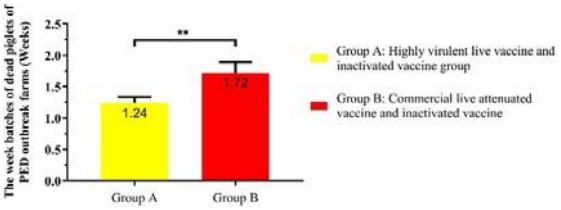

Figure 3. The week batches of dead piglets of PED outbreak farms with different immunization strategies.

Acknowledgments

Data-knowledge driven intelligent diagnosis of typical livestock and poultry diseases (2021ZD0113803).

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PCV3-associated disease in neonatal piglets in Brazil

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Introduction

Porcine circoviruses (PCVs) are endemic worldwide, and species 2 and 3 are known to infect pigs. PCV3 was associated with cardiac and multi-systemic inflammation, fetal mummification, abortions, and pneumonia in aborted fetuses (2,7). In Brazil, PCV3 has been detected through PCR in serum samples from sows with a history of stillborn piglets and in tissue samples from mummified fetuses (3,8). However, no disease in piglets has been described. This study aims to describe the pathological and molecular findings of PCV3-associated clinical disease for the first time in South America.

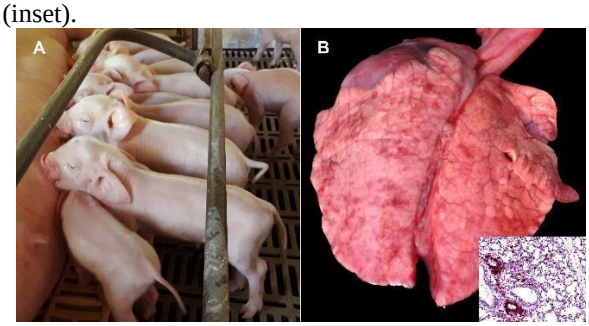
Materials and Methods

Gross, histological and molecular evaluation of 42 piglets was performed, and findings were recorded. Conventional PCR was performed to PCV3 For the phylogenetic analysis, ORF2 region from one positive sample per farm was amplified. Formalin-Fixed Paraffin-Embedded (FFPE) tissue specimens from seven cases with marked lesions were selected and submitted to ISH assay in order to detect transcriptionally active PCV3 in association with histological lesions, following a described protocol (9).

Results

A total of 42 piglets from 16 different sows, from five different sow farms, were submitted for routine diagnostic investigation. All farms were located in different cities in the state of Santa Catarina, Brazil. All piglets showed signs of large and caudally rotated ears ("Dumbo-like piglets") (Fig. 1a), weakness, and dyspnea. Most of them were from gilts (77%), with a birth average of 4.8 "Dumbo-like piglets" per litter. Piglets died 1–5 days after birth. At postmortem examination, all piglets had similar gross findings, including large and caudally rotated ears, the lungs did not collapse, and had marked interlobular edema (Fig. 1b). Microscopically, vasculitis was observed in the organs. There was also lymphohistiocytic interstitial pneumonia; multifocal areas of myocarditis, myositis, and gliosis. A total of 17 out of 42 samples tested positive for PCV3. PCV3 capsid gene alignment showed high nucleotide identity between all the sequences analyzed (97%–99%). The sequences shared more than 99% identity among them, with the most related sequence being PCV3/CH/Beijing-2/2018 from China. The phylogenetic tree showed three separated clades (PCV3a and the putative PCV3b and PCV3c) (4,5). In all tested cases, replication of PCV3 was most commonly observed in lymphocytes and plasma cells in perivascular areas, alveolar septa and in the smooth muscle of arteries (Fig.1b).

Figure 1. a) 1-day-old piglets presenting large caudally rotated ears and healthy piglets with normal size and correctly inserted ears. **b)** Non-collapsed lungs, with a shiny red appearance, due to interlobularedema. Lung with multifocal staining representing PCV3 mRNA, inflammatory cells mainly in perivascular areas. ISH (inset).



Discussion and Conclusion

The diagnosis of PCV3-associated clinical disease in neonatal piglets was based on the molecular findings in association with the detection of PCV3 mRNA in microscopic lesions. In our study, a consistent gross lesion observed in PCV3 positive piglets was the large caudally rotated ears. This finding has been previously observed in positive PCV3 wasting pigs (1) and in PCV3 positive neonatal piglets (6). However, the pathogenesis for this remains unclear and the correlation between ear malformations and PCV3 infection has not yet been proven conclusively. Affected piglets also had dyspnea, ear malformation, weakness, systemic vasculitis, interstitial pneumonia, myocarditis, and encephalitis. The sequences from this study clustered within the most common PCV3a genotype and corroborated with the limited nucleotide diversity of PCV3 across many countries worldwide (4). This finding demonstrates the worldwide distribution of the virus, justifying additional research and the implementation of control measures, to prevent economic losses.

Acknowledgments

CNPq, CAPES and FAPERGS.

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Precise elimination and restocking of African swine fever

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Introduction

In China, before the first half of 2019: African swine fever occurred without any effective measures. After the first half of 2019: The technology for the precise elimination of wild viruses is mature, with a high success rate and small losses. Through the measures of accurate culling and reinstatement after theoccurrence of ASF in three pig farms, the key points of precise elimination and restocking were summarized, and the successful practice of more pig farms was provided.

Materials and Methods

The three pig farms all adopt the same precise elimination and restocking ideas.

Precise elimination process: 1. The first confirmation of positive ASF pathogen PCR test; the first confirmation is that after the onset of ASF, the wholegroup is sampled for ASF pathogen detection,including personnel, personnel dormitories, environment, vehicles, pig sales platforms, etc. . Sows use cotton swabs for single-head sampling, nursery and fattening pigs use cotton ropes for saliva sampling, one cotton rope per column; personnel, personnel dormitories, environment, vehicles, and pigs are sampled using sterile gauze dipped in physiological Saline sampling; also increase the frequency of testing, with testing every 4 days until two consecutive negative tests. 2. After the whole group is tested, evaluate the overall positive infectionsurface, and then specify a feasible and precise elimination plan. 3. Improve biosafety measures, stopthe transfer and breeding of pigs, and increase the disinfection of the whole farm; personnel outside the pig farm are prohibited from entering the farm. All the main roads and the passages of the buildings are all sprayed with quicklime + caustic soda water, onceevery 2 days. All buildings are equipped with water shoes at the entrance, and a foot basin is also set up. After changing the water shoes, you can only walk inthe caustic soda water. The water shoes at the entrance of each house are only used in this house, and it is strictly forbidden to run around to avoid cross-infection. The commuting routes of each department are fixed, and the flow of people, logistics, vehicles, and pigs must be cut off, cut off, and cut off again. Immediately cut off all direct and indirect connections between the sick pig house and other buildings, and sort out possible connections such as personnel and materials as soon as possible.

4. The pigs with a positive test are electrocuted to be harmless. The aisle for pulling dead pigs should be paved with colored strips or film paper in advance. Dead pigs should follow the principle of not falling to the ground during transfer to prevent positive pigs from polluting the environment. All personnel involved in pulling dead pigs should wear protective clothing, water shoes, and disposable rubber gloves

After handling dead pigs, burn relevant items. Water shoes should be replaced in time and placed in a disinfection basin for immersion. 5. Use quicklime + caustic soda water to spread the empty columns or buildings. It is strictly forbidden to flush the columns to prevent the spread of viruses in the environment. At the same time, stick seals or color strips at the door of the unit, and people are strictly prohibited from entering.

Operation process of restocking: the real cause of African swine fever before the resumption; third-party biosafety audit to find loopholes in biosafety and improve the existing biosafety system; biosafety upgrade and transformation of pig farms; cleaning and disinfection: manure Soaking in caustic soda for roads and pens; rough washing; sampling, testing, and evaluation; covering with foam cleaning agent; fine washing, if necessary, wipe the pens and other parts with steel wool before rinsing; water line disinfection; after passing the inspection, proceed to the next step; For disinfection, the amount ofdisinfectant should be calculated according to the area of the pen; the buildings with ASF in the fieldare sterilized by flame, and the normal pen may notbe sterilized by flame; after the disinfection is completed, sampling and testing are conducted.

Results

After the occurrence of African swine fever in the three pig farms, Precise elimination and restocking was successful. So far, the pig farms are all producing in a safe and orderly manner.

Pig farm	Scale	Farming mode	Precise eli ination		Hut renovat	Cleanin g and	Restocking starting	Current state
			Starting time	End Time				
Case 1	800	One-point breeding	16-Jul-20	25-Jul-20	3months	1month	December	safe
Case 2	800	One-point breeding	13-Oct-20	31-Oct-20		2months	January	safe
Case 3	1000	One-point breeding	17-Oct-20	25-Oct-20		1month	December	safe

Conclusions and Discussion

Precise elimination and restocking can be successful. In the post-ASF era, Biosafety is still the only way out; regional joint prevention and control based on common interests; reliable testing; grounded emergency drill plans; strong execution and supporting reward and punishment mechanisms; biosafety education and training for all staff; Batch production management and intelligent pig raising.

Acknowledgments

PIG PEACE (Hangzhou) Technical Service Co., Ltd.
Prairie Diagnostic Services (PDS) in Canada

PRRSv 1-4-4 L1C Observations from Outbreaks

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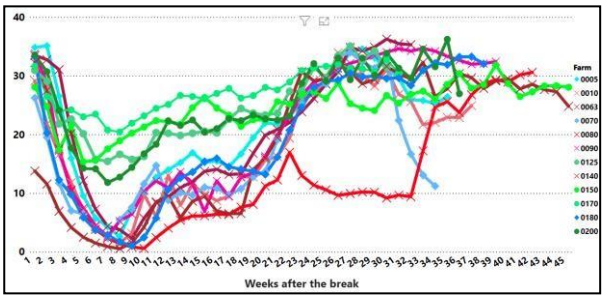
Introduction:
PRRSv continues to be a challenge for the US swine industry, costing the industry from $580.68 – $663.91/year [1,2]. With approximately 24% incidence of PRRSv in sow farms being monitored in Morrison Swine Health Monitoring Project (MSHMP) [3].
Laboratory data shown as part of the Swine Disease Reporting System (SDRS) verified from a diagnostic perspective[4], identifying the PRRS virus of RFLP 1-4-4 from Lineage 1C variant as a strain with rising prevalence and associated with highly virulent cases.
Biosecurity and bio-exclusion particularly are important piece of PRRSv control to avoid herd infection. Sow herds located in increasingly pig dense areas break at a greater frequency which has been associated with the number of surrounding finishing populations[5]. Filtration has been shown tosuccessfully reduce the frequency of PRRSv. [6] Feedmitigants have been used to reduce the risk of bringing new viruses into herds.

Objective:
Objectives of this study were to characterize the virus PRRSv 1-4-4 L1C variant that has recently circulated in Midwest of United States industry in 12 breeding herds. Description included both the clinical and diagnostic aspects as well production implications of affected herds.

Results:
The 12 breeding herds had outbreaks from November 2020 to July 2021. Clinical signs of this strain were consistent with what has been previously described for PRRSv virus, including increased incidence of abortions, lower farrowing rate, increased sow mortality, stillborn and mummies and preweaning mortality. In the wean to finish flows, it caused reduced average daily gain (ADG) and increased mortality particularly in the nursery phase but in finishing as well in lateral breaks of these sites. The difference with the PRRSv 1-4-4 L1C is the magnitude and length of signs observed. There does appear to be cross protection from previous exposure and vaccines, it appears to allow herds to stabilize and return to negative pig flow faster.
Diagnostically this virus is like previous viruses with the PCR-based test working well. The one thing that has been different with these viruses is the much lower CT (cycle threshold) values observed in some herds, indicating relatively higher viral loads in these affected animals.
Control measures include allowing the farms to stabilize and then move forward with field virus elimination utilizing a load, close and expose protocol. Farms with an onsite gilt developer are set up to do this the easiest. Ongoing monitoring of the process has become easier and better at detecting lowprevalence herds with the use of processing fluids.
Due to wean pigs need to continue to be monitored tobe sure that you don't miss infection occurring in the farrowing barn. Herds have returned to negative status and with an increased time to low prevalence.
The figure shows changes in annualized pigs weaned/mated female over time following PRRSv1-4-4 L1C variant outbreak on 12 different farms,
demonstrating the dip in productivity taking up to 30 weeks to recover.



Conclusions and Discussion:
It appears that PRRS 1-4-4 1C variants act similarlyto other PRRS viruses we have delt with in the past.Severity of the outbreak depends on existing herd immunity, how quickly the virus moves through theherd, and amount of time from outbreak to closure. Have eliminated this virus successfully and returnedto baseline production.

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Prevalence of Verotoxin-producing *Escherichia coli* (VTEC) on Korean swine farms

Introduction

Verotoxin-producing *Escherichia coli* (VTEC) causes swine oedema disease by producing verotoxin 2e (VT2e), which induces degenerative angiopathy in susceptible pigs. It is well established that Swine Oedema Disease (SOD) has a huge negative impact on the productivity of the swine industry. Sudden deaths, neurological symptoms, and retarded growth followed by large economic losses can be caused by SOD. However, up until now, the prevalence of VTEC on Korean swine farms has not been well studied due to the limited sensitivity of traditional diagnostic methods. The objective of the present study was to investigate the prevalence of VTEC with a new method using oral fluid samples and FTA (Flinders Technology Associated) cards.

Materials and Methods

From November 2019 to April 2021, a total of 959 oral fluid samples were collected from 196 farms distributed in all provinces of South Korea. The screened farms were selected randomly, without knowing the VTEC infection status of the farms and without SOD being suspected in the majority of cases. *Verocheck*, a commercial test developed by HIPRA, was used (1). The process consisted of collecting oral fluids, transferring them to FTA cards, and evaluating the presence of VT2e genes by real-time PCR assay. Positive cut-off was established at 38.5 CT, and other semi-quantitative values were established (see Table 1).

Table 1. Designation according to Ct value by real-time PCR assay targeted to *Verotoxin 2e* genes

CT value	<30	30-35	35-38.5	>38.5
Designation	Positive+++	Positive++	Positive+	Negative

Regarding the analysis and interpretation of the data, the laboratory results were further correlated with farm information such as herd size and sampling age. Logistic and linear regressions were performed in both cases.

Results

Overall, 132 farms out of 196 were positive for VTEC (see table 2).

Table 2. Nationwide positivity of *VTEC* at farm level in South Korea

	Positive	Negative	Total
No. of farms	132	64	196
Proportion	67.3%	32.7%	100%

There was a significant correlation (P<0.001, r=0.26) with sampling age (see figure 1). The mean percentage positivity was significantly higher in samples from animals older than 12 weeks (77.4%[a]) than animals 9-12 weeks old (60.0% [b]), 6-9 weeks old (47.1% [c]) and younger than 6 weeks (33.9%[d]).

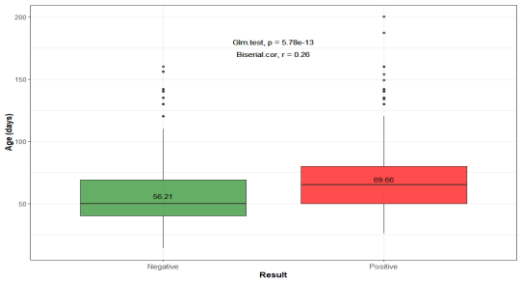

Figure 1. Correlation between VTEC positivity and sampling age.

When positivity was analyzed according to herd size, a negative correlation was observed (P=0.042), the herds with fewer than 600 sows having a higher rate of positivity (see figure 2) and the semi-quantitative positivity increasing as herd size was reduced (see figure 3).

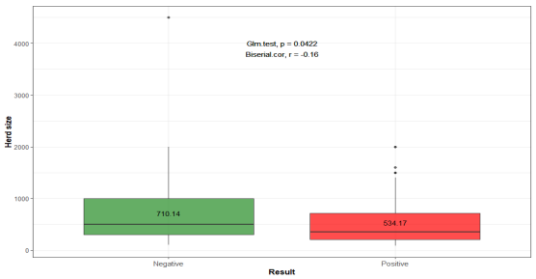

Figure 2. Correlation between VTEC positivity and herd size.

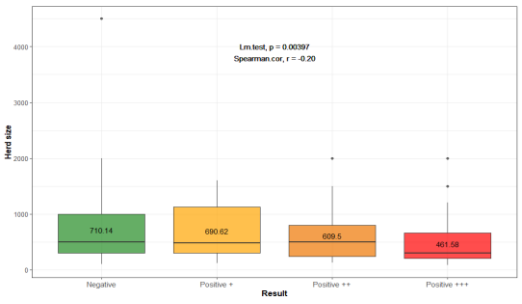

Figure 3. Correlation between herd size and semi-quantitative positivity.

Discussion and Conclusion

The results of the current study indicate that VTEC is present nationwide in South Korea, with a high prevalence at farm level. Moreover, herd size and age of the animals significantly affects positivity.

Acknowledgments

This study was financed by HIPRA.
The authors would like to acknowledge all the vets and farmers who supported them in obtaining the samples.

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Re-learning old lessons: PRRSV in Poland

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Introduction

This study describes the introduction of PRRSV into a negative site and the procedures implemented to control and eliminate it. At 11 months post-outbreak, the farm is classified as "provisionally negative" as per the most recent PRRSV herd classification system. Overall, this report highlights the importance of close attention to external and internal biosecurity: "*ufaj ale sprawdzaj*" (trust but verify!).

Materials and methods

In December 2020, a 3150 sow farrow-to-wean multiplication farm in Poland received 59 replacement gilts from a PRRSV-negative supplier outside of Poland. The gilts were shipped on a truck equipped with air filtration and placed into quarantine in an isolation facility adjacent to the gestation barn. Serum samples collected on arrival (15 animals) tested negative for PRRSV antibody, but one of 2 serum pools was RT-PCR positive. The result was confirmed by re-test and all gilts were culled. Subsequent testing of animals in buildings adjacent to the quarantine facility produced PRRSV-positive results in January 2021. ORF5 sequencing identified a PRRSV-2 lineage 5.1 virus with 98.2% homology to a commercial MLV (GenBank AF66183). The decision was made to eliminate the virus and the following steps were taken:

1. The herd was loaded to capacity (gilt replacements) after which all breeding females on the farm were exposed to the farm-specific virus by diluting viremic piglet serum (2.5 ml of serum in 100 ml of normal saline) and spraying nares, oral cavity, and face using a standard garden sprayer.
2. Strict McRebel™ procedures were implemented in farrowing facilities.
3. PRRSV monitoring was implemented using processing fluids (all litters) and blood samples (n = 30) from each group of weaned pigs.

Results

Monthly productivity data for 2020 and 2021 are given in Tables 1 and 2. Exposure to PRRSV had little impact on conception (data not shown) or farrowing rates, but did affect born alive per sow, piglet mortality, and total pigs weaned. Notably, the highest piglet mortality occurred 3 months after exposure (39.8%), followed by a significant decrease in weaned pig numbers 4 months after exposure. Born alive per sow was lower 4-5 months post-exposure as compared with 2020. Production stabilized and returned to baseline approximately 6 months after the incursion was noted, with the exception of elevated piglet mortality (not associated with PRRSV infection) in 2021 month 9.

Table 1. Farrowing rate and born alive by month

Month post-PRRS	Farrowing (%)		Born alive/sow	
	2020	2021	2020	2021
1	87.2	86.7	15.7	15.7
2	89.5	86.9	15.47	15.2
3	87.6	90.6	15.4	14.6
4	***75.9***	***91.7*** [a]	***15.7***	***14.5*** [a]
5	87.3	91.1	***16.0***	***14.6*** [a]
6	88.8	90.0	15.9	15.7
7	87.5	89.3	15.7	15.6
8	90.6	90.7	15.5	16.1
9	88.1	89.2	15.6	15.8
10	87.8	--	15.6	16.1
11	88.2	--	15.7	--
12	87.8	--	15.1	--
x̄	***86.9***	***89.7*** [a]	***15.7***	***15.4*** [a]

Table 2. Piglet mortality and pigs weaned by month

Month post-PRRS	Piglet mortality (%)		Pigs weaned	
	2020	2021	2020	2021
1	22.4	17.7	1650	2120
2	17.8	22.6	1947	1700
3	***17.1***	***39.8*** [a]	2018	1560
4	17.6	27.9	***1900***	***1280*** [a]
5	20.2	20.6	1500	1663
6	19.6	15.3	1736	1650
7	17.8	19.6	1836	1800
8	17.1	17.3	1813	1800
9	16.4	21.2	***2013***	***1375*** [a,b]
10	16.3	27.4	1852	2105
11	18.8	--	2275	--
12	16.9	--	1688	--
x̄	***18.2***	***22.9*** [a]	1829	1705

[a] Significant difference 2020 vs 2021 (T-test, $p < 0.05$).
[b] Enteric disease outbreak

Discussion

This experience highlights the importance of external and internal biosecurity. Notably, even reliably PRRSV negative sources may experience problems. Thereafter, internal biosecurity was not sufficient to contain the virus. Notably, procedures developed to control and eliminate PRRSV (load, close, expose and McRebel™) proved to be highly effective. In this case, on-going monitoring initially produced negative PRRSV PCR results in processing fluids and weaned piglet serum samples at 5 months post-outbreak with results consistently negative through 11 months post-outbreak. Thus, the expectation is that on-going monitoring will show that the virus has been eliminated from this site.

Reduction of *Salmonella* levels in an organic pig farm by probiotic supplementation

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Introduction

Hindgut pathogens as *Salmonella spp.* are commonly carried by fomites in organic open farm environments and are pathogenic in the cecal-colonic intestinal segment. Combined with Public health restrictions, this has significant economic impact in organic pig production with limited access to treatment by antimicrobials. A *Salmonella* control program with a probiotic strain of *Clostridium butyricum* (Miya-Gold®) has previously been described in a conventional pig farm (1). This case report describes a *Salmonella* control program in an organic pig farm with clinical dysentery. *Clostridium butyricum*probiotic strain provides an anaerobic environment in the caecum-colon intestinal segment (2) supporting the development of a healthy microbiome, while inhibiting the growth of pathogenic populations.

Materials and Methods

An organic pig farm with clinical bloody and mucous diarrhea, diagnosed *Brachyspira hyodysenteria* positive by PCR, and with a high seroprevalence of *Salmonella* in carcasses (Salmonella level 2, index >40) was enrolled in a program with Miya-Gold®-O supplementation at 1 kg per tonne of feed from 10- 40 kg and 0,5 kg per ton of feed from 40 kg till slaughter. All pigs received one batch of antibiotic treatment (lincomycin-spectinomycin) at entry (at 10 weeks of age) during 5 days. As per organic label rules, no further treatment was possible. Despite antibiotic treatment mucous diarrhea was present at entry. *Salmonella* serum titers were monitored before and after the probiotic inclusion (Table 1).

Table 1. *Salmonella* index evolution

	Month	Samples	Positive	Salmonella index (level 2 > 40)	Salmonella level
Miya-Gold® -	January	5	3	32,3	1
	February	6	4	54,2	2
	March	5	1	62,5	2
	April	14	7	33,3	1
	May	6	2	49,3	2
Miya-Gold® +	June	5	1	36,7	1
	July	7	2	31,1	1
	August	7	2	28,2	1
	September	7	0	27,6	1

Results

The prevalence of Salmonella seropositive samples was 47% from January till May (17 weeks, 1301 pigs), when the program was started. From June till September the prevalence of Salmonella seropositive samples dropped to 20% after supplementation in the full growing period (17 weeks, 1565 pigs), (P=0,03 Fischers exact test, 2 tailed) (3) (Table 2; Figure 1). Clinical diarrhea was still present but without mucus and blood, and with lower prevalence.

Table 2. *Salmonella* serum titers

	Miya-Gold® +	Miya-Gold® -
Salmonella Positive +	5 (0,20)	17 (0,47)
Salmonella Negative -	21 (0,80)	19 (0,53)
Total	26	36

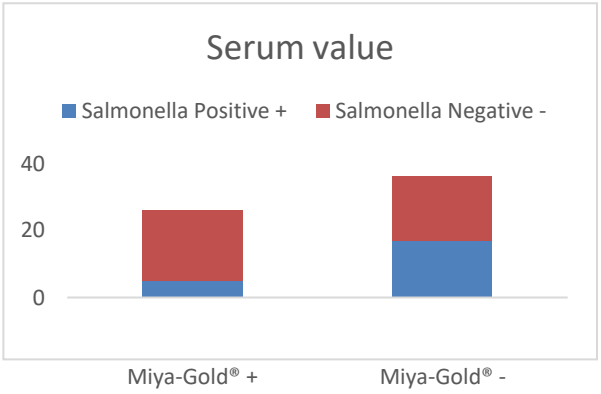

Figure 1. *Salmonella* serum values

Discussion and Conclusion

Inclusion of Miya-Gold-O® lowered the risk of a carcass being classified salmonella seropositive (OR=0,27). The clinical score of feces improved.
This case describes a consistent stabilizing effect of Miya-Gold® O on gut health creating a less favorable environment for *Salmonella spp.*

Acknowledgments

Danish Crown Ejerservice

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Saliva sampling as an alternative method besides pooled faeces samples for measuring qPCR Lawsonia levels

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Introduction

Lawsonia intracellularis bacterium is still causing significant economic damage in pigs with pooled faeces sample tested by qPCR as an ordinary measure of the disease level. Saliva sampling method is recently getting more attentions due to its advantage as being more user-friendly by veterinarians.
The aim of this study is to investigate whether a saliva sampling can be adapted as an alternative method in the field using statistical correlation analysis compared to the pooled faeces sampling.

Material and Methods

In a Dutch finishing farm samples were taken at different time points from 12 different compartments. At sampling point, in different pens a pooled faeces sample was collected from different fresh faeces present in that pen with a small spoon in a small container and stirred for making a homogenous sample. At the same time in that same pen, a saliva sample was collected by a chewing rope offered to the same pigs (1).
In total 195 times both individual samples of faeces and saliva were tested by qPCR Lawsonia in the BactoReal Lawsonia kit of Ingentix at the CDS in Boxmeer, The Netherlands. The samples were also pooled by 3 to represent the measurements per compartment side. Statistics calculations were conducted using Spearman's correlation and inter-rater reliability by Cohen's kappa (on Lawsonia status with 38.5 as the cut-off of Saliva sampling and 0 or not 0 as the cut-off of faeces sampling).

Results

A significantly strong correlation on individual samples (r=-0.804, p<0.001) was detected between both sampling methods using the Spearman's correlation. The Kappa value was 0.49 with p<0.001 to show the concordance of both measurements (110/195 being positive by both sampling methods; table 1). Also between the pooled samples a strong correlation (r=-0.818, p<0.001) was shown in the Spearman's correlation.

Table 1. Calculation of the Interrater reliability (individual samples)

		Faeces		Total
		POS	NEG	
Saliva	POS	110	28	138
	NEG	16	41	57
Total		126	69	195

Figure 1. Comparison of LI qPCR BactoReal Ingenetix Saliva rope samples (Ct) vs faeces (log copies/ µl) (individual samples)

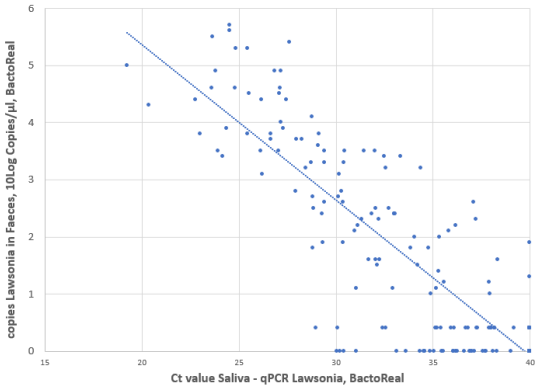
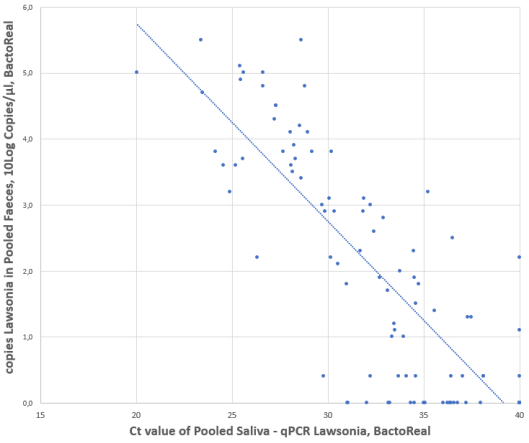


Figure 2. Comparison of LI qPCR BactoReal Ingenetix pooled Saliva rope samples (Ct) vs Pooled faeces (log copies/µl)



Discussion and conclusion

This study presents a strong correlation between both sampling methods for qPCR Lawsonia. It indicates that saliva sample is a reliable alternative sampling method for practical use in the field. Lawsonia is not excreted by the saliva; but it corresponds with the pen contamination. Saliva sampling outperforms faeces sampling mainly because that with a rope more pigs are sampled compared to some fresh faeces of that same pen from unknown sources. In addition, with rope sampling a veterinarian can collect a sample without entering the pen and when necessary, conduct additional PCR testing on the same sample on for instance PRRS, Flu or mycoplasma. Last but not least, compared to individual sampling, for practical reasons the use of pooled samples can be recommended as very similar strong correlation was shown using our data.

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Scrotal hemangiosarcoma in a Large White boar: Case report

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Introduction

Tumors are rarely diagnosed in swine specie because of the short lifespan of production animals. Normally, these tumors do not present any clinical signs and are often detected at the time of slaughter (1,2). Moreover, since necroscopic examination is often not performed when animals are found dead, determination of the prevalence of neoplasms in the species is more difficult (3). Therefore, this study was to describe a case of hemangiosarcoma of the scrotum in a Large White boar.

Materials and Methods

A 2-year-old Large White boar, used in the reproductive management of a pig farm without a history of pre-existing problems, was examined because of lesions on the skin of the scrotum. During physical examination, testicular asymmetry and miliary skin tumor masses ranging from millimeters to 2 cm were observed. The samples were collected from the testicular parenchyma, epididymis, ductus deferens, and skin segments containing lesions for histopathological (HE) and immunohistochemical (monoclonal antibodies Ki67, CD31 and factor VIII) diagnoses. The skin lesions were multiple, verrucous in appearance, and some were pedunculated. The collected material was stored in identified jars containing neutral buffered formalin and sent to the Histology Laboratory of the Biomedical Institute of the Universidade Federal Fluminense. The skin slides were analyzed in the Laboratory of Pathological Anatomy of the Centro Estadual de Pesquisa em Sanidade Animal of Rio de Janeiro, Pesagro-Rio.

Results

After the routine histological processing, no alterations were observed in the testicles, epididymis, or ductus deferens. Microscopically, the nodes in the scrotum consisted of poorly demarcated, highly cellular, expansile, and multifocal invasive neoplasms (muscle and superficial and deep dermis) composed of immature endotheliocytes organized in neovascular formations. The tumor cells were pleomorphic, slightly oval to spindle-shaped with eosinophilic cytoplasm and hyperchromatic nuclei with one to three nucleoli. Anisocytosis and anisokaryosis were moderate, with three mitoses per 10 fields of high magnification.

Discussion and Conclusion

All nodules analyzed were compatible with hemangiosarcoma. Tumors with cellular pleomorphism and mitotic activity are considered hemangiosarcomas (4). After immunohistochemical evaluation for the quantification of tissue angiogenesis, the neoplastic cells immunoexpressed the monoclonal antibodies CD31 and Factor VIII, through the identification of proteins expressed on the surface of endothelial cells. The Ki67 cell proliferation marker was positive in approximately 10% of the neoplastic cells, demonstrating a high degree of malignancy. The most identified tumors in cardiovascular and hematopoietic systems of swine are lymphoma (5), hemangiosarcoma, hemangioma, and cardiac rhabdomyoma (1). Hemangiosarcoma in swine species has been identified in various organs and tissues such as skin, testis, ovary, liver, spleen, and meninges (6, 7, 8, 9, 10). However, to the best of our knowledge, this is the first study to demonstrate this condition on the skin of the scrotum. The presence of hemangiomas in the scrotal region of boars usually occurs between 1 and 4 years of age, and that it has no influence on libido, litter size, and return to estrus (11). In this study, despite the diagnosis of hemangiosarcoma, the same was observed in boars,both with respect to age and reproductive performance. Therefore, it is expected that this report will contribute to the knowledge of the frequency of neoplasms reported in swine species.

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Senecavirus A detection in processing fluids after a Senecavirus A outbreak in breeding herds

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Introduction

Senecavirus A (SVA) has been responsible for a rampant increase in foreign animal disease investigations in the United States (1). Besides vesicles in adult animals, neonatal mortality and diarrhea have been associated with SVA infection in sow herds in different countries (2). However, both the pathogenesis and epidemiology of this disease are still unclear. Information regarding the presence and dynamics of SVA in processing fluids in sow farms during an outbreak is scarce. Furthermore, data on the within-herd transmission dynamics in the suckling pig population is not available. Additionally, the role of heat-check boars on the persistence of the virus in the breeding herd after an outbreak is also unknown. The objectives of this study were to 1) estimate the time to SVA-negativity in processing fluids after an outbreak; 2) assess the role of heat-check boars in the persistence and transmission of SVA within a farm, and 3) to estimate the production losses associated with an SVA outbreak.

Materials and Methods

This study was designed as an observational study, where ten conveniently selected sow farms undergoing an SVA outbreak were invited to participate in the study. A total of 310 processing fluids from participating sow farms were collected weekly or bi-weekly and tested for SVA RNA by rRT-PCR. If available, PF samples collected before the outbreak was detected were also tested. Farms were invited to collect semen from heat-check boars. Samples collected were shipped to the University of Minnesota (UMN) Veterinary Diagnostic Laboratory (VDL) for testing.

Results

The PF follow-up time ranged from 16 to 30 weeks in all farms, with an average follow-up of 22.5 weeks. Some farms were SVA-positive by PF testing even before clinical signs of SVA were detected, with the earliest detection of the virus in PF up to three weeks before the outbreak. Sow farms had at least one positive PF for an average number of positive weeks of 11.8 (Std Dev = 5.2) after the outbreak. Most farms had a varying number of consecutive negative weeks between positive PF results, ranging from 1 to 10 weeks.

Only one farm agreed to collect semen samples from heat-check boars. In the first semen collection at week 7 after the outbreak, four and three boars (out of nine collected boars) had PCR positive and suspect samples, respectively. In a second semen collection at week 18 after the outbreak, one out of sixteen collected boars yielded a PCR-suspect result. The boar yielding this result was euthanized at week 22, and tissue samples were sent to the laboratory. Surprisingly, the tonsil and testicle samples were RT-qPCR positive for SVA, with Ct values of 30.5 and 17.8, respectively. At the time of writing, only one farm had provided weekly production data, and pre-weaning mortality (PWM) was the only production indicator that suffered a significant change. PWM quickly increased from a historical 14.3% to 18.1%, 23%, and 42.7% in the first three weeks after the outbreak.

Discussion and Conclusion

Data from this study confirms that SVA can be detected in processing fluids and can aid as a monitoring tool. The 11.8-week average of PF-positivity after SVA outbreak detection in the ten sow farms indicates that the neonatal and suckling pig population may be exposed to this virus for an extended period after clinical disease resolution. Factors related to the variability of SVA detection in PF are currently unknown. Heat-check boars can potentially be a source of SVA to naïve gilts and sows. The high viral RNA levels, especially from the testicular sample, suggest that heat-check boars might act as carriers of SVA after an outbreak in a sow farm. In general, caution should be taken if weaning SVA negative piglets is desired as the number of consecutive negative weeks needed to consider the sowfarm as stable requires more investigation. Understanding the within-herd epidemiology of thisdisease can lead the industry to implement aggressive interventions to eliminate the virus at the system level. This project provides novel information about SVAinfection dynamics, serving as the foundation for building control and elimination programs.

Acknowledgments

The authors would like to acknowledge participating veterinarians and pig farms staff for agreeing to participate in this study and help collect samples. This study was funded by the American Association of Swine Veterinarians Foundation.

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Sow mortality: A practical approach to early intervention to reduce sow mortality

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Introduction

Sow mortality has significantly increased over the past 5 years in the U.S. swine industry. One database suggests that there has been a 50% increase in annualized sow mortality between 2015 and 2020[1].

As breeding herds have increased in size and theavailable labor force has decreased, one area that is often neglected is critically evaluating breedingfemales, particularly in the breeding and gestation phase, even though they typically spend 85% of their reproductive lives in this phase.

The objective of this study was to determine if early identification and treatment of at-risk sows in the breeding and gestation phase could reduce weekly sow deaths on a commercial sow farm in the Midwestern United States, a high swine dense region.

Materials and Methods

A 4,000 head, commercial breeding herd was used in this evaluation. The herd is endemic for Porcine Reproductive and Respiratory Syndrome virus, *M. hyopneumoniae*, and Influenza A virus. The breeding herd is housed in individual gestation stalls where breeding and gestation occur. Breeding females were fed once per day at 6:00h, via a drop feeding automated system. The methodology to improve the early identification and treatment of at-risk sows was to inspect each sow in breeding and gestation as feed was dropped. Two teams of two people were deployedfor a 2-week time period (Monday-Friday), which took place on weeks 24 and 25 of 2021. One Iowa State University (ISU) veterinarian was teamed with a gestation barn manager and walked one gestation rowat a time, with one person in the front of the stalls andthe other person behind the stalls. The primary indicator of an at-risk sow was one that was not up andeating at the time of feeding. Sows were encouraged or assisted to stand and then the evaluators performeda physical exam, including taking a rectal temperature.At-risk sows were flagged by hanging a card in front of the gestation stall with the appropriate reason, notedon the card. Information regarding the at-risk sow wasrecorded onto a daily form. The farm staff would return later to re-evaluate the sow, review the reasons they were flagged, consult the farm's sow treatment protocol, then provide and record the appropriate treatment. The time that the two teams started and finished the evaluation process was recorded each day.

Weekly sow deaths were the outcome measured. Weekly sow deaths from weeks 1-23 of 2021 (baseline period) were compared to weeks 24-48, 2021 using statistical process control charting. The Exponentially Weighted Moving Average (EWMA) was calculated for each period and then plotted on a EWMA chart using a lambda of 0.4 and a S.D of 3.0 (Table 1). To compare the change in % annualized sow mortality between the two time periods a chi-squared test for trend in proportions was performed using R program.

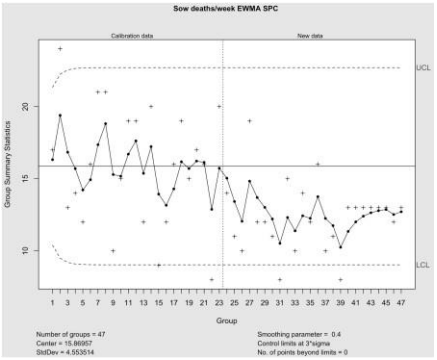
Results

When evaluating weekly sow deaths, a significant reduction was detected following training on identification and treatment of at-risk sows in breeding and gestation. The staff was able to maintain and improve on this reduction in sow mortality well beyond the two weeks of training. The breeding herdinventory during the entire evaluation period remained static, therefore weekly sow deaths were a good proxy for annualized % sow mortality. When comparing weeks 1-23 to weeks 24-48, there was a 4.25 percentage points reduction in sow mortality (from 16.75% to 12.50%). There was a linear trend suggesting a decrease on the % annualized sow mortality between the two time periods (p=0.007). Allof this occurred independently of any additional effortin the farrowing rooms, even though the weekly sow deaths included deaths from both breeding, gestation and farrowing.

Discussion and Conclusions

This evaluation demonstrates the opportunity toreduce sow mortality by prioritizing the identification and treatment of at-risk breeding females. For many health conditions, one of the primary signals of illness and discomfort is anorexia. In a system where all breeding/gestation breeding females are fed once per day at the same time, this can be used as an early signal to identify a breeding female that may need more thorough evaluation and treatment. The gestation barnstaff can flag at-risk sows during feeding and come back later in the day to perform the follow-up assessment and treatment.

Table 1. EWMA chart of weekly sow deaths before and after training to detect and treat at-risk sows in weeks 24 and 25 of 2021.



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Swine Erysipelas risk on non-vaccinated farms in China

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Introduction

Swine Erysipelas (SE) is an economically significant disease capable of affecting all stages of production. In sows, SE can cause abortions at any moment of gestation, but also acute death, skin lesions, fever, or lameness (1). The protective role of specific antibodies against SE enhanced through vaccination, is the key to controlling infectious reproductive problems in sows (2). Recently SE has been categorized as a potential disease to cause outbreaks in China (3). Serological assays such as ELISA have been used to detect antibodies against *Erysipelothrix rhusiopathiae* in pigs. The contact with the bacterium or after vaccination develops a humoral immune response (4).

The objective of this study was to evaluate whether *E. rhusiopathiae* is present or not on Chinese farms that are not vaccinating against it, as a prospective causative agent of reproductive disorders, evaluating through the immune status of the animals if the bacterium is circulating on the farms.

Material & Methods

A total of 2681 sows were included in the study (parity 0 to 7) from 68 different farms located in 13 provinces from North and South China. Farm size varied between 564 to 35,000 sows. None of these farms include the immunization against *Erysipelothrix rhusiopathiae* in their vaccination schedule. Blood samples were collected from January 2020 to December 2020.

Serum samples were tested using a commercialized ELISA kit (CIVTEST® SUIS SE/MR; Cut off-IRPC: 40). The ability of this kit to detect anti-SE antibodies without bias has been previously reported (5).

Results

From the total number of animals, 22.7% were SE positive (IRPC >40). Sows of parity 1 (29%) and parity ≥2 (24%) had a higher positivity rate than parity 0 (15%), with these differences being statistically different ($p<0.05$, Mann-Whitney U test).

The number of farms within each range of positive animals was: 3 (0% positivity), 12 (2%-9% positivity), 12 (10-19% positivity), 24 (20-36% positivity), 5 (37-54%) and 12 (55-70% positivity) (Table 1).

Table 1. Number of farms within each range of positive animals

Range of positivity	Nº of farms
0%	3
2-9%	12
10-19%	12
20-36%	24
37-54%	5
55-70%	12

Discussion & Conclusion

Based on these results, *E. rhusiopathiae* is circulating in 95.5% (65/68) of the nonvaccinated Chinese farms, as a different percentage of animals have developed an immune response against the bacterium. So, SE is a bacterial disease that could be causing different reproductive disorders on Chinese swine farms that could be going unnoticed. Furthermore, the positivity in sows of parity ≥1 is higher than in parity 0, which indicates that the prevalence in the production units is higher.

As prevention of SE is best accomplished by immunization programs (1) the inclusion of vaccines including antigens of *E. rhusiopathiae* , which elicits a strong immune response, must be used to prevent reproductive disorders.

Acknowledgments

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The reasonable entry process for personnel in the post-ASF era

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Introduction

Since August 2018, African swine fever has always been one of the most difficult problems for pig farming enterprises. Its existence has forced pig farms to spend more on biosecurity; how can people safely enter pig farms in the post-ASF era It is a point that many pig farm owners and veterinarians are very concerned about. By comparing the personnel entry process of several different pig farms, a reasonable personnel entry process in the post-ASF era was concluded.

Materials and Methods

The entry process of a large-scale 300-sow farm in Fujian, China: personnel directly arrive at the gate of the pig farm, enter the atomization room for atomization for 90 seconds, and directly enter the living area and production area.

The entry process of a large-scale 500-sow sow farm in Fujian, China: personnel directly arrive at the entrance of the pig farm, put on slippers on the farm, and go to the door of the dressing room outside the field; enter the atomization room for atomization for the 90s, and then enter the dirty area of the locker room and carry them with them. Take off all your clothes and put them in the designated cabinet; after showering, enter the clean area to dry your body and put on work clothes on the field. Enter the living area and enter the production area after 24 hours of isolation.

The entry process of a 1,000-sow farm of a group company in Zhejiang, China: personnel arrive at the designated centralized isolation point, put on slippers at the isolation point, and conduct sampling and testing; after passing the test, shave their hair, enter the centralized isolation point to take a bath, change the clothes at the isolation point, and isolate for 24 hours; isolation After 24 hours, they will be sent to the gate of the pig farm by a special vehicle from the isolation point, and at the gate of the pig farm, put on slippers on the field, and go to the door of the dressing room outside the field; trim their nails, and carry out ozone fumigation and disinfection on their belongings; enter the shower area for a shower; Enter the production area after 48 hours of regional isolation.

Results

There is a certain degree of biosecurity loopholes in the entry process of the personnel of Pig Farm 1 and Pig Farm 2, and the entry process of the personnel of Pig Farm 3 is relatively reasonable. Reasonable entry process: No pork is allowed 24 hours before returning to the arena. Before returning to the arena, trim your fingernails and change into clean clothes before returning to the arena. Sampling and testing at the designated isolation point. After the ASF test is negative, take a bath and change clothes and isolate at the isolation point for 48 hours; if the ASF test is positive, soak the clothes in disinfectant water, take a bath and change clothes, and re-sample for testing until the test is negative; Quarantine for two nights. Samples were taken early on the third day. After the ASF test was negative, a test certificate was issued. The isolation point is responsible for picking up the personnel to drive a special car to the first disinfection point of the pig farm. Put on the shoes on the field at the first disinfection point, put on disposable shoe covers, and the off-site disinfection vehicle will be taken to the locker room at the gate of the pig farm. (All personal belongings except mobile phones and computers should be placed in the first disinfection point or centralized isolation point for packaging and sealing.) Enter the dirty area of the locker room at the gate of the pig farm, take off all the clothes you wear and put them in a disinfection bucket for soaking and disinfection. After bathing, go to the clean area to change the sterilized clothes in the farm and enter the isolation point in the pig farm for 24 hours. After 24 hours of isolation on the farm, the ASF test was negative, and after taking a bath and changing clothes, they entered the living area of the pig farm for 24 hours of isolation. After wiping the mobile phone and computer with alcohol cotton balls, put them in the transfer window for ozone fumigation for 1-2 hours or UV for 1-2 hours. After 24 hours of isolation in the living area, you can enter the locker room of the production area to take a bath and change into the production area.

Conclusions and Discussion

After ASF testing and bathing, the biosafety of personnel entering the farm can be ensured, the risks brought by personnel entering the venue can be reduced, and losses to pig farms can be reduced.

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Prairie Diagnostic Services (PDS) in Canada

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The use and sharing of a boar semen lysate

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Introduction

PRRS virus can be transmitted vertically through boar semen, and the boar semen must remain PRRS negative, otherwise, it will lead to PRRS infection and disease in sow farms. Daily monitoring of PRRS infection status in boar studs is an important means of preventing PRRS. Semen is extracted by different methods, and the extraction method with the highest sensitivity is selected.

Materials and Methods

1. Sample classification: Using 4 bottles of different semen, nucleic acid extraction was performed by four different methods. Method A: Extract with manual nucleic acid extraction kit; Method B: First use RealPCR TL-60 for extraction and then with manual nucleic acid extraction kit; Method C: Extract with automatic nucleic acid extraction kit; Method D: Firstuse RealPCR After TL-60 treatment, use an automatic nucleic acid extraction kit for extraction.

	Boar semen1	Boar semen2	Boar semen3	Boar semen 4
A	A1	A2	A3	A4
B	B1	B2	B3	B4
C	C1	C2	C3	C4
D	D1	D2	D3	D4

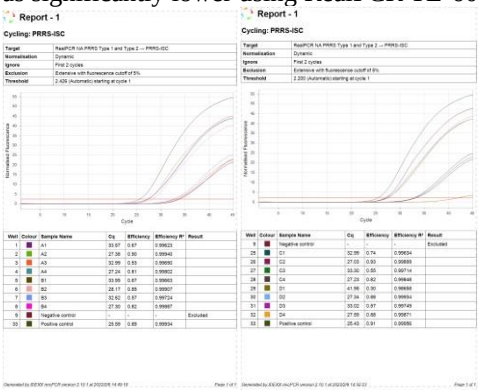
2. Sample processing: 1ml semen samples were taken from the four groups of ABCD and centrifuged at 12000rpm for 4min. In groups B and D, thesupernatant was discarded; 400ul RealPCR TL-60buffer was added, mixed with a pipette tip, and incubated at 70°C for 10min; the lysed samples were centrifuged at 15,000rpm for 1min, and 400ul of the clarified lysate was added to a fresh centrifuge tube.

3. Nucleic acid extraction: In group A, 200uL of supernatant was added to a new 1.5mL centrifuge tube, and then 500uL of lysate from tomorrow's DNA/RNA virus extraction kit was added, shaken, and mixed for the 30S, and allowed to stand at room temperature for 5min. Transfer all the solutions of A and B to the purification column, centrifuged at 12000rpm for 1min, and discard the liquid in the collection tube; add 500uL of rinse solution 1 to the purification column, centrifuged at 12000rpm for 1min, and discard the liquid in the collection tube; add 500uL to the purification column Rinse solution 2, centrifuge at 12000rpm for 1min, discard the liquid in the collection tube; put the purification column back into the collection tube, centrifuge at 12000rpm for 2min; transfer the purification column to a new 1.5ml centrifuge tube; add 50ul of eluent to the purification column, After incubation at room temperature for 2 min, centrifuge at 12,000 rpm for 1 min, and collect the DNA/RNA eluate into a 1.5 mL centrifuge tube. Groups C and D were extracted using an automatic nucleic acid extraction kit. First, the deep-well plate was taken out, and the magnetic beads were resuspended by inverting and mixing several times. Then, the liquid on the wall was tapped on the table to slow down, and then the aluminum sealing film of the kit was carefully removed. ; Take out the proteinase K solution, and after brief centrifugation, use a pipette to add 20uL to the first 4 wells of the first column; take the samples of group C,and use a pipette to add 300uL to the corresponding 4 wells of the first column; take the samples of group D, use a pipette to add the last 4 sample wells in the first column without proteinase K; put the deep-well plate into the automatic extractor for nucleic acid extraction; after the extraction, the eluate in the fifth column is transferred to the EP tube, for the extracted nucleic acid.

4. Configuration of the reaction system: use a 25uL system (20ul reaction solution with 5ul nucleic acid) for configuration.

Results

The semen PRRS in the FAM channel was negative; the Ct value of the endogenous gene in the HEXchannel was significantly lower using RealPCR TL-60 buffer.



Conclusions and Discussion

After the semen is first treated with RealPCR TL-60 buffer, the lysis will be more thorough and the sensitivity of pathogen detection will be increased. At the same time, monitoring of PRRS in boar semen will help to better prevent PRRS.

Acknowledgments

PIG PEACE (Hangzhou) Technical Service Co., Ltd.

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Third-party biosecurity audit of Chinese pig farms

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Introduction

At present, the main measures to prevent and control ASF in pastures are achieved through reasonable, effective, and strict biosecurity measures. With the normalization of ASF epidemics and the fall in pig prices, some pastures have experienced a decline in awareness of epidemic prevention, lax systems, and supervision mechanisms. In this situation, it is particularly important to carry out biosecurity audits of pig farms. The purpose of biosafety audit: to help pig farms find biosafety problems, analyze the causes of problems, and provide reasonable solutions.

Materials and Methods

1. External biosafety inspection of the ranch: The inspection of the external environment should focus on the inspection and analysis of people, vehicles, objects, disinfection points, transfer-out pig platforms, and public areas (roads).
2. Internal biosecurity inspection of the ranch: material disinfection room, storage room, entryprocess, and degree of implementation.
3. Inspection of infrastructure equipment and its functions: laboratories, decontamination points,transfer stations, drying rooms, material disinfection rooms, shower rooms, etc. are essential epidemic prevention infrastructure for ranches. The pressure onthe next link increases, and the probability of infection increases; of course, there are also ranch facilities and equipment, but it does not endow them with functions and there is no standard operating procedure. The final result is the same as if there is no.

Results

It is difficult to ensure cleanliness and incomplete disinfection at the transfer point of material elimination and transfer; neither of the two pig sheds has been thoroughly cleaned and disinfected after use,and the vehicles shuttle back and forth between the two places. risk is very high. The materials in the disinfection room are cluttered and unattended, and the hygiene is poor. This is not a disinfection room, but a "drug storage room" in a sense.

Conclusions and Discussion

1. Regular biosecurity audits are necessary, which will help the ranch find existing problems and correct them in time, especially third-party biosecurity audits.
2. There must be a standard process, supervision, reward, and punishment mechanism for each link of the operation of the pasture biosecurity.
3. In the construction of biosafety facilities, rationality should be considered and given their due functions. Facilities without substantial functions are decorations.
4. There is no best biosecurity, so managers cannotbe satisfied with the status quo.
5. Persistent high-intensity and repeated execution ofa process will lead to paralysis and carelessness. Biosecurity is a philosophy or attitude that focuses onmaintaining and improving the health of the herd and preventing the introduction of new pathogens.

Acknowledgments

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FOOD SAFETY & PARASITES

Designing a minimum- intervention strategy for the control of Neurocysticercosis in the Eastern Cape province of South Africa

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Introduction

Neurocysticercosis (NCC) is a human neurological disease caused by the tapeworm, *Taenia solium*. The natural life cycle of *T. solium* includes humans as final hosts (known as taeniasis) and pigs as intermediate hosts (cysticercosis). The transmission from pig to human is through the ingestion of encysted larvae in undercooked pig meat; however, NCC develops when humans become accidental intermediate hosts through the ingestion of eggs via a faecal-oral route or from a contaminated environment, allowing the larval stage to develop and migrate to the nervous system.

This is a disease of resource-poor, rural communities and the Eastern Cape Province of South Africa has a high number of clinical cases presenting as epilepsy in children and adults. (1)

Afrivet, a South African animal health company, initiated and funded a pilot intervention project to tackle cysticercosis and NCC in an Eastern Cape village, with the recommendation that the region's state veterinary services continue with the protocol in the long term.

Materials and Methods

A population of free-ranging pigs was identified in the Upper Gxulu community in the Keiskammahoek region of the Eastern Cape Province of South Africa. Several community-engagement meetings were held to educate the pig owners and obtain their consent for the proposed intervention project (2), and to provide feedback and recommendations afterwards. A team, which included Afrivet representatives, the state veterinarian andanimal health technicians from the local state vet officeas well as members of the Upper Gxulu village, embarked on an NCC- intervention program in June of 2021. 105 pigs that met the criteria for eligibility for vaccinations (2) were given a 1ml of Cysvax (a vaccineagainst *T solium* in pigs) via deep intra-muscular injection and subjected to lingual palpation to inspect for the presence of *T. solium* cysts. Five weeks later, in August 2021, the team returned to the village to administer a second dose of Cysvax to the pigs, as wellas a single dose of Paranthic, which is an oxfendazole product effective against the encysted larvae of *T. solium*, at an oral dose of 30mg/kg. (3) The pigs were marked with an ear tag at each treatment so that fully vaccinated pigs were easily identifiable. A further 76 pigs had reached the age of eligibility for the firstvaccination while the team was in the community in August 2021, so this was administered and the state veterinarian committed to return to the village to followup with the second dose of vaccine and the dewormer. In June 2021, before the program commenced, ten pigs were selected at random from willing sellers and purchased by Afrivet for slaughter at the East London abattoir. The carcases were inspected on the slaughter line using routine meat inspection protocols. This was repeated in February 2022 with 9 pigs that were marked with 2 ear tags each- indicating that they had received both doses of vaccine plus the deworming dose. It should be noted that the gold standard for diagnosing cysticercosis in pigs is necropsy, with multiple incisions throughout the musculature of the carcass. (4) Logistics prevented the use of this technique at the time of the project.

Results

During lingual inspection of the 181 pigs that received the first vaccination, 7 of the pigs each had a single, viable cysticercus in the tongue. One carcass from the unvaccinated group that was slaughtered at East London abattoir before the commencement of the program in June 2021, was condemned with focal cysticerci in the tongue and triceps muscles. Incidentally, all but 1 of these unvaccinated pigs had multiple hydatid cysts in the livers and lungs from *Echinococcus granulosus* and *T. hydatigena* infections.

In February 2022, 9 treated pigs were purchased for slaughter and meat inspection. There were no *T. solium* cysts noted in the vaccinated pig carcasses inspected, and only a small number of non-viable hydatid cysts in the livers and lungs of 5 of the pigs.

Discussion and Conclusion

Between 4% and 10% of the pig population that was subjected to lingual or meat inspection was positive for cysticercosis. It can be concluded that *T. solium* is a problem in the pigs in this community.

It has been proposed that an animal health strategy aimed at treating only cysticercosis in the pig population - if carried out responsibly and consistently - can effectively eliminate the associated human NCC disease in a community in 3 years. (5) The apparent reduction in viable cysts in pigs treated with the vaccine and oxfendazole product confirm the efficacy of the proposed protocol, and, if the Eastern Cape veterinary services can incorporate this protocol into a primary animal health care program, a positive impact on human health can be expected in the near future.

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Efficacy of a new oral anticoccidial to control and prevent coccidiosis (*Cystoisospora suis*) in piglets compared with a commercially available product

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Introduction

The use of anticoccidials in commercial swine farms increases the animal's health, reducing the use of antimicrobials and increasing profits (1,2). This study aimed to evaluate the efficacy of a new oral anticoccidial formulation on preventing and controlling *Cystoisopora suis* through the evaluation of oocysts per gram feces and diarrhea in piglets

Materials and Methods

The study was carried out an experimental swine farm. Fifty newborn piglets were distributed into five groups: Negative Control Group (NCG, n = 10), animals treated with 0.4 mL/Kg BW of 0.9% saline solution; Positive Control Group (PCG, n = 20), piglets received 0.4 mL/Kg BW of toltrazuril (Baycox®, Bayer Animal Health); and New Anticoccidial Group (NAG, n = 20), piglets treated with 0.4 mL/Kg BW of a new oral anticoccidial (MSD Animal Health). Piglets were treated at their third day of life (considered as D0). Animals from PCG and NAG weredivided into Preventive Group (PG) which were inoculatedwith 100,000 sporulated oocysts of *C. suis* 2 days after treatment and Therapeutic Group (TG) the animals were inoculated with the same number of oocysts one day beforetreatment, totalizing 10 piglets per group. The NCG animals were also inoculated with the same dose of oocysts, one day before treatment with saline. In 14 times,between 4 and 27 days post-treatment, rectal swab sampleswere collected from all piglets and a quantitative estimation of oocyst infection was performed (oocysts pergram feces, OPG). In the same days, the animal's feces were classified according to fecal score (1 as solid feces to4 as liquid feces). Piglets with scores higher than 2 were considered as presenting diarrhea. The results were compared to determine the effect of the treatments on the prevention or control of coccidiosis through analysis of variance (ANOVA) followed by either T-Student test for the OPG or through Fisher test for the number of piglets with diarrhea at each day. The significance level considered was $p \leq 0.05$.

Results

Mean OPG for each group are shown in Tables 1 and 2. In the whole period, new anticoccidial group piglets regardless to preventive or therapeutic had lower mean OPG than those from NCG ($p < 0.001$) and had similar results in comparison to PCG ($p > 0.05$). At D+4, D+5, D+6, D+7 piglets NCG had higher values of fecal score than those that received preventively or therapeutically thetoltrazuril or new anticoccidial formulation ($p < 0.05$).

Table 1. Mean OPG for preventive groups and negativecontrol[1].

Study Day	New anticoccidial	Positive Control	Negative Control
D+4	0^a	0^a	480^b
D+5	10^a	70^a	1190^b
D+6	20^a	0^a	2330^b
D+7	40^a	0^a	4190^b
D+8	10^a	0^a	63330^b
D+9	40^a	10^a	161000^b
D+10	0^a	0^a	25040^b
D+12	0^a	0^a	6280^b
D+16	0^a	0^a	1640^b
D+18	0^a	0^a	300^a
D+20	0^a	0^a	30^a
D+22–D+27	0^a	0^a	0^a
	0^a	0^a	

Table 2. Mean OPG for therapeutic groups and negativecontrol[1].

Study Day	New anticoccidial	Positive Control	Negative Control
D+4	0^a	0^a	480^b
D+5	10^a	0^a	1190^b
D+6	0^a	20^a	2330^b
D+7	40^a	0^a	4190^b
D+8	10^a	200^a	63330^b
D+9	30^a	10^a	161000^b
D+10	0^a	0^a	25040^b
D+12	0^a	0^a	6280^b
D+16	0^a	0^a	1640^b
D+18	0^a	0^a	300^a
D+20	0^a	0^a	30^a
D+22–D+27	0^a	0^a	0^a
	0^a	0^a	

[1]Different letters in a row indicates statistically significantdifferences within main effect

Discussion and Conclusion

The new anticoccidial formulation was able to keep the decrease the number of oocysts per gram feces and to preventdiarrhea for the entire challenge period compared to negative control group, no harming the growth performance. These results were similar to reported by Maes et al., 2007; Hiob et al., 2019 which the use of anticoccidials in swine farms increases the animal's health and profits.

In conclusion, the new oral anticoccidial has high efficacy on the prevention of coccidiosis on piglets, as well as on the treatment of established infections.

Acknowledgments

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Family farms for raising pigs: research and extension action to control parasites in a rural community in the state of Rio de Janeiro, Brazil

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Introduction

The family pig properties have diversified breeding system, with parasites being a problem in their production (1,2). Pig parasites can cause interference in their development, which can cause economic losses to the producer (1). Some parasites have zoonotic potential, with pigs being the main reservoir (2). Therefore, the objective of the present study was to evaluate pig for sites and their producers in family-type systems located in Cachoeiras de Macacu, RJ, developing extension actions.

Materials and Methods

Technical visits were carried out on 10 family farms between 2020 and 2021. In these the fecal samples were collected from pigs, as well as skin scrapings from the ears. In addition, stool specimens from farmers and their families were analyzed. The collected material was processed by direct examination, sedimentation and flotation coproparasitological techniques. This study involved three technical visits to each farm. Information on the handling of pigs and people participants were recovered through questionaries. Moreover, extension activities were developed to mediate information about parasites highlighting prophylaxis.

Statistical analyses were performed to determine the significance of the frequency the parasites among the family farms and to ascertain if there was any significant association with the information obtained from the forms and animals' sex and age with the parasite positivity. The pigs were classified in: Initial stage – from one to two months of age, Growing stage – from two to four months old, and Fattening stage – four months and older. A univariate exploratory data analysis was initially performed to select variables $p \leq 0.05$ based on the chi-squared test or Fisher's exact test. Then, a multivariate logistic regression analysis of the significant variables was then made, with a significance level of 5%, in which possible risk factors with Odds Ratio (OR) and their respective 95% confidence intervals. All analyses were performed using Epi InfoTM software.

Results

Feces and scraps from the ear were collected from 180 and 142, respectively pigs and also feces from 34 family farmers. Gastrointestinal parasites were detected in 86.1% of the pigs, especially forms compatible with Phylum Ciliophora (70.5%), strongyles (56.7%), *Strongyloides ransomi* (44.4%), coccidia (38.3%), *Ascaris suum* (32.2%) and *Trichuris suis* (17.2%) with statistically significant positivity ($p<0.05$). *Sarcoptes scabiei* var. *suis* was identified in 3.5% of the ear skin scraping samples. An analysis of infections by age group revealed that the general frequency of parasite, Phylum Ciliophora and strongyles were statistically significant ($p<0.0003$; $p<0.0096$; $p<0.0109$). Pigs in the growing age group had 3.9 times more likely to be parasitized when compared to the other age categories. Other factors were also associated with the frequency of specific parasite taxa, including type of food provided to animals, form of washing facilities, care of piglets at birth, type of facility and floor ($p<0.05$). The pig farmers participated actively in extension activities, interacting dynamically in the lecture on "Parasites and the importance of their control", "happy pig and sad pig", the "field day" and "homework checking".

Discussion and Conclusion

It is clear that the family systems that participated in this study did not have the financial or technical conditions to handle the animals in a sanitary manner, minimizing their parasitic infections. This situation reinforces the need for the creation of programs by the government to support these small family producers who need of these animals as a source of income and subsistence.

Acknowledgments

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Formaldehyde and organic acids based formulations on the reduction of *Salmonella* in feed and its impact in nursery

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Introduction

Salmonellosis is one of the most common food-borne diseases and pork is the third source of human contamination among animal foods (1). Several factors are related with the *Salmonella* infection in the farm and feed plays an important role. Brazilian feed mills produce a large amount of feed, which supply many farms; therefore, a contaminated batch has a potential impact of reaching many herds (3). Once infected, pigs shed high concentrations of *Salmonella* through feces contaminating the environment and other pigs. Thus, the aim was to evaluate the control of *Salmonella* by including formaldehyde and organic acid based products in the diet and its performance in nursery.

Materials and Methods

Four different commercial products based on formaldehyde and blends of organic acids were tested against feed contaminated with *Salmonella* Senftenberg. The product D (Table 1) presented the best performance (3 $\log_{10}$ reduction and the lowest concentration) and was selected for inclusion in an experimental field trial in a nursery farm. In this study, 336 male pigs of the same genetic, with 21± 2 days-old and body weight of 6,2± 0,37 kg, were distributed in 28 pens through a complete randomized blocks design. Seven replicates were performed with 12 piglets per pen, for each treatment (0%, 1%, 2% and 3% of in feed inclusion by liquid spray). Animals and the leftover feed were weighed on the placement day and at each feeding change at 7, 18, 27 and 43 days. Weight gain, daily weight gain, feed intake and feed conversion of pigs were evaluated in the same sampling events.

Results

The results are presented in Table 1 and Table 2.

Discussion and Conclusion

The commercial formulas composed of formaldehyde and organic acids presented better performance in the control of *Salmonella* than the products only constituted by organic acids. These results can be explained by the high level of disinfectant activity of formaldehyde, as well as by not being affected by organic matter.

Althoug the selected product presented a good magitude of *Salmonella* reduction in vitro, the higher the inclusion level, the worse the pig performance in the farm. After 7 days of treatment, a significant difference ($p<0.05$) was observed only in feed conversion. However, at the end of the nursery phase (43 days of placement), feed intake, feed conversion, daily weight gain and weight gain were significantly ($p<0.05$) influenced by diet. This can be explained by the fact that formaldehyde has a negative impact on the proteins availability in treated feed and food (2).

Table 1. Percentage of feed samples positive for S. Senftenberg, according to treatment and contact time (h).

Treatment	Composition	Contact time (h)			
		4	24	168	360
Product A 0,2%	30% F, 10,8% PA	0	0	0	0
Product B 0,8%	6,8% LA, 9,3% PA, 48% FA	100	100	0	0
Product C 0,2%	30 % F, 3% SA, 6% CP ,1% PS	10	0	0	0
Product D 0,1%	30% F and 5% OA	20	0	0	0

F – Formaldehyde; PA - Propionic Acid; LA - Lactic Acid; FA - Formic Acid; SA - Sodium Acetate; CP - Calcium Propionate and PS - Potassium Sorbate; OA – Organic Acids.

Table 2. Adjusted means for performance variables as a function of treatments and length of stay.

Time (days)	Treatment 1	2	3	4	P>F
	Daily weight gain - DWG (kg/day)				
7	0.050± 0.0045^{a}	0.042± 0.0045^{ab}	0.033± 0.0045^{bc}	0.024± 0.0045^{c}	0.008
18	0.237± 0.0053^{a}	0.227± 0.0053^{ab}	0.216± 0.0053^{bc}	0.205± 0.0053^{c}	0.004
27	0.335± 0.0054^{a}	0.323± 0.0054^{ab}	0.311± 0.0054^{bc}	0.299± 0.0054^{c}	0.001
43	0.420± 0.0053^{a}	0.409± 0.0053^{ab}	0.399± 0.0053^{bc}	0.389± 0.0053^{c}	0.005
	Feed conversion				
7	1.108± 0.0559^{a}	1.288± 0.0559^{b}	1.404± 0.0559^{b}	1.693± 0.0559^{c}	<.0001
18	1.041± 0.0109^{a}	1.065± 0.0109^{ab}	1.073± 0.0109^{b}	1.094± 0.0109^{b}	0.021
27	1.083± 0.0056^{a}	1.099± 0.0056^{a}	1.116± 0.0056^{b}	1.135± 0.0056^{c}	<.0001
43	1.307± 0.004^{a}	1.319± 0.0044^{a}	1.335± 0.0044^{b}	1.350± 0.004^{c}	<.0001

* Means followed by distinct letters in the lines differ significantly by the t test ($p\leq0.05$).

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Intestinal helminths in swine raised in the Northern Pioneer region of Paraná

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Introduction

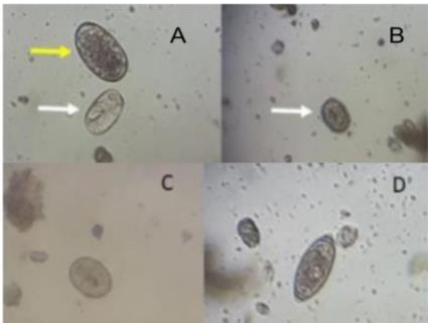
Swine gastrointestinal parasites are importance from extensive breeding at intensive breeding with clinical or subclinical consequences of mild to severe severity in production such as increased feed conversion to organ condemnation. Predisposing factors can be: system, sanitation, feeding management, age of the animals, quarantine and the origin of the water like your treatment (1). Zoonotic character must be taken account mainly in underdeveloped regions and in the use of waste for irrigation (2).

Materials and Methods

Approved by Committee on Ethics in Use of Animals (CEUA) (registration n° 08/2020). There was maked a survey and an analysis of the creation system of the 887 properties in the North Pioneiro region. Of these, a visit to 16 properties located in 7 municipalities was authorized. A comprehensive and epidemiological questionnaire was applied to the producer, containing questions about sanitary and environmental conditions. After the acception by producer, to realize the collections the animals were manually contained by a pipe or moved inside of your own pigsty for the natural stimulation of the defecation the collection was directly inside the rectal ampoule and stored in isothermal boxes with recyclable ice. The faeces was analyzed by the Gordon and Whitlock Technique.
Consideration was given to the number of samples and not to ownership. The questions about the epidemical quiz was associated with the results and were verified by the Chi-Square-Test with a 5% level.

Results

From the analysis of the 887 properties, the creation of subsistence stood out with 87.59%. 571 samples were collected for laboratory results, which were 7.5% positive and 92.5% negative. Of the positive samples, nematode eggs were found as shown in Figure 1 with their respective frequencies: Strongylids (48.8), Ascaris suum (27.9%), Trichuris suis (7.0%) with associations between Strongylids and Ascaris (11 .6%) and Strongylids, Ascaris and Strongyloids (4.7%).
Some variables (characteristics) with p value <0.05 correlated with positive OPG are highlighted: termination category with 48.83%, subsistence creation with 69.76%, fecal deposit above the water source with 65.11% and liquid and normal consistency with 74.41% (48.83% for diarrhea).



Font: (OGAWA, 2021)

Figure 1. Intestinal helminths identified in granules from swine from Norte Pioneiro, 2021. A. Strongylid eggs (yellow arrow) and Strongyloides ransomi (white arrow); B. Ascaris suum egg (white arrow); C. Egg of Metastrongylus sp.; D. Egg of Trichuris suis.

Discussion and Conclusion

In view of the Northern Pioneer region, where subsistence farming is highlighted over commercial farming, we obtained a low number of positive samples. In different breeding systems, they presented from high to low postive samples in less technified to more technified breedings (3,4). The same parasites were found by (3,5). The frequency with less developed regions and less technified creations is related, so water is suggested as an important contaminant when the manure is above the water source (6). Therefore, it is important to guide producers and employees on the importance of swine nematodes, as the low number of positive samples in this case may be related to the parasite survey and not to a follow-up, and there may be false negatives. Fecal consistency, it is argued that clinical signs of helminthiasis are usually mild or similar to other enteric causes, but it is associated with the frequency of helminthiasis in diarrhea (48.83%) highlighting the importance of subclinical infection (7).

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Microbiological data of meat of weaned piglets fed a silage containing cheese whey, grape pomace, and olive oil wastewater

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Introduction

Recently, there is an increasing interest for the development of functional feeds rich in bioactive compounds, that are considered important both for the health of animals and the consumers of animal products. Agro-industrial byproducts that often have a high pollution load, could potentially be used as sources of bioactive compounds in animal feeds, benefiting the protection of the environment and the circular economy. In the present study, a silage which contained olive mill waste water, grape pomace and deproteinizedcheese whey was used as a feed additive in order to investigate the effects on microbiological parameters of the meat of weaned piglets.

Materials and Methods

All experimental procedures were in accordancewith the National guidelines for animal trials (1).

The examined silage was evaluated at three inclusion levels: 0%, 5% and 10%. Each dietary treatment included 15 weaned and individually ear-tagged piglets. The trial lasted 40 days. Meat cuts were collected after slaughter from 6 animals per treatment and the microbe counts were measured for the differentmeat cuts (ham, shoulder and pancetta), using appropriate agar cultivation methods.

The collected data were subjected to one-wayANOVA using SPSS v.20 Statistical Package. Microbiology results were log-transformed (log10 cfu/g)prior to analysis. Data homogeneity was tested using Levene's test. Significance was set at 5% ($P<0.05$).

Results

As shown in Table 1, sulphite reducing *Clostridium*were significantly reduced in the ham, in the group fed with 5 and 10% silage ($P\leq0.05$), while TVC was also reduced in the group fed with 10% silage. Sulphite reducing *Clostridium* populations were also decreased in the belly cuts after a 10% silage treatment ($P<0.05$). Total viable counts and the populations of *E. coli*, *S. aureus*, *Staphylococcus spp.* and *C. jejuni/coli* did not differ significantly between the groups in the pancetta and shoulder cuts, as well as *Clostridium* populations inthe shoulder ($P>0.05$).

Table 1. Microbial load of meat cuts of ham, shoulder, and pancetta

	Silage inclusion				
	0%	5%	10%	SEM	P
Microbial load of Ham (Log cfu/g)					
TVC	6.68	6.20	5.71	0.18	0.09
E. coli	4.09	4.18	4.28	0.09	0.74
S. aureus	3.20	2.99	3.12	0.12	8.13
Staphylococcus spp.	4.34	3.59	3.80	0.15	0.11
Clostridium spp.	4.17[b]	2.05[a]	2.06[a]	0.28	0.05
C. jejuni	3.86	3.46	3.76	0.07	0.09
Microbial load of Shoulder (Log cfu/g)					
TVC	6.43	6.59	6.64	0.09	0.65
E. coli	3.99	4.02	4.23	0.09	0.54
S. aureus	3.21	3.11	3.14	0.13	0.95
Staphylococcus spp.	4.47	4.18	3.96	0.15	0.44
Clostridium spp.	4.24	4.05	3.78	0.12	0.33
C. jejuni	3.83	3.83	3.78	0.07	0.94
Microbial load of Pancetta (Log cfu/g)					
TVC	6.71	6.84	6.78	0.08	0.83
E. coli	4.25	3.80	3.95	0.09	0.18
S. aureus	3.38	3.19	3.15	0.12	0.70
Staphylococcus spp.	4.36	4.15	4.11	0.13	0.75
Clostridium spp.	4.38[b]	4.35[b]	3.70[a]	0.12	0.01
C. jejuni	3.83	3.84	4.03	0.07	0.73

[a,b] Mean values with different superscripts differ significantly

Conclusions and Discussion

Based on these results, the microbiota of different meat cuts was improved in the 5% and 10% silage treatments. Reduction in sulphite-reducing clostridia (which contain both pathogenic and spoilage organisms)was seen in all meat cuts, especially in the 10% silage treatment. Also, a tendency for lower TVC was observed in the ham cuts of pigs fed 10% silage.

Moreover, the present study indicated that the use ofa silage containing byproducts of the Greek agro- industry sector did not negatively affect the microbiological parameters of the meat of weaned piglets, particularly reduced *Clostridium* counts in all meat cuts.

Acknowledgments:

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Microbiological quality of pig carcasses in a slaughterhouse under risk-based inspection system

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Introduction

Meat product inspection procedures are adopted to guarantee food quality and safety for consumption (1). Due to technologies and regulations advancement for farming and slaughtering pigs, a change in zoonotic profile attributed to pork has been identified (2,3). Frequently detected lesions during inspection procedures have no impact on public health, while the palpation and incision techniques used favor bacterial cross-contamination (2,4,5,6,7). This change in the zoonotic profile turns out necessary to review the inspection procedures according actual public health hazards based on risk analysis. Thus, a global movement began to establish inspection parameters based on epidemiological risk profiles, culminating in the publication of Normative Instruction 79 in Brazil in 2018 (8). In order to assess microbiological contamination when adopting a risk-based inspection system, the occurrence of *Salmonella* spp. and the quantification of enterobacteria and mesophiles were compared in pig carcasses slaughtered under traditional and risk-based inspection systems.

Materials and Methods

Swab samples were collected for five days from pig carcass inspected under the traditional system and for five days under the risk-based system, always at 5:30 am, 8:30 am, 11:30 am, and 2:30 pm. At each time and date, samples of five carcasses were collected, achieving 20 carcasses per day per inspection system and on total 200 carcasses throughout the experiment.

The sampling procedure was carried out based on Brazilian legal requirements by rubbing a sterile sponge on four points of each carcass (ham, belly, loin, and axillary region), totaling 400 cm^2 (9). Each sample was tested for enterobacteria and mesophile counts and Salmonella enterica presence. (10,11,12).

A Fisher's exact test was performed to compare Salmonella enterica results between the two inspection systems. Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darling tests were performed to assess the normality of the enterobacteria and mesophiles results and Wilcoxon test to compare the inspection systems. The sample collection times were compared using Kruskal-Wallis test, followed by Wilcoxon test when the former presented significant results ($p \leq 0.05$) (13).

Results

A statistical reduction was identified for the quantification of enterobacteria (log 0.47 to 0.23 CFU/cm^2) and mesophiles (log 1.87 to 1.55 CFU/cm^2) in pig carcass inspected under risk-based system. The occurrence of *Salmonella enterica* did not show statistical significance (4% to 5.3%). There was no statistical significance when comparing time effect.

Discussion and Conclusion

Pig carcass inspected under risk-based system showed lower enterobacteria and mesophiles counts when compared to traditional system. It can be suggested that these results reflect the reduction in carcass handling, less exposure of contaminated tissues due to the complete removal of the head, and the suppression of cuts in carcass and head lymph nodes. Regarding *Salmonella* spp., no differences were found between the inspection systems. Both inspection systems rendered results within the legal accepted limits (9).

The results allowed us to conclude that adopting risk-based inspection systems improves food safety through enterobacteria and mesophile reduction. Future studies using similar analyses methods are indicated after the official implementation of this new inspection system.

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New approach on *Salmonella* detection by sock and environmental swab samples on 105 sow farms in Germany

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Introduction

Salmonellosis is the second most reported gastrointestinal infection in humans after campylobacteriosis in the EU. As pork consumption is a major source of human salmonellosis outbreaks, *Salmonella (S.)* spp. monitoring assessing the prevalence in slaughter pigs via serology has been implemented in Germany since more than 10 years. However, this program has not managed to reduce the number of positive fattening herds. Studies have shown that *S.* status of the fattening farm is strongly related to the *S.* status of the sow farm (1). To assess the role of the sow farms on the *S.* introduction into fattening farms with a high prevalence, and to see if the use of sock and environmental swab samples, like shown in poultry (2) is a suitable method, 105 sow farms (sized 50-10.000 sows) were sampled between 2015 and 2021 and analyzed for *Salmonella* via microbiological culture.

Materials and Methods

All sampled farms were linked to fattening farms with increasing serological prevalence, identified through the obligatory monitoring. For the sock samples each boot was covered with a plastic overshoe before entering the pen. The sock was placed over one plastic shoe to collect fecal content from the floors at each pen within the sampled compartment.

Picture 1: Taking a sock sample

For the environmental swabs a synthetic dust cloth was used to sample dust, on various lines and on pipes within the pens above the belly button. For each sample a new pair of non-sterile gloves was used to make sure not to cross contaminate the samples. The number of samples depended on the size of the farm, at least one sample in all areas of production. The samples were analyzed for *Salmonella* ssp. via microbiological culture. Detected *Salmonella* ssp. isolates were typed. An average of 12 socks and 10 swabs per farm resulted in a total of 1256 socks and 1099 environmental swabs. Statistical test (two-sided, significance level 0.05) was performed by Wilcoxon-Mann-Whitney-U Test on the samples of the three mainly sampled areas farrowing, nursery, other areas (insemination, gestation, gilt quarantine).

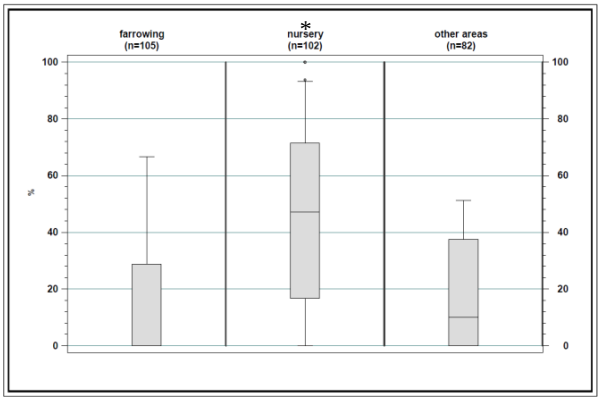
Results

On 97 farms (92.4%) Salmonella spp. was detected in at least one sample. In total 38.2% (n=480) of the socks and 27.1% (n=298) of the swabs were positive for *Salmonella* ssp. On 61% of the farms only one serotype could be detected, in 25.7% two and in 5.7% three or more serotypes could be found. Most frequently detected was *S.* Typhimurium in 77.1%, followed by Salmonella Derby in 18.1% of all farms. In farrowing, nursery, and other areas there were 18.9% (n=125), 47% (n=456) and 21.7% (n=98) respectively of the samples positive for *Salmonella*. Significant more farms showed the highest number of positive samples in the nursery compared to the other areas of production (graph 1). There was no significant difference regarding farm size or region.

Discussion and Conclusion

Sock and environmental swabs have proofed to be a valid, noninvasive and cheap tool to identify *Salmonella* present on farms regardless the farm size. They could be a suitable addition to serological monitoring. These results underline the role of sows in the *Salmonella* control (1), as already farrowing units were positive for *Salmonella*. As the detection rate was highest in nurseries as already shown by (3), stopping the infection dynamics in nursery piglets promises a big progress in Salmonella control.

Figure 1. Percentage of positive samples per farm/age group (other areas sums up: insemination, gestation and gilt quarantine)



* indicate significant differences (p <0.001)

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Pig coccidiosis in industrial type farms and a comprehensive program for the control of exogenous and endogenous stages of their development

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Introduction

In recent years, both in our country and in many countries of the world where pigs are engaged, parasitic protozoa - coccidiosis - Isocpora suis, Eimeria spp., Balantidium coli become especially relevant among diseases (1, 2). They affect pigs of different ages, but they have the greatest negativeeffect on the body of pigs in young animals. Pigs of older age groups suffer from coccidiosis and balantidiasis in a mild form, and sows are often the main sources of noted invasions for born piglets (3- 5).

We set ourselves the task of studying the spread of pig coccidiosis in industrial-type farms and compiling a comprehensive program for controlling the exogenous and endogenous stages of their development.

Materials and methods

In the course of the work, piglets of 3-20 days of age were examined for invasion by Isocpora suis using the Mac Master method. Fecal samples for research were taken in pig farms in different regions of Russia together with the veterinary service monthly in the amount of 10-20 from each farm. Infection of piglets with Eimeria spp. and Balantidium coli were established in the study of fecal samples from piglets 20; thirty; 60; 90 and 120 days old according to the combined Darling method. The intensity of coccidiosis invasion was determined by counting oocysts in 1 g of pig feces using a Mac Master chamber.

To control the exogenous stages of coccidia during the preparation of breeding pigsties for disinfestation,the complex agents Kenokoks 4% and Eimeriotsid5% were used at a dose of 0.5 l/m2 at an exposure of 2 hours, and 4% as the base variant hot sodium hydroxide solution.

To control the endogenous stages of coccidia, piglets on the 3rd day of life were given a single dose of Toltrazuril 5% at a dose of 30 mg/kg of body weight according to the AI.

The effectiveness of the complex use of drugs against exogenous and endogenous stages of coccidia in piglets was evaluated by the results of studies of fecalsamples using the McMaster method before administration, then 5, 10, 15, 22, 30, 45 and 60 days after the administration of the drug.

Research results

The conducted studies have shown that in piglets of7-30 days of age, the extensinvasion by isosporesranged from 10 to 60% with the intensity of invasionfrom 2 to 50 ind. in the field of view of themicroscope. On average, Isocpora suis oocysts wereisolated in 15.5% of piglet faecal samples. According to veterinary laboratories, the average prevalence ofeimeriosis in pigs in the country was 25.2%, withfluctuations in federal districts from 10.5 to 35.25%. The results of coproscopic studies of samples from pigsties, where disinvasion against exogenous stagesof coccidia was carried out with complex meansKenokoks and Eimeriotsid, and piglets at 3 days ofage against endogenous stages of coccidia were giventhe noted dose of Toltrazuril up to 45 days of agewere free from coccidia and balantidia. In the studyof samples from piglets of 60 days of age, coccidiaoocysts were isolated in two samples, EI was 10%,balantidia cysts were absent. Whereas piglets from the control pigsty were infested with coccidia duringall periods of the study (10-60 days), and with balantidia from 30 to 60 days.

Discussion and conclusion.

Our studies have shown that coccidiosis of young pigs is widespread in industrial-type farms in Russia. The complex implementation of measures against exogenous and endogenous stages of porcine coccidiahad a positive impact on the level of biosecurity ofthe pigsty-mother liquor on production and economic indicators. The safety of piglets of the experimental pigsty after complex measures against exogenous and endogenous stages of porcine coccidia was 7.1% higher compared to the control group, and the increase in live weight in experimental piglets was 13.9% higher. Feed costs per unit of live weight gain in the experimental piglets were 12.1% less than in the control.

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Seroprevalence of *Toxoplasma gondii* and Hepatitis E virus (HEV) in free-ranging wild boars hunted in six Brazilian states

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Introduction

Wild boars, including Eurasian wild boars (*Sus scrofa* Linnaeus), feral pigs (*Sus scrofa domesticus*), and hybrids between the two, have increased dramatically around the world. Currently, subpopulations of this invasive exotic species are present in more than 20 Brazilian states (1). In Brazil, wild boars are classified as exotic invasive species, with nationwide hunting officially permitted for control (2). These free-living populations can harbor many infectious agents transmissible to domestic pigs and other animal species including human (3). Considering the high dissemination of these populations and the increasing popularity of consumption of wild boar meat and meat products, the foodborne pathogens transmission is an increasing threat to human and animal health.

Toxoplasma gondii and Hepatitis E virus (HEV), are multi-hosts zoonotic foodborne pathogens that may be transmitted through the consumption of raw or undercooked infected meat and meat products.

The aim of this study is to provide an overview of the seroprevalence of *Toxoplasma gondii* and Hepatitis E virus in free-living wild boar hunted for population control and surveillance proposes, in six Brazilian States during the year 2019.

Materials and Methods

During the year 2019, a total of 245 wild boar sera samples were collected with collaboration of hunters licensed for population control of these specie and by research team.

Blood samples were collected by puncture in the cavernous sinus or heart, by exsanguination (cervical major veins) or from the thoracic cavity, immediately after death. Then were transported to the laboratory for centrifugation and stored at −20°C until serological analysis.

The samples were tested for detection of antibodies against *T. gondii* using a commercial indirect enzyme-linked immunosorbent assay (ELISA; ELISA ID Screen Toxoplasmosis Indirect Multi-species, IDvet, Grabels, France) and the apparent seroprevalence was estimated. The screening to detect anti-HEV specifics IgG antibodies was performed using the commercially available indirect enzymatic immunoassay (ELISA; PrioCHECK® HEV Antibody porcine ELISA Kit. Thermo Fisher ScientificTM, Waltham, MA, USA). The HEV seroprevalence was estimated considering the sensitivity (91,0%) and specificity (94%) of the test. The serological tests were performed according the manufacturer's instructions.

Results

The Tables 1 and 2 summarize the distribution and apparent seroprevalence for *Toxoplama gondii* and HEV seroprevalence in wild boar in six Brazilian states.

Table 1. Seroprevalence of *Toxoplasma gondii* in wild boar hunted during 2019 in six Brazilian States.

States	N° Positive/ Tested	*Toxoplasma gondii*	
		Prevalence (%)	(CI 95%)
MS	0/11	0,0	(0,0% - 0,5%)
MG	27/80	33,8	(23,2% - 44,2%)
SP	20/49	40,8	(27,0% - 54,7%)
PR	18/27	66,7	(48,7% - 84,6%)
SC	14/25	56,0	(36,3% - 75,7%)
RS	21/53	39,6	(26,4% - 52,9%)
Total	**100/245**	**40,8**	**(34,6% - 47,0%)**

Table 2. Seroprevalence of Hepatitis E virus in wild boar hunted during 2019 in six Brazilian States.

States	N° Positive/ Tested	HEV	
		Prevalence (%)	(CI 95%)
MS	5/11	46,4	(156,5% - 76,3%)
MG	11/80	13,8	(6,1% - 21,4%)
SP	18/49	36,7	(23,1% - 50,3%)
PR	3/27	11,1	(0,0% - 23,2)
SC	5/25	20,0	(4,1% - 35,9%)
RS	6/53	11,3	(2,7% - 19,9%)
Total	**48/245**	**19,6**	**(14,6% - 24,6%)**

Discussion and Conclusion

The seroprevalence of *T. gondii* and HEV detected in this investigation indicates that there are a potencial risk for human and animals health by consumption of wild boar meat products.

Acknowledgments

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HERD MANAGEMENT

Adenosine triphosphate (ATP) bioluminescence assay for biosecurity verification

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Introduction

ATP (Adenosine triphosphate) is the basic energy component molecule of all plant and animal cells and is present in all microorganisms and organic residues. Due to this, its presence on a surface can be used as an indicator of organic contamination. When ATP reacts with luciferin in the presence of luciferase it produces a release of energy in the form of light, this is called bioluminescence (also mechanism for firefly light in abdomen). Therefore, the presence of ATP can be detected as a light output being emitted. Therefore, the measurement of light intensity provides a visual indicator of ATP present and of the total organic contamination from the surface tested.[16]

ATP bioluminescence is used extensively in the food industry as a tool for monitoring and maintaining cleanliness.

ATP bioluminescence is a rapid and simple method to assess surface cleanliness and microbiological contamination in food facilities, dairy industry, hospitals, animal transports and so forth.[1] ATP bioluminescence detects, among others, microbial cells, food residues, bacteria, fungi and yeast, which might persist after inadequate cleaning.[2] It is measured in Relative Light Unit (RLU) values, which is directly proportional to the amount of ATP present. Several studies have demonstrated strong correlations between RLU values and bacterial microbe counts or surfaces which were judged to be dirty.[1,3,4,5]

Personal hygiene, such as changing clothes and footwear as well as handwashing, prior to entering a farm, are important biosecurity measurements to prevent the entering and spreading of pathogens.[6] Several studies, especially from pigs, show the effectiveness of personal hygiene. Pathogens such as *Escherichia coli*[7], foot and mouth disease virus[8], classical swine fever virus[9], influenza A virus[10] and porcine reproductive and respiratory syndrome virus[11] have been shown to be transmitted indirectly through people from infected pigs to other pigs when no personal hygiene measures were implemented.

Therefore, the use of an ATP bioluminescence assay to control personal hygiene on farms would be a useful and fast method

Materials and Methods

BioChek has undertaken field studies using the Hygiena EnSURE Touch luminometer and ATP bioluminescence products in the South African pork industry. The purpose of these projects was to evaluate whether the ATP technology can be adopted at the farm level as a tool to test and validate on-farm hygiene protocols. These projects officially started on August 1, 2021, at one of the primary genetic suppliers for the South African industry. Other farms then followed this initial farm with protocols and hygiene guidelines were adjusted as experience with the products and the labor force was gained. This study was undertaken to both show the potential of biosecurity verification and its effect on worker adherence to the protocols plus to prove that the products can be effectively utilized in a farm environment. The studies focused on farm hygiene and biosecurity verification in the following areas:

- Employees and visitors pre-shower and post-shower
- Employee lunch containers brought intofacility
- Outside containers of semen deliveries
- Outside containers of pest control equipmentand supplies delivered to the farm
- Outside containers of veterinary products delivered to the farm
- Waterers, feeders, fencing, and pen slats post cleaning and disinfection

For each of the items or areas to be sampled detailed instructions with diagrams were presented to the employees and lead employees were trained to perform the sampling on a consistent basis.

RLU result provides information on the level of contamination within seconds. The higher the RLU number the more ATP present and the dirtier the surface tested.

Results

All three pilot farms showed significant improvements in the average shower values as awareness on the farm increased under its employees. The swabs also identified employees who required additional training on the shower procedure and once trained, showed significant improvement. Farms where clean coveralls were not supplied daily was more challenging due to cross contamination with dirty clothes increasing ATP values post shower. As the pre-shower values decrease, the risk arriving at the farm also decreases, due to workings being more cautious on hygiene overall as seen in Table 1.

Table 1. Average Pre and post shower values

Date	Pre-Shower	Post Shower
02-Aug-21	554	390
06-Aug-21	589	242
10-Aug-21	586	409
11-Aug-21	326	206
17-Aug-21	385	246
26-Aug-21	670	232
01-Sep-21	147	79
02-Sep-21	86	53

When comparing the shower hygiene of Farm 1 to Farm 2 one can also see a significant difference. With the highest value being 238RLU while farm 2 had a high value of 3607 RLU. Which poses a biosecurity risk for the farm.

Table 2. Shower hygiene comparison between two pilot farms.

	Farm 1	Farm 2
Shower 1	71	3607
Shower 2	238	473
Shower 3	83	2995
Shower 4	104	1466

Farm 1 tested farrowing pens after only a two-step washing process. Twenty-five samples were taken, and the average value was 2660 RLU. After a disinfection step was added, the same 25 areas were tested again, and the average was 446 RLU, showing the significant impact of the disinfection step. Farm 3 was a farm with scouring issues in the farrowing crates. The first farrowing house that was tested had an average value of 2728 RLU (9 samples were taken). The second farrowing house had a high rate of scouring and the average of the 22 samples were 2999 RLU. After retraining the employees responsible for washing and disinfection and changing the current processes to be more effective, the average of the 33 samples taken in the third house were 1267 RLU.

Discussion and Conclusion

Several studies have demonstrated that ATP bioluminescence is a fast and easy to use method to monitor microbial contamination and the effect of hygiene interventions for a variety of surfaces (e.g., milking equipment, animal transporters, stable-surfaces, and hands). No general thresholds for the differentiation between clean and dirty exist, the differentiation needs to be defined based on the surface tested, the environment and the desired degree of cleanliness. There is no standard method for the definition of thresholds. Methods include reference values based on objects/surfaces with a low bacterial count, calculation of RLU-thresholds based on existing thresholds of microbiological assessments, or the relative drop of ATP levels after a hygiene intervention. Limitations of the method mainly concern the specificity of the assay, since high ATP-levels can also be caused by non-pathogenic or dead microbes, as well as interactions with certain disinfectant materials.

People and vehicles are major risk factors regarding the introduction of pathogens into a farm. This risk can be reduced by certain biosecurity measurements, such as proper body washing or cleaning and disinfecting vehicles before entering a farm. ATP bioluminescence is a suitable tool to monitor these measurements in real time. Additionally, hand washing improves over time when ATP tests are implemented, due to the increased awareness of the personnel.[12]

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Analysis of pace of vaccination and labour costs in two different intradermal vaccination protocols

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Introduction

Porcine circovirus type 2 (PCV2) is a small, non-enveloped member of the family *Circoviridae*. The virus is ubiquitous in domestic swine and causes a wide range of syndromes collectively referred to as porcine circovirus-associated diseases (PCVAD) (1, 2). *Mycoplasma hyopneumoniae* (*Mhyo*) is one of the smallest free-living prokaryotic microorganisms and induces enzootic pneumonia, a disease characterised by coughing, significantly decreased feed efficiency and poor growth performance (3, 4).

Immunisation of piglets against both pathogens has become one of the most important procedures in swine production (5, 6, 7). Moreover, development of needle-free systems enables swine producers to administer vaccines without exposing pigs to stressful intramuscular injection. Other features of the system include increased work safety and convenience.

The objective of our study was to compare the pace of work and labour costs between two intradermal vaccination protocols with Mhyosphere® PCV ID (Laboratorios Hipra, Amer, Spain), applied using Hipradermic 3.0 (Laboratorios Hipra, Amer, Spain) before or after weaning.

Materials and Methods

The study was carried out in 2021 during 8 consecutive weeks in northern Poland on 2 farms vaccinating against *Mhyo* and PCV2 associated diseases using different vaccination protocols in 6 kg DanBred piglets: 2 days before weaning (BW), or 1 day after weaning (AW). The total number of pigs immunised BW and AW was 8736 and 9017, respectively.

All the animals were reared under conditions meeting the requirements of Council Directive 2008/120/EC of 18 December 2008, laying down the minimum standards for the protection of pigs. Owing to obvious technical limitations relating to the construction of the farrowing pens (one litter in one pen, presence of a sow, and farrowing rails blocking direct access to piglets running on the other side of a sow), the BW team consisted of 5 employees, i.e. 4 people handling suckling piglets in 2 neighbouring farrowing pens, and 1 man standing outside and operating the device. On the farm using the AW protocol (with approximately 30 weaned piglets in each pen) the same tasks were completed by 1 person equipped with the device mounted on a bracket.

The data on the pace of work were collected in real time by Hipradermic® 3.0 using commercial software (HIPRAlink® Vaccination Laboratorios Hipra, Amer, Spain). The statistical difference in the pace of vaccination was analysed using t-test (R software v4.0.3, R Core Team).

Results

The BW team needed 13 sessions to vaccinate 8736 animals at a pace of work equal to 604 doses/hour, whilst the AW team used 20 sessions to immunise 9017 piglets, achieving 469 doses/hour (Table 1). Even though the pace of vaccination was significantly higher in the BW protocol (p=0.008), the total labour cost per applied dose was 3.9 times higher than the value observed in the AW system.

Table 1. Comparison of results obtained during intradermal vaccination of piglets against *Mhyo* and PCV2 associated diseases using different protocols.

Variable	Vaccination protocol	
	Before weaning (BW)	After weaning (AW)
Number of sessions	13	20
Number of employees	5	1
Number of doses	8736	9017
Pace of work (doses/h)	604[a]	469[a]
Labour cost[1]	3.9	1

[1] Labour cost is expressed as the ratio of total cost of manpower needed to apply one dose of the vaccine using two different protocols; (a) superscripts indicate statistically significant differences ($p \leq 0.05$)

Discussion and Conclusion

Our data indicate a high level of effectiveness of both vaccination strategies in terms of proper manpower utilisation; however, owing to obvious technical limitations relating to the specific infrastructure of typical commercial sow farms, vaccination after weaning seems to be the most profitable in terms of labour costs calculated per one applied dose of the vaccine.

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Benchmarking PRRS Outbreak Management Strategies

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Introduction

Only 40% of the US breeding herd is negative for porcine reproductive and respiratory syndrome virus (PRRSV). Many veterinarians have anecdotal evidence that is taking longer to control or eliminate PRRSV from breeding herds. It has also been reportedthat the severity of contemporary outbreaks is higher than compared to 10 or 20 years ago.

The objectives of this study were to benchmark practices implemented to manage PRRSV infection in breeding herds, and describe whole-herd metrics of success associated with key practices.

Materials and Methods

This was a prospective cohort study following 32 breeding herds that reported a PRRSV outbreak. Herd demographic characteristics as well as practices implemented as part of the disease management were captured. The eligibility criteria consisted of breeding herds in the USA facing a PRRSV outbreak between 2019-2021, and willing to share weekly diagnostic results of suckling pigs, and key productivity parameters including herd size and number of piglets weaned per week.

Herds were considered as reaching time-to-low-prevalence (TTLP) when achieved 8 consecutive weeks of PCR-negative results on processing fluids testing (n=1 sample/week) or piglet serum testing (n=30 piglets/week). Time-to-baseline-productivity (TTBP) was calculated based on exponentially weighted moving average using 3 sigmas and 0.4lambda and 21 weeks of baseline prior to the outbreak.TTBP, thus, represented the number of weeks it took for each herd to recover the same level of productivity(pigs not weaned) as it had prior to the outbreak. Totalloss per 1,000 sows was calculated as the area under the curve of piglets weaned below expected (i.e., below baseline average) from the outbreak, until TTBP was reached. Total Loss, therefore, representedthe severity of the outbreak in terms of loss in piglet production throughout the course of the PRRSV outbreak. Descriptive statistics were used to compare TTLP, TTBP and TL between different exposure groups.

Results

As described in the online project dashboard (www.fieldepi.org/POMP), there was a wide variability of herd demographic characteristics of enrolled herds. Herd size ranged from 2000-12000 sows, 9 (28%) herds were previously naïve, 22 (69%) implemented herd closure, 2 (6%) implemented batch- farrowing system, there were 10 distinct PRRSV genotypes defined as RFLP patterns, 20 (63%) herds implemented whole-herd deliberate exposure using either modified-live virus (MLV) vaccine, live-virus inoculation (LVI) or a combination of these. There was also variability on timing to initiate bio-management practices in the farrowing house/suckling pig population.

The average and interquartile (25^{th} – 75^{th} percentile) for TTLP and TTBP were 35 (29-49) weeks and 22 (16-26) weeks respectively. The median and interquartile range for total loss was 4,092 (2,326- 6,834) per 1,000 sows. When comparing those numbers to a previous study following a cohort of breeding herds facing PRRSV outbreak between 2009-2012, the TTLP observed in this study was 8 weeks longer. This can be attributed to change in virus characteristics, management practices, and moreefficient surveillance systems implemented in this study. For TTBP, the contemporary herds had an increase is 6 weeks in average as compared to that historic database, and total loss increased 1,303 pigs per 1,000 sows. The methodology used in the present study was the same used in the previous study, supporting the perception that contemporary outbreaks are more severe than previously experienced (Linhares et al., 2014).

Factors associated with shorter TTLP and less severe production impact (shorter TTBP and lower total lossper 1,000 sows) (p-value 0.1) included prior immunity(naïve herds took longer to recover), implementation of herd closure, implementation of whole-herd exposure, operating a batch-farrowing system, and implementing bio-management practices sooner to prevent virus transmission between piglets, crates, androoms.

Herds reporting to seek elimination had longer TTBPand higher total loss and reported 40% higher percentage points on the success on achieving TTLP within 1 year as compared to herds reporting to seek control rather than virus elimination.

Within herds that did whole-herd exposure, herds using MLV achieved TTBP sooner and had a less severe total loss as compared to herds using LVI. Thiswas consistent with the literature (Linhares et a., 2014). On the other hand, herds using LVI had a shorter TTLP compared to herds using MLV – also consistentwith historic data.

The number of PRRSV strains, as defined by whole PRRSV genome sequencing analysis, was positively correlated with TTLP, TTBP, and total loss.

Conclusions

This study reports factors associated with PRRSV management in sow farms, comparing with historic data. It confirms the importance of herd closure, whole-herd exposure, prior immunity, batch- farrowing system, and timing of bio-management practices.

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Biocheck.UGent quantifies biosecurity levels on livestock farms

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Introduction

Biosecurity is considered to be the basis of any disease control program. High biosecurity standards are essential for sustainable animal production and healthy animals as the occurrence of disease can be limited and therefore also the need for antimicrobial use can be lowered (1,2).

Although the importance of biosecurity is generally well known, many studies show that the current level of implementation of biosecurity measures is insufficient. Therefore, the Biocheck.UGent scoring system was developed to gain insights into current biosecurity practices and to act as a tool for improving disease prevention.

Materials and methods

The Biocheck.UGent scoring system for pig production was developed in 2008, providing a tool to quantify on-farm biosecurity levels. Since then, the system has been continuously improved. It takes the different transmission risks of infectious diseases into account, leading to an objective and risk-based scoring (3). All relevant components of both external (all measures preventing the introduction of pathogens in the farm) and internal (all measures taken to prevent spread within the farm) biosecurity are considered and scored. Scores range from 0 to 100, with the latter representing the implementation of the highest biosecurity standards. Worldwide and national (starting at 40+ entries per country) benchmarks are provided in a personalized report.

Results

The scoring system is freely available on www.biocheck.ugent.be and has already more than 34.000 entries in over 70 countries. Specifically for pig production, the database contains almost 27.000 entries. The majority of data entries are from Belgium (n=5604) and Ireland (n=480), two countries where Biocheck.UGent is a mandatory monitoring tool on a national level. There are also lots of data entries from China (n=450) and Vietnam (n=294).

The subcategory with the lowest scores on average is feed, water, and equipment supply (49). The best scoring subcategory was the purchase of animals (89).

Table 1 Average external biosecurity scores for pig production based on entries in Biocheck.UGent (4)

External biosecurity	Score
Purchase of breeding pigs, piglets, and semen	89
Transport of animals, removal of carcasses and manure	81
Feed, water, and equipment supply	49
Visitors and farmworkers	74
Vermin and bird control	77
Location of the farm	65
Total score	**75**

Table 2 Average internal biosecurity scores for pig production based on entries in Biocheck.UGent (4)

Internal biosecurity	Score
Disease management	76
Farrowing and suckling period	62
Nursery unit	70
Finishing unit	79
Measures between compartments, working lines, and use of equipment	56
Cleaning and disinfection	74
Total score	**68**

Discussion and conclusion

The scores for the different subcategories suggest there is ample room for improvement for most aspects of biosecurity. Although more attention is needed for measures linked to internal biosecurity.

Measuring on-farm biosecurity levels is a first step in more sustainable animal production for the future.

Biocheck.UGent can further support people in the field with additional features and services.

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Economic evaluation of PRRS pig farm based on the return-on investment per sow per year (RSY)

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Introduction

Since 2006, the outbreak and impact of highly pathogenic porcine reproductive and respiratory syndrome virus (PRRSV) disease had dealt a heavy blow to the pig industry. Consequently, China's pig industry had gradually recovered and grwon into a rapid large-scale production mode. However, evaluation of effect of pig diseases on pig farm management were still based on productive factors, such as mortality, slaughter rate, feed conversion ratio (FCR) and other production indicators. while there was limited research on economic (including return-on investment) factors. The management practices of pig farms mainly stem from Europe and America, which did not effectively reflect the current pig breeding structure and model in China. The existing economic return-on-investment model was greatly affected by the current market and prices, and can not explain the daily management practices clearly. Return-on investment per sow per year (RSY) refers to an evaluation method of sow productivity and benefit after covering all production costs. This study intends to establish a mathematical model of RSY to process and analyze the economic loss of PRRS in pig farms and the evaluation of strategies.

Materials and Methods

The calculation process of RSY was: on the premise of solidifying all costs, summarize and define the main structural costs of pig farms, complete the standardized generation of fixed assets and biological fixed assets, decompose the costs of pigs (biological assets) in each stage, calculate the prices of products in different stages (weaning piglets, nursing pigs and fattening pigs), solidify and calculate the comprehensive total cost, RSY was generated by combining the simulated sales amount and the total comprehensive cost.

Example: According to the date of the outbreak and strategy adopted in sow farm, the proportion of RSY contribution apportioned in the two months before and after the closure of the herd was evaluated. Taking the annual RSY as the target, the assessment took the first day the year as the dividing point to compare the RSY.

Results

This method was applied to a 2,200 sow farm in Hubei Province. Before and after the occurrence of PRRS disease, herd closure strategy was adopted to evaluate the economic effect of this strategy for two years. According to the database and accounting method set by the model, the RSY evaluation results were shown in Table 1.

Table1. Two annual RSY factors before and after PRRS closure in a sow farm in Hubei Province

Factors	2015 (before)	2016 (after)
Stock of sows	1,520	2,262
Costs of birth piglet (¥)	266.17	235.05
Costs of weaned piglet (¥)	307.57	276.45
Costs of nursing piglet (¥)	495.25	464.13
Weight gain cost of suckling piglet (¥)	8.67	7.67
Weight gain cost of nursing piglet (¥)	15.90	13.90
Mortality in farrowing	3,607	3,190
Average weight of deaths in farrowing (kg)	3.2	3.1
Losses of mortality in farrowing (¥)	1.025.709.00	798,737,45
Mortality in nursing	1,947	1,370
Number of nursing pigs sold	34,122	53,448
Losses of mortality in nursing (¥)	769,120.40	502,542,07
Total sales of nursing pigs (¥)	23,912,697.60	38,429,112.00
PSY	21.3	24.1
RSY	20.10%	30.48%

Discussion and Conclusion

RSY for 2015 and 2016 were 20.10% and 30.48%, respectively. After the comprehensive measures were taken, this indicator increased by 10.38%. Finance system of sow farm showed that in 2016, the profits directly increased were ¥ 2,653 and ¥ 852.44, respectively. Therefore, the direct excess loss caused by PRRS was ¥ 792,627.20. According to the RSY result of the current year, the total loss went as high as ¥ 952,000. At the same time, it indirectly affected the economic value of ¥ 1,428,000 through the profit generated by the sales of binary breeding pigs.

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Effect of sow parity order, piglet birth order and weight at birth, on the volume of colostrum intake and the quality of maternal antibody transference

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Introduction

Colostrum is a source of protective antibodies against certain diseases such as neonatal diarrhoea or atrophic rhinitis, cellular immunity, growth factors tointestinal maturation and an energy source fornewborn piglets (1). An early and high intake of colostrum after birth is a major determinant of pigletsfor ensuring the health and growth of piglets at least until weaning (1,2).
When colostrum intake occurs at the right time after birth, there is a high correlation between sows and their offspring's Swine Erysipelas (SE) titers in the first week post farrowing (3), the method to calculate the maternally derived antibodies transfer is called MDA TT. The aim of this study was to evaluate the effect of sow parity order and piglet birth order and weight, on the volume of colostrum intake and quality of maternal antibody transference, by two different methods, the difference of weight between birth and 24 hours later, and the MDA TT.

Materials and Methods

The study was conducted on a commercial farm with 4100 sows in South of Brazil, using ERYSENG® PARVO/LEPTO including antigens of SE, Porcine Parvovirus and 6 serovars of *Leptospira* spp. A total of 49 sows from parity 1 to 8 (OP1 to OP8) were included. Birth order and weight at birth were collected from 660 of their piglets. All piglets were identified with ear tags and weighed 24 hours after birth. The volume of colostrum intake per piglet was calculated based on the formula from Devillers (2). To calculate the quality of maternal antibody transference, 3 piglets from each litter were selected according to birth weight (low, medium and high) and birth order (first, middle and last). 7 days after farrowing, blood samples were collected from thepiglets and their mothers, to evaluate SE antibody titers against using an ELISA kit (CIVTEST® SUIS SE/MR; Cut off-IRPC: 40) according to manufacturing instructions. The data was submitted to parametric (ANOVA), or non-parametric (Kruskal-Wallis) analysis of variance and the results were significant when $p<0.05$.

Results

Piglet weight at birth had a linear increase with reference to sow parity ($p<0,01$) but was not affected by birth order (P=0,34). The volume of colostrum intake was affected by piglet weight at birth ($p<0,01$). Piglets weighing >1.7 kg or more at birth ingested an average of 266 gm of colostrum during the first 24 hours of life and piglets with a lower weight at birth (<1.3 kg) consumed 189 gm.

Thecolostrum intake had a linear decrease with reference to birth order ($p<0,01$). Another variable that affected colostrum intake was parity order ($p<0,01$). Piglets born from sows with OP1-3, OP4-5 e OP≥6 had on average 249 gm, 205 gm and 265 gm of colostrum intake, respectively. The SE antibody titters transference between sows and their offspring had a high correlation, but the transfer of maternal antibodies was affected by birth weight and parity order ($p<0,01$). Piglets weighing >1.49 kg at birth had a higher antibody concentration when compared with the piglets with a lower weight (<1.3 kg). Sows with OP≥6 had the highest SE antibody titers but the lowest transference rate to their piglets, meanwhile OP1 had the lowest titers but a good transference rateto their piglets. Regarding birth order, piglets born after the seventh, had lower titers than the previous ones.

Conclusions and Discussion

The colostrum intake in low-birth-weight piglets was below the physiological limit of these animals which is 25.1% of their body weight (1). Piglets that intake less than this amount of colostrum are exposed to a higher risk of mortality during lactation (1). Although the volume of colostrum intake was strongly influenced by weight at birth, piglets born after the first 6, had lower serum titers, even in piglets with a birth weight >1.3 kg. Sows OP≥6 had higher serum titers and the volume of consumption intake of colostrum in their litters was higher than those observed in other parity orders, however, their piglets had lower titers, regardless of birth order or weight. This could be explained by older femalesproducing a greater volume of colostrum while their immunoglobulin G concentrations are lower when compared to younger females (1,2). Based on these results, the MDA TT allows the quality of the colostrum intake to be measured objectively giving information about the transference of immunity from sows to piglets.

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Immunocastration of cryptorchid boars using the GnRH vaccine Improvac®

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Introduction

Castration of male pigs is usually done by surgery within the first week of life to avoid boar taint. In some boars, however, testicles may not descent, i.e. remain intraabdominally. Boars with uni- or bilateral cryptorchidism will develop boar taint as in intact boars (1). Therefore, abdominal testicles have to be removed by a costly surgery or boars have to be sold at lower prices.

Vaccination against boar taint with the GnRH analogue Improvac® has been proven to be a safe and effective method to avoid boar taint in intact boars (2). The aim of this study was to evaluate the effect of Improvac® on the development of boar taint, sexual function and testes size of cryptorchid in comparison to intact boars.

Materials and Methods

Ten intact (IN) and 11 cryptorchid boars (one with both and ten with one cryptorchid testis; CR), all Duroc hybrids, were included. Boars were 14 weeks of age at the beginning of the study and were vaccinated twice with Improvac® at 17 and 24 weeks of age. All animals were slaughtered at the age of 29 weeks. Prior to both vaccinations and five days before slaughter, blood samples were taken to be analyzed for estrone sulfate (ES) and testosterone (T) by a competitive radioimmunoassay. Furthermore, the circumference (CIRC) and cross-sectional area (CSA) of both testes were determined by ultrasound. Carcasses were tested for boar taint.

Results

Both CR and IN had measurable concentrations of ES and T prior to both vaccinations, but concentrations decreased to almost zero levels after the second vaccination immediately prior to slaughter (Tab. 1). In one IN, both ES and T concentrations increased after second vaccination (not included in Tab. 1).

As for CIRC and CSA, the following observations were obtained (Tab. 2): Abdominal testes were smaller than scrotal tested in CR. Scrotal testes of CR and both testes of IN increased in size between first and second vaccination, but then decreased. Abdominal testes of CR remained relatively unaffected post vaccination. The one IN that still had measurable ES and T concentration post vaccination also showed a concomitant increase in testes size. None of the CR and IN exhibited boar taint.

Discussion and Conclusion

Vaccination against boar taint in uni- and bilateral cryptorchid boars using Improvac® led to an abolishment of ES and T production similarly as oberseved in intact boars. Also, sizes of the decend testes decreased similarly in CR and IN, while the chryporch testes remained relatively unchanged in this regard. Since none of CR had boar taint at slaugther, the recommended treatment regimes of two vaccinations with the last 4–5 weeks prior to slaughter proved appropiate also in CR. Why one IN did not respond to treatment remains however unanswered.

Table 1. Estrone sulfate (ES) (ng/ml) and testosterone (T) (ng/ml) concentration ranges (Min-Max) in serum of cryptorchid (CR; n=11) and intact boars (IN; n=9) before and after Improvac® application

Para-meter	Boars	Time points[1]		
		1	2	3
ES	CR	0.2-3.26	0.5-3.2	<0.1-0.3
	IN	0.64-2.53	0.26-2.89	<0.1-0.1
T	CR	0.4-2.9	0.5-5.5	<0.1-0.2
	IN	<0.1-1.7	<0.1-2.0	<0.1-0.4

[1]Before first (1) and second (2) vaccination and five days before slaughter (3).

Table 2. Testes circumference (CIRC in cm) and cross-sectional area (CSA in cm^2) range of cryptorchid (CR; n=11; scrotal and abdominal testes) and intact boars (IN; n=10) before and after Improvac® application

Para-meter	Boar	Time points[1]		
		1	2	3
CIRC	CR scr.	10.5-19.1	19.5-26.0	17.0-22.1
	CR ab.	7.0-13.6	5.1-11.9	6.6-10.9
	IN	10.1-16.2	16.6-21.7	15.6-22.8
CSA	CR scr.	7.9-27.0	28.3-47.3	20.9-36.8
	CR ab.	3.7-7.7	2.0-10.7	3.6-8.9
	IN	7.3-19.6	20.7-36.2	15.6-38.3

[1]Before first (1) and second (2) vaccination and five days before slaughter (3).

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Movements and working lines of farm staff to optimize internal biosecurity on pig farms

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Introduction

It is generally recommended to house different age groups in a pig farm separated from each other, in order to limit or prevent the transmission of pathogens from one group to another. Furthermore, the farmers should follow a specific sequence when doing their work in the compartments with different animal categories. Ideally, one should go from young to old and from health to sick animals, to avoid spreading pathogens on the farm. This means that the following sequence should be followed: 1) dressing room, 2) farrowing unit, 3) gestation unit, 4) nursery unit, 5) fattening unit, 6) quarantaine unit, 7) cadaver storage.(1) In Europe, pig breeders often apply a batch management system (BMS) for the sows. This implies that the sow population is divided into several batches according to their reproductive stage; and that insemination, farrowing, and weaning only take place at specific and fixed timepoints.(2) The present observational study aimed to determine the movements of staff in pig farms, and to investigate possible differences in movements between the different weeks of the BMS.

Materials and Methods

Five commercial sow farms were enrolled in the study. On each farm, a monitoring system for movements of farm staff within the buildings was installed.(3) Detection points were installed in all rooms of the farm, and farm workers had to wear a personal beacon that sent a Bluetooth signal to the detection points. Movements were registered in an online platform. Movement data were collected from 1 December 2019 until 30 November 2020. Movements according to the principles described in the introduction were considered as safe, other movements were considered as risky, unless a dressing room was visited before going to another age group. A time filter was set on the detection points of the dressing rooms, as an insurance that farm workers actually changed their clothing.

Results

The results on the movements of farm staff are shown in Table 1. The distribution of the total number of movements significantly ($P<0.05$) differed for the different weeks of the BMS for farms B, C, D, and E. On farm A, there was no significant difference in total number of movements in the different weeks of the BMS. The distribution of the proportion of risky movements significantly differed for the different weeks of the BMS for farms C and D.

Table 1. Median (min.-max.) number of daily movements and proportion of risky movements in the different weeks of the batch management system

	Total (*n*)	Risky (%)
Farm A		
e	32 (2-76)	11 (0-30)
f	32 (9-75)	9 (0-32)
g	33 (13-90)	11 (0-35)
Farm B		
a	32 (2-633)	33 (0-52)
b	53 (2-348)	33 (0-46)
c	33 (1-349)	33 (0-45)
d	38 (2-320)	32 (0-50)
Farm C		
a	58 (21-244)	38 (10-46)
b	64 (14-236)	35 (17-47)
c	49 (14-166)	36 (20-44)
d	41 (13-103)	36 (8-50)
Farm D		
a	71 (10-247)	16 (6-27)
b	85 (8-210)	14 (4-27)
c	66 (2-173)	15 (0-32)
d	52 (7-273)	13 (0-28)
Farm E		
a	43 (5-487)	14 (0-44)
b	57 (2-351)	10 (0-60)
c	31 (1-629)	12 (0-40)
d	35 (1-544)	14 (0-40)

Weeks of the BMS for farm A, working in a 3-week system (e: weaning; f: insemination; g: farrowing) and farms B, C, D, and E, working in a 4-week system (a: weaning; b: insemination + farrowing; c: no main activities 1; d: no main activities 2).

Discussion and Conclusion

The proportion of risky movements was high on all farms, with daily median values ranging from 9 up to 38%. This study also showed that the behaviour of farm workers is different for the different weeks of the BMS. On the farms working in a 4-week system, more movements were made in the insemination + farrowing week, compared to other weeks. In conclusion, internal biosecurity in pig farms can be increased by optimizing staff movements, in particular during specific weeks of a BMS.

Acknowledgements

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Real time sound based monitoring supports timely and intelligent data-based interventions based on theactual respiratory health status (ReHS) in nursery facilities

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Introduction

Coughing is a well-recognized sign of respiratory disease that provides no definitive information regarding causative pathogens and no clear intervention guidance. Furthermore, due to limited daily periods of observation, the onset and duration of coughing is not fully appreciated. Often, standardized treatments are implemented that are notnecessary nor applied at the optimal time.

SoundTalks® (ST), a sound-monitoring system that transforms pig respiratory sounds into a Respiratory Health Status (ReHS) metric, provides an alarm system for producers to intervene quickly in respiratory events that involve coughing (ReHS+ status). This promotes optimally timed diagnostics and the application of treatments when needed. The objective of this study was to describe the ReHS in a sound-monitored commercial farm and to improve the accuracy of interventions in monitored rooms compared to non-monitored rooms.

Material and methods

13 out of 21 rooms in a 2400 head nursery were sound-monitored by ST for 20 months (1 monitor/room). The nursery received weaned piglets into 2-3 rooms weekly. Diagnostics and treatments in ST rooms were performed based on ReHS+ alarms. ReHS+ triggered intervention data from ST monitored rooms were compared to intervention data from non-ST monitored rooms.

Results

Continuous monitoring of the studied farm demonstrated that coughing events tended to occur simultaneously in more than one room at the time, see blue square in Fig 1. Whereas other events were covering only few rooms, red square Fig. 1.

Coughing episodes appeared at variable periods within the first 4 week after placement. The OF sampling at the time of ReHS+ revealed the presence of Influenza A Virus (IAV), Glaesserella parasusis, Pasteurella multocida and Streptococcus suis at every coughing event. Results from our study demonstrated that sound-monitoring led to a more objective and accurate identification of the onset of actual coughing events that required an intervention (i.e. diagnostics and therapies). Additionally, there was a 30% reduction in the number of batches that required an intervention in ST monitored rooms (7 out of 10 batches) when compared to standardized therapy at a specific post-placement interval in non-ST monitored rooms (10 out of 10 batches). Fig. 2 shows an example of a period of almost 2 months where 4 out of the 13 ST monitored batches did not receive any treatments.

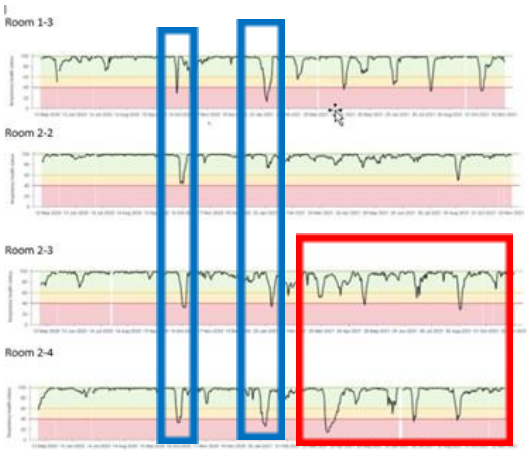

Fig 1. Respiratory index curve for 4 of the rooms from May until November 2021. Respiratory health status is indicated by colors. Green indicates optimal respiratory health status; Yellow is warning against increasing respiratory problems; and red indicates high level of cough.

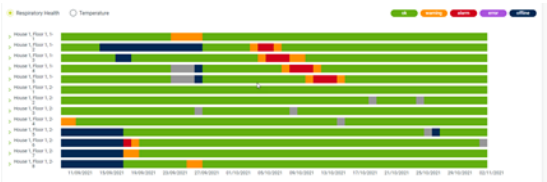

Fig 2. The heatmap is showing the daily status of all monitors (i.e. green, yellow and red) over a 2-month period for each of the monitored rooms. Blue color indicates lack of WIFI connection.

Conclusions

Sensor-based respiratory health monitoring effectively identifies nursery facility coughing events and optimizes the application of both diagnostic sampling and treatment interventions. Moreover, the absence of ReHS alarms is an excellent indicator of pig respiratory health and provides confidence that no treatment was needed, and antibiotic usage was effectively decreased.

Return on investment (ROI) in pigs vaccinated with Porcilis Ileitis in a commercial farm.

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Introduction

Ileitis or Proliferative Enteropathy is caused by an obligate intracellular bacterium, *Lawsonia intracellularis*, and manifests clinically in several ways; The chronic form is known as Porcine Intestinal Adenomatosis (PIA), the acute form is known as Proliferative Hemorrhagic Enteropathy (PHE) and the Sub-clinical form is mainly characterized by poor performance (1); Sub-clinical disease is the most common form of presentation in Colombia with a high economic impact due the low performance of the animals in some productive parameters. In a 2006 study where 19 veterinarians from large companies in the United States were interviewed to classify and quantify productivity and economic losses due to major health problems, in 14 of these companies, ileitis was classified as the main health challenge (2); In this same study, it is estimated that productivity losses and increased production costs associated with Ileitis per marketed pig are estimated at USD 4.65 (unpublished data). The objective of this study was to evaluate the impact of vaccination with Porcilis Ileitis on the technical and economic results in the fattening stage in a commercial farm using the Porcilis Ileitis vaccine (containing inactivated *L. intracellularis* bacteria in XSOLVE adjuvant), without restricting the use of antibiotics.

Materials and methods

The trial was conducted on a farm with 2.100 sows located in the region of Antioquia (Colombia) with a history of Ileitis, diagnosed by Elisa (Svanovir® L. intracellularis/Ileitis-Ab) and clinical signs associated with intestinal hemorrhagic syndrome at the end of the fattening. For this study, 4085 piglets were selected at the beginning of the fattening phase with an average age of 75.3 days of life, which were distributed in two treatments, 1942 piglets vaccinated with Porcilis Ileitis (Treatment 1) with 25 replicates and 2143 unvaccinated piglets (Treatment 2) with 30 replicates. The Porcilis Ileitis group was vaccinated at 21 days of age (weaning), while the control group was not vaccinated. The environmental and management conditions were the same for both groups. The 1942 piglets vaccinated with Porcilis Ileitis were distributed in 25 groups or experimental units and the 2143 non-vaccinated piglets were distributed in 30 groups or experimental units. The total of each treatment was weighed at the beginning of the evaluation (75.3 days of life +/- 0.81 days), then it was weighed randomly, selecting 20% at 103 and 133 days of life, age at which the harvest was made or sale of pigs to the market begins. All pigs had ad libitum access to feed and water throughout the trial. The economic results of this study were evaluated through statistical simulation and predictions of the analysis models adjusted according to the case. All the analysis was performed in R software (3). For the economic evaluation of this study, the following values were considered; Kg fattening feed USD 0,482, Kg live weight pigs to market USD 1,899 (average sales 2021 according to source www.porkcolombia.co) and treatment cost (Porcilis Ileitis) USD 1,04. The Colombian peso to US dollar exchange rate used for this study was $3,982.60/1 USD as of January 31, 2022, according to Banco of the Republica.

Results

The group vaccinated with Porcilis Ileitis obtained better results in the productive parameters compared to the non-vaccinated pigs (summary in table 1).

Table 1. Evaluated parameters

Parameter	Vaccinated group (PI)	Control group
Final Age	132,4	133,6
Final weight (kg)	100,57	98,8
ADG (grs)	1109[a]	1078
FCR	1,96	2,06
FC (kg)	124,72[a]	128,10
M (%)	0,41	0,65

a: statistically significant difference

The P. Ileitis treatment groups presented on average a lower cost per pig in the feed (feed consumption - FC) value of USD 1,629. Assuming a stable sale price for all the animals, the pigs of the Porcilis Ileitis treatment presented a higher sales value in USD 3.36 per pig. Under these conditions, the Porcilis Ileitis treatment generated a return on investment (ROI) of 3.2:1 Considering the moment in which this study ends (beginning of the harvest), the result may be better considering that Ileitis is a disease that generates a greater impact in the last weeks of life of pigs.

Conclusion and Discussion

In this study, it was observed that the implementation of a program with Porcilis Ileitis in piglets generates a positive ROI (Return on Investment), a value that can have a greater impact if we consider a strategic management of medications in addition to obtaining a better gut integrity.

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Risk factors associated with sow mortality under the condition of a production system in the Midwestern United States during 2019-2021

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Introduction

Sow mortality has significantly increased in the last three years in the U.S. swine industry. Although well known by the producers, veterinarians, academia, and swine industry partners, this epidemic issue has persisted and worsened.

The execution of hypothesis-driven research related to this topic is difficult, in part due to the lack of fundamental understanding of the root of the causes contributing to this problem.

The objective of this study was to assess the risk factors associated with sow mortality in a large swine production system located in the Midwestern United States, a high swine dense region.

Materials and Methods

A cohort study was performed using 48 breeding herds (breed-to-wean), with an average of 4671 sows (varying between 1397 and 7946), 16 being stall and 32 open pen gestation, 17 with internal gilt development unit (GDU), and 31 receiving breeding gilts, from one production system totaling approximately 240,000 sows. Herd demographics, weekly productivity data, selected practices implemented as part of the daily management, disease status, and decisions made related to sow farm health (e.g. health interventions) & production management factors were captured.

The eligibility criteria to enroll the production system consisted of managing sow farms facing an increase in sow mortality in the past three years, and willing to understand the risk factors associated with sow mortality in the whole system.

A binomial mixed regression model using PROC GLIMMIX in SAS® (SAS Institute, Inc., Cary, NC) was used to build a multivariable model using sow farm as random effect. The final model was obtained using a stepwise approach. Differences in means were compared using a t-test with Tukey-Kramer adjustment and p-values <0.05 were considered significant. Farm ventilation type was retained in the model as a confounding variable.

Results

Table 1 describes the risk factors associated with sow mortality (multivariable model). Breeding herds facing porcine reproductive and respiratory syndrome (PRRS) outbreaks (epidemic), endemic and epidemic herds for PRRSV and positive for *Mycoplasma hyopneumoniae* (Mhyo), PRRS outbreaks with no feed meds added, open pen gestation farms and younger herds were associated with higher sow mortality in this study.

The mainly causes of sow mortality in this study were sudden deaths (30.89%), lame deaths (29.10%), prolapses (26.96%), and others causes (13.05%), respectively.

Discussion and Conclusions

The sow mortality in this observational study has significantly increased in the past three years as well as reported in the past five years by the U.S swine industry. Although several causes and associations, the available literature is lacking, and additional research is needed to clarify this multifactorial problem.

Table 1. Final multivariable model with the risk factors associated with sow mortality.

Risk factors	Categories	Annualized Mortality	P-value
Year	2019	14.19%[a]	<0.001
	2020	17.28%[b]	
	2021	19.39%[c]	
Quarter	Jan-Mar	16.84%[a]	<0.001
	April-June	16.49%[b]	
	July-Sept	16.40%[b]	
	Oct-Dec	17.56%[c]	
Herd parity	1	17.25%[a]	0.016
	2+	16.39%[b]	
Gestation type	Pens	19.80%[a]	<0.001
	Stalls	14.28%[b]	
PRRS status	Negative-Neg	15.55%[a]	<0.001
	Endemic-End	16.30%[b]	
	Epidemic-Epi	18.76%[c]	
Feed Medication	Med	15.73%[a]	<0.001
	Non-Med	17.97%[b]	
Interaction between Feed medication and PRRS status	Med - Neg	14.83%[a]	0.001
	No Med - Neg	16.30%[b]	
	Med – End	15.41%[c]	
	No Med - End	17.24%[d]	
	Med - Epi	17.03%[e]	
	No Med - Epi	20.67%[f]	
Interaction between PRRS and Mhyo status	Neg - Neg	15.79%[ab]	<0.001
	Neg - Pos	15.31%[ac]	
	End - Neg	16.58%[d]	
	End - Pos	16.03%[b]	
	Epi - Neg	18.10%[e]	
	Epi - Pos	19.44%[f]	

The causes of sow mortality in this study support the data about the U.S industry related to sow mortality; a recent report from 104 sow farms representing approximately 400,000 sows shows a prolapse incidence of 21% of the total mortality.

Acknowledgments

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Sow mortality: Removal reasons and factors associated with increased mortality

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Introduction

Sow mortality has been a growing concern over the last decade. It is estimated that about 3 parities are necessary to achieve break-even in the replacement production return for the producer [1,2]. Still, average annual replacement rate is around 50%, with most removals occurring on parity-zero or parity one females [3–5], before a positive net present value is achieved. Most studies assess mortality within a limited time frame of a couple of years, at the most. Thus, the main goal of this study was to describe sow mortality over the course of nine years and investigate factors possibly associated with high sow mortality weeks and individual sow risk of dying.

Materials and Methods

Historical production data recorded on PigChamp from April 2009 to October 2018 (totaling 470 weeks) were obtained from four commercial farms from one production system located in the U.S. Midwest region representative of current production practices. Deaths and culls were compared based on their lifetime contribution time (the sum of the female life days between the first service to the removal date) and parity at removal. Deaths were described by removal reasons, by month, days from last service, days from last farrow, and parity at removal. Risk factors associated with mortality were assessed using two different models: 1) a Poisson model to estimate factors associated with the number of deaths per week (week as a panel variable, farm as a cluster variance estimator, and total number of sows in inventory as the exposure); and 2) a multilevel Poisson regression to model the sows individual risk of dying throughout their lifetime (sows-year contribution time as the exposure, farm as a random effect).

Results

We obtained 357,425 service records of 85,608 sows. Of these, 70,467 sows were removed during the study period (11,852 died and 58,615 were culled). The average annualized mortality per month ranged from 1.79% to 3.29% for all farms combined. Deaths occurred at a median of 116 days from last service, or 26 days post-partum. The median parity upon death was two. Overall, the main reasons for death were locomotion (27%) and reproduction (24%). A higher weekly number of deaths was associated with spring and summer (incidence rate ratio (IRR) 1.27 and 1.37, respectively, compared to winter). Sows had a higher mortality rate when they have been exposed to at least one PRRS break during their lifetime (IRR 1.55) and when housed in groups (pens) during gestation (IRR 1.32); and lower mortality rate when housed in filtered farms (IRR 0.76), accounting for an interaction term between parity at removal and PRRS outbreak exposure.

Discussion and Conclusion

The majority of deaths occurred during summer months and there was a higher probability of a high mortality week during warmer weeks, likely related to factors aggravating thermal stress along the lines of ventilation equipment malfunction and/or power outages. We found most deaths occurred at younger parities during peripartum, which hinders cost-competiveness of the sow farms operations. We found an effect modification between mortality and PRRS exposure and mortality and farm filtration status comparing the bivariate with the mutlivariate model. This suggests that a decrease of other infectious health challenges with filtration is partially captured by PRRS exposure.

Efforts to monitor a myriad of diseases through time as for PRRS would allow a more comprehensive understanding of the relative contribution of each disease to the sow mortality burden. Likewise, environmental and other factors presumed to affect mortality risk, such as barn temperature and humidity levels, need to be more accurately recorded to truly assess their association. Prospective data collection targeting these factors are needed for a more in-depth analysis of factors involved in sow mortality in order to generate interventions and thus reduce sow mortality.

Acknowledgments

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Survey on Claw Lesions of Sows in the Republic of Korea

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Introduction

Leg lameness in sows is one of the main reasons for culling, causing huge economic losses in pig farms. Sows with lameness cannot compete with normal sows for water and feed intake in the group of gestation housing, resulting in decreased productivity.

There are various causes of lameness, and one of the main causes of lameness is the claw lesion, which is frequently found in pigs. The purpose of this study was to investigate the claw lesions of sows on domestic farms and to find out how the claw lesions affect the productivity of sows.

Materials and Methods

This survey was conducted on 906 sows in five ordinary farms from November 2018 to May 2019. 189 sows in the first parity, 183 sows in the second parity, 97 sows in the third parity, 123 sows in the fourth parity, 67 sows in the fifth parity, and 252 sows in the sixth-twelfth parities were surveyed. The claw lesions observed in the study included the cracked wall vertical(CWV), cracked wall horizontal(CWH), differences in the length of dew claws(D), and differences in the length of toes(T). Each claw lesion of the sows was given 0-3 points in proportion to the degree of the lesion, and each sum of the claw lesion scores of the limbs was indexed as 0, 1-5, 6-10, 11-15, 16-20, ≥21.

Results

One or more claw lesions were found in 95.6% of the sows surveyed. The highest percentage (38.7%) of the sows had 6-10 points and 1.0% of the sows had more than 21 points(Table 1).

Forelimb claw lesions were found in 86.1% of the sows and hindlimb claw lesions in 91.8%(Table 2).

Table 1. Comparison of No. of sows showing claw lesions with claw lesion score index

Claw lesion score	0	1-5	6 -10	11 -15	16 -20	≧21
No. of sow showing/ No. of sow tested (%)	40/ 906 (4.4)	317/ 906 (35.0)	351/ 906 (38.7)	152/ 906 (16.8)	37/ 906 (4.1)	9/ 906 (1.0)

Table 2. Results of sows showing claw lesions at the front and hind legs in sows tested

Claw lesion	Number of sows showing claw lesions (%)			
	Front leg	Hind leg	Total	Claw lesion score
CWH	431/906 (47.6)	574/906 (63.4)	706/906 (77.9)	2.01
CWV	642/906 (70.9)	651/906 (71.6)	812/906 (89.6)	2.92
DC	242/906 (26.7)	472/906 (52.1)	538/906 (59.4)	1.76
T	37/906 (4.1)	299/906 (33.0)	312/906 (34.4)	0.59
Total	780/906 (86.1)	832/906 (91.8)	866/906 (95.6)	7.28

The incidence rate of claw lesions was 90.2% in the first parity, and 99.6% in the sixth parity or above(Table 3).

Table 3. Average claw lesion score index of sow according to parity of sow

Parity	Front leg	Hind leg	Avg
1	2.09 (±1.97)	2.67 (±2.18)	4.76 (±3.30)
2	2.84 (±2.42)	3.80 (±3.10)	6.64 (±4.45)
3	2.90 (±2.34)	4.39 (±3.20)	7.29 (±4.15)
4	3.56 (±2.51)	5.25 (±3.33)	8.81 (±4.33)
5	2.61 (±2.09)	4.49 (±2.89)	7.10 (±3.23)
6~12	3.35 (±2.51)	5.94 (±3.90)	9.29 (±5.29)

* $p<.05$(post hoc: scheffe test)

Conclusion

One or more claw lesions were found in 95.6% of the sows surveyed.

The cracked wall vertical (CWV) was observed in 812 head (89.6%) of the sows and was the most frequently observed claw lesion.

The difference in the length of toes (T) was found in 312 head (34.4%), showing the lowest rate of claw lesions.

The incidence rate of claw lesions of sows gradually increased in proportion to the parity. The most common claw lesion in the sixth parity was CWV in the right forelimb, which was found in 65.2% of the sows.

Veterinarian assessment of influenza A virus impact on swine populations in the United States: perceptions, prevention, and control

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Introduction

Influenza A virus in swine (IAV-S) is consistently reported within the top 3 most frequent etiologies diagnosed from respiratory porcine tissue cases according to the Swine Disease Reporting System (www.fieldepi.org/sdrs-reports). Swine veterinarians are on the front line of diagnosing and managing healthdecisions to control IAV-S, but no survey and synthesisof information from this source has been reported. The objective of this study was to assess the veterinarians' perceptions towards IAV-S prevention and control in United States (US) swine.

Materials and Methods

A survey was distributed to a list of 343 veterinarians obtained from the Iowa State University Veterinary Diagnostic Laboratory (VDL) database. The survey included: a) Demographic variables: veterinary practice, geographic region, size of breeding herds, and grow-finisher sites; b) Veterinarian perceptions: importance of IAV-S, if IAV-S challenges are increasing or not, IAV-S economic impact, veterinary and clients' concern regarding the presence of IAV-S, and estimatedcost per market hog of IAV-S in clients' swine operations; c) Control measures: breeding herds using IAV vaccines, replacement gilt isolation use of IAVvaccines, nursery and grow-finisher sites using IAV-S vaccines; d) Prevention measures: vaccine type for breeding herd, recommended time of vaccine administration in the breeding herd, mass vaccination, primary sources for introducing IAV-S, and employee's annual human influenza vaccine recommendations. Descriptive statistics and Fisher's exact test were performed to assess associations ($P \leq 0.05$) between veterinary practices vs. perception, control, and prevention measures. The analyses were performed using R program v 4.1.0 (1).

Results

A total of 194 (56.55%; 194/343) veterinarians completed the survey (Table 1). The states of Illinois, Indiana, Iowa, Kentucky, Michigan, Minnesota, Ohio, and Wisconsin represented 76% (147) of the responses. For IAV-S perceptions, 173 (89%) considered IAV-S one of the top three health challenges in the swine industry. In addition, 130 (67%) perceived that IAV-S challenges are increasing (P=0.03), mainly between theswine exclusive group vs. mixed animal group (P=0.04). Moreover, 128 (66%) considered a moderatelevel of economic impact regarding the presence of IAVin swine (P=0.001), and 129 (67%) considered an estimated cost per market hog of IAV in their clients' production systems between $1 and $5. Eighty-eight veterinarians (45%) said their clients were somewhat concerned with IAV-S.

To control IAV-S in the breeding herd, 81 (42%) indicated IAV-S vaccines were used in 76-100% of herds.

Table 1. Veterinarian's answers to herd size and veterinary practices.

Herd size	Veterinary practices[1] 1	2	3	4	5	Total n (%)
Breeding herd (number of sows)						
≤1000	8	2	8	8	1	27 (14)
1000:5000	81	14	3	19	1	118(61)
5,000:25,000	14	3	1	1	0	19 (10)
> 25,000	19	1	0	1	0	21 (11)
Not Applicable	4	0	1	0	4	9 (5)
Nursery and grow finish herd (number of pigs)						
≤100,000	10	2	7	2	10	25 (62)
100,000:500,000	31	9	4	18	31	62 (32)
500,000:1000,000	32	7	1	8	32	48 (25)
> 1000,000	51	2	0	1	51	54 (28)
Not Applicable	2	0	1	0	2	5 (3)

[1]Primary veterinary practices: 1-swine exclusive; 2- large animal; 3- mixed animal; 4- mixed and predominantly larger animal; 5- other primary veterinary practice.

Regarding vaccine use in gilt development, 101 (52%) veterinarians reported the percentage was 76-100%, and 86 (44%) stated their clients use the vaccine in the nursery phase. To prevent IAV-S, 159 (84%) used commercial vaccines in the breeding herd (P=0.02), 94 (49%) of the veterinarians used autogenous vaccines. For IAV-S vaccine use during gilt isolation, 156 (82%)of the veterinarians reported they suggested vaccinationduring this phase (P<0.01). Forty-nine (27%) reported that they recommend quarterly vaccination, and 71 (38%) recommended biannual mass vaccination in the breeding herd. Finally, 83 (43%) encouraged farm employees to receive an annual human influenza vaccine.

Discussion and Conclusion

This study assessed veterinarians' perceptions regarding the presence of IAV-S in swine production systems and control and prevention methods from the breeding herdto grow-finisher stages of production. This study presented different IAV-S perceptions in veterinary practices. This study described current practices to assess IAV-S mitigation's success in making strategic decisions.

Acknowledgments

Veterinarians surveyed, and Iowa State University Presidential Interdisciplinary Research Initiative.

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IMMUNOLOGY & VACCINOLOGY

A bivalent PCV2a-PCV2b vaccine offers biologically superior protection compared to monovalent PCV2 vaccines

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Introduction

Porcine circovirus type 2 (PCV2) vaccines represent a success for the industry. Nonetheless, while PCV2a vaccines have limited the prevalence of PCV2a viruses, they, along with PCV2's high evolutionary rate and propensity to recombine, have likely supported a growing divergence in epitopes shared amongtraditional vaccines and wild type strains (1,2,3). The growing dissimilarity in epitopes may be contributing to decreased efficacy of PCV2a vaccines in the field (4). While PCV2 vaccines are generally considered to offer cross-protection against a variety of genotypes, vaccine-induced protection is best when the vaccine and challenge strain are closely matched (5). Taken together, modernizing PCV2 vaccines to encompass a broader repertoire of epitopes may offer broader protection to diverse field strains. The objective of these studies was to evaluate monovalent and bivalent PCV2 vaccines in terms of homologous and heterologous efficacy.

Materials and Methods

Three studies were conducted; only PCV2 naïve pigs were enrolled. Pigs in Study A were allotted to four groups (Table 1). Pigs in Study B and C were allotted to three groups (Table 2). Vaccines were experimental chimeric PCV1-PCV2a (cPCV2a), cPCV2b, or bivalent cPCV2a-cPCV2b; all vaccines were inactivated whole viruses adjuvanted with MetaStim (Zoetis Swine adjuvant). Vaccines were administered as a 2 mL dose at 3-4 weeks of age. Virulent PCV2b or PCV2a was administered about 3 weeks post vaccination. Efficacy parameters were evaluated as described (6) but included viremia, fecal shedding, lymphoid colonization (IHC), lymphoid depletion (LD) and histiocytic replacement (HR), PigMatrix was used to identify T cell epitopes in the capsid proteomes of the vaccine and challenge strains. Epitopes of vaccine and field strains predicted to bind common class I and class II SLA alleles were identified, compared, and an EpiCC score calculated. Study protocols were approved by the Zoetis Institutional Animal Care and Use Committee.

Results

In Study A, PCV2a and PCV2b challenge control animals experienced similar levels of PCV2 infection and disease. The cPCV2a vaccine protected pigs post PCV2a or PCV2b challenge. However, a biologically relevant improvement in protection was observed when the vaccine and challenge strain were matched for viremia, fecal shedding, LD and HR (Table 1). Clinical efficacy correlated to T cell epitope similarity in that the cPCV2a vaccine offered greater coverage (36.46%) to PCV2a than PCV2b challenge viruses. All monovalent and bivalent vaccines in Studies B & C induced protection, however, the bivalent vaccine induced biologically superior efficacy (Table 2).

Table 1. Quantitative summary of Study A results[1]

Tx	Vaccine	Challenge	% Improvement of homologous vs heterologous protection (T02 v T04)1					EpiCC analysis
			Viremia	Fecal Shedding	LD	HR	IHC	% Coverage
1	Saline	PCV2a	28.6	10	100	100	-20.1	
2	cPCV2a	PCV2a						100
3	Saline	PCV2b						
4	cPCV2a	PCV2b						73.28

[1] %Improvement=(heterologous-homologous)/ heterologous

Table 2. Studies B & C quantitative summary[1]

Tx	Vaccine	Challenge	% Improvement bivalent vs mono-valent protection (T02 v T03)					EpiCC analysis
			Viremia	Fecal Shedding	LD	HR	IHC	% Coverage
Study B								
1	Saline	PCV2a	28.3	29.9	68.5	NA	53.3	
2	cPCV2a	PCV2a						100
3	cPCV2a-cPCV2b	PCV2a						100
Study C								
1	Saline	PCV2b	21.9	4.5	25.1	100	37.5	
2	cPCV2b	PCV2b						100
3	cPCV2a-cPCV2b	PCV2b						100

[1] %Improvement=(monovalent-bivalent)/monovalent

Discussion and Conclusion

PCV2a vaccination induced cros-protection to PCV2b but efficacy was better when the vaccine and challenge strain were closely matched. T cell epitope similarity correlated to protection and may be helpful as a tool to predict protective efficacy. The bivalent vaccine induced biologically superior efficacy compared to either monovalent even when the vaccine and challenge strains were matched. The bivalent vaccine's induction of protective immunity was greater than the epitopes shared among the vaccine and challenge strains. The bivalent vaccine's unique and conserved epitopes likely added to broader/more complete coverage of PCV2 challenge, supports positive outcomes following exposure to diverse PCV2 field strains.

Acknowledgments

Zoetis Animal Research Support veterinarians and staff. Andres Gutierrez for EpiCC analyses.

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A bivalent vaccine containing PCV2a and PCV2b enhances T-cell epitope coverage against a large and geographically diverse population of recent PCV2 field isolates

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Introduction

Widespread vaccination, continued viral presence, and a high capacity for genetic change, are all conditions that can enhance vaccine-driven evolution, and all apply to PCV2. Genetic analyses show that change is occurring, genotype shifting being an example (1). Although PCV2a-based vaccines still appear generally effective, there is concern about current and future performance. Broadening the vaccine-induced antigenic coverage is a way to mitigate this risk. Based on *in silico* predictions, for a selected set of common class I and class II SLA alleles, the PCV2 capsid protein contains around 168 separate antigenic epitopes potentially recognizable by T-cells. EpiCC is a computer algorithm that allows the antigenic similarity of two proteins to be predicted from a comparison of their putative T cell epitope content. A previous study has shown that a bivalent PCV2a/PCV2b vaccine (Fostera® Gold PCV, Zoetis) expands epitope coverage and protection compared to PCV2a or PCV2b monovalents (2). This work is now extended with a larger and more recent sample of geographically diverse PCV2 isolates.

Materials and Methods

746 PCV2, ORF2 nucleotide sequences from global locations dating from 2017 to 2021 were obtained directly from veterinary diagnostic laboratories (n=684) or from GenBank. The 24 countries were classified into N. America (n=235), S. America (n=57), Asia (n=185) and Europe (n=269). Sequences were classified to PCV2a (129), PCV2b (109) and PCV2d (508). DNA sequences were converted to amino acid sequences, screened for T cell epitopes, and, using the EpiCC algorithm, compared to those of four commercial vaccines: bivalent Fostera Gold PCV (AB), containing PCV2a and PCV2b as chimeric PCV1-PCV2 viruses, the PCV2a component alone (A1), and two baculovirus-derived PCV2a vaccines (A2 and A3). EpiCC scores were converted into % T-cell epitope coverage for each vaccine to field virus comparison. The non-parametric Wilcoxon test for paired samples was used to compare vaccine coverage within genotype. For vaccine AB the relative contributions of the a and b components against each genotype were also calculated.

Results

Phylogenetic classification confirmed the continuing field relevance of all 3 genotypes but with a predominance of PCV2d in all regions. The mean T-cell epitope coverage provided by AB was consistently higher than that provided by the monovalent vaccines in all regions and for all genotypes (Table 1), with results for genotype within region also statistically significant (not shown). Consistent differences in field strain T-cell epitope coverage were also seen between PCV2a monovalent vaccines with A1 > A3 > A2.

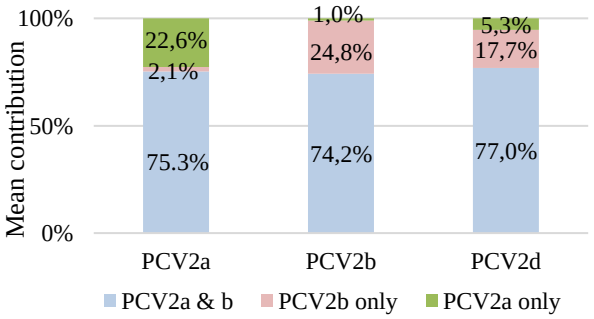
Table 1. Mean % ORF2 T-cell epitope coverage provided by different PCV2 vaccines.

		Vaccine			
	No.	AB	A1	A2	A3
Global	746	82.1	69.1	63.2	66.3
Region					
N. Amer.	235	82.9	72.3	66.5	70.0
S. Amer.	57	82.1	67.7	61.6	64.9
Asia	185	80.5	67.3	61.1	64.2
Europe	269	82.4	68.0	62.0	64.9
Genotype					
PCV2a	129	82.2^{a}	80.8^{b}	76.9^{c}	80.1^{d}
PCV2b	109	93.2^{a}	70.0^{b}	64.2^{c}	67.4^{d}
PCV2d	508	79.7^{a}	66.0^{b}	59.4^{c}	62.6^{d}

Superscripts indicate significant differences between vaccines. All were highly significant (P<0.001) except between vaccines A1 and A3 for PCV2a (P<0.01).

The PCV2a and PCV2b antigens contributed common and unique T-cell epitopes to vaccine AB in terms of coverage to field strains (Figure 1). Most field strain epitopes were found in both, but PCV2a made a substantial unique contribution against PCV2a, and PCV2b against PCV2b and d.

Figure 1. Contribution of the PCV2a and PCV2b components to T-cell epitope coverage of vaccine AB



Discussion and Conclusion

The inclusion of PCV2b and PCV2a in a single vaccine enhanced T-cell epitope coverage against a, b and d field strains, reflecting the value of an additional genotype in countering genetic diversity within, as well as between, genotypes. The coverage impact was consistent across regions, supporting the idea that the bivalent vaccine may provide enhanced and preserved efficacy in the face of continuing PCV2 virus evolution.

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A safety study in pregnant sows vaccinated with Porcilis® PCV ID

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Introduction

Porcine circovirus type 2 (PCV2) is an economically important pathogen of pigs associated with a range of clinical manifestations, including post-weaning multi-systemic wasting syndrome and PCV2-associated reproductive failure[1]. Co-infection with PCV2 and other viruses is associated with worsened clinical outcomes[2]. Effective vaccination is key to the management of PCV2-associated disease. Prevention of early infection or reproductive-linked PCV2 may necessitate vaccination of pregnant sows. Porcilis® PCV ID is an effective single-injection intradermal vaccine that protects against PCV2. In this study, the safety of Porcilis® PCV ID in pregnant sows was examined.

Methods

50 pregnant sows at 7 months – 6 years of age, with no or a low PCV2 antibody titre (i.e. < 6.0 $\log_2$) and negative for PCV2 antigen by qPCR were treated with Porcilis® PCV ID. All groups received 3 consecutive doses at days 0, 14 and 28 of the study. Vaccine was administered using the IDAL® 3G Mono. The animals were followed to farrowing and zootechnical parameters were observed.

Group 1 (n=10): Placebo group, 0.2. ml Isotonic Saline Solution, Intradermal in the neck at 21 ±6 days of gestation.
Group 2 (n=10): 0.2. ml Porcilis PCV ID, Intradermal in the neck at 21 ±6 days of gestation.
Group 3 (n=10): 0.2. ml Porcilis PCV ID, Intradermalin the perianal region at 21 ±6 days of gestation.
Group 4 (n=10): 0.2. ml Porcilis PCV ID, Intradermalin the neck at 63 ±6 days of gestation.
Group 5 (n=10): 0.2. ml Porcilis PCV ID, Intradermalin the perianal region at 63 ±6 days of gestation.

Results and Discussion

No vaccine-related clinical abnormalities or mortality, or increase in rectal temperature was observed. No vaccine-related differences in frequencies per sow of live, healthy, weak, stillborn, mummified or crushed/mortality piglets, on in abortion or length of gestation were observed.

The results show that Porcilis® PCV ID is a safe vaccine that can be used at all stage of gestation in swine.

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Group	Mean litter size	Mean live per litter	% live (mean)	% Stillborns (mean)	% Mummies (mean)	Mean body weights live piglets	Length of gestation
1	19.2	16.3	84.9	10.7	4.4	1.35	116.8
2	17.8	15.9	87.5	10.6	1.9	1.21	118.6
3	17.8	16.2	92	6.7	1.5	1.31	118.2
4	19.8	16.3	78	14.2	2.8	1.37	116.5
5	17.9	15.8	92.3	9.9	1.1	1.31	117.9

Action of oral bromhexine on the immune response of the respiratory mucosa of piglets

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Introduction

Bromhexine is a synthetic derivative of Vasicin that acts to normalize mucus in the respiratory tract. Its use increases the secretion of immunoglobulins A (IgA) previously produced by cells of the immune complex in the respiratory system of pigs (3). Only IgA and IgM are effectively secreted on mucous membranes, with IgA corresponding to the majority of antibodies at these sites, this form of antibody being the main responsible for the local adaptive immune response (2). Marked-double T lymphocytes ($CD4^+$ $CD8^+$) are a peculiarity of pigs seen very rarely in other mammals.With maturity, these cells become increasingly important for local immunity, being present in large quantities in the peripheral lymphoid organs (6). The present study aimed to evaluate the immune response in the respiratory mucosa of pigs treated with oral bromhexine.

Materials and Methods

The experiment was carried out in the nursery phase.
48 piglets were separated into two treatments, bromhexine treated group (TG) and negative control group (CG). The TG received a 1% bromhexine solution (1 mg/kg bodyweight) by drinking water for 5 days. 48 blood samples and 48 nasotracheal lavage samples were collected, half for each treatment, divided into 2 stages: 48 hours and 96 hours after the beginning of the experiment. Leukocytes were extracted from whole blood by histopaque and centrifugation, followed by washing with bufferedsolution. Specific antibodies were added to the obtained cells, incubated for 30 minutes and fixed with paraformaldehyde for further analysis on the flowcytometer FACSC alibur (Becton and Dickinson). Nasotracheal lavages were collected from animals anesthetized with ketamine and xylazine. In theseanimals a volume of saline solution was injected, laterrecovered using a syringe and urinary catheter. Marked-double T lymphocytes and phagocytosis wereanalyzed by the flow cytometry method. IgAs were quantified by the Bradford method for total protein quantification. The statistical tests used are presented below each graph and the software used to perform the analysis was GraphPad Prism 6.

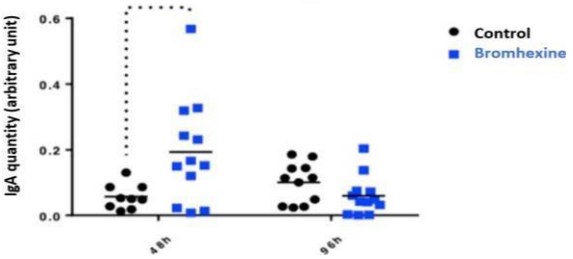
Results

The total IgA concentration was increased by bromhexine at 48 hours (Figure 1), and the number of marked-double T lymphocytes was increased (P = 0.09) in the treated animals (Figure 2).

Conclusions and Discussion

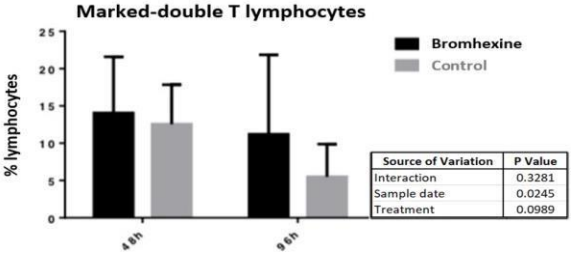
Bromhexine increases mucus turnover, increasing the secretion of IgA previously produced by the immune cells of the respiratory system (5). In the 96h collection, both groups obtained similar results, since bromhexine does not increase IgA production, but rather its secretion. The marked double T lymphocytes are important in tissue immunity, and their elevation after bromhexine may be consistent with this premise (6). Bromhexine appears to be able to activate the cellular immune response by reducing local inflammation (1,4). By controlling the respiratory burst that is common after phagocytosis, bromhexine inhibits the oxidative stress associated with the inflammatory process (3). Bromhexine increased the concentration of IgA and marked-double T lymphocytes in the respiratory tract of piglets 48 hours after the start of treatment. Therefore, based on the results obtained in this work, it is concluded that there was a reduction in the inflammatory process in the respiratory mucosa of piglets treated with bromhexine.

Figure 1. Relative quantitation of total IgA[1].



[1]Each dot represents an animal. The lines and bars represent mean and standard deviation. Statistical analysis of Student's t (P <0.05).

Figure 2. Percentage of leukocytes in the nasotracheal lavage[1].



[1]The vertical axis shows the percentage of leukocytes found. Statistical analysis of Student's t (P <0.05).

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Can the treatment of sows during lactation help in the PPE control? A case report.

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Background and objectives

Porcine Proliferative Enteropathy (PPE), caused by *Lawsonia intracellularis* (L.i.), is one of the most prevalent enteric diseases in Spain (1) with a major impact on pig performance. Pigs are usually infected subclinically and therefore undetected by farmers, but the economic impact of the infection has been estimated between 1.3€ and 18.5€ per affected pig (2,3). Most studies have analyzed the results of vaccination or antibiotic treatment of pig. However, there is lack of information about the antibiotic treatment of the sows during lactation combined with additional vaccination of the piglets for the control of L.i.. Therefore, the objective of this study was to evaluate the impact of this combination.

Materials and Methods

This study was conducted in a multi-site farm with 500 Iberian sows. Pigs at fattening were suffering from subclinical Ileitis confirmed by qPCR in fecal samples. A cohort of 60 dams received Lincomycin (110 ppm) in the feed during 24 days of lactation period while 120 dams were non-treated. There was no cross-fostering between treatment groups. Pigs from the treated dams were vaccinated with a modified live L.i. vaccine, Enterisol® Ileitis (Boehringer Ingelheim Vetmedica GmbH) or left unvaccinated. A total of 240 fattening pigs split in three groups were included in the study of which 16 lost the ear tag or died and 224 were weighed at 18 and 28 weeks of age (WOA): Non-treated dams/Non-vaccinated pigs (NoTreat-NoVac), N=70), Non-Treated dams/Vaccinated pigs NoTreat-Vac, N=83), Treated dams/Vaccinated pigs (Treat+Vac, N=71). Pigs were vaccinated by a drench after weaning (4 weeks of age). Pigs were raised in a room containing 4 batches of 125 each.). Weight (kg) and average daily gain (ADG, kg/d) were assessed.

Results

Results are summarized in Table 1 and Figure 1

	Number	Weight at birth	Weight at weaning	Weigh at 18 WOA	eight at 28 WO	ADG born-28 WOA
NoTreat-NoVac	70	1,34±0,28	7,57±1,74[a]	42,74±5,50[a]	71,14±6,88[a]	400,41±51[a]
NoTreat-Vac	83	1,39±0,13	6,17±1,01[b]	51,56±4,54[b]	78,31±6,19[b]	418,35±36[b]
Treat+Vac	71	1,31±0,27	6,29±1,31[c]	40,71±4,75[c]	74,65±6,17[c]	412,74±40[a]
			p<0,0001	p<0,05	p<0,05	p<0,05

Table 1 Weight at birth, at weaning, at 18 WOA, at 28WOA and ADG born-28WOA

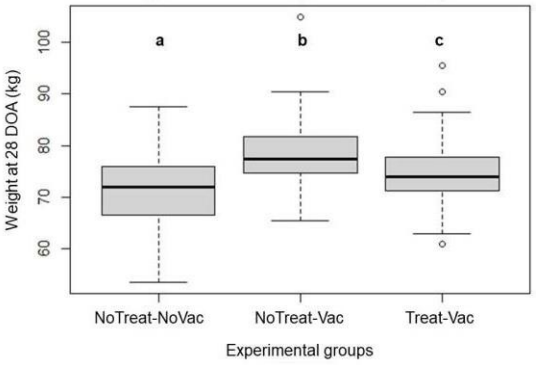

Figure 1. Weight of pigs at 28 weeks of age

Discussions and Conclusions

L.i. vaccination of pigs led to a greater ADG from weaning to 28 WOA. Furthermore, in spite of the treatment of dams during lactation, this practice does not seem to have a positive impact on the ADG of pigs. Indeed, the microbiota of pigs mirrors that of their dam, and a negative impact of antibiotics on microbiota has been described (4). Therefore, this could be the reason why Vac_Trat pigs didn´t grow better. More studies are warranted to understand the role of sows in the severity of PPE and the strategies to reduce its economic impact.

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Case report: Improvement of animal health and performance parameters in a German fattening farm after introduction of an intramuscular *Lawsonia intracellularis* vaccination at the beginningof fattening

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Introduction:
The pathogen Lawsonia intracellularis (LI) is widely distributed in pig herds (1). The clinical picture of a Lawsonia infection is strongly dependent on theimmune status of the animals, the pathogen dose, and the age of the animals. Acute diseased pigs show massive bloody diarrhea with aversion to feed, pallor and apathy. This disease leads to a mortality up to 50% (2) and strongly reduced performance in the fattening period.

Material and methods:
This report refers to a fattening farm with 1988 fattening places in western Germany (all-in all-out management for 3 animal groups on his farm). After the change of the piglet origin in 2015, clinical problems with acute Lawsonia infections in the fattening phase occurred repeatedly. Most of the time clinics started with 50-60kg. The animals showed bloody diarrhea became pale and some were growth retarded. Selected animals for necropsy macroscopically showed porcine hemorrhagic enteropathy (PHE) (Fig.1) and PCR examination of the ileum confirmed the Lawsonia infection.

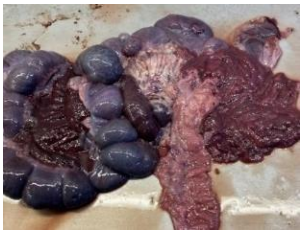

Figure 1: View of opened ileum with marked PHE, the colon is also affected

Losses due to LI were estimated between 0.2-0.5%. In almost every group, oral group treatments with Tylosin, as well as individual animal treatments with Tylosin were sometimes carried out repeatedly. In total, the farm came up with 10.81 treatment days/animal. Due to the farmer's targeted treatments, the pigs grew veryhomogeneously, and a high health status could be maintained. Furthermore, the farmer reported that the time required for animal observations and treatments was immense. In 2020, the farmer started to vaccinate his pigs intramuscularly with Porcilis® Lawsonia at the beginning of fattening with 27 kg. To test the success of the vaccination, he divided the pigs into vaccinated groups (2 groups, 1533 pigs) and unvaccinated comparison groups (3 groups, 1983 pigs). The fatteningperformance of each group was used for comparison.

Results:
Already while fattening, the animals appeared more vital and fit. The LI clinic could be controlled and the fattening performance improved. Losses decreased from0.97% to 0.4%. Feed conversion improved from 1: 2.58to 1: 2.51. Daily weight gain increased by 40.3 g and veterinary costs were reduced to 0.25€ (excl. vaccine costs) (Fig. 2). Furthermore, the fattening period was reduced by 2.67 days. Here, the low purchased weight with - 0.85kg and the higher sales weight with + 0.9kg of the vaccination group must be mentioned (Tab. 1). Asa result of the improved biological performance an economic benefit of 4.42€/fattening pig could be achieved. (Vaccination costs not considered).

Table 1: Number of animals evaluated and weight development

	non vaccinated	vaccinated
Number of animals	1983	1533
weight, start of fattening (kg)	26.70	25.85
weight, end of fattening (kg)	121.93	123.20

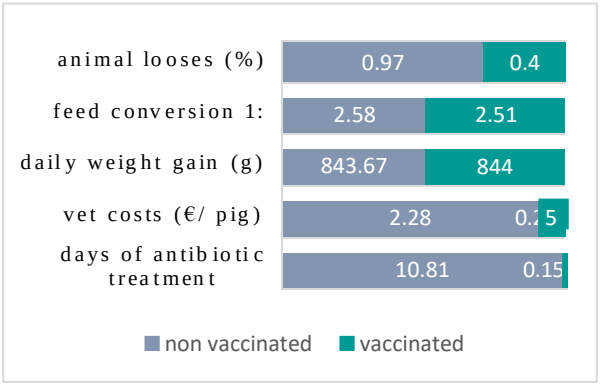

Figure 2: Performance parameters with and without Porcilis® Lawsonia vaccination

Conclusion:
The results summarized here show that vaccination with Porcilis® Lawsonia at the beginning of the fattening period can significantly reduce acute Lawsonia infection in the mid- and end of fattening, produce an improvement in their biological performance as well asthe economic outcome and significantly minimize the therapeutic use of antibiotics.

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Cellular and humoral immunity elicited by an Influenza A polyvalent virosomal vaccine in pigs

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Introduction

Influenza A virus (IAV) causes economic losses to the swine industry and public health concerns. The IAV is endemic in pigs and genetically and antigenically distinct virus lineages of subtypes H1N1, H1N2 and H3N2 circulate in different geographic regions with limited cross-protection (1, 2). The co-circulation of distinct IAV lineages associated with rapid viral evolution challenges the development of effective vaccines. The aim of this study was to evaluate the immunogenicity of an adjuvanted virosome-based influenza vaccine containing the hemagglutinin (HA) of H1N1pdm, H1N2 and H3N2.

Materials and Methods

Forty-three SPF pigs were randomized into three groups: G1: 10 non-vaccinated; G2: 30 vaccinated; and G3: 3 vaccinated – for long term immunity evaluation. Pigs from G1 received PBS injection, and pigs from G2 and G3 were vaccinated intramuscularly with a virosomal IAV vaccine on D0 and D14. Pigs were daily monitored for clinical signs, or any adverse effects related to vaccination. Blood and nasal swab samples were collected from all pigs on D0, D14 and D28. Pigs from G3 were also sampled on D60 and D90. Necropsy was performed on D28 for G1 and G2 and on D90 for G3. Nasal swab samples were evaluated by RT-qPCR for IAV (3). Serum samples were evaluated by hemagglutination inhibition (HI) (4) and serum virus neutralization (SVN) (5), using vaccine homologous viruses (H1N1pdm, H1N2 and H3N2) as antigens. For flow cytometry, splenocytes were labeled with CFSE for *in vitro* stimulation by the three vaccine viruses and stained with fluorochrome-labeled monoclonal antibodies RPE-CD8alpha and PerCP-Cy5-IFN-γ. Different cytokines (GM-CSF, IFNγ, IL-1α, IL-1ra, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18 and TNFα) were evaluated with MILLIPLEX MAP Porcine Cytokine/Chemokine Magnetic Bead kit (Merck Millipore), by Luminex platform. Differences between groups were evaluated using Kruskal-Wallis and Wilcoxon tests by SAS (6).

Results

No clinical signs were observed in pigs during the experiment. All nasal swabs collected on D0, D14, D28, D60 and D90 were negative for IAV. IAV antibodies were not detected in non-vaccinated pigs (G1). IAV antibodies for the three virus antigens were detected in the vaccinated group (G2) as follows: 20.7% of pigs developed antibodies for H1N1 (titers of 40 to 160), 48.3 % for H1N2 (titers of 40 to 160) and 100% for H3N2 (titers of 160 to 640). Antibodies to H1N2 (titer 40) and H3N2 (titer 160) were detected in one out of three G3 pigs sampled on D90. Neutralizing-antibodies were detected in all vaccinated pigs (G2) with titers of 40 to 1280 for H1N1, 10 to 320 for H1N2 and 320 to 5120 for H3N2 viruses. On D90, one out of three pigs from G3 had antibodies to H1N1 (320) and H1N2 (20), and all three pigs had antibodies for H3N2 (titers of 40 to 320). Vaccinated pigs (G2 and G3) had increased $CD8^+$ $IFNy^+$ expression when compared to non-vaccinated pigs (G1) ($p<0.0001$). The $CD8^+$ $IFNy^+$ expression was higher for H3N2 virus, followed by H1N1 and H1N2. All cytokines evaluated by Luminex were expressed, but there was no difference between vaccinated and non-vaccinated pigs.

Discussion and Conclusion

A robust humoral and cellular immune response was induced in pigs through vaccination with a virosomal vaccine containing the HA genes of the most prevalent virus subtypes in pigs. Virosomal vaccines closely mimic the native virus, binding and fusing with host cells, contributing to a robust immunity (7). Specific HA antibodies and neutralizing activity for H1N1, H1N2 and H3N2 viruses were detected, which persisted for at least three months. Cellular immune response, with high expression of $CD8^+$ $IFNy^+$ T lymphocytes was elicited. HA-specific antibodies block virus attachment and entry into the cells (8) and cellular immune responses contribute to eliminate infected cells and reduce virus shedding, thus playing an important role during IAV infection (9). Vaccination of pigs with a polyvalent virosomal vaccine containing representative virus strains circulating in Brazil may reduce the IAV impact on the swine production and at human-animal interface.

Acknowledgments

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Clinical safety of the combination of Suiseng® Coli/C, Suiseng® Diff/A andRhiniseng® in sows under field conditions.

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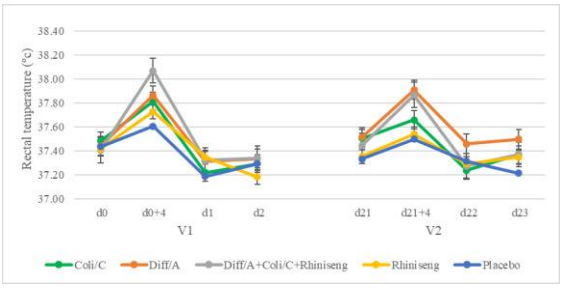
Introduction:
Vaccination of pregnant sows before farrowing is a key practice to boost the immune response and thus, the concentration of protective immunoglobulins in the colostrum (1). Piglets can then be passively protected by colostrum if they are nursed properly and prevent future cases of disease or event enhancing the piglet performance and body weight after weaning (2). Combination of vaccines is commonplace in order to minimize the pain of the sow and improve animal welfare during the third part of gestation. The objective of this field study was to assess the safety of Suiseng® Diff/A (Diff/A), Suiseng® Coli/C (Coli/C) and Rhiniseng® when administered together .

Materials and Methods:
A randomized, blinded, and controlled study was performed on a commercial farm in Brazil. A total of 75 pregnant sows were randomly distributed into 5 groups (n=15/group): Coli/C, Diff/A, Rhiniseng®, Coli/C + Diff/A + Rhiniseng®, and Placebo (Phosphate Buffered Saline). Animals in all groups were administered the corresponding product intramuscularly (2 ml) twice: one dose 6 weeks prior to farrowing (V1) and a second one 21 days later (V2). Sows in group Coli/C + Diff/A + Rhiniseng® received all products together (6 ml). Local and systemic reactions, and body temperature were monitored individually from the day before each vaccination, up to 2 days post-vaccination. Reproductive parameters were recorded from vaccination to farrowing.

Results:
A clinically irrelevant transient rise in rectal temperature was observed in all groups at 4h after each dose administration, returning to normal values after 24h. The maximum individual temperature was observed in group Coli/C + Diff/A+ Rhiniseng® at 4h after the 2nd dose (39.04ºC) with no statistically significant differences being detected between groups at any timepoint (Figure 1). Mild local inflammatory reactions (≤3cm) were observed in some animals in all groups. The highest incidence was observed in the Rhiniseng® group with 2/15 animals (13%) showing local reactions (Figure 2). These reactions were transient and had totally disappeared after 4 days. Finally, reproductive parameters showed no statistically significant differences between groups in the mean number of live born, stillborn and mummified piglets ($p > 0.05$)(Table 1).



No statistically significant difference (p>0.05)
Figure 1. Mean (± SEM) rectal temperatures after V1 and V2 doses.

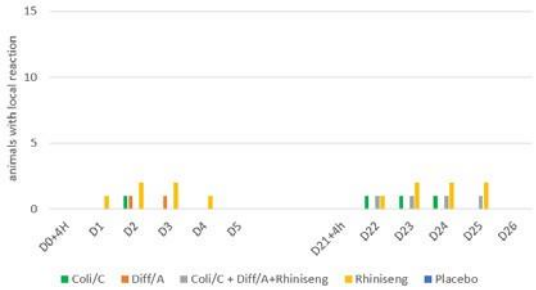


Figure 2. Number of sows with local reaction at inoculation point after V1 and V2 doses.

Table 1. Reproductive parameters.

Group	Live-born	Stillborn	Mummified
Coli/C	**13.3**	**0.2**	**0.0**
Diff/A	**14.0**	**0.4**	**0.4**
Diff/A+Coli/C+ Rhiniseng®	**12.0**	**0.7**	**0.1**
Rhiniseng®	**13.1**	**0.8**	**0.0**
Placebo	**13.5**	**0.6**	**0.4**
***p-value*[1]**	***0.267***	***0.367***	***0.689***

[1] *Kruskal Wallis test*

Conclusion:
The results obtained in the present study demonstrate optimal safety of Suiseng® Diff/A, Suiseng® Coli/C and Rhiniseng® combination under field conditions in sows. Consequently, although this practice is entirely under the responsibility of the veterinarian, there are no safety concerns that may prevent it.

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Clostridioides difficile control through vaccination. First Brazilian field experience.

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Introduction

Clostridioides difficile (C. difficile) is considered a significant pathogen causing diarrhoea in piglets between 1 and 10 days (2). C.difficile infection is clinically characterized by colitis, mesocolon oedema, and the reduction in weight gain in piglets (1,2). Previous exposure of piglets to antibiotic therapy is considered a risk factor which triggers the bacteria colonization and the disease (1,3,4). In addition, the stress of pregnancy and farrowing predisposes the sow to bacterial shedding in her faeces (1). The aimof this study was to evaluate the use of the new vaccine SUISENG® Diff/A, under field conditions.

Materials and Methods

The present study was carried out on a pig farm (10,000 sows) in Southern Brazil. 50 sows and their litters were divided into 2 groups. Group 1 (G1) (n=25) was immunized with two doses of SUISENG® Diff/A containing C. difficile toxoid A and B, and Clostridium perfringens alpha toxoid following the manufacturer's instructions. Control group (G2) (n = 25) was not vaccinated. Vaccine safety was assessed by local and systemic reaction after vaccine inoculation. Piglets from females of both groups (G1, n=364; G2,n =357) wereindividually weighed at birth and weaning; and monitored twice a day for the presence of diarrhoea until weaning. Colostrum intake was also evaluated according to De Cleer et al., 2020 (5). Every dead piglet was necropsied and macroscopic lesions suggestive of enteric pathogens infections wereevaluated. In addition, faecal samples from diarrhoeic piglets during the first 10 days of life were evaluated by Polymerase Chain Reaction technique (ENTEROCHECK) to detect various genes encoding for pathogenicity factors of Escherichia coli, Clostridium perfringens type C, C. difficile and C. perfringens type A. Moreover, two piglets per litter that had diarrhoea were euthanized and necropsied to assess macroscopic lesions caused by enteric pathogens with the aid of histopathology. The use of injectable antibiotics to treat diarrhoea, prevalence of scours, mortality due to diarrhoea and average daily weight gain (ADWG) were compared between groups. Statistical analyses were performed with the software R.

Results

No female had a local or systemic reaction after vaccination. Piglets from both groups that died without diarrhoea did not show macroscopic lesions suggestive of enteric pathogens. The diarrhoea rate in piglets and litters from G1 were 2% (08/364) and 8% (2/25), respectively; and none of the piglets died. On the other hand, 14% (50/357) of the piglets and 48% (12/25) of the litters from G2 had diarrhoea and 24% (12/50) piglets died after having diarrhoea. 20% (4/12) of G2 piglets with diarrhoea, which finally died, showed mesocolon oedema and "volcanic eruption" lesions under histopathology diagnosis. Faeces were positive for C. difficile toxoid A and B in 0% (0/2) and 25% (4/12) of the samples evaluated in G1 and G2, respectively. Total mortality of piglets in the nursery period was statistically reduced with the vaccine (G1, 5,68%(b); G2, 7.98%(a); p- value<0.001). To control diarrhoea in piglets, 180 and 1125 mg of active ingredient (injectable antibiotic) were used in piglets from groups 1 and 2, respectively. There was an 84% statistically significant reduction in the use of antibiotics in piglets from vaccinated sows (p value <0.001). Moreover, those piglets also showed greater uniformity and had an increase of 17 grams in the average daily weight gain than piglets in control group during the lactation (p-value<0.001) (Figure 1).

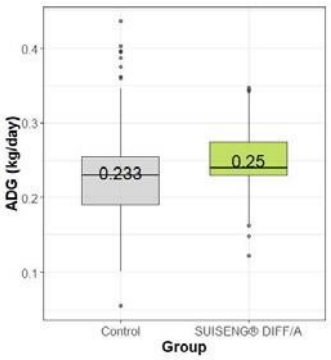

Figure 1. Average daily gain (ADG) during the maternity period of piglets from G1 and G2.

Conclusions and Discussion

The present study reveals the great efficacy of thefirst and only vaccine to control C. difficile infection in piglets. Multiple productive parameters such as mortality, diarrhoea clinical signs, ADGW and weight homogeneity have been improved thanks to SUISENG® Diff/A. Furthermore, a reduction in the antibiotic usage has also seen. Further studies areneeded to reinforce the benefits of vaccinationagainst C. difficile.

References

Available from the authors.

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Colostrum antibodies level assay against PCV2 as a possible tool to improve vaccination

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Introduction

The interference between maternally derived antibodies (MDA) and post-vaccinal seroconversion is well known for PCV2, though its clinical impact is still debated (1). Successive studies described here have been performed to assess the interest of assaying antibodies in colostrum as a tool to improve PCV2 vaccination program.

Materials and Methods

A PCV2 ELISA test (BioChek) used for serum samples was adapted for colostrum samples. Antibodies against PCV2 are expressed by this test as the S/P ratio of the assayed sample by reference to positive and negative reference samples. This test has been previously correlated with immunoperoxidase monolayer assay (IPMA) considered as a reference test for PCV2 humoral immunity (2). Adaptation of the test to colostrum allowed to check its reproducibility and stability of the colostrum samples before assay.

The correlation between the S/P ratio in sow colostrum and issued piglet serum was assessed from 2 studies in the Netherlands and in Taiwan (44 sows of various parities and 141 issued piglets). Colostrum samples were taken within the farrowing process and blood samples at 7 days of age, after careful follow up of colostrum intake by the piglets. The correlation was assessed from the coefficient of determination (R^2 = 0.719) of the regression line plotting the piglet serum S/P against the sow colostrum S/P. The MDA depletion was then estimated by calculating the mean slope (β) of the log linear decline of piglet serum S/P ratio over time from 3 studies performed in the Netherlands, Taiwan and Korea (183 piglets who were blood sampled 3 times between 1 week of age and the day of vaccination). The mean MDA half-life ($T_{1/2}$) was calculated as $T_{1/2}$ = (log(2))/β = 25 days. The threshold of interference between MDA and post-vaccinal seroconversion was finally calculated by plotting the difference of piglet serum S/P ratio between post and pre-vaccination times against the pre-vaccination value. The regression line was determined from 5 trials in Taiwan and Korea totalizing 115 piglets vaccinated with a PCV2 whole inactivated virus (Suigen® PCV2, Virbac). The interference threshold was estimated as the intercept between the regression line and the X-axis (corresponding to an average nil seroconversion). These findings allowed to establish a relation between the colostrum S/P ratio and the estimated piglet age at which its serum S/P ratio would fall below the threshold of interference between MDA and post-vaccinal seroconversion. As this investigation could be done at farm level, it was recommended to take colostrum samples from a number of sows per farm representative of parity distribution. Then it was necessary to define a criterion reflecting the colostrum S/P distribution in the concerned herd. The 90th percentile of the colostrum samples S/P ratio was selected, corresponding to 90% of the cumulated frequency for colostrum S/P ratio. By selecting the vaccination age corresponding to this 90th percentile, only 10% of litters would require theoretically a later vaccination age. The PCV2 ELISA test was then used routinely in 91 farms in Taiwan (owning between 100 and 5500 sows) where at least 10 colostrum samples per farm could be assayed.

Results

The threshold of interference between MDA and post-vaccinal seroconversion was calculated as S/P = 1.1. This threshold was consistent with a threshold of protection against PCV2 infection from previous correlation between antibody levels (assayed by IPMA) and viremia (3), and from the correlation between the ELISA S/P ratio and the antibody levels by IPMA (2). The vaccination age calculated from the 90th percentile of the colostrum S/P was rounded up per week, for practical implementation at farm level. Additionally, the 10th percentile of the colostrum S/P was calculated to check if it is below or above 2. As this value corresponds to a duration of protection around 4 weeks of age, if at least 10% of sampled sows have a colostrum S/P < 2, it would be recommended to vaccinate the sows to improve the maternal immunity transferred to piglets. The predicted vaccination age ranged from 3 to 7 weeks of age in the 91 Taiwanese farms investigated. The colostrum 10th percentile and mean S/P ratio were significantly higher in the farms vaccinating gilts or/and sows against PCV2, reflecting the potential impact of dams vaccination on immunity transfer to piglets.

Table 1. Colostrum S/P ratio and predicted piglets vaccination age from 91 farms in Taiwan

Criteria (m ± SD or range)	Farms without gilts nor sows vaccination[1]	Farms with gilts and/or sows vaccination[2]
10th percentile S/P	1.1^{a} ± 0.5	1.7^{b} ± 0.5
Mean S/P ratio	1.9^{a} ± 0.2	2.1^{b} ± 0.2
90th percentile S/P	2.4 ± 0.4	2.5 ± 0.4
Vaccination age	3-7 weeks	4-7 weeks

[1]: 69 sampled farms. [2]: 22 sampled farms. [a,b]: means in the same row with different superscripts differ (p < 0.005).

Discussion and Conclusion

This colostrum test could be a practical tool to revisite PCV2 vaccination program for piglets and sows, taking into account vaccine used and proper colostrum intake.

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Combination of attenuated and killed PRRSV vaccines to enhance immunity and pig performance

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Introduction

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is a global viral pathogen in pigs that causes economic losses in every country where it is present (1). Currently, a first good immunization is achieved using a modified live vaccine (MLV) to control the PRRS. In the field, Thai veterinarians recommend the use of a killed vaccine (KV) in the gestation phase to boost the immunity of sows previously vaccinated with a MLV.
The combination of MLV and KV has several benefits, namely an increase in neutralizing antibodies and cell-mediated immunity responses (2) and a remarkable improvement in the PRRSV status in breeding herds. For several reasons, as part of the immunization strategy to generate more profit in sow herds, KV in combination with MLV has been commercially available for a long time in Thailand. However, there are limited data available regarding the impact of a KV in combination with a MLV on sows in a PRRSV-infected herd.
In this study, the combination of a MLV and a KV (SUIPRAVAC® PRRS, HIPRA) to maintain a highlevel of immunity against PRRSV in sows and to keep producing quality piglets was studied in Thai piggeries.

Materials and Methods

A smallholder contract farm (480-sow farrow-to-finish unit) in Nakhon Si Thammarat province (Thailand) was enrolled in the trial during 2021. This farm had a vaccination programme against US and EU PRRSV coinfection that consisted of routine mass vaccination with a MLV (MLV group) (3). Since 2021, all pregnant sows had been routinely mass vaccinated with a MLV and additionally they were vaccinated at 4 weeks before farrowing with SUIPRAVAC® PRRS (2 ml dose, IM) (MLV+ KV group).
A safety assessment of gestating sows was carried out after vaccination to check for local reactions. Colostrum (volume 1 mL) was collected from the first front teats of sows in the MLV group (n=5) and the MLV+KV group (n=5) within 1 hour after farrowing. All samples were sent to the Kamphaengsaen Veterinary Diagnostic Center (KVDC, Kasetsart University, Thailand) for evaluation of neutralizing antibodies against EU and US field isolates of PRRSv. The production performance of the piglets was statistically compared between groups by t-test (paired two sample for means) using the SPSS statistical program (version 22.0).

Results

No serious long-lasting side effects were observed in any gestating sows after MLV+KV vaccination. The MLV+KV group had significantly higher antibody titres against both EU and US PRRSV in the colostrum compared to the MLV group (Table 1). Piglets born from the MLV+KV group had a significantly better production performance than the MLV group (Table 2, p<0.05).

Table 1. Colostrum neutralizing antibody titres in sows against EU and US PRRSV.

EU		US	
MLV+KV*	MLV	MLV+KV*	MLV
1:160	1:160	1: 640	1:80
1:160	1:80	1:320	1:40
1:320	1:80	1:80	1:80
1:320	1: 160	1:160	1:80
1:160	1: 80	1:80	1:40

*Significantly higher titres of neutralizing antibodies against EU and US PRRSV in the MLV+KV group (p-value 0,04)

Table 2. Performance of piglets born from treated sows

Production parameter	MLV + KV	MLV	Diff (%)	p-value[ab]
No. of piglets (n)	539	559	-	0.57
Mortality (%)	1.11[a]	3.22[b]	-65.53	<0.001
Culling rate (%)	1.48[a]	5.72[b]	-74.13	<0.001
Weaning weight (kg)	7.39[a]	6.96[b]	+6.18	0.047
Age of piglets at weaning (d)	22[a]	25[b]	-12	<0.001

[ab] P<0.05 indicated statistical differences between group.

Discussion and Conclusion

This preliminary study demonstrates that SUIPRAVAC® PRRS is safe in gestating sows. Additionally, it is accepted that the highest immune responses against PRRSV were achieved after the combination of MLV and KV. This suggests that SUIPRAVAC® PRRS helps to reach higher levels of neutralizing antibodies in the colostrum after a booster dose before farrowing, leading to the achievement of better production data in piglets from these sows.

Acknowledgments

The authors would like to thank to farm owners for providing pig production information and support.

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Comparative efficacy evaluation of six PCV2 vaccines in a wean-finish farm with subclinical PCV2 infection

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Introduction

Porcine Circovirus type 2 (PCV2) is considered one of the most important viral pathogens of pigs even in Naïve PRRS farm (1). Commercial PCV2 vaccines were initially developed to control PCVAD but are now also used to improve performance in subclinical infection (2). The objective of this negative controlled, blinded study was to compare the efficacy of six different PCV2 vaccines in a subclinical PCV2 infection farm in Thailand.

Materials and Methods

The wean-to-finish, replacement gilt multiplier farm, received weaned pigs from a 2,100-sow source with naïve PRRS status. At weaning (18-21 days of age), 143 female piglets were ID-tagged, weighed, and randomly allocated to seven treatment groups (one control and six vaccination groups, Table1). Pigs in the six vaccination groups were each vaccinated with an assigned PCV2 vaccine per label indication.

Table1. Study design and results of each group protocol

Group	No. of pig	Vaccine	ml/dose	LS means (SE)[2]	
				ADG (g/day)	Weight out (kg)
Control	23	No	No	602.39 (11.99)^{bc}	68.86 (1.25)^{bc}
T1	19	Chimera	2	636.68 (13.21)^{ab}	72.43 (1.37)^{ab}
T2	19	Whole cell	0.5	624.45 (13.20)^{abc}	71.15 (1.37)^{abc}
T3	20	Subunit	2	611.33 (12.87)^{abc}	69.79 (1.34)^{abc}
T4	20	Subunit	2	616.19 (12.91)^{abc}	70.30 (1.34)^{abc}
T5	19	Subunit PCA	1	662.42 (13.19)^{a}	75.10 (1.37)^{a}
T6	23	Subunit	0.2 ID	595.18 (12.31)^{c}	68.11 (1.28)^{c}

*** a-c mean in a column without a common superscript letter differ (P < 0.05)**

All pigs were housed in the same house, kept on a same feed and under the same caretaker. The vaccinated pigs and control pigs were comingled in each pen (blinded and randomized). Serum samples were collected individually of 5-10 pigs per group biweekly at 4-18 weeks of age, pooled at 5:1 and tested by RT PCR for PCV2 viremia. The study ended prior to pigs being sold to the GDU. Individual pig was weighed to calculate ADG, which was used for measuring the difference of each vaccine protocol by least squares (LS) means (indicated as SE in parentheses) as the primary parameter. The total days of this study was about 104 days. The comparison between groups was tested by ANOVA. The Multiple comparison was assessed by Tukey's Honest Significant Difference (HSD) test. The economic benefit indicated by return of investment was evaluated by BECAL, Boehringer Ingelheim Economic Calculator.

Results

The RT PCR results aligned to clinical and performance parameters indicate that PCV2 was one of the causative agents for the subclinical PCVAD in this farm with no influence by PRRSV, resulting in reduced growth performance.

Table 2. The presence of viremia from the blood samples throughout the experimental period.

Group	4 wk	6 wk	8 wk	10 wk	14 wk	16 wk	18 wk
Control	Negative	Positive	Positive	Positive	Negative	Positive	Negative
T1	Positive	Negative	Negative	Negative	Negative	Positive	Positive
T2	Negative	Positive	Positive	Positive	Positive	Positive	Positive
T3	Negative	Negative	Negative	Negative	Positive	Positive	Negative
T4	Negative	Positive	Positive	Positive	Positive	Positive	Negative
T5	Negative	Negative	Negative	Negative	Negative	Negative	Negative
T6	Negative	Negative	Negative	Negative	Negative	Positive	Negative

After PCV2 vaccination, there was significant improvement in the growth rate in all PCV2 vaccinated groups when compared with control group except in Group T6. The highest growth rate was seen in Group T5 (662.43 g/d) and 75.1 kg at final weight with no viremia detected throughout the study.

Discussion and conclusions

In this study, high-health pigs vaccinated with different PCV2 vaccines were protected against natural PCV2 challenge defined by the presence of viremia and the reduction of performance in the control group. In all PCV2 vaccination groups, growth improvement was seen except for the T6 group. In addition, T5 (Subunit PCA) group has shown superior efficacy under these conditions and bring advantages to the pig multiplier farm in terms of weight gain and overall herd health status.

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Comparative efficacy evaluation of two CSF C-strain vaccines in the field

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Introduction

Classical swine fever (CSF) is an important swine disease in China. Sporadic outbreaks with mild clinical signs were reported despite massivevaccination. One possible reason for vaccine failure could be inference from maternally-derived antibodiesat vaccination in the field [1]. In our previous study, we demonstrated that the overall performance of our Ingelvac CSF MLV vaccine was superior to that of two commercial ST-based CSF MLV vaccines in the presence of maternal-derived antibodies (MDAs) in acontrolled experiment[2]. The aim of this study was toevaluate the efficacy of these two vaccines in commercial pig farms and optimize the vaccination scheme to minimize MDA impact.

Materials and Methods

Two groups of 60 piglets each were intramuscularly vaccinated with Ingelvac CSF MLV (C-PK, Lot. FC2020001) either at the age of 30 ± 3 days or 45 days. Another group of 60 piglets, following the routine vaccination program at site, were intramuscularly administered the C-strain vaccine produced in the ST cell line (C-ST, Lot. 20200903) at the age of 28 days and boosted 1 month post-first vaccination. The CSF antibody were monitored at intervals. At 65 days post-first vaccination, five pigs from vaccination groups were randomly selected. Together with a challenge control group, they were transported to the challenge facility and received 1 mL of highly virulent Shimen virus ($10^{6.0}$ $TCID_{50}$/mL) intramuscularly. Rectal temperatures and clinical scores (CSs) were recorded daily[3]. The subcuti, tonsil, lymph node, lungs, kidney, bladders and button ulcers in the colon and spleen were examined for CSF lesions at necropsy. The presence of the CSFV genome in blood and nasal swab samples at 3, 7, 10 and 16 days post challenge was determined by real-time RT–PCR.

Results

Among three vaccination groups, pigs vaccinated once with Ingelvac CSF MLV at the age of 45 days had thebest clinical and virological protection (Table. 1).

Notably, no CSFV viral RNA was detected in the nasal samples of pigs vaccinated with Ingelvac CSF MLV, which implied that vaccinated pigs will not shed fieldvirus to the environment and spread virus to neighbouring pigs after infection. The CSF antibody response also supported the efficacy results, in which pigs in Group 3 had the highest virus neutralizing titers at challenge and the IDEXX E2 antibody response before slaughter among the three groups (data not shown). The blocking rate of the IDEXX E2 antibodyresponse was also monitored at different intervals. The average blocking rate was above 50% at the age of 28 days before vaccination in Groups 1 and 3. The antibody in Group 3 did not increase throughout the study regardless of boost.

Conclusions and Discussion

The results demonstrated that a single shot of theIngelvac CSF MLV vaccine had superior efficacy over two shots of ST-based C-strain vaccines routinelyapplied in the field in the presence of MDAs, which is consistent with our previous efficacy evaluation in thecontrolled experiment. Statistical analysis was not performed in this study due to limited number ofanimals. Further large-scale studies will be carried out to confirm the finding. Our results also emphasize theimportance of monitoring MDAs to guide vaccinationschemes to achieve solid protection in the field.

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Notes to Table 1

[1]Average clinical score = Total CS in the group/(No.of piglets*days recorded before death or necropsy) [2]Average pathological lesion = Total gross lesions ofthe group/(number of piglets*7 tissues observed) [3]Viremia and virus shedding = Total frequenciesdetected positive of the group/(number of piglets*4time points tested)*100%

Table 1. Efficacy results after challenge

Group	No. of piglets	Fever	Average clinical score[1]	Average pathological lesion[2]	Mortality	Viremia[3]	Virus shedding[3]
1. C-PK (1 shot at the age of 28 days)	5	0/5	0.26 (21/80)	0 (0/35)	0% (0/10)	20% (4/20)	0% (0/20)
2. C-PK (1 shot at the age of 45 days)	5	1/5	0.06 (5/80)	0 (0/35)	0% (0/10)	20% (4/20)	0% (0/20)
3. C-ST (2 shots at the ages of 28 and 58 days)	5	3/5	1.41 (113/80)	0.06 (2/35)	0% (0/10)	45% (9/20)	20% (4/20)
4. Challenge control	5	3/5	7.03 (204/29)	0.60 (21/35)	100% (5/5)	100% (7/7)	100% (7/7)

Comparative efficacy of different PRRS vaccination strategies on piglets using PROGRESSIS at different days of life.

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Introduction

It has been postulated that piglet vaccination would be an option to reduce the negative impact of PRRS. While piglets are usually vaccinated with modified live vaccine (MLV) [1], few reports are available about the efficacy of killed vaccine (KV) [2]. In this study we report results obtained after piglet vaccination with KV at different ages.

Materials and Methods

In a three-site farm with a pig population of about 100,000 pigs a PRRS vaccination schedule aimed to control an acute outbreak was set up in sows, based upon revaccination during pregnancy with a live vaccine (60 days) and PROGRESSIS (90 days).

Later on, it was decided to implement piglet vaccination with the KV vaccine at different ages: 24 days (T1), 31 days (T2) and 34 days (T3) in 20, 50 and 26 weaning batches, respectively, weaned between March and October 2020.

The parameters used to compare the vaccination methods were average daily gain (ADG) and mortality rate during the post-weaning phase. Results were analysed by means of an ANCOVA model including ADG or %mortality as independent variables, entrance weight as covariate and treatment and technician as factors.

Results

According to our model, body weight at entrance and the technician were not significant parameters considering average daily gain (ADG) as the response variable.

ADG (g/d) results (mean±SD) were: 340±48.2, 348±43.3, 390±33.3 for T1, T2 and T3 respectively. α=0.05, pT1-T2=0.998, pT1-T3=0.062, pT2-T3<0.001

Mortality (%) results (mean±SD) were: 8.99±4.61, 10.1±5.28, 5.91±3.48 for T1, T2 and T3 respectively. α=0.05, pT1-T2=0.958, pT1-T3=0.900, pT2-T3=0.135

Discussion and Conclusion

T3 group showed better mortality results yet these were not statistically significant. Conversely, there was a significant increase of ADG. These better results could be due to a better immunoresponse in animals with lower levels of maternal derived immunity[3]. While in the other treatments the inoculation time is too soon and there is maternal antibody interference[4]. The better performance of T3 might be the result of a better stabilization with time.

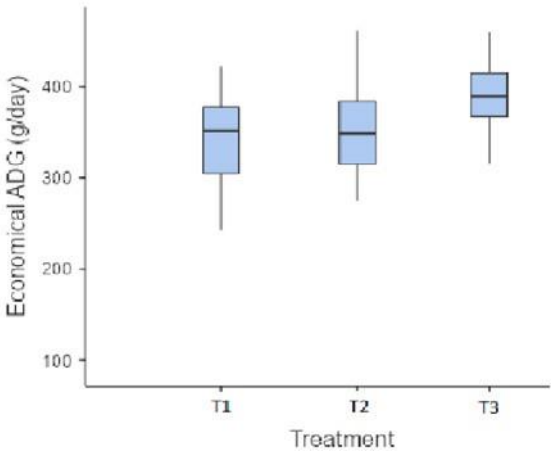

Figure 1. Economical ADG (g/day) boxplots for T1, T2 and T3 pigs.

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Comparative efficacy of two commercial *Actinobacillus pleuropneumoniae* (APP) vaccine in Thailand

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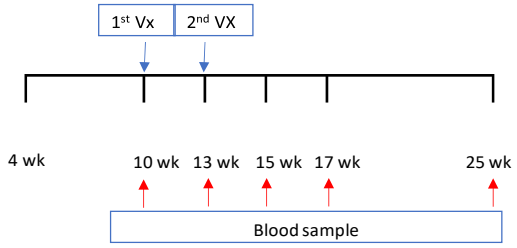
Introduction

Actinobacillus pleuropneumoniae (APP) is a gram-negative and toxin-producing organism that causes severe pleuropneumonia in swine (1).
The most common serotypes reported in Thailand based on surface polysaccharides are 18, 1, 9 and 12 (2). The serotype 5 is the commonly detected by a local laboratory). The outbreak causes a severe decline in the farm's economics due to reduced growth in the chronic infection and high pig losses due to acute death, increasing FCR in heavy weight pigs. Sustainable control of APP requires integrated good management, pig flows, medication and vaccination program together with understanding disease burdens related with other primary factors. The objective of this study is to evaluate the immuneresponses and the pig performance after vaccination with 2 vaccines.

Materials and Methods

A 5,000 sows Farrow to finish farm, located in Ratchaburi province has been selected to conduct this study. Before implementing the vaccination, the APP status was confirmed by serology, bacterial isolation, and the clinical manifestations. The 8 weeks old, 1,519 pigs were allocated in to 2 groups, the Control and the Ingelvac H group. The 2 commercial APP vaccines were applied at 10 weeks and 13 weeks old. Blood samples were collected at 10, 13, 15, 17 and 25 weeks of age and tested with ELISA APP serotype 5 shown as figure 1. The sero-responses was compared by Boxplot, Minitab, LLC USA. The finishing performance of two study groups were analysed by Paired T-test.

Figure 1: The method of vaccination and sampling protocol



Results

The seroresponses of 2 groups is shown in figure 2. The Ingelvac H group has the antibody response to serotype 5 faster than the control group at 3 week post the first shot of vaccination as well as % of seropositive pigs (73% vs 53%) and reached 100% at 2 weeks following the second shot of vaccination. There were no statistic differences of the pig performance from these two groups shown as table 1.

Figure 2: The sero responsed to APP serotype 5

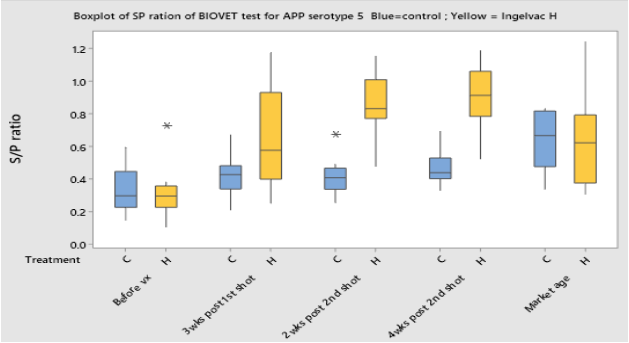


Table 1: Growth performance and Mortality

Performance Index	Control	Ingelvac H	Diff
Pig move in	759	760	-1.0
% Total loss	3.28	3.30	0.02
ADG (g/day)	756	755	-1.0
FCR	2.4	2.37	-0.03
FCG (USD/Kg)	1.16	1.15	-0.01
Lung lesion score	15%	14%	-1.0

Discussion

Selecting of APP vaccines should be based not only on the efficacy, safety and price but also needs to consider the farm's infection dynamic. In the farm that requires an early immune response to address an early infection. In those cases, Ingelvac H may be able to provide the faster immunity, although there was no difference of efficacy between the 2 commercial APP vaccines.

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Comparative efficacy study of PORCILIS® PCV M HYO in a Japanese commercial farm

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Introduction:
Porcine Circovirus Type 2 (PCV2) and *Mycoplasma hyopneumoniae* (*M.hyopneumoniae*) are common pathogens affecting swine production worldwide. qPCR can be used to monitor for PCV2 viremia and hence vaccination efficacy. PCV2 viremia is directly correlated to decreased Average Daily Gain (ADG)[1]. PCV2 infections later in life have greater economic consequences for swine as this coincides with high growth phases. The aim of this study was to observe and compare the efficacy of Porcilis® PCV M Hyo against a competitor combination ready to use (RTU) vaccine in Japan.

Materials and Methods:
This study was performed in a commercial farm in a swine dense region of southern Japan. The farm used a RTU PCV2b vaccine from May 2020 to Jan 2021. From Feb 2021, they began to use Porcilis® PCV M Hyo. A retrospective analysis was conducted to understand the efficacy of PCV2 control. Cross-sectional blood sampling of the herd was conducted in Feb 2021, just before the switch, to understand the background PCV2 viremia level. Subsequently vaccination with Porcilis® PCV M Hyo was conducted and monthly sampling was conducted from Feb – Aug 2021. Samples were pooled 5-1 and tested for qPCR

Group	Age Groups (N)	Date of Sampling	Treatment
A	30/60/90/120/150/180 (5 per group)	2nd Feb 2021	competitor RTU PCV2b vaccine
B	30/60/90/120/150/180 (5 per group)	Monthly from Feb to Aug 2021	Porcilis® PCV M Hyo

Results:
Viremia was detected at day 150 (pooled average = 3.6 x 10^5 copies/uL) and day 180 (pooled average = 2.4 x 10^4 copies/uL) in Group A. In contrast, viremia was not detected at either of these days of production in Group B.

Conclusions:
The viremia measurement results suggest that Porcilis® PCV M Hyo provided excellent control of PCV2 viremia, with viremia not found in vaccinated pigs after use.

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Comparative field efficacy study between a freshly mixed PCV2/*M. hyopneumoniae* vaccine and a ready-to-use vaccine in a Korea

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Introduction

Porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* (M. hyo) are two major pathogens causing Porcine Respiratory Disease Complex (PRDC). These two pathogens result in serious economic losses in Korean swine industry (1). To mitigate economic losses, two different kinds of commercial combination vaccines are available for the less injections and better efficacy. In this study, the efficacy was evaluated based on performance and physiological responses for the improvement of farm productivity.

Materials & Methods

Overall, 998 pigs out of two farrowing batch in a commercial herd in South Korea were randomly assigned to two treatment groups. Group 1 (n = 498) was vaccinated with 2 ml Porcilis® PCV M hyo (Intervet international B.V., Netherlands). Group 2 (n = 500) was vaccinated with 2 ml FLEXcombo® (freshly prepared mixture of Ingelvac CircoFLEX® and Ingelvac MycoFLEX®, Boehringer Ingelheim Vetmedica GmbH). Piglets were vaccinated at 21 days of age according to label recommendation. Body temperature was measured prior to vaccination and 6, 24 and 48 hours post vaccination from behind the ear using a contactless infrared thermometer. A subset of 15 piglets of each group was subjected to blood sampling prior to vaccination as well as 24 and 48 hours post vaccination for determination of the acute phase proteins haptoglobin and C-reactive protein using Life Diagnostics ELISA kit. To evaluate the performance of the pigs in the two different treatment groups, mortality & cull rate from wean-to-finish and average market weight were recorded for each group. Statistical analyses were performed by Fisher's exact test and two-way ANOVA.

Results

The wean-to-finish mortality was statistically higher in Group 1 compared to Group 2. The average mortality rate was 11.4% for Group 1 and 8.2% for Group 2 ($p<0.05$). Average market weight was 113.1 kg in Group 1 and 113.8 kg in Group 2. Both treatment groups showed increase of acute phase proteins in serum as well as body temperature compared to basal levels (Figures 1 and 2). However, this increase in acute phase proteins and body temperature were significantly higher in Group 1 ($p<0.05$).

Figure 1. Mean serum concentrations of haptoglobin and C-reactive protein in piglets: baseline, 24 and 48 hours after vaccination, significant difference between groups at 24 hours post vaccination. (by Tukey's test)

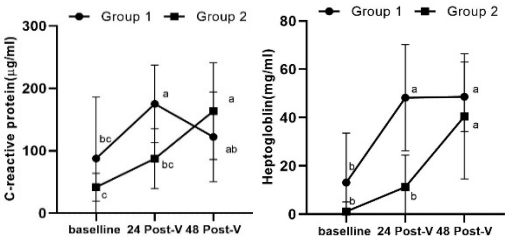
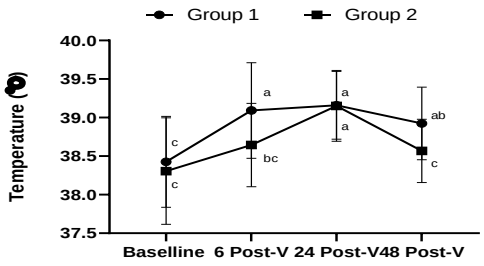


Figure 2. Mean body temperature in piglets: baseline, 6, 24 and 48 hours after vaccination, significant difference between groups on 6 and 48 post vaccination. (by Tukey's test)



Conclusions and discussion

FLEXcombo® demonstrated greater reduction in mortality rate, resulting in a higher percentage of marketed pigs and total volume of pork sold. FLEXcombo® also proved to be less reactive, evidenced by the lower expression of acute phase proteins and fever after vaccination. The results of this study are in line with other studies, demonstrating that vaccine selection should be based on its efficacy as well as its effect on piglet welfare (2).

Acknowledgement and references

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Comparative safety study of PORCILIS® PCV M HYO in a Japanese commercial farm

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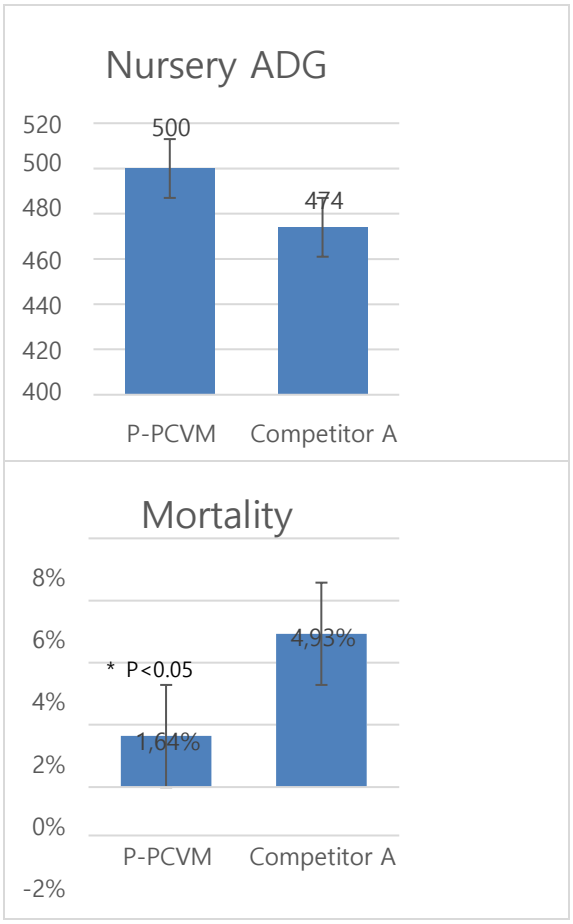
Introduction:
Apart from immediate effects such as swelling or tenderness at the site of vaccination, other commonly observed effects of vaccine reactogenicity in swine are a period of feed aversion, possibly leading to decreased Average Daily Gain (ADG). Use of a safe vaccine would thus not affect nursery ADG relative to a control group. The aim was to compare and observe the field safety of Porcilis® PCV M Hyo (P–PCVM) against a mixed PCV2 and Mycoplasma hyopneumoniae (M.hyo) vaccine in field conditions in Japan.

Materials and Methods:
This study was performed in a 1150 sow level commercial farm in a swine dense region of Southern Japan. Pigs were randomly allocated within litter to one of 2 treatments – 1) 610 piglets vaccinated at 3 weeks of age with Porcilis® PCV M Hyo 2) 609 piglets vaccinated at 3 weeks of age with a competitor product. Safety parameters observed were average daily weight gain and mortality from weaning to nursery exit. Starting weights between groups showed no statistical differences (Group 1: 6.23kg, Group 2: 6.36kg, p>0.05). Results were analyzed using Microsoft Excel Data Analysis Toolkit. ADG was analyzed by T Test and mortality was analyzed by Chi-Square test.

Results:
No local or systemic reactions were observed in the 14-day post-vaccination period, across both vaccines used in the study. Group 1 (P-PCVM) showed statistically improved mortality (p=.002453) and numerically better ADG (p= 0.1468).

Conclusions:
The post vaccination observations support the fact that P-PCVM had a similar safety profile in swine compared to a competitor vaccine on the market in Japan. Improved ADG is presumed that fewer adverse event prevents from growth retardation during nursery.



Comparative study of the safety of Oedema Disease vaccines

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Introduction

Oedema disease (OD) in piglets caused by Verotoxin 2e (VT2e), produced by *Escherichia coli*, is an enterotoxaemic disorder that produces neurological signs and subcutaneous oedema (1). Vaccines are the preferred alternative for the control of this pathology, as they have been shown to be useful for the prevention of OD at weaning when piglets are vaccinated during the first days of life (2, 3). Two commercial vaccines are available on the market, one containing a purified recombinant VT2e antigen and the second one containing a non-purified recombinant VT2e.
The aim of this study was to compare the safety of these two commercial vaccines against oedema disease.

Materials and Methods

Piglets without VT2e neutralizing antibodies were distributed between 3 groups of 12 animals each: the VEP group was vaccinated with 1mL of VEPURED® (purified vaccine), the VA group was vaccinated with 1 mL of vaccine A (non-purified vaccine), and the control group was given 1 mL of phosphate-buffered saline (PBS).
Local reactions and temperature were evaluated before and at 4, 6, 24, 48 and 72 hours after vaccination.

Results

The body temperature of the piglets increased from 0 to 4 hours after vaccination. The VA group had a significantly higher increase in temperature than the control group, whilst there was no significant difference between the VEP group and the control group. Twenty-four hours after vaccination the temperature returned to baseline in all the groups (Table 1).

Table 1. Temperature increase after vaccination.

Group	$\bar{X}$ / SD	Temperature increase /Study day				
		D0+4h	D0+6h	D1	D2	D3
VEP	$\bar{X}$	0.38^{a,b}	0.29	0.00	0.30	0.12
	SD	0.34	0.46	0.30	0.34	0.25
VA	$\bar{X}$	0.49^{b}	0.43	0.04	0.23	0.07
	SD	0.33	0.28	0.40	0.41	0.29
Control	$\bar{X}$	0.05^{a}	0.18	-0.05	0.11	-0.16
	SD	0.38	0.22	0.36	0.34	0.32

Significant differences are represented with different superscript letters (ANOVA test; $p<0.05$). SD: standard deviation

After vaccination, local reactions were observed in some animals included in the study, (2/12) in the VEP group, (5/12) in the VA group and (1/12) in the control group (Figure 1).

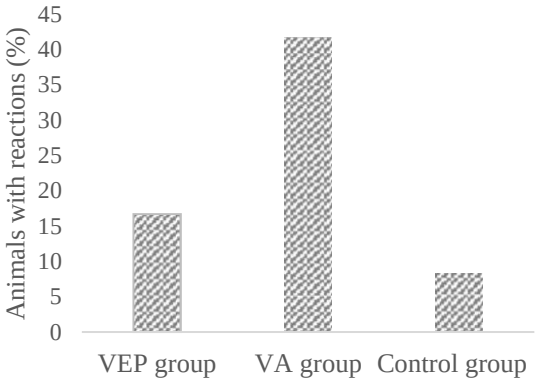

Figure 1. Percentage of animals with local reactions at any time during the study

The VEP group had only two animals with slight inflammation, whilst the VA group had animals with slight inflammation (3) and moderate inflammation (2). Only the VA group had animals with local reactions at day 3. However, no significant differences were observed between groups with regard to any of these local parameters.

Conclusions

The results obtained in this study show that VEPURED® is the safest vaccine alternative, as animals vaccinated with that vaccine showed less temperature increase and fewer local reactions than commercial vaccine A.

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Comparative trial of lung lesions associated with *Mycoplasma hyopneumoniae* at slaughter in pigs vaccinated with different *Mhyo* vaccines in Peru

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Introduction

Mycoplasma hyopneumoniae (Mhyo) is the primary agent involved in enzootic pneumonia (EP) and one of the leading agents involved in Porcine Respiratory Disease Complex (PRDC). EP directly impacts the productive and economic performance of swine farms, reducing the average daily weight gain and increasing the conversion ratio, and consequently increasing the days that the pigs have to stay on the farm to reach the final slaughter weight[1].

The aim of this study was to compare lung lesions associated with *Mhyo* at slaughter when applying different Mhyo/PCV2 vaccines in pigs from Peru.

Materials and Methods

A farrowing-to-finish farm of 1,500 sows in Peru, with circulation of *Mhyo* that was using an intramuscular vaccine against Mhyo of 2 ml per dose (Vaccine A) and switched to Mhyosphere® PCV ID (Vaccine B), an intradermal vaccine against *Mhyo* and PCV2, all in one, in 0.2 ml per dose. Apart from the change in the vaccine used, there were no other notable changes in management or treatment on the farm.

The lung lesions of pigs given Vaccines A and B were monitored at the slaughterhouse. The assessment of the lesions was done by the same person and with a blinded method, so that the evaluator did not know which vaccine had been administered in each case. The following parameters were analyzed: lesion incidence (lungs [%] with lesion), mean lesion (mean lesion grade among all lungs), and lesion rate (mean grade among all affected lungs). The modified Madec systemwas used to evaluate the lung lesions[2,3]. Moreover, the economic cost was calculated based on the number of lungs on each grade based on Straw *et al.*[4]

Statistical analysis was performed using the R software program.

Results

The mean lesions consistent with *Mhyo* were reduced from 0.82 to 0.24 with Vaccine B ($p<0.001$) (Table 1), which means a reduction of 70.7 %. Moreover, thelesion rate decreased from 1.37 to 1.14, and the incidence from 59% to 21% ($p<0.001$) with a significant reduction of 64.4%. Finally, the extra cost per pig due to Mhyo lesions, went from 1.97 € to 0.58 €

Table 1. Lung parameters by vaccine treatment and statistical analysis.

Parameter	Vaccine A	Vaccine B	P-value
Nº of lungs	390	100	
Mean lesion	0.82	0.24	<0.001
Lesion rate	1.37	1.14	0,10
Incidence	59%	21%	<0.001
Extra costper pig (€)	1.97€	0.58€	

The distribution of the lung lesion grades (Figure 1) was different with the two vaccines, being lower with Vaccine B, with less than 25% of animals on grade 1, 2 or 3. Furthermore, Vaccine B had no animals on grade 4.

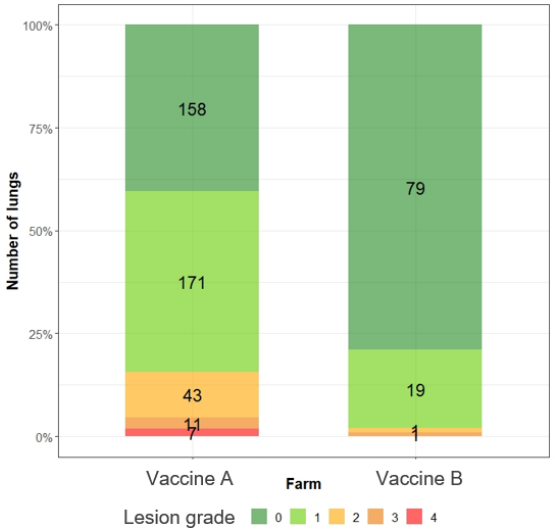

Figure 1. Distribution of the animals by lesion grade and vaccine.

Discussion and Conclusion

The new intradermal vaccine against *Mhyo* and PCV2, Mhyosphere® PCV ID (Vaccine B), significantly reduced the mean lesion and the incidence of *Mhyo*-lung lesions up to the time of slaughter compared to Vaccine A, and consequently decreased the extra cost per pig associated with *Mhyo*, which is very important to keep the economic productivity of swine farms.

Acknowledgments

The authors wish to thank the company GRANJA ISAMISA - PERU

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Comparative trial of Mhyosphere® PCV ID safety under field conditions in Canada

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Introduction

Porcine respiratory disease complex (PRDC) is a multifactorial disease resulting from the interaction of different infectious agents, management and environmental conditions, and is considered a major health concern. *Mycoplasma hyopneumoniae* (*Mhyo*) and Porcine Circovirus type 2 (PCV2) are among the main causes of PRDC[1]. Currently, control of these disease is mostly accomplished by improvement in management practices, good environmental conditions and immunizing prophylaxis by vaccination. These vaccines are mostly applied after weaning, a stressful and critical moment for piglet's performance. Therefore, the safety of these vaccines could affect the performance of these animals after weaning.

The aim of this study was to demonstrate the safety, through growth performance and mortality, of Mhyosphere® PCV ID and different *Mhyo*-PCV2 RTU vaccines under field conditions in Canada.

Materials and Methods

A farrow-to-finish farm of 325 sows located in Canada positive of *Mhyo*, PCV2 and PRRS was selected. A total of 484 healthy piglets of 3 weeks of age from different parity sows were distributed in 4 different groups (day 0) taking into account gender and weight. At day 2, group A (n=121) was administered with 0.2 ml of Mhyosphere® PCV ID, an intradermal needle-free vaccine against *Mhyo* and PCV2 related diseases, all in one. Group B (n=121) was administered with 2 ml of a commercial intramuscular vaccine against *Mhyo* and PCV2, ready-to-use (RTU), Group C (n=121) was vaccinated with 2 ml of another different commercial intramuscular vaccine against *Mhyo* and PCV2, RTU, and group D (n=121) were pigs no vaccinated used as a control group.

The safety of each vaccine was measured through the weight of the pigs during the first 15 days. The pigs were weighed at day 9 and 15 of the study. The mortality was also recorded during these 15 days.

The statistical analysis was done by using R program.

Results

In order to know if there were significant differences of growth between the control non-vaccinated group and the different vaccines, a linear regression model was performed with vaccine and initial weight as factors.

Regarding the average daily weight gain (ADWG) between days 0-9, the initial weight was a significant factor as well as the vaccine. Considering the control group as the reference (Table 1), there was a significant and relevant reduction of ADWG with Vaccine B (-23.66 g/day), and relevant but not significant differences with Vaccine C (-16.70 g/day). Neither significant nor relevant differences with MHYOSPHERE® PCV ID (+2.51 g/day).

	Estimate	Std. Error	P-value
Intercept	131.32	20.68	<0.001
Weight	7.61	3.47	0.029*
Mhyosphere® PCV ID	2.51	10.17	0.805
Vaccine B	-23.66	10.17	0.02*
Vaccine C	-16.70	10.17	0.101

Table 1. Statistical analysis of ADWG during the first 9 days of the study.

Non-significant differences on ADWG (9-15 days) between groups were observed. Regarding mortality, there were no significant differences between groups during the studied period.

Discussion and Conclusion

MHYOSPHERE® PCV ID was the only vaccine without significant nor relevant differences on ADWG compared to the control group during the first 9 days after weaning; therefore, this vaccine demonstrated to be a solution to immunize against *M.hyo* and PCV2 related diseases without interfering with the growth of pigs during the entry into the nursery phase.

The reason behind the observed differences was not investigated in this study, however, it is known that the route of administration, intradermal, that has already demonstrated an advantage in terms of animal welfare[2], but also the formulation of the vaccine (active substance and adjuvant) could influence the higher performance of piglets vaccinated with MHYOSPHERE® PCV ID.

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Comparing side effect of two vaccine products for Atrophic rhinitis in Korean swine farm

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Introduction

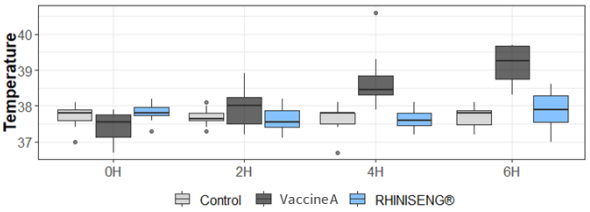
Atrophic rhinitis in swine caused by *B.bronchiseptica* (Bb) and *P.multocida* (Pm) causes damage to a pig's nasal turbinals, which makes it easier for pathogens such as *Streptococcus suis*, *Mycoplasma hyorhinis*, *Haemophilus parasuis* to infect through the pig's respiratory tract[1]. To prevent atrophic rhinitis and the following respiratory infection, vaccine against Bb and Pm are widely applied to pregnant sows on Korean swine farms, to promote piglets' passive immunity through colostrum intake[2]. However, it is frequently observed that certain vaccine products cause side effects such as anorexia and abortion following increased sow body temperature after vaccination[3]. The objective of present study was to compare the side effects of two types of vaccine that represents vaccine containing oil-based adjuvant and vaccine containing aqueous adjuvant, respectively, which are two major forms of vaccine for atrophic rhinitis in South Korea.

Materials and Methods

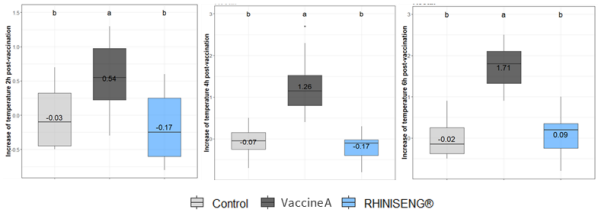
To compare safety of two different vaccines, a commercial farm that had 400 sows (Chungnam province, South Korea) with a healthy breeding herd was selected. 30 pregnant sows in similar parity and in similar reproductive cycles, 4-5 weeks before farrowing, were divided randomly into three groups consisting of 10 sows. "Vaccine A" group and "Rhiniseng" group were vaccinated with vaccine product containing oil-based adjuvant with α-tocopherol and Rhiniseng® (Laboratorios Hipra, Spain) containing HIPRAMUNE G®, respectively, following manufacturers' instruction. The "Control" group was left unvaccinated. To estimate side effect after vaccination, rectal temperature of all sows in each group was measured just before vaccination(0H), 2 hours(2H), 4 hours(4H) and 6 hours(6H) after vaccination. When the measured rectal temperature was over 40℃, it was interpreted that the sow had a fever. To analyze the difference of body temperature by each group and timing, linear regression model and Anova(turkey) as a post-hoc test were performed.

Results

At 0H and 2H, there were no differences in rectal temperature between groups and none of sows showed to have a fever. However, a higher average rectal temperature was observed in Vaccine A group (38.71℃) in comparison to Rhiniseng® group (37.64℃) and control group (37.65℃). One sow in A group showed fever (40.6℃) at 4H. At 6H average rectal temperature was still higher in A group (39.16℃) than Rhiniseng® (37.90℃) and control (37.7℃) group respectively. Overall, increased average rectal temperature over time was seen in Vaccine A group (Figure 1). Regarding average increase of rectal temperature between 0H and each timing (2H, 4H and 6H), higher increases in temperature were observed in Vaccine A group (0.54℃ at 2H, 1.26℃ at 4H, 1.71 at 6H) than in Rhiniseng® (-0.17℃ at 2H, -0.17℃ at 4H, 0.09℃ at 6H) and control group(-0.03℃ at 2H. (Figure 2 and Table 1).



[Figure 1]. Rectal temperature by time after vaccination in each group



[Figure 2]. Average increase of rectal temperature between 0H and each timing (2H, 4H and 6H)

[Table 1]. Summary of average rectal temperature compared in each group and timing

Time	Control	Vaccine A	RHINISENG®	P-value*
0H	37.72 ± 0.34	37.45 ± 0.41	37.81 ± 0.25	0.064
2H	37.69 ± 0.25	37.99 ± 0.57	37.64 ± 0.36	0.145
4H	**37.65 ± 0.41**	**38.71 ± 0.77**	**37.64 ± 0.27**	**<0.001**
6H	**37.7 ± 0.3**	**39.16 ± 0.54**	**37.9 ± 0.49**	**<0.001**
Increase 0_2H	**-0.03 ± 0.44**	**0.54 ± 0.55**	**-0.17 ± 0.53**	**0.010**
Increase 0_4H	**-0.07 ± 0.39**	**1.26 ± 0.76**	**-0.17 ± 0.34**	**<0.001**
Increase 0_6H	**-0.02 ± 0.44**	**1.71 ± 0.54**	**0.09 ± 0.53**	**<0.001**

Discussion and Conclusion

The late gestation period (4-5 weeks before farrowing) is of the upmost importance as this is when fetal daily weight gain becomes substantial in a sow's uterus. Therefore, if vaccination in this period causes an increase in sow body temperature, not only will the risk of abortion increase, but also the lack of sow appetite could decrease birth weight and vitality of newborn piglets. For this reason, efficacy of vaccine, in conjunction with the safety of vaccine should be considered when choosing a vaccine against Atrophic Rhinitis.

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Comparison of immune response against Swine Erysipelas by ERYSENG® PARVO and a Korean live attenuated vaccine against Swine Erysipelas + Classical Swine Fever

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Introduction

Immune protection against Swine Erysipelas (SE) is commonly carried out on most of the South Korean swine farms with a live attenuated vaccine combining antigens against *Erysipelothrix rhusiopathiae* and Classical Swine Fever virus (SE + CSF) (1). For the prevention of reproductive problems, almost every gilt is vaccinated at least once in the acclimation period and then a booster is administered in every cycle (1).
The objective of the present study was to compare the immune response elicited by a local live attenuated vaccine against SE + Classical Swine Fever (CSF) and, a novel inactivated bivalent vaccine against SE and Porcine Parvovirus (PPV), ERYSENG® PARVO.

Materials and Methods

A total of 30 healthy seronegative to SE gilts, from a Korean farm with 350 sows located in Gyeongnam province, were randomly divided into 3 groups. 'Control' group, non-vaccinated against SE during the trial. 'Competitor' group, vaccinated with a combined local live attenuated vaccine against SE+CSF. And a third group, vaccinated with ERYSENG® PARVO, a bivalent vaccine recently registered in South Korea, containing inactivated antigens against SE and PPV, and adjuvanted with HIPRAMUNE® G. Both groups were vaccinated on the 3rd week after arrival on the farm, during the quarantine period, according to the manufacturer's instruction with 2 doses apart. Blood samples from the 3 groups were collected 3 weeks after the second dose, and the quantity of antibodies against SE were measured using an indirect ELISA kit (CIVTEST® SUS SE/MR) in a Korean laboratory. The ELISA results were interpreted as positive when IRPC value were > 40. The ability of this kit to detect anti-SE antibodies without bias has been previously reported (2). To compare positivity rate and IRPC titer of each group, logistic regression and t-test were performed respectively.

Results

The positivity rate in the ERYSENG® PARVO group was higher than in the competitor group (100% and 30%, respectively, *p-value*=0.005) (Figure 1). The average IRPC titer in each group showed a similar pattern. ERYSENG® PARVO group had higher average IPRC titer (96.85) than competitor group (30.97) (*p-value*<0.001) (Figure 2). All the animals in "Control" group remained negative at the moment of blood sampling, which proves that there was no exposure to the field bacterium, and the immune response in both vaccinated groups was exclusively due to the vaccines.

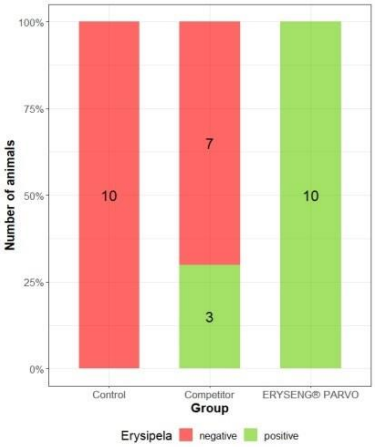

Figure 1. Columns representing the positivity rate in each group

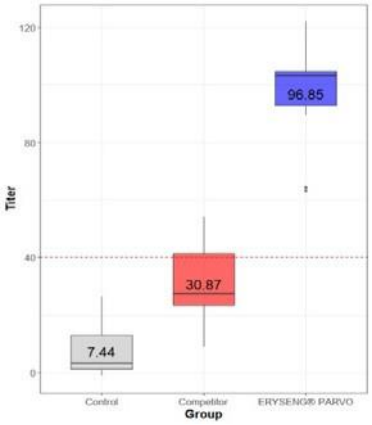

Figure 2. Boxplots of SE IRPC titer in the 3 different groups.

Discussion and Conclusion

Based on these results ERYSENG® PARVO elicits higher immune response against SE, which is key to controlling infectious reproductive problems in sows (3) These differences could be explained by the type of vaccine (live attenuated vs inactivated), the paper of the adjuvant and the quantity or type of antigen.

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Comparison of PRRS vaccination strategies of sows

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Background and Objectives

The objective of this study was a comparison of different PRRS vaccination strategies to stabilize the disease in one farm experiencing PRRS outbreaks in spite of a MLV vaccination program.

Material and Methods

The trial was realized in an industrial pig farm (farrow to finish), with a population of 100 000 pigs, in Russia under a PRRS disease outbreak. PRRS status of farm was Positive Unstable (I-A) by Holtkamp, et al. (2021).
T1 Group: a modified live vaccine (MLV), mass vaccination with one dose every three months two times.
T2 Group: a modified live vaccine (MLV) at 60 days of gestation with revaccination by inactivated vaccine "PROGRESSIS" (IV) at 90 days of gestation.
Treatments were applied according to an alternate pattern.
The parameters under scrutiny were average daily gain (ADG) and mortality rate obtained in different batches of the post-weaning: 36 (T1), 42 (T2), weaned between 29 October 2020 and June 2021. Results were analysed by means of an ANCOVA model including ADG or %mortality as independent variables, entrance weight as covariate, treatment and technician as factors.

Results

According to our model, body weight at entrance and the technician are not significant parameters considering average daily gain (ADG) as the response variable.
ADG (g/d) results (mean±SD) were: α=0.05, 304±43.3, 342±50.0 for T1 and T2 respectively, $p<0.001$
Mortality (%) results (mean±SD) were: α=0.05, 10.3±5.43, 6.49±6.92 for T1 and T2 respectively, $p=0.474$.

Discussion and conclusion

Using a combination of MLV and IV led to a significative increase of ADG at postweaning ($p<0.001$), while mortality did not have a significative reduction ($p=0.474$)
These differences could be because of the dual effect induced by the KV vaccination on MLV vaccinated sows[1,3]. Analogous treatment performed better with time, what can be a sign of stabilization[2,4,5].

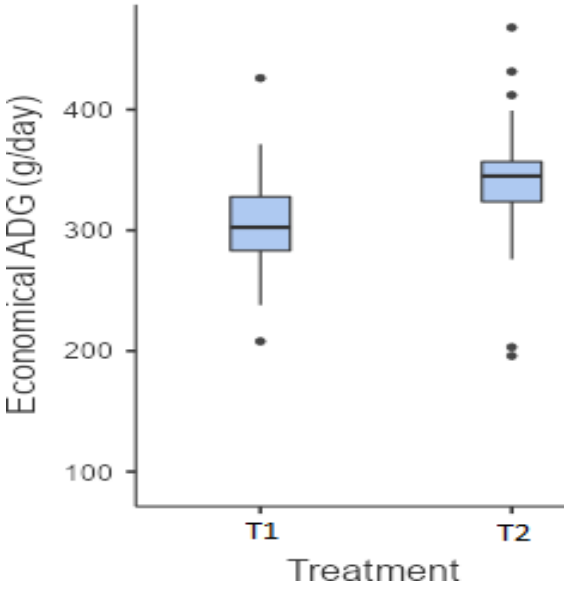

Figure 1. Economical ADG (g/day) boxplots for T1 and T2 pigs.

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Comparison of the efficacy of two PCV2 vaccines in a PRRSV and PRV free farm

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Introduction

Porcine circovirus type 2 (PCV2) is ubiquitous in swine farms around the world, causing growth retardation, decreased average daily gain (ADG) and increased mortality. Efficient vaccination is an important way to control the disease (1). PRRSV and PRV infection will interfere with the evaluation of PCV2 vaccine in the field. The aim of this study is to compare the efficacy of two PCV2 vaccines in a pig farm where both PRRS and PR are negative.

Materials and Methods

The trial was implemented in a large-scale pig farm in Central China with two-site production model in 2021. 885 piglets from a fattening house around 28 days old were selected for a randomized double-blind trial. The piglets were separated by gender with double ear tags and divided into 3 groups, vaccinated at 28 days old: group A= PCV2 vaccine A (0.5 ml/dose), group B =PCV2 vaccine B (1 ml/dose), and group C= Control group (no PCV2 vaccination). Piglets were weighted at the time of vaccination and reweighed before slaughtering. Average daily gain (ADG) and mortality were compared among groups, analyzed using student's t-test. During the age of 100-170 days, the S/P value of PRRS and gE S/N value of PR were detected. The antibody and qPCR of PCV2 were also measured to assess the infection pressure by serum or saliva sampling.

Fig.1 S/P of PRRS and gE S/N value of PR were detected

Results

During 100-170 days of age, the gE S/N value of PR and the S/P value of PRRS were both negative, ruling out the interference of these two diseases. After 120 days old, the PCV2 antibody level and viral load increased, some sample's viral load was higher than 10^5 or even 10^7 copies/ml (data not shown), indicating that the pig herds were undergoing a certain pressure of PCV2 infection. The ADG of control group was 851.63g, lower than the two vaccine groups, statistical significant ($p<0.01$). The ADG of group A and group B were 883.62g and 890.51g, respectively, no significant difference ($p>0.05$) (Table 1).

Table 1: ADG (g/day) in different groups

	Group A （n=273）	Group B （n=268）	Control （n=269）
ADG	883.62[a]	890.51 [a]	851.63[b**]

**Statistical significance between a and b ($p<0.01$）

The mortality rate of two vaccine groups was lower than control group, and group A was 0.73% lower than group B (Table 2).

Table 2: Mortality rate in different groups

	Group A （n=294）	Group B （n=290）	Control （n=301）
Mortality	2.72%	3.45%	3.99%

Discussion and Conclusion

PCV2 is the primary causative agent of PCVD. The evaluation of the efficacy of piglet's vaccination will be affected by herd selection, environments, especially PRRSV and PRV infections. This trial compares ADG and mortality of two PCV2 vaccines in a PRRSV and PRV free pig farm. It shows that whether vaccine A or vaccine B, can provide high-efficiency protection for the herds, and the efficacy is very closed. This is consistent with previous research (2).

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Comparison of the immune response against Swine Erysipelas elicited by ERYSENG® PARVO andlocal live vaccines in China

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Introduction

Prevention of Swine Erysipelas (SE) is best accomplished by immunization programs (1). It hasbeen previously reported that the immune response elicited by vaccines against SE can vary (2). The presence of SE seronegative breeding animals on vaccinated farms, means that these negative animals are susceptible to suffering clinical signs related to SE, which could cause major reproductive losses with a huge economic impact (3).

The aim of this study was to compare the immune response against SE elicited by different commercial reproductive vaccines (inactivated vs live vaccines) under field conditions in China.

Material & Methods

A total of 33 farms and 1,105 animals were included in the study. 11 farms and 362 sows (parity 0 to 8) that were vaccinated with ERYSENG® PARVO (Group A), an inactivated bivalent vaccine against SE and Porcine Parvovirus with Hipramune® G[d], a ginsenoside base adjuvant. The other 22 farms, 743 animals (parity 0 to 8) were vaccinated with 7 different Chinese live vaccines (Group B) including *Erysipelothrix rhusiopathiae* antigens. All the samples were collected from March 2020-June 2021.

Sera samples were collected at similar moments of the cycle in both groups and tested using a commercialized ELISA kit (CIVTEST® SUIS SE/MR; Cut off-IRPC: 40). The ability of this kit to detect anti-SE antibodies without bias has been previously reported (4). An anova regression model with the Dunnett post-hoc test was performed for the titer analysis, an anova logistic model with the Dunnett post-hoc test for the positivity analysis, using a *P-value=0.05* for both.

Results

The percentage of positive animals in group A was 93% compared to 45% in group B, with this difference being statistically significant (*p-value < 0.001*) (Figure 1). The positivity rate varied depending on the farm from 83% to 100% in group A, being in all the farms higher than 80%. In group B, only 1 of the 22 farms had a positivity rate higher than 80%, the rest varied between0% to 71% (Figure 1). The average ± SD titers of farms in group A was 107 ± 35 while in group B it was 45 ± 34 (Figure 2), with this difference between groups being statistically significant (p-value<0.001).

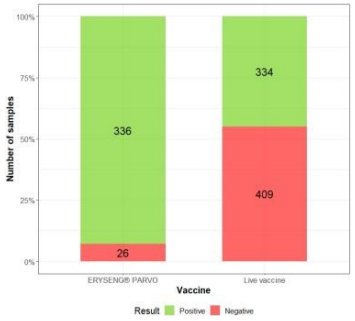

Figure 1. Columns representing positivity rate using ERYSENG® PARVO and Chinese live vaccines

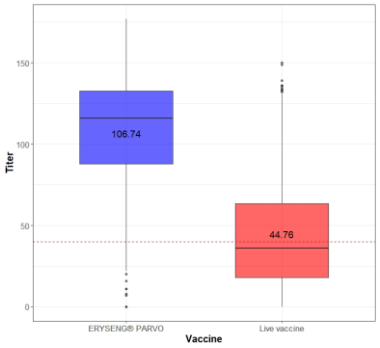

Figure 2. Boxplots of SE serology of farms using ERYSENG® PARVO and Chinese live vaccines.

Discussion & Conclusion

The humoral immune response against *E. rhusiopathiae* with ERYSENG® PARVO was much higher than the one from Chinese live vaccines. This could be due to a different recognition of the antigen by the animals, or a different effect of the adjuvants. Having higher SE titers suggests lower negative subpopulations (5), meaning a lower risk for the breeding herd. Based on these results, the use of novel vaccines in China such as ERYSENG® PARVO can help the protection against SE. It has been recently reported in Chinese studies, that the traditional inactivated vaccines and attenuated vaccines commonly used there, have many shortcomings and defects, resulting in an unsatisfactory protection against SE (6).

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Comparison of the protection against PCV2 or *Mycoplasma hyopneumoniae* experimental infections with the injectable or intradermal PCV2/M.hyo vaccines

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Introduction

PCVD due to PCV2 virus and Enzootic pneumonia due to primarily *Mycoplasma hyopneumoniae* (M.hyo) are the most common diseases in current swine herds causing huge and economic losses. Vaccination against those two pathogens helps to reduce their clinical manifestation and decrease of performance (1,2). Several commercial mono- or bi-valent vaccines are available. The aim of this study was to compare the efficacy of injectable PCV2 and M.hyo RTM vaccines with the intradermal(ID) PCV2/M.hyo RTU product.

Material and Methods

Piglets were vaccinated either with Circovac® mixed with Hyogen®(DUO) - both Ceva or an ID vaccine containing inactivated recombinant M.hyo expressing cpPCV2(Mhyo/PCV ID) at 3 weeks of age(WOA). Vaccinated and positive control pigs were challenged at 7(WOA) either with the PCV2d strain or with the M.hyo culture. Serum samples were collected prior to vaccination, challenge, and slaughter, and measured and IDVet Mycoplasma hyopneumoniae ELISA kits. Pigs were always euthanized 28 days post infection (DPI) and either PCV2 loads in lymph nodes (Lnn) or lung lesions scores (LLS) according to the EuPh 9.0 were measured to assess the efficacy.

Results

The PCV2 viremia at 28DPI was 0.93 and 0.69 log copy#/microL for DUO and Mhyo/PCV ID respectively(p>0,05). The PCV2 loads in mediastinal, mesenterial Lnn and tonsillar swabs were 5.99, 5.19 and 2.80 log copy#/microL for DUO and 6.02, 5.05 and 2.59 log copy#/microL for Mhyo/PCV ID respectively(p>0,05).

Fig.1. PCV2 indicators for the DUO group

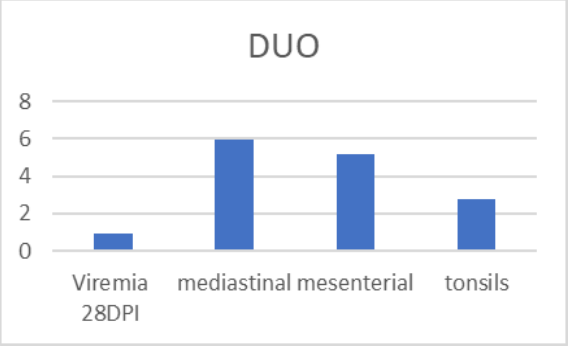
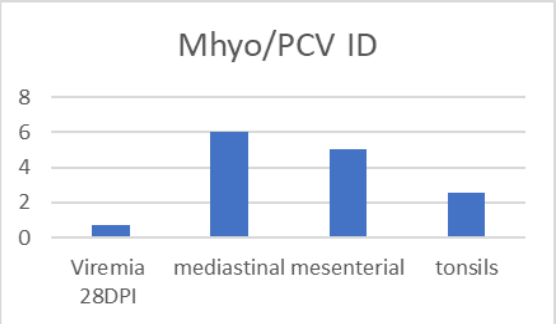
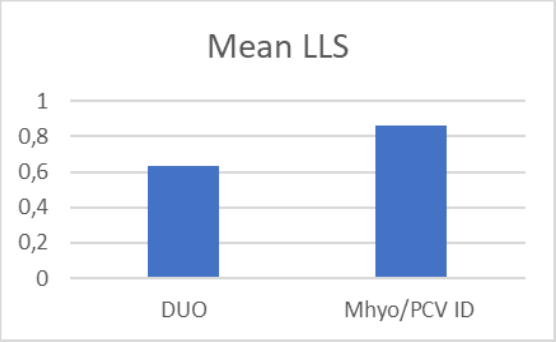


Fig 2. PCV2 indicators for the Mhyo/PCV IDgroup



DUO induced M.hyo seroconversion before the challenge (97% seropositivity), while Mhyo/PCV ID did not (0%) and those pigs became seropositive only after the M.hyo infection. Mean LLS were 0.63 and 0.86 for DUO and Mhyo/PCV ID respectively(p<0,05 for DUO vs any other groups).

Fig 3. Mean Lung lesion scores



Only DUO differed significantly from the positive non-vaccinated control in LLS.

Discussion and Conclusion

This study demonstrated that whole virus and bacterin based injectable vaccine provided equal PCV2 protection as Mhyo/PCV ID. DUO however significantly outperformed the recombinant intradermal vaccine in the potency to induce strong immune response and in the protection against the development of lung lesions due to M.hyo.

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Construction and Stable Expression of Mammalian Cell-based Secretory Classical Swine Fever Virus Envelope Glycoprotein E2 to Enhance Antigenicity and Performance of Enzyme-linked Immunosorbent Assays

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Introduction

Classical swine fever virus (CSFV) belongs to the family *Flaviviridae* and the genus *Pestivirus* and is a single positive-strand RNA virus that can be divided into three major genotypes and each have 4-7 sub-genotypes. The major envelope glycoprotein E2 of CSFV plays an important role in cell attachment, eliciting immune responses, and vaccine development (1, 2). In this study, to produce the secretory viral glycoprotein with the most native structure, bioactivity, and fully post-translational modifications, a mammalian cell-based system was used for expressing ectodomain of different genotypes of CSFV E2 proteins. The antigenicity of these mammalian cell-based CSFV E2 glycoproteins was compared with a commercial E2 based ELISA using a panel of IFA-confirmed serum derived from naïve and Lapinized Philippines Coronel (LPC) strain live attenuated vaccine (LAV) immunized pigs.

Materials and Methods

Sequences of E2 encoded region from G1.1, G2.1, G2.1d, and G3.4 were modified by truncation of the transmembrane domain and replacement of the original signal peptide with part of human tissue plasminogen activator sequence. The modified sequences were ligated onto pcDNA™ 3.1/V5-His TOPO® TA mammalian expression vector (Invitogen, Carlsbad,CA, USA) The expression of CSFV E2 proteins by HEK-293 cell line was determined by ICC and western blot and purified by resin affinity binding (Thermo Scientific, MA, USA). The mammalian cell-based CSFV E2 glycoproteins were used to develop inhouse ELISAs and the performance of ELISAs was compared with a commercialized CSFV E2 (Com. E2) based ELISA using a panel of serum samples (n=192) derived from naïve and LPC vaccinated pigs characterized by IFA in LPCV infected PK-15 cells.

Results

Four HEK293 cell lines stably expressing different genotypes of CSFV E2 glycoproteins were successfully generated. These proteins were secretory and expressed yield about 3-4.5 mg/L in the supernatant.

The result of different CSFV E2 ELISA as compared to IFA staining result are shown in table 1. Using IFA result as standard, the CSFV genotype 1.1 E2 based ELISA had a cut-off value of 0.71 with sensitivity of 82.8% and specificity of 97.6%. The CSFV 2.1 E2 based ELISA had a cut-off value of 0.71 with sensitivity of 86.0%, and specificity of 97.6%. The CSFV 2.1d E2 based ELISA had a cut-off value of 0.70 with sensitivity of 82.8% and specificity of 95.3%. The CSFV 3.4 E2 based ELISA had a cut-off value of 0.66 with sensitivity of 86.0% and specificity of 97.6%. All inhouse E2 glycoproteins-based ELISA showed higher sensitivity and specificity as compared with the commercially available CSFV E2 ELISA.

Discussion and Conclusion

In this study, secretory CSFV E2 glycoproteins with the most native structure and fully post-translational modifications have been successfully constructed, expressed, and purified using a mammalian cell-based system. Using these E2 proteins as antigens, the performance of these inhouse CSFV E2 ELISAs was higher than the commercialized ELISA kit.

Using the mammalian expression system of HEK-293 had provided us with the unique opportunities for the E2 proteins to process complex multi-dimensional folding and post translation modifications. By removing the transmembrane domain of E2 glycoproteins, it allows the glycoproteins to be released into the expression medium, increasing the yield, and eliminating the complex process of cell lysate.

The results indicate the CSFV E2 is a suitable candidate for ELISA production and the better antigenicity of the mammalian cell-based E2 secretory glycoproteins could be potential subunit vaccines.

Table 1. The summary of the result of serological assays in each group. (+): positive; (-); negative; Com. E2: commercialized CSFV E2 ELISA

	ELISA Result	Com. E2	1.1 E2	2.1 E2	2.1d E2	3.4 E2
IFA + n=93	+	71	77	80	77	80
	-	22	16	13	16	13
IFA - n=99	+	16	8	9	13	11
	-	83	91	90	86	88
Sensitivity		76.3	82.8	86.0	82.8	86.0
Specificity		83.8	91.9	90.9	86.9	88.9
Accuracy		80.2	87.5	88.5	84.9	87.5

Acknowledgments

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Convenience and economic benefit of early one-shot *Mycoplasma hyopneumoniae* vaccination at 3 days of age in a commercial sow farm

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Introduction

Mycoplasma hyopneumoniae (*M. hyopneumoniae*) isthe primary pathogen of enzootic pneumonia, a chronic respiratory disease in pigs. Vaccination of piglets to protect against *M. hyopneumoniae* can be performed at several ages, depending on product label specifications (1). Early intervention in the first week of life may have advantages, since piglets can already become infected with *M. hyopneumoniae* during the suckling period, resulting in a significant percentage of *M. hyopneumoniae*-positive piglets around weaning (2-5). The current study compared convenience and economic benefits of *M. hyopneumoniae* vaccination in piglets of 3, 7 and 14 days of age.

Materials and Methods

The study was performed on a 2000-sow unit operating in 1-week batch management system with approx. 100 sows per week batch. Each study group (vaccination at 3, 7 and 14 days of age) included 20 sows and their respective suckling piglets. At 3 days of age, piglets were vaccinated during the regular processing activity, whereas at 7 and 14 days of age, piglets had to be vaccinated during a special vaccination moment. Total time to vaccinate the litter was recorded, including the number of piglets in the litter. Duration of vaccination per piglet at each specific vaccination moment was calculated. Based on these results, an economic calculation of vaccination cost at each age was performed taking into account pre-weaning mortality, labor cost, cost of vaccine doses and time needed to perform the vaccination.

Results

Duration of piglet vaccination at 3 days of age was significantly ($P < 0.05$) shorter (2.64 ± 0.08 seconds) as compared to 7 days of age (4.90 ± 0.18 seconds) and 14 days of age (6.04 ± 0.22 seconds). Economic calculation in a 1000-sow unit, using a vaccination convenience calculator, demonstrated that although the total number of piglets vaccinated is lower (- 443 and - 838 at 7 and 14 days of age, respectively) at a later vaccination age, the related increase in vaccine cost in the early vaccination group (3 days of age) was largely compensated by the decrease in cost of overall vaccination time ($ 1,115.61 and $ 1,461.00 lower at 3 days of age as compared to 7 and 14 days of age, respectively).

Discussion and Conclusion

In conclusion, *M. hyopneumoniae* vaccination at 3 days of age has several advantages over later vaccination at 7 or 14 days of age. Besides the benefits in convenience of piglet handling at that age, we could also demonstrate economic benefits of early *M. hyopneumoniae* vaccination.

Table 1. Vaccination convenience calculator output generated with the following performance imput variables: 1,000 sows, 33.5 piglets weaned per sow per year, 13.1% pre-weaning mortality.

	Group 1	**Group 2**	**Group 3**
Vaccination age of piglets	Day 3	Day 7	Day 14
Sow production parameters			
Number of sows	1,000	1,000	1,000
Weaned pig/sow / year	33.5	33.5	33.5
Total pigs weaned / year	33,500	33,500	33,500
Total pigs born / year	37,889	37,889	37,889
Piglet mortality			
Dead pigs (d 0-weaning)	4,389	4,389	4,389
Dead pigs (d 1-3)	3,348	3,348	3,348
Dead pigs (d 4-7)	443	443	443
Dead pigs (d 8-14)	395	395	395
Dead pigs (d 15-weaning)	202	202	202
Dead pigs vaccinated	1,040	597	202
Piglet vaccination parameters			
Total pigs vaccinated	34,540	34,097	33,702
Duration (s) vaccination / pig	2.64	4.90	6.04
Total duration (h)	25.33	46.41	56.54
Total working days (1 person)	3.33	6.11	7.44
Cost vaccination time ($ / worker	633.23	1160,24	1413.61
Total cost vaccination time ($ for total workforce)	1,899.70	3,480.72	4,240.82
Total cost vaccination time / weaned piglet ($) for total workforce	0.570	0.104	0.127
Total cost of vaccination (incl. vaccine & labour)			
Total vaccine cost ($ / year)	36,267.08	35,801.68	35,386.96
Cost vaccination dose / weaned piglet ($)	1.083	1.069	1.056
Total vaccination cost (time + vaccine dose) / weaned piglet ($)	1.139	1.173	1.183
Total vaccination cost / year ($)	**38,166.78**	**39,282.40**	**39,627.78**

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CSF vaccine based on E2 recombinant glycoprotein and adjuvanted with oil-in-water emulsion induces a full protection in a pig field trial

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Abstract
Classical swine fever (CSF) is a highly contagious viral disease which causes important economic losses in the pig industry. Even if many countries have succeeded in eliminating CSF, it remains endemic in South and Central America, Eastern Europe and Asia. Systematic prophylactic vaccination is the most effective strategy to control CSF in the endemic zones. In this aim, live attenuated vaccines have been widely used to control the disease but these conventional vaccines fail to identify infected from vaccinated animals (Differentiating Infected from Vaccinated Animals). Thus, a new generation of CSF DIVA vaccines are needed. In this study, a recombinant vaccine, based on the highly immunogenic structural glycoprotein E2 of CSF virus, is assessed in a pig trial.

Two groups of 7 pigs are intramuscularly injected in the neck with 2 ml of the E2 recombinant CSF vaccine at day 0 (D0) and D21. The vaccines are either adjuvanted with a carbomer or with an oil-in-water emulsion (Montanide™ ISA 28R VG ; 15/85 w/w). A third control group is left unvaccinated. After each vaccination, the body temperature and the local reactions at the injection site are monitored. The body weight gain is also controlled at D21 and D35. Moreover, blood samples are taken at different dates in order to assess the E2 specific antibodies by ELISA. At D35, a lethal dose of Shi-Myn strain of the CSF virus is intramuscularly injected into the posterior femoral muscle. After challenge, the vaccine protection is evaluated by calculating the survival rate and by PCR detection of CSF virus in the blood samples and in the spleens and lymph nodes.

In terms of safety, the 2 adjuvanted vaccines were very well tolerated: no abnormality was noticed in the injection site, the body temperatures did not exceed one celsius degree after injections and the body weight gains were normal. Regarding the efficacy, vaccine groups showed a similar antibody profile with a positive threshold reached after the boost at D21. After challenge, only the vaccine adjuvanted with the Montanide™ ISA 28R VG induced a survival rate of 100% while that based on carbomer failed to fully protect the pigs (57%). The monitoring of the viremia also underlined an early and total clearance of the virus with the emulsion based vaccine compared to the carbomer vaccine.

Taken together, these results showed that E2 recombinant CSF vaccine associated with an oily adjuvant as Montanide™ ISA 28R VG is a good vaccine candidate and adapted to protect pigs from the disease while maintaining a good safety profile.

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Current situation against Swine Erysipelas in gilts and sows in Thailand evaluating antibody immune status

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Introduction

Swine Erysipelas (SE) is caused by *Erysipelothrix rhusiopathiae*. Erysipelas problems occur sporadically in the pig population, but there is evidence that more severe outbreaks have occurred in recurring intervals of approximately 10 years due to the restriction of antibiotic usage as a feed additive in pigs (1). In Thailand, research on the evidence of SE is rare and additional information is needed. The objective of the present study was to investigate the current situation of SE antibody status in gilts and sows in modern commercial swine herds in Thailand, vaccinating or not against SE.

Materials and Methods

The evidence of SE infection in 19 commercial swine herds in Thailand was investigated by analyzing serological data of serum samples submitted to HIPRA Diagnos between 2016 and 2020 (n = 2,229 samples). Antibody titer against SE was determined by using indirect ELISA (CIVTEST® SUIS SE/MR, Laboratorios Hipra S.A., Amer (Girona), Spain). The percentage of animals with positive antibody titers for SE (i.e., IRPC value >40) was analyzed in association with group of animals and herds. Serological profiles were collected from six groups of swine commercial herds in Thailand: A, B, C, D, E and miscellaneous. Herds A and B did not vaccinate gilts and sows against SE, while herds C, D and E vaccinated gilts and sows against SE. Miscellaneous herds performed different vaccination schedules. The statistical analyses were carried out by using SAS version 9.4.

Results

Of all the samples (n = 2,229), 40.3% (n = 898) were sero-positive for SE. The sero-negative pigs had an average antibody titer of 17.4 ± 10.5 (0 – 40), while the sero-positive had an average antibody titer of 78.4 ± 26.9 (41–155). The percentage of SE sero-positive animals was 48.4% in sows and, 35.4% in gilts. (P < 0001). The percentage of sero-positive sows was higher than gilts (P<0.001). In addition, the prevalence of SE was not different between herds vaccinated against SE and herds that did not vaccinate (43.1%, 203/444 samples versus 45.7%, 615/1427 samples, respectively, P=0.330).

Table 1 Prevalence of Swine Erysipelas sero-positive gilts and sows by herd

Herds	Vaccine	Gilts	Sows
A	No	21.7	73.3
B	No	61.6	NA
C	Yes	21.7	NA
D	Yes	62.7	59.6
E	Yes	28.2	NA
Misc.	Variety	21.8	25.4
Total		35.4	48.4

NA = not available

Conclusions and Discussion

Of all the tested samples from 19 commercial swine herds in Thailand, the prevalence of swine erysipelas was 40.3%. This is the first report on the prevalence of SE among commercial swine herds in Thailand during the past 20 years. The prevalence of SE was not different between herd vaccinated against erysipelas and herd that did not vaccinate against SE (43.1% versus 45.7%). The present study indicated a relatively high prevalence of SE in non-vaccinated herds in Thailand. In previous studies, in Lao PDR, the prevalence of Swine Erysipelas among small holder pig farm was 45.2% (2). This percentage is in a close agreement with the present study. This indicates that the control program of SE in commercial swine herds in Thailand should be raised. The presence of positive animals on farms that are not vaccinating is indicating that the bacterium is present on the farms and could be causing undiagnosed clinical problems. In those farms vaccinating against SE the type of vaccine and protocol of vaccination should be reviewed, as there is still a high percentage ofseronegative animals.

Acknowledgements

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Cytokine´s gene expression profile in tissues of piglets supplemented with *Saccharomyces cerevisiae boulardii* CNCM I-1079 and vaccinated against *Actinobacillus pleuropneumoniae*

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Introduction

Cytokines are inflammatory biomarkers critical for humoral and cell mediated immunity. Their expression may vary with factors like weaning, vaccines, or probiotic supplementation. The live yeast *Saccharomyces cerevisiae boulardii* CNCM I-1079 (LSB; Levucell®SB; Lallemand SAS, France) has effects on immune modulation. We studied the effect of LSB supplementation to sows and piglets on performance and cytokine's expression in piglets after vaccination against *Actinobacillus pleuropneumoniae* (APP).

Materials and Methods

Seventy-two mixed parity sows were supplemented (LSB) or not (CON) 2×10^9 CFU/kg during lactation. At weaning, piglets from each group were allotted to a control (CON) or LSB (CON + 2×10^9 CFU/kg and 1×10^9 CFU/kg in phase 1 (18 days) and 2 (days 19-42))diet. The piglets were vaccinated against APP (Coglapix, Ceva Santé Animale, France) on days 26 and 49 post-weaning. Fifteen pigs/treatment were individually weighted at the moment of each vaccination and 3 weeks after the second vaccination, when the pigs were humanely euthanized, and samples from lungs, lymphnodes and jejunum were taken. The RNA was isolated using a commercial kit (RNeasy Micro kit, Qiagen, Germantown, USA) and cDNA was synthetized to perform q-PCR using SYBR-green chemistry. The relative quantification of IFN-α, IFN-γ, TNF-α, IL-12p35, IL-12p40, IL-10, TGF-β, IL-8, IL-1α, IL-1β and IL-6 was quantified in tissues using β-actin as housekeeping gene. Data were analyzed in SPSS Statistics 26 (IBM). Body weight and growth were analyzed by a general linear model with sow diet, piglet diet, and their interaction as main effects; cytokine`s expression with a Kruskal-Wallis test for k unrelated samples. The experimental unit was the pig. Differences with a *P*-value <0.05 were considered as significant, and *P*-values between 0.05 and 0.1 were considered a trend.

Results

There was no interaction between sow diet and piglet diet. Piglets fed LSB grew faster between vaccinations and were heavier at the second vaccination compared to CON-fed piglets ($P<0.05$). In parallel, piglets from LSB-fed sows tended to grow faster and be heavier ($P<0.1$; Table 1) than piglets from CON sows in the same period. Besides, all the cytokines analyzed were less expressed in the lungs of the piglets from LSB-fed sows ($P<0.05$). There was no effect of piglet diet on cytokine´s expression.

Discussion and Conclusion

The lower expression of the cytokines in the lung indicates a reduced anti-inflammatory reaction in piglets from LSB-fed sows. We can attribute the effect to the sow diet because (1) reported that an infection with APP increased lung concentrations of IL-1β, IL-6, IL-8 and IFN-γ in piglets. Therefore, the lower response suggests that those piglets were less challenged and did not need to produce cytokines. Additionally, the improved performance of the piglets from LSB-fed sows are in line with (2), who reported that the expression of the immune biomarkers is not necessarily correlated with an improvement in performance, since their production may involve an increase in energy requirements. (3) reported that inflammatory response after weaning is transient, reverting later to a pre-weaning situation. Accepting weaning as an acute stressful event for the animals, and the administration of the vaccine as an acute management stress comparable to weaning, we can hypothesize that LSB supplementation to sows may have helped their piglets to speed up the process to recover the basal inflammatory status after the vaccine. Besides, LSB supplemented to sows is proven to affect piglet microbiota after weaning (4). Given that gut microbiota plays a role against respiratory infection in mice (5), the gut microbiota modulation in the piglets from LSB-fed sows may have affected their inflammatory response in the lungs. We conclude that feeding *Saccharomyces cerevisiae boulardii* CNCM I-1079 to sows may help their piglets to keep a basal inflammatory status in the lung after vaccination against APP, and to improve their performance, suggesting a maternal imprinting effect.

Table 1. Growth performance of piglets between the 1st and the 2nd vaccination

	CON	LSB	SEM	*P*-value
BW 1st vaccine (kg)	14.42	14.46	-	-
BW 2nd vaccine (kg)	**26.57**	**27.69**	**0.317**	**0.090**
ADG 1-2 (g/d)	**527**	**576**	**13.79**	**0.090**

Acknowledgments

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Detection of *Clostridium perfringens* Epsilon toxin induced antibodies as a method to check compliance in sows vaccinated against *Escherichia coli and Clostridium perfringens.*

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Introduction

The passive protection induced by vaccination of sows pre-farrowing against *Escherchia coli* and *Clostridium perfringens* on their progeny is well known (1). Of all commercial vaccines available, only one that includes *Clostridium perfringens* type D Epsilon toxin (2).

The objective of the present study was to measure the serological response to *Clostridium perfringens* type D Epsilon toxin contained in a *E. coli & Clostridium* commercial vaccine for sows and gilts as potential tool for vaccination compliances.

Materials and Methods

"Arauzo" farm, with 2,500 Iberian breed sows, in a production system producing weekly batches of 120 animals (sows and gilts). A laboratory test was carried out in serum and feces from piglets with neonatal diarrhea to ensure the absence of Epsilon toxin in the farm and confirm the presence of the pathogen in the piglets.

Afterwards, three batches were enrolled in the study; Group A, unvaccinated, Group B vaccinated with commercial vaccine without Epsilon toxin, and Group C vaccinated with commercial vaccine including Epsilon toxin (Gletvax 6).

The administration of the vaccine was carried out according to the label instructions.

Same sampling protocol was implemented in the three groups. Colostrum was collected from 10 sows at farrowing and 20 piglets (2 piglets per-litter) were bled one week after birth.

Epsilon toxin antibodies were detected by Bio K 222 Monoscreen AbElisa test from Bio-X Diagnostics (3). The interpretation of results is based sample´s positivity using inhibition percentages (<20 is negative, 20-40 +, 40-60 ++, 60-80 +++ and >80 ++++)

Results

Group A, colostrum and piglet blood samples were negative.

For Group B, 8/10 of colostrum samples and 17/20 of serum samples were negative, and all with lowinhibition percentages (20-41).

Group C, all samples were positive (colostrum andpiglet serum) showing high inhibition percentages.

Discussion and Conclusion

Presence of *Clostridium perfringens* Epsilon toxin is not common in pig farms. A Clostridium vaccine with Epsilon toxin induced specific antibodies, detectable by an ELISA test.

Under the conditions of this study, we found that the use of a ELISA test might be a useful tool to monitor sow vaccination compliance with Gletvax 6 and also a method to potentially measure adequate colostrum intake in piglets born to vaccinated sows

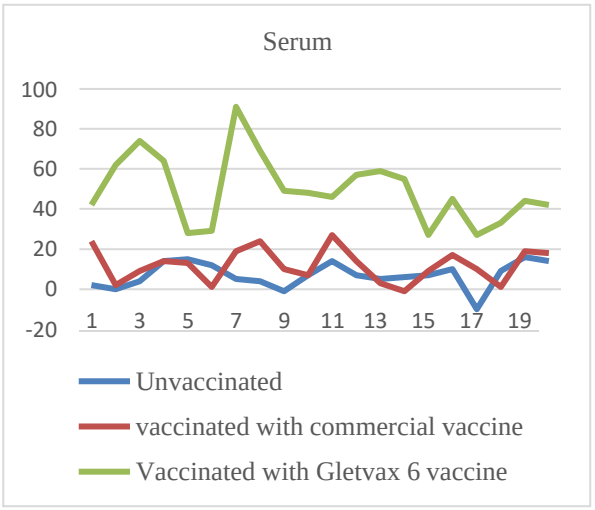

Table 1. Epsilon toxin induced antibodies were detected using inhibition percentages in serum, on three batches.

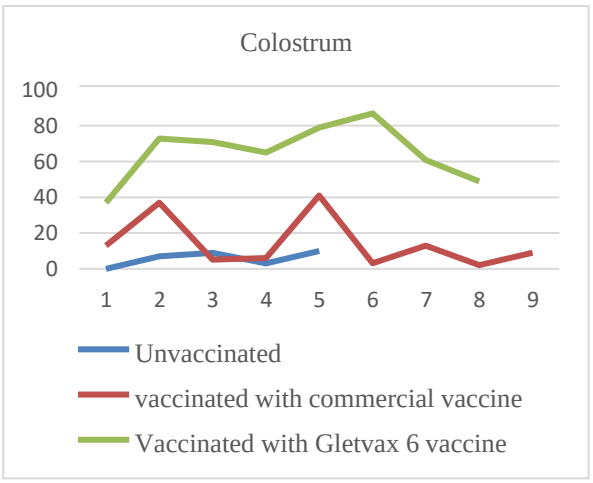

Table 2. Epsilon toxin induced antibodies were detected using inhibition percentages in colostrum, on three batches.

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Development of an ELISA for the detection of IgG antibodies against PCV2 Cap protein

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Introduction

The PCV2 belongs to the *Circoviridae* family, and their ORF2 encode for the capsid protein (Cap), the primary structural component target from the immune system. The PCV-associated diseases (PCVAD) are caused by the PCV2 and have high economic importance to the swine industry due to the losses generated and control measures costs (1). Vaccination against PCV2 has been widely used in the swine industry as an efficient strategy for reducing the incidence of PCVAD, and the determination of antibodies against Cap protein can help design better vaccination programs (2). This work aimed to producea recombinant Cap protein of PCV2 and develop an ELISA to detect IgG antibodies against PCV2.

Materials and Methods

Chemically competent cells *E. coli* BL21 Gold (DE3) were transformed by heat shock with 100 ng of plasmid pET28b(+)/t2ORF2 (encoding PCV2a Cap protein). They were seeded on LB agar plates with kanamycin and incubated for 24 h at 37°C. One colonywas selected to prepare the inoculum in 20 mL of LB medium and incubated for 16 h, at 37°C, at 220 rpm. Then, 10 mL cultures were used to inoculate 1000 mL of fresh LB medium with kanamycin. The expressionof the PCV2a Cap protein was induced by adding IPTG. Subsequently, 1 gram of bacterial pellet wastaken and resuspended by adding 4 mL of TE lysis buffer.

The cell suspension was sonicated on ice centrifuged, and the supernatant was filtered through a 0.22 µM membrane and loaded into an IMAC column (Histrap HP Cytiva). Elution was performed using the ÄKTAprime plus equipment through an elution gradient of 0-100% elution buffer. Fractions of 1 ml were collected and analyzed by SDS-PAGE at 12% and protein quantification by Bradford assay.

High adherence 96-well ELISA plate was coated with recombinant PCV2a Cap antigen (2 µg/mL), diluted on carbonate buffer at 4°C overnight. After incubation, the plate was washed once with PBS 1X. Then, the plate was blocked for one h at room temperature with 2% of bovine serum albumin on PBST. Swine sera samples were diluted 1:100 in PBS with 25% goat sera, and 50 µL of diluted samples were added to the ELISA plate. Serum samples were incubated for 30 min at room temperature and washed five times with PBST buffer.

50 µL of HRP-conjugated goat anti-porcine IgG H+L was added per well, incubated for 30 min, washed with PBST, and 50 µL of TMB was added to each well. After 10 min of incubation, 50 µL of H_2SO_4 (1M) wereadded to stop the colorimetric reaction. Finally, optical density was measured using a microplate reader at 450 nm.

Results are expressed as AU (arbitrary units); the cut-off was determined with serum negative to PCV2 Cap protein. Samples with AU >20 are considered positive.

Results

Figure1 shows the PCV2a Cap expressed the *E. coli* BL21 Gold (DE3), we obtained a final yield of 1 mg/g dry weight. The above could be scaled to 4.4 mg/1000 mL of bacterial culture, thus being a high performance considering the cost of system maintenance

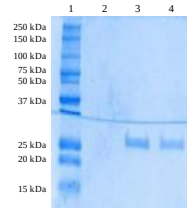

Figure 1. PCV2a Cap antigen expressed in E. coli BL21 Gold (DE3).

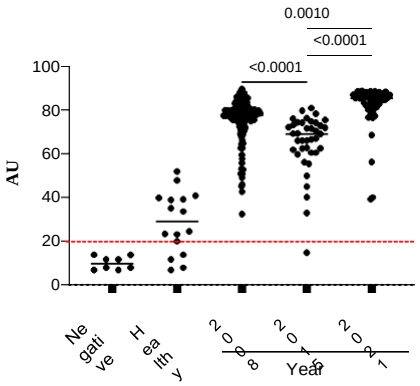

Figure 2. Determination of IgG antibodies against PCV2 Cap protein.

Serum from pigs negative to PCV2 were used to determine the cut-off, and samples with AU>20 are considered positive. Healthy pigs represent animals from a high herd health status, which showed low or negative IgG antibodies against PCV2 Cap protein. 2008, 2015, and 2021 represent conventional pigs sampled at the indicated year. Results showed significant differences between years.

Discussion and Conclusion

We expressed and standardized an ELISA to detect IgG antibodies against PCV2 Cap protein in this work. This ELISA can be used to specifically distingue negative samples from those with low or high IgGlevels.

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Development of performance parameters and economic results after changing from oralvaccination against *Lawsonia intracellularis* to intramuscular vaccination in combinationwith a PCV M Hyo RTU vaccine

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Introduction

Lawsonia intracellularis (*LI*) can be detected in alarge proportion of pig herds (1). Entry into herds usually occurs through the purchase of infected pigs (2). Lawsonia is ingested orally from the pigs' environment (3). While the infectious dose is relatively low, infected pigs can excrete high levels of the pathogen in their feces (3). After infection various clinical courses are described: Besides fulminant acute cases, chronic and subclinical disease with reduced growth performance play an important role (2). Registration trial data shows high efficacy of intramuscular Porcilis® Lawsonia vaccine (3). The present field case investigates the effect of changing Lawsonia vaccination from an oral vaccine to an intramuscular vaccination scheme.

Materials and Methods

Incoming pigs to the observed fattening farm (1400 places) previously were vaccinated with a PCV Mhyo RTU vaccine and against PRRSV (24th day of life). Additionally, an oral Lawsonia vaccine was used at the beginning of fattening. Farmer and vet classified the herd as subclinical LI-infected whereby losses due to LI were described in the finishing group. Homogeneity of the animals appeared inconspicuous and only individual animals were treated. Then, Lawsonia vaccination scheme was changed to Porcilis® Lawsonia in combination with Porcilis® PCV Mhyo at 24th day of life. Performance data between both Lawsonia vaccination schemes was compared in two groups.

Results

Intramuscular vaccinated animals remained clinically healthy despite pathogen excretion in early and mid-fattening. Performance parameters showed improvements in IM vaccinated group compared to orally vaccinated group (FCR -0.2 to 2.8, ADWG +44 g to 826 g/day, losses -1.7% to 0.5% total, veterinary costs -0.05 Euro/fattening pig).

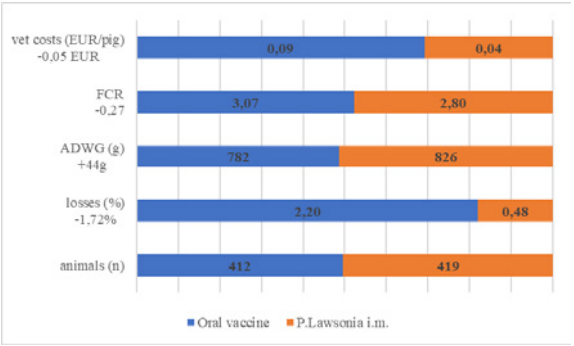

Figure 1: Group size and performance data in oral vaccinated and intramuscular vaccinated pigs

Comparing both groups in total an economic advantage of 8.14 €/fattening pig was achieved (vaccination costs not included).

Table 1: Comparison of production costs in oral vaccinated and intramuscular vaccinated groups in €/pig produced

	Oral vaccine	P.Lawsonia i.m.	Delta
Feed expenses (€/pig)	72,71	66,32	-6,39
Animal losses (€/pig)	2,21	0,47	-1,74
Lost profit (€/pig)	-0,05	-0,01	0,04
Vet costs (€/pig)	0,1	0,04	-0,06
Total Production costs (€/pig)	**74,97**	**66,82**	**-8,15**

Conclusions and Discussion

The use of the intramuscular Lawsonia vaccine in combination with a PCV Mhyo RTU vaccine resulted in a clear economic advantage. Overall, especially due to the improvement of FCR, additional 8.14 €/fattening pig in the intramuscular group compared to the orally vaccinated group were earned.

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Dynamics and chronology of *Mycoplasma hyopneumoniae* shedding capacity in piglets submitted to an innovative oral immunization protocol

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Introduction

Mycoplasma (M.) hyopneumoniae is the mainetiological agent of Porcine Enzootic Pneumonia. Its prevention is of great interest to the productive system, whereas its colonization in the lung tissue leads to intense productive losses. An oral vaccine against *M. hyopneumoniae*, capable of stimulating the mucosa-associated lymphoid tissue (MALT) was developed.The oral vehicle was porous silica and a polymer with specific pH solubility (Mechler-Dreibi et al., 2021).The present study aimed to evaluate the efficacy of a newly developed oral vaccine in reducing *M. hyopneumoniae* shedding by experimentally infectedpigs, comparing its efficacy with an intramuscular vaccine widely used in pig farming.

Materials and Methods

Thirty 21-days old weaned *M. hyopneumoniae*-free piglets were randomly distributed into three groups (n=10), with Group 1 (CV) of piglets vaccinated with a single dose commercial vaccine at 24 days of age, Group 2 (OV) of piglets vaccinated with the oral vaccine at 24 days of age, and Group 3 (CONT) constituted the control group. All animals were challenged at 70 days of age with 5 mL of culture medium containing 10^6 CCU/mL of *M. hyopneumoniae*, administered by the tracheal route. Samples of oral fluids were collected from each pen every three days, while laryngeal swabs were collected weekly to monitor the presence and shedding of the pathogen by qPCR.

Results

At 7 days post-infection (dpi), it was possible to detect *M. hyopneumoniae* DNA in at least 70% of the laryngeal swab samples in all groups. All animals fromall groups had positive samples until the end of the experimental period, with the exception of the OV group at 49 dpi, which had 50% of positive piglets (Table 1). The quantification of *M. hyopneumoniae*DNA by qPCR in oral fluid samples showed that in theCV group, there were positive animals at all collection dates, except at 17 dpi, while the OV group had negative samples on 7, 10, and 14 dpi. The CONT group was negative only at the 10 dpi (Fig. 1).

Table 1: Percentage of *Mycoplasma hyopneumoniae* positive animals in laryngeal swab samples collected from the 7 to 56 days post-infection (dpi) by this pathogen

	7 Dpi %	14 dpi %	21 dpi %	28 dpi %	35 dpi %	42 dpi %	49 dpi %	56 dpi %
VC	80	80	90	100	100	100	100	100
VO	70	80	100	100	100	100	50	100
CONT	70	100	100	100	100	100	100	100

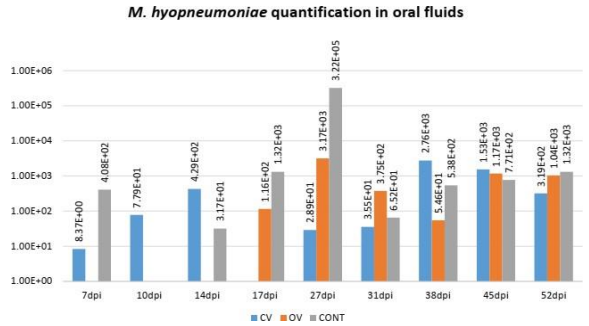

Fig. 1: Mean estimation of *Mycoplasma hyopneumoniae* quantification by qPCR in oral fluid samples collected per stall of each group.

Discussion and Conclusion

Animals vaccinated by the oral route had the peak of bacterial load in the laryngeal swabs at 28 dpi, later than the group vaccinated with the comercial vaccine (21 dpi) and the control group (14 dpi). Specific locallysecreted IgA may play a protective role, preventing or decreasing the pathogen's adhesion to the ciliated epithelium (Martelli et al., 2014). Oral fluid samples from the OV group showed a lower detection of *M. hyopneumoniae* when compared to the others, being negative in the first three collection points. The oral vaccine may have influenced the low detection of the pathogen in oral fluid samples due to the stimulation ofMALT, as reported by Mechler-Dreibi et al. (2021). Detection and shedding of *M. hyopneumoniae* in laryngeal and nasal swabs and oral fluid samples were influenced by the immunization of the piglets, since lower bacterial loads were observed in the vaccinated groups.

Acknowledgments

FAPESP #2020/08028-5; 2018/12742-5; 2019/19710-4; 2017/17844-8

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Early live vaccination for Lawsonia intracellularis control in Colombia

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Introduction

Lawsonia intracellularis (LI) infection is prevalent worldwide and a commonly underdiagnosed problem with two clinical forms of proliferative enteropathy (PE): acute and chronic. The chronic form is observed in weaned and growing pigs less 6-20 weeks of age. Affected pigs have mild to moderate diarrhea and failure to sustain growth. The acute form of the disease occurs more commonly in young adults and is characterized by sudden death associated with anemia and hemorrhagic diarrhea (PHE) (1). Alongside biosecurity and proper husbandry, the use of vaccines isa common practice in the industry (2). The objective of this trial was to assess the efficacy of an early onset of immunity after oral live LI vaccination to provide long lasting protection against clinical or subclinical manifestations of the disease and economic loses under field conditions in Colombia.

Materials and Methods

A total of nine batches of approximately 900 healthy piglets were randomly assigned to the vaccinated and unvaccinated groups: 4 and 5 batches, respectively. Husbandry and management (feed, premises, and sanitary protocols) were equivalent for both groups with the sole difference of a 14-day old dose of a live L. intracellularis (Enterisol Ileitis® Boehringer Ingelheim) 2-ml dose administered by drench for the vaccinated pigs. The efficacy of the vaccination was assessed comparing productive parameters among groups (mortality, final weight, feed conversion rate and return of investment). Statistical analysis of the data was performed using the Stata 17 program with the t-student or the Mann Withney U tests for comparison. Only numeric differences were detected in the analysis.

Results

Mortality was decreased from 4.84% to 4.44% for the vaccinated pigs as can be seen in table 1. The average daily growth (ADG) and final weight showed better performance in the vaccinated group leading to a 190 grams difference among the two groups. The FCR was 0.1 lower for the vaccinated animals. The vaccination strategy gave an overall return of investment rate (ROI) of 4.88:1.

Parameter	Vaccinated	Unvaccinated	Difference
Mortality %	**4.44**	**4.80**	**0.360**
Finish (Kg)	**114.16**	**113.97**	**0.190**
FCR	**1.9**	**2.0**	**-0.1**
ROI			**4.88 : 1**

Table 1. Comparison of productive parameters Wean to Finish (137 days) in vaccinated (Enterisol Ileitis®) vs non vaccinated. The marketvalue of 0.1 FCR points in Colombia is of 3.5 $.

Conclusions

Live vaccination against L.I. as early as 14-days of age can control the clinical and subclinical effects of this fastidious bacteria field challenge without the need to use of antibiotics to control this disease (3). Live vaccines have proven to induce a long-lasting protectionbased on local and systemic induction of both cellular and humoral immune response against L. intracellularis (1, 2). The healthier the animal the more able they are tofulfil their genetic potential. The results obtained in thistrail under field conditions prove that a control programincluding early live L.I vaccination can be successful. This is demonstrated by, improved ADG, a decreased mortality and a lower FCR. These improvements resultin a ROI of 4.88.

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Effect of antibiotic usage on Swine Erysipelas seroconversion after vaccination with attenuated live vaccines in South Korea

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Introduction

It is well known that the simultaneous administration of antibiotics and live attenuated Swine Erysipelas (SE) vaccines can compromise the development of the immune response, so it is recommended to discontinue antibiotic treatment 8-10 days before vaccination (1). On the one hand, in South Korea, vaccines against SE are usually combined with Classical Swine Fever antigen, both being live attenuated vaccines (CSF+SE). As CSF vaccination is mandatory in the country, all animals in the breeding herd are vaccinated with CSF+SE in quarantine period and during every lactation.On the other hand, antibiotics are broadly used, so interference between them and bacterial live attenuated vaccines could occur.

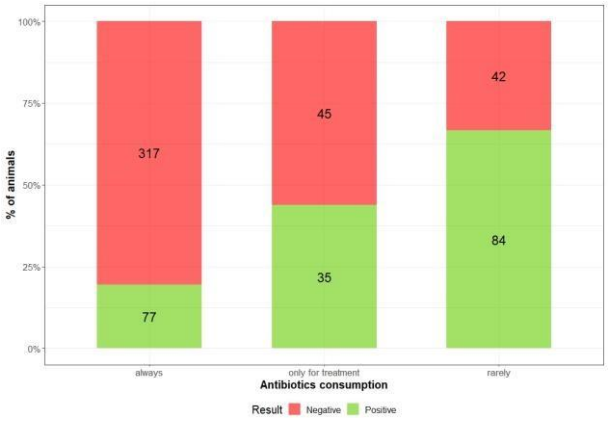
The objective of the present study was to verify if the humoral immune response against SE induced by live attenuated CSF+SE vaccines is compromised by antibiotic usage on Korean farms.

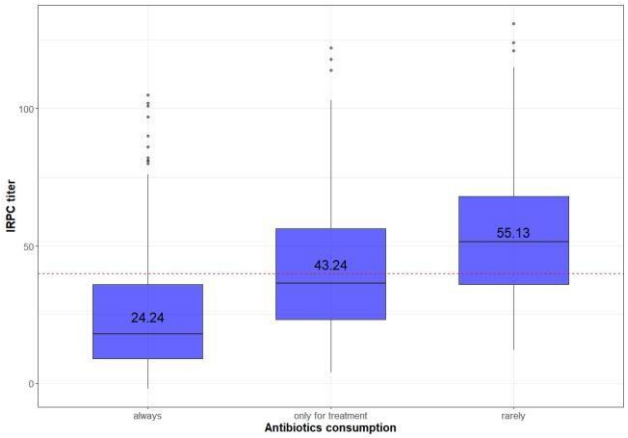
Materials and Methods

A total of 600 serum samples from sows of different parities were collected from 26 Korean swine farms. All these farms were vaccinated with a CSF+SE live attenuated vaccine, following manufacturers' instructions. Information of the usage of antibiotics was collected from each farm by interviewing farm managers or veterinary consultants. Based on the antibiotic consumption in gilts and sows, the farms were divided into 3 categories from higher to lower: 'always' (N=17), 'only for treatment' (N=4) and 'rarely' (N=5). Antibody titers against SE were measured using an indirect ELISA kit (CIVTEST® SUS SE/MR) in a Korean laboratory. The ELISA results were interpreted as positive when Cut-off IRPC value was > 40. The correlation between the positivity rate and antibiotic consumption, and between the average titer and antibiotic consumption was analyzed by logistic regression or linear regression with a Turkey Post-hoc test, respectively.

Results

The positivity rate of SE and average titers was statistically significantly different (*P-value* < 0.05) among the three categories of antibiotic consumption. The highest values were on those farms in the "rarely" category, being respectively 66.7% and 55.13, followed by "only for treatment" 43.8% and 43.24 and finally "always" with 19.5% and 24.24 (Figures 1 and 2).





Figures 1 and 2. Positivity rate and average IRPC titer according to type of antibiotic usage.

Discussion and Conclusion

Based on this study, the usage of antibiotics results in a lower humoral immune response against SE after vaccination with live attenuated CSF+SE vaccines. Even though the antibody level in 'rarely' usage is superior than in the other 2 categories, it remains low when compared to the one elicited by SE inactivated vaccines (2). In order to avoid vaccination failures, other type of vaccines should be evaluated to achieve a better immune protection, as the antibodies seem to play a key role in the protection against SE (3).

Acknowledgments

HIPRA wish to thank all the Korean farmers that collaborated in this project.

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Effect of oral vaccination against *Escherichia coli* combined with a nutritional tool during nursery. Performances and intestinal integrity

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Introduction

Intestinal health has arisen a major concern in swine industry. In fact, in 2022, zinc oxide, one of the most valuables preventive measures against diarrhea, is being banned in the European Union.

During the last years a wide range of alternatives to zinc oxide has been proven. This work describes the results of a trial combining vaccination with symbiotic and compared to zinc oxide.

Materials and Methods

Three hundred and eighty-eight piglets were individually weighed from weaning to start of finishing, and the feed conversion rate and feed consumption was calculated on a pen basis. Two preventive strategies were assyed: oral vaccination against *E.* coli with Coliprotect® F4/F18 (Elanco Animal Health), and feed supplementation with Reforce, a symbiotic (Farm Faes Nutrición Animal). Four groups were allotted: vaccinated+ ZnO (VZn), vaccinated+Reforce (VR), reforce (R) and ZnO (Zn).

At the end of nursery period feces samples were obtained to perform a panel of intestinal integrity by gene expression analysis of calprotectin, occluding, zonulin, IFN-γ and TGF-β. The comparison of data was performed using ANOVA for physical performances and Kruskal-Wallis and Mann-Whitney's U test forgene expression of intestinal integrity panel. To reduce intestinal integrity data, a discriminant function analysis was performed.

Results

The physical performance appears in table 1 and 2.

Table 1. Physical performance for prestarter feed

Prestarter feed							
Batch	IW	FW	N	WG	FP	FCR	ADG
VR	6.79	9.61	89	2,82	3,82	1,35	235
VZn	6.84	9.44	88	2,6	3,86	1,48	217
R	7.27	9.18	102	1,91	3,33	1,74	159
Zn	6.69	8.55	104	1,86	3,27	1,76	155

Table 2. Physical performance for starter feed

Starter feed							
Batch	IW	FW	N	WG	FP	FCR	ADG
VR	9.61	15.69	86	6,08	8,17	1,34	405
VZn	9.44	15.56	84	6,12	7,07	1,16	408
R	9.18	13.92	100	6,17	7,11	1,15	411
Zn	8.55	8.55	99	5,37	6,6	1,23	358

Where IW= initial weight, FW= final weight, WG= weigh gain, FP= feed per piglet (Kg), FCR= feed conversion rate (Kg/Kg), ADG= average daily gain (g/d).

As regards intestinal integrity assessment, there was a higher mRNA quantification in VR for calprotectin, occluding and IFN-γ and the highest quantification for Zonulin for Zn and VR groups. The lowest quantification was observed in R groups.

The discriminant functions analysis allowed to assign correctly 90% of the samples to each putative group. The Figure 2 shows the plot of the two main functions.

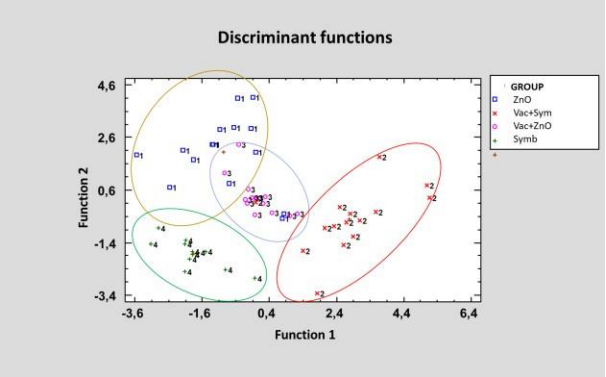

Figure 2. Canonical plot for discriminant functions analysis

The plot demonstrates the separation of each treatment, showing a clear and different effect from the different strategies.

Discussion and Conclusion

The prevention of enteric diseases during nursery is today one of the hardest issues for veterinarians and producers. The use of vaccine and symbiotics offers an adequate solution for prevention of diseases, especially those related to *E. coli*. The efficiency of oral vaccination against *E. coli* has been previously recorded (1) The effect of symbiotics on intestinal integrity has previously been investigated: a low quantity of cytokines in piglets and mices (2,3,4) supplementedwith symbiotics has been observed. In our trial, the R groups showed the lowest quantification for all themarkers, indicating an intestinal healthier status. The increased quantification of calprotectin and IFN-γ in VRgroup is consistent with a vaccination effect. In this trial, the results from vaccination with symbiotic or alone were better than ZnO group. A higher weight increase and better FCR in prestarter period suggest better intestinal function. More research is needed to describe the synergistic action of Coliprotec® F4/F18 and Reforce in field conditions.

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Effect of vaccinating pigs with an intramuscular *Lawsonia intracellularis* vaccine on animals' performance and economy during fattening period

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Introduction

One of the most important bacteria causing enteric disorders in pigs is *Lawsonia intracellularis* (LI). Animals can be infected during different stages of live. Clinical signs vary from acute bloody diarrhoea to chronical or subclinical forms (2). Some risk factors as well as the animals age at infection seem to have an impact on the clinical outcome of the disease (1, 3). The presence of LI lowers animals' performance and causes economical losses mainly caused by poor performance: reduced weight gains, increased mortality, and higher feed conversion ratio (4).

Materials and Methods

The field observation was made in a fattening farm in North East Germany. Piglets were intradermally vaccinated against PCV and orally against Lawsonia (oral vacc) pre weaning. LI related symptoms were seen at about 13-14 weeks of age: bloody diarrhoea, runts, and increased total losses. Vaccination was changed to the intramuscular vaccination with Porcilis® Lawsonia (i.m. vacc) at 3 weeks of age. Performance data from both groups were collected and compared on farm base. On this base an economic output of the vaccination change was calculated.

Results

With introducing the intramuscular inactivated LI vaccine, the clinical signs were reduced. These groups did not need antibiotic treatment via feed anymore. Homogeneity in the groups vaccinated with the intramuscular LI vaccine was enhanced noticeable. Daily weight gain (ADWG) increased by 43 g. Animal losses decreased by 2.3 % from 4.5 to 2.2 % and premature sales by 1.4%. Feed conversion ratio (FCR) was improved by 0.03. Vet costs diminished massively due to the reduced use of antibiotics. In totala benefit of 3.90 €/pig excluding vaccination costs could be calculated.

Table 1. Number of animals and weights in the differently vaccinated groups

	Oral vacc	*i.m. vacc*
Number of animals (n)	15161	4142
Weight at stabling (kg)	28.4	28.4
Weight at slaughter (kg)	119.4	122.3
Duration of fattening (d)	98	96

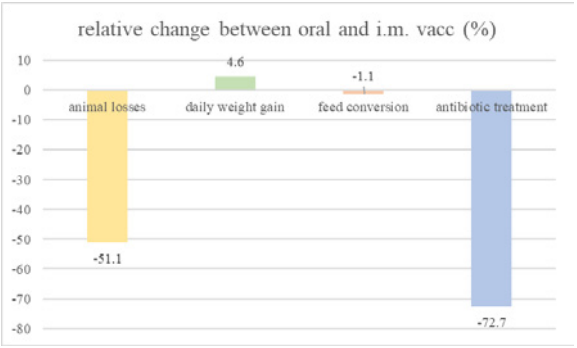

Figure 1: Relative change in animals' performance in oral vacc and i.m. vacc group

Conclusions and Discussion

The infection with *Lawsonia intracellularis* can cause severe clinical disorders and reduce economic results. Vaccination with Porcilis® Lawsonia helped to control clinical symptoms and led to enhanced performance sustainably. Premature sales, antibiotic treatments and management of underperforming pigs were decreased. In conclusion the intramuscular Lawsonia vaccination ensured healthier pigs, better performance, and an improved economic result through lower production costs.

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Effects of intradermal needle-free vaccination against Mycoplasma hyopneumoniae and Porcine circovirus type 2 on piglet performance and lung lesions at slaughter in Brazil

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ıtroduction

[ycoplasma hyopneumoniae is the causative agent of ızootic pneumonia (EP) and is one of the most prevalent ıd important agents associated with the porcine respiratory sease complex. EP is characterized by causing significant oblems in the swine industry through endemicity, ıronicity, decreased average daily gain, poor feed ɔnversion, and increased days to market weight (1,2). nother agent associated with the porcine respiratory sease complex is Porcine Circovirus type 2 (PCV2) which characterized by clinical or subclinical infections among gs. The most representative symptoms of the diseases clude porcine dermatitis and nephropathy syndrome, hich mainly occur during the growing or finishing stages pigs; post-weaning multisystemic wasting syndrome, hich affects nursery and growing pigs; and porcine spiratory disease complex (PRDC), which usually occurs pigs 14–20 weeks of age (3,4). Both diseases are ɔntrolled by vaccination at weaning, and the aim of this udy was to evaluate the effect of intradermal needle-free ıccination on piglet performance and lung lesions at aughter.

laterials and Methods

total of 1342 piglets, intact males and females, were dividually weighed at weaning at 21 days old, identified ith ear tags and distributed between two groups according sex and weight: competitor with intramuscular vaccination M) and Mhyosphere® PCV ID with intradermal needle-ee vaccination (ID). After weaning, they were allocated to e nursery phase, and vaccinated against Mycoplasma ɣopneumoniae and Porcine circovirus type 2 associated seases via the IM route with two different commercially ɣailable vaccines (n=671) or with Mhyosphere® PCV ID ı=671), an ID vaccine administered with Hipradermic® 0. The body temperature was measured at 0 (time of ıccination), 6 and 24 hours after vaccination. The piglets ere individually weighed at 7 days and 42 days after eaning to assess weight gain in the nursery phase and at 30 days at the end of the finishing phase. At slaughter, the ngs were evaluated for the prevalence of macroscopic ıeumonic lesions through the artificial intelligence ɔplication Hipralink Diagnos® (Hipra) with the MADEC odified system.

esults

he increase in body temperature after vaccination was fected by the administration route and vaccine used. The ɔdy temperature of the IM animals was 0.18 degrees higher an that of the ID animals in the first 6 hours after ıccination. The first week of nursery performance was fected by the vaccination, the IM animals having a lower ody weight [BW] and Average Daily Weight Gain ADWG] than the ID piglets. No statistical differences were observed in the nursery phase, but in the finishing phase, the ID animals had a higher ADWG than the IM piglets, resulting in a 4.36 Kg increase in BW (Table 1). At slaughter, the ID animals had significantly (p<0,05) fewer lung lesions and a lower prevalence of pneumonic lesions than the IM animals (Figure 1).

Figure 1. Prevalence of different grades of lung lesions between Mhyosphere® PCV ID (ID) and competitor (IM).

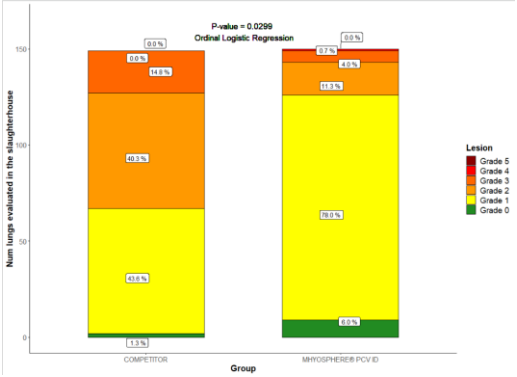

Table 1. Temperature and performance in the finishing phase and pneumonic lesions lung score.

Temperature after vaccination	Mhyosphere® PCV ID (ID)	Competitor (IM)	*p-value*
0h	40.01	39.95	0.51
6h	41.05	41.23	0.04
Temp. Increase 6h	1.04	1.27	0.027
24h	39.59	39.63	0.36
Temp. Increase 24h	-0.42	-0.32	0.36
Weaning weight	6.46Kg	6.41Kg	0.606
Body weight 7d	6.37Kg	6.14Kg	0.002
ADWG 7d	0.011	-0.019	0.005
Body weight 42d	20.31Kg	19.91Kg	0.107
ADWG 42d	0.374	0.368	0.32
Body weight 180d	133.8Kg	129.44Kg	< 0.001
ADWG 180d	0.820	0.792	< 0.001
Average lung lesion	1.15	1.68	0.02

Conclusions and Discussion

This study demonstrated that intradermal needle-free vaccination against *Mycoplasma hyopneumoniae* and Porcine circovirus type 2-associated disease with MHYOSPHERE® PCV ID is safe to administer in weaned piglets and effective in preventing losses caused by these agents, improving weight performance, and reducing pneumonic lung lesions.

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Efficacy and immune response in pigs vaccinated at three days and/or three weeks of age (with and without PCV2-specific maternally derived antibodies) with PCV2b and challenged with PCV2d

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Introduction

Vaccination of pigs at a young age has several advantages, e.g., ease of handling, and early induction of protective immunity. However, neonatal vaccination (< 7 days of age) poses potential problems including interference by maternally derived antibodies (MDA) and the relative immaturity of the immune system. The effectiveness of PCV2 vaccination in neonatal pigs may be affected by age related factors other than MDA (1). It is noteworthy that PCV2 vaccination at 3 weeks of age is effective even in the face of high MDA levels (2). In this study (3) we investigated the effects of age (3 days and/or 3 weeks) and level of MDA (low to high) on PCV2 vaccine immunity and efficacy.

Materials and Methods

Sows were prescreened (4);12 sows with a range of PCV2 antibody levels were selected. Two sows were vaccinated with a commercial PCV2 vaccine (5) to further increase levels of MDA. Pigs were allotted into four groups, T01-T04 (Table 1). Each group contained pigs from sows with a range of PCV2 antibodies. Vaccines were an experimental chimeric PCV1-PCV2b, inactivated whole virus vaccine adjuvanted with MetaStim (6) and were administered as a 2 mL dose at 3 days of age (T02, study day 0), 3 weeks of age (T03, study day 21) or split into 2 one mL doses given at 3 days and 3 weeks of age (T04, study days 0 and 21). A control group (T01) received placebo (MetaStim adjuvant) at 3 days of age (study day 0). Pigs were challenged with PCV2d on study day 49 and were necropsied on study day 59. Immune and efficacy parameters were evaluated as previously described (7) and included serology, IFN-γ ELISpot, PCV2 viremia, PCV2 fecal shedding, histopathology and immunohistochemistry. Statistical analysis was as previously described (7), except the IFN-γ data was only summarized. The study protocol was approved by the Zoetis Institutional Animal Care and Use Committee.

Results

On study day -1 (pre-vaccination) the MDA levels in pigs varied by litter, ranging approximately between 0 and 1 (S/Nc ratio); ≤0.5 S/Nc is positive. On Day 42 (before challenge), only groups receiving a vaccine at 3 weeks of age (T03 and T04) had a significant serological response. Following challenge all vaccinated groupshad a serological response, but T03 and T04 remained higher than T02 until study end. There was a significant co-variate (MDA levels, SERELISA value day Day 0) effect on serological response, with higher levels of MDA reducing the serological response to vaccination. All vaccines (T02-T04) reduced viremia on days 7 and 10 post challenge. However, on day 7 post challenge T02 had higher levels of viremia than T03. All vaccines reduced fecal shedding on day 10 following challenge. There was no significant effect of the co-variate on viremia or fecal shedding. All vaccines reduced PCV2 colonization (immunohistochemistry, day 10 following challenge). There was no difference between groups with respect to lymphoid depletion, and no histiocytic replacement was noted in any groups. There was no significant effect of the co-variate on tissue outcomes. All vaccines induced a PCV2-specific IFN-γ ELISpot response (group averages of 100-500 PCV2-specific IFN-γ secreting cells per million PBMC), however the response in T03 and T04 was 2-3 times higher than T02 on the day of challenge (study day 42). The effect of the co-variate on IFN-γ ELISpot response was not determined.

Discussion and Conclusion

While all vaccine schedules induced immunity and reduced viremia and fecal shedding, both groups that received a vaccination at 3 weeks of age (T03 and T04) had better efficacy in several variables (Table 1). There was an effect of MDA level on the serologic response to vaccination but not to other efficacy endpoints (viremia, fecal shedding and tissue lesions). These results suggest higher MDA levels may impact serological responses but still allow for effective immunization in 3 week of age pigs and that immune endpoints other than serology may be needed to predict vaccine efficacy.

Table 1. Qualitative summary of study results[1].

Trt	Vaccination	Relative ranking of efficacy outcomes			Relative ranking of immune outcomes		Overall Rank
		Viremia	Fecal Shedding	Tissue Lesions	SERELISA	IFN-γ ELISpot	
T01	Adjuvant control	4	4	4	4	4	4
T02	3 days of age	3	2	3	3	3	3
T03	3 weeks of age	1	1	2	1	1	1
T04	3 days and 3 weeks of age	2	2	1	1	1	2

[1]Relative ranking of study outcomes for each vaccine schedule. 1=best result, 4=lowest result.

Acknowledgments

Zoetis Animal Research Support veterinarians and staff. ELISpot assay, Traci Godbee; Vaccine potency testing, Fabrizio De Mattia; Vaccine formulations, KarlUpman.

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Efficacy and immunological parameters upon vaccination with different *Mycoplasma hyopneumoniae* bacterins

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Introduction

Mycoplasma hyopneumoniae is the primary pathogen causing enzootic pneumonia (EP) in pigs and one of the primary agents involved in the porcine respiratory disease complex (1). To date, vaccination against this pathogen is carried out worldwide (2). However, vaccination triggers only partial protection with varying results between pigs and herds. It is believed that the adjuvant, which enhances the immunogenicity of the vaccine, also plays a role in eliciting systemic immune responses. Therefore, the present study was conducted to assess the protective efficacy of two different, commercial bacterins and the effect of an adjuvant (and immunostimulant) without an antigen on innate immune responses using an experimental infection model.

Materials and Methods

Upon arrival at the experimental facilities, fifty-three 4-week old piglets that were free of *M. hyopneumoniae* were randomly divided into 5 groups: V1 Hyogen® (n=12), V2 another commercial bacterin (n=12), V3 same as V1 but without the antigen (n=12), PC non-vaccinated challenged control (n=12), NC non-vaccinated non-challenged control (n=5) that served as a sentinel group. After an acclimation period of 9 days, pigs in groups V1, V2 and V3 were vaccinated intramuscularly on D0, while the animals in the PC and NC group received a physiological saline solution. On D21, all animals except those in NC were endotracheally inoculated with 2 different *M. hyopneumoniae* strains (2 times 7 mL of 10^7 CCU/mL/pig). Clinical signs were assessed daily using a respiratory disease score (RDS) (score 0 - 6) and after euthanasia (D49), macroscopic lung lesions (MLL) were evaluated (score 0 - 35). Also, blood samples weretaken on D0, D7 and D21 to isolate monocytes, and subsequently these monocytes were stimulated with LPS to measure cytokine levels (IFN-γ, IL-1β, IL-6 and IL-10) (pg/mL) in the cell culture supernatant. On D7, D35 and D49, broncho-alveolar lavage (BAL) samples were taken to determine *M. hyopneumoniae*-specific IgA levels with an in-house indirect ELISA (OD values),cytokine levels (IFN-γ, IL-1β, IL-6 and IL-10) (pg/mL), and *M. hyopneumoniae* DNA load (log organisms/µL) measured with a dPCR assay targeting the P102 gene.

Results

The results of the RDS, MLL, monocyte IL-10 secretion, BAL IgA, BAL *M. hyopneumoniae* DNA load, BAL IL-1β and IL-10 levels are shown in Table 1. Only results from immunological assays with significant differences between the groups are shown.

Table 1. Results for RDS, MLL, monocyte IL-10 production, BAL IgA, BAL *M. hyopneumoniae* DNA load, BAL IL-1β and IL-10 levels (mean ± standard deviation). Groups that have no superscript in commonare significantly different from each other ($P \leq 0.05$).
NA: not analysed

		V1	V2	V3	PC
RDS		0.24^{a} ± 0.83	0.60^{a} ± 1.39	0.46^{a} ± 1.15	0.60^{a} ± 1.36
MLL		0.05^{a} ± 0.10	3.52^{b} ± 3.91	4.52^{b} ± 5.02	7.60^{b} ± 4.89
IL-10 Mono	D7	2281^{a} ± 1212	*NA*	1935^{a} ± 1921	4970^{b} ± 1987
BAL IgA	D35	0.61^{a} ± 0.80	0.26^{ab} ± 0.39	0.09^{b} ± 0.10	0.12^{ab} ± 0.10
dPCR	D49	2.42^{a} ± 0.86	3.31^{ab} ± 0.97	4.45^{c} ± 0.47	3.84^{bc} ± 0.60
BAL IL-1β	D49	233^{a} ± 60	361^{ab} ± 194	576^{b} ± 322	344^{ab} ± 108
BAL IL-6	D49	25^{a} ± 10	56^{b} ± 39	67^{ab} ± 67	33^{ab} ± 13
BAL IL-10	D49	74^{a} ± 62	79^{a} ± 75	5^{b} ± 13	33^{ab} ± 45

Discussion and Conclusion

Although vaccination did not reduce the clinical signs (RDS) in our study, less macroscopic lung lesions were detected in V1 compared to the other groups. The presence of local IgA in BAL two weeks after challenge (V1) might indicate priming of the immune system (3). Also, less pro-inflammatory (V1) and more anti-inflammatory (V1 and V2) cytokines were detected in BAL, which indicates that vaccination modulates the cytokine responses in BAL upon infection. *Mycoplasma hyopneumoniae* DNA load on D49 was lower in V1 compared to V3 and PC, and also in V2 compared to V3. Interestingly, LPS-stimulated blood monocytes from V1 and V3 produced less IL-10 (compared to PC), which might be beneficial to trigger protective immune responses. However, further research including more parameters related to the innate immune responses is needed to elucidate the importance of the adjuvant in vaccines against *M. hyopneumoniae*.

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Efficacy of different PCV2 and Mhyo combined vaccines against PCV2 or *Mycoplasma hyopneumoniae* experimental infections.

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Introduction

Enzootic pneumonia due to primarily *Mycoplasma hyopneumoniae*(M.hyo) and PCVD due to PCV2 virus remain a severe health problem in pig farms (1). Vaccination against those two pathogens helps to reduce their clinical manifestation and corresponding losses. Several commercial mono-or bi-valent vaccines are available. The aim of this study was to evaluate the efficacy of different PCV2 and M.hyo vaccines in experimental challenge models.

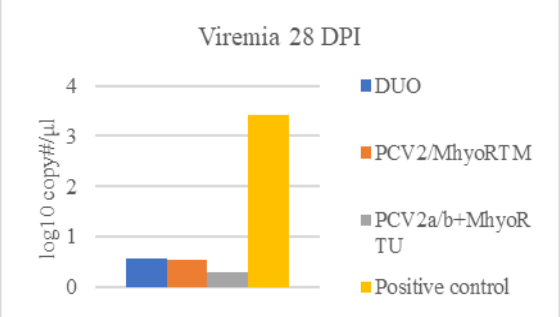
Material and Methods

In two different experiments three weeks-old piglets were vaccinated either with Circovac® with Hyogen®(DUO)-both Ceva mixed as RTM or other PCV2/M.hyoRTM vaccine or a PCV2a/b+M.hyo RTU vaccine. In the trial 1) vaccinated and positive control pigs were challenged at 9 weeks of age(WOA) with the PCV2d strain. In the trial2) vaccinated and positive control pigs were challenged at 8 WOA with M.hyo. Serum samples were collected prior to vaccination, challenge, and slaughter, and measured and IDVet *Mycoplasma hyopneumoniae* ELISA kits. Pigs were always euthanized 28 days post infection(DPI) and either PCV2 loads in lymph nodes (Lnn)(trial1) or lung lesions scores(LLS) according to the European Pharmacopoeia 9.0 and Hannan et al., 1982, (trial2) were measured.

Results

In the trial 1) the viremia at 28DPI was 0.55, 0.54 and 0.28 log copy#/microL for DUO, PCV2/MhyoRTM and PCV2a/b+MhyoRTU, and 3.41 log copy#/microL for positive control-respectively.

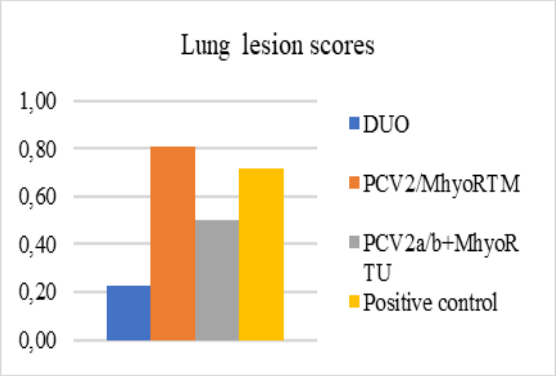
Fig 1. Viremia 28 days post infection



The PCV2 loads in mesenterial and inguinal Lnn were 5.28, 4.94, 4.84,7.68 log copy#/microL and 4.80, 4.40, 4.26 and 6.49 log copy#/microL for DUO; PCV2/MhyoRTM,PCV2a/b+MhyoRTU and positive controlrespectively. All treatment groups differed significantmly from the control, but not among themselves.

In trial 2) the mean LLS were 0.23, 0.81, 0.50 and 0.72 for DUO, PCV2/MhyoRTM, PCV2a/b+MhyoRTU, and positive control respectively ($p<0,05$ for DUO vs any other groups). Only DUO differed significantly from the positive control.

Fig 2.Weighted Lung lesions scores



Discussion and Conclusion

This study demonstrated that DUO provided equal PCV2 protection as PCV2/MhyoRTM and PCV2a/b+MhyoRTU. DUO however outperformed both PCV2/MhyoRTM and PCV2a/b+MhyoRTU vaccines concerning the protection against the development of lung lesions due to M.hyo. Some of the combined PCV2 and M.hyo vaccines may provide in such a challenge model insufficient protection against M.hyo infection.

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Efficacy of PCV2a vaccines administered separately or mixed with M.hyo component in the protection against an experimental PCV2d infection.

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Introduction

PCVD (Porcine Circovirus Diseases) remain a common problem in most of swine farms (1). Strains of different genotypes of PCV2 are circulating in the herds. The PCV2d seems predominating particularly in farms with clinical PCVD problems (2). Different mono-valent or combined vaccines are available on the market. The aim of the study was to compare the efficacy of PCV2a vaccines against PCV2d infection.

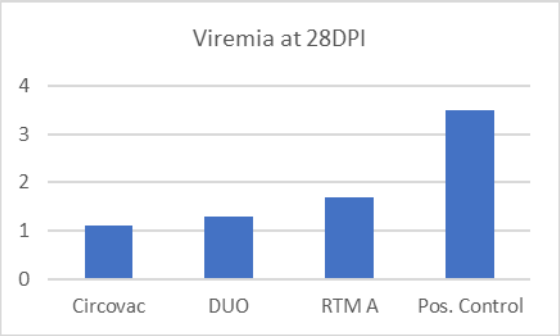
Material and Methods

Conventional weaned piglets (20per group) were vaccinated at 3 weeks of age (WOA) either with Circovac®0.5ml(PCV2a vaccine, Ceva – Group C) separately or mixed with Hyogen®2ml (M.hyo vaccine, Ceva – Group DUO) or with PCV2a Vaccine A 1ml mixed with M.hyo vaccine B1ml (Group RTMA), a group of non- vaccinated pigs served as controls. All were challenged at 12 WOA (D0) with a PCV2d isolate. Pigs were sampled weekly and sacrified 4 weeks (D28) post-challenge (DPI). Virus loads were measured by qPCR for efficacy evaluation.

Results

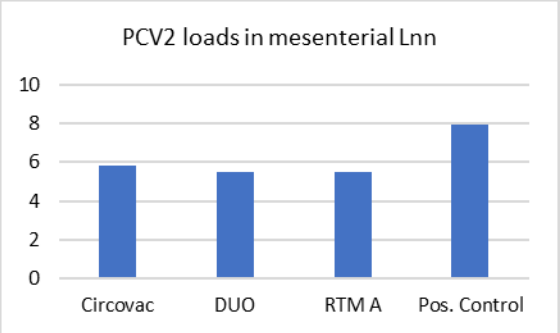
Viremia at 28 DPI was 1,1; 1,3; 1,7 and 3,49 copy#/µl for groups C, DUO , RTMA and pos. control respectively.

Fig 1. Viremia 28 days post challenge



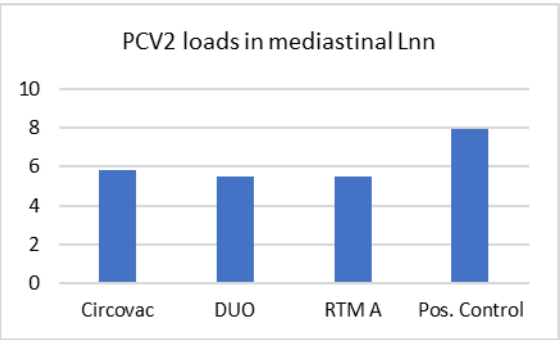
The PCV2 loads in mesenterial were 5,8; 5,5; 5,5 and 7,97 copy#/µl for groups C, DUO, RTMA and pos. control respectively.

Fig 2. PCV2 loads in mesenterial lymph nodes



The loads in mediastinal lymph nodes were 3,8; 3,5; 4,4 and 6,21 copy#/µl for groups C, DUO, RTMAand pos. control respectively ($p<0,05$ treatmentgroups vs pos. control).

Fig 3. PCV2 loads in mediastinal lymph nodes



Discussion and Conclusion

This study demonstrated that Circovac® administrated separately or mixed with a corresponding M.hyo vaccine provided equal protection against the epidemiologically most important genotype of PCV2. The reduction of viremia and viral loads in lymphoid tissues were also very similar to the other PCV2/M.hyo RTM products. These results confirm it's convenience being used in the control of PCVD single or together with M.hyo.

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Efficacy of the synergy between live-attenuated and inactivated PRRSV vaccines against a NADC-like strain of porcine reproductive and respiratory syndrome virus in four-week piglets

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Introduction

This study evaluated the cross-protective efficacy of synergy between live-attenuated and inactivated PRRSV vaccines compared with a single vaccination with PRRS modified-live virus (MLV) vaccine against challenge with NADC30-like strain. Serological tests in MK (primed intramuscularly with MLV and boosted with killed vaccine three weeks later) groups revealed no differences in both anti-N and anti-GP protein antibodies compared with M1 (single MLV dose) group and failed to provide further alleviation on virus load, shedding as well as protection against, gross lesions and ADG. Thus, as a booster, the killed vaccine confers minimal additional protection in NADC30-like infected piglets.

Materials and Methods

The effect of sampling material and collection order on the ELISA sample-to-positive (S/P) ratio were analyzed using a mixed effect two-way ANOVA model followed by Tukey-Kramer adjusted t-test (6). A total of 45 PRRSV-free pigs were randomly divided into five groups: 1) strict control (SC); 2) positive control (PC); 3) single MLV dose (M1); 4) primed intramuscularly with MLV and boosted with killed vaccine A three weeks later (MK1); and 5) intramuscular prime MLV boosted subcutaneously with killed vaccine B three weeks later (MK2). piglets in the M1, MK1, and MK2 groups were intramuscularly immunized with a label dose of Ingelvac PRRS® MLV at 4 weeks of age. After three weeks, the piglets in the MK1 and MK2 groups received a second intramuscularly vaccination with 2 mL two different KV vaccines. Three weeks later, piglets in SC group were given MEM medium, whereas the other groups were intramuscularly injected with 2 mL 4.5 Log^{10}(TCID50) of v2016/ZJ/09-03 PRRSV. All pigs were euthanized and necropsied at 15 days post-challenge (DPC).

Results

No significant differences in the antibody titer, virus load, lung lesion and ADG were observed between the MK groups compared with singular MLV vaccination group.

Discussion and Conclusion

The data in this study demonstrate that MLV can still provide partial protection against NADC30-like strain infection. However, two commercial PRRS KV vaccines cannot provide further protection against NADC30-like virus infection in naïve piglets within 15 days post-infection base on MLV prismed vaccination. There is no difference in the efficacy between a single injection with the MLV vaccine and MLV plus KV boost vaccination scheme.

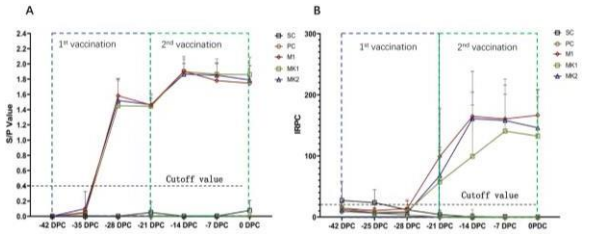

Figure 1. Serological reaction before challenge. (A) anti-N protein antibody titer; (B) anti-GP proteins antibody titer.

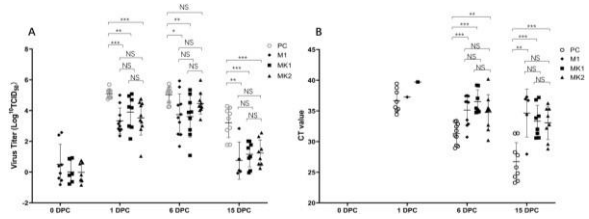

Figure 2. Viremia and virus shedding. (A) Viremia; (B) Virus shedding Data are shown as mean ± standard error (error bars). (*p<0.05; **p<0.01;***p<0.001). NS (Not Statistically Significant).

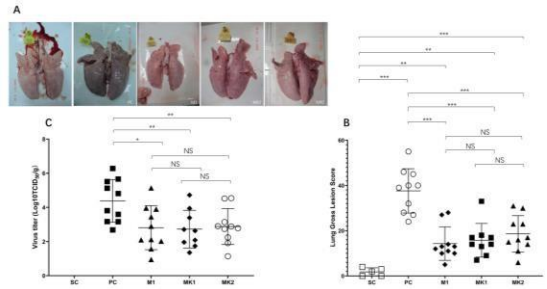

Figure 3. Gross lung lesion scores and virus load inlungs at 15 DPC. (A) Gross lung lesion examination; (B) Gross lung lesion scores; (C) Thevirus load in lungs at 15 DPC.

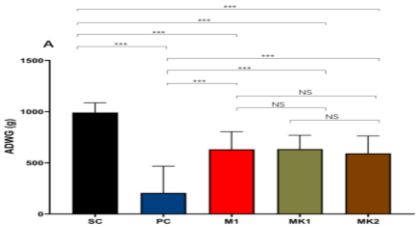

Figure 4. Average daily weight gain measurements.

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Evaluation of a recombinant vaccine against *Lawsonia intracellularis* in swine

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Introduction

Proliferative enteropathy (PE) is one of the main bacterial infections (*Lawsonia intracellularis*) that affect swine worldwide. The disease has a high prevalence (57 - 100%) (1) and is characterized by bloody diarrhea, loss and decrease in weight gain, stillbirths and death of animals (2) causing significant economic losses in the sector (3). The vaccines available for the control of PE have limitations related to the levels of protection conferred (4), which highlights the need to develop new vaccines that protect animals against infection and induce sterilizing immunity. In a previous study we confirmed the ability of *L. intracellularis* recombinant protein, fused with TT-Th carrier molecule (rLiTT), expressed in *E. coli* system to stimulate specific immune response in mice. Therefore, in the present study we evaluated the experimental vaccine in swine and characterized the immune response.

Materials and Methods

The antigen used in the vaccine development was selected through bioinformatics analysis and obtained through expression in a heterologous system. Recombinant protein (rLiTT) was purified by affinity chromatography and quantified by BCA Protein Assay kit (GE Healthcare). The vaccine contained the 400 µg dose of rLiTT adsorbed in 10% aluminum hydroxide [Al(OH)3; Sigma Aldrich)]. To evaluate rLiTT potential as vaccine antigen, swine were allocated in three groups and inoculated by intramuscularly injection on days 0 and 21. Group 1. rLiTT experimental vaccine; group 2. Porcilis® Ileitis vaccine (MS&D – Saúde Animal); group 3. Control group, saline solution plus 10% aluminum hydroxide. Blood samples were collected by cranial vena cava puncture on days 0, 10, 21, 30, 45 e 60 to evaluate the humoral immune response by indirect ELISA. The indirect ELISAs was carried out using microtitre plates coated with rLiTT protein (100 ng/ well) or commercial vaccine, both diluted in 0.1 M carbonate bicarbonate buffer (pH 9.6) at 4 °C overnight. The plates were washed 5× with PBS-T (PBS, 0.05% Tween 20) and incubated for one hour at 37 °C with swine sera diluted (1:100) in PBS-T with dry skin milk (3%). After 5 was hes with PBS-T, anti-pig IgG HRP-conjugated antibody (Sigma, USA), diluted 1:5000, was added and incubated for one hour at 37°C. After 5 washes with PBS-T, 100 µl of substrate solution (10 mg ortho-phenylenodiamine (OPD, Sigma-Aldrich) was added and reaction was and the plate was read in a microplate reader. All protocols were approved by the Ethics Committee on Animal Experimentation (CEEA No. 28134-2019) of the Federal University of Pelotas (UFPel).

Results

The vaccinated animals (group 1) showed high levels of specific antibodies anti-rLiTT, especially from day 30, demonstrating the effect of the second vaccine dose, administered on day 21 of the experiment (Fig 1). The level of antibodies induced by the experimental vaccine was equal to or greater than the commercial vaccine during the period. No specific antibodies were detected in the control group.

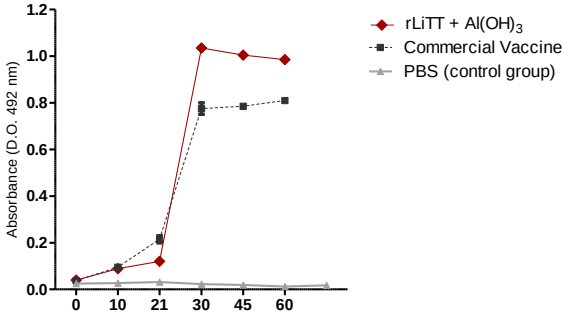

Figure 1. **Humoral immune response.** Total IgG produced in swine vaccinated with rLiTT and commercial vaccine. Pool of sera were characterized at a single serum dilution (1:100). The mean optical density ($OD_{492\ nm}$) ± standard deviation (bars) from triplicates test is shown.

Conclusions and Discussion

In this study we tested a recombinant vaccine against EP in swine model. The vaccine induces significant humoral response, showing the potential to protect against *L. Intracellularis* infection. Future studies aim to challenge vaccinated animals in order to determine the effectiveness of the vaccine and verify the presence of the bacteria and its viability in the animals' feces.

Acknowledgments

Coordination for the Improvement of Higher Education Personnel (CAPES) and National Council of Technological and Scientific Development (CNPq).

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Evaluation of different vaccination strategies for sows and their piglets.

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Background and Objectives

The objective of this report is to describe the stabilization of a PRRS outbreak using two different vaccination strategies and comparing both[1].

Material and Methods

For this trial we used an industrial three-site farm in Russia with a population of about 100 000 pigs.
It consists on an implementation and comparison of different vaccination strategies of sows, using only modified live vaccine (MLV) or combination of MLV with an inactivated vaccine (IV) PROGRESSIS, with or without piglet's vaccination.
T1 group: sows were vaccinated with a MLV, mass vaccination inoculating one dose every three months two times. Piglets without any vaccination.
T2 Group: a MLV at 60 days of gestation with revaccination by PROGRESSIS (IV) at 90 days of gestation. Piglets from the T2 group were vaccinated at 34 days old using PROGRESSIS.
The parameters used to compare the vaccination strategies were average daily gain (ADG) and mortality rate obtained in the post-weaning of 17 (T1) and 26 (T2) batches of piglets weaned between September 2019 and June 2020. Results were analysed by means of an ANCOVA model including ADG or %mortality as independent variables, entrance weight as covariate, treatment, and technician as factors.

Results

Our results show that the mean body weight at entrance and the technician were not significant parameters considering both ADG and % mortality as response variable. Our results show that the only significant parameter playing a role, both in ADG and mortality of piglets, was the treatment (vaccination strategy).
ADG (g/d) results (mean±SD) were: 258±72.9, 390±33.3 for T1 and T2 respectively. $\alpha = 0.05$, $p<0.001$.
Mortality (%) results (mean±SD) were: 19.8±8.71, 5.91±3.48 for T1 and T2 respectively. $\alpha = 0.05$, $p<0.001$.

Discussion and conclusion

The use of PROGRESSIS both in sows and piglets led to a significant increase of ADG and reduction of mortality ($p<0.001$) [1,2,3,4]. It could obey to the combined effect of a better colostral immunity and the effect of piglet vaccination [5,6].

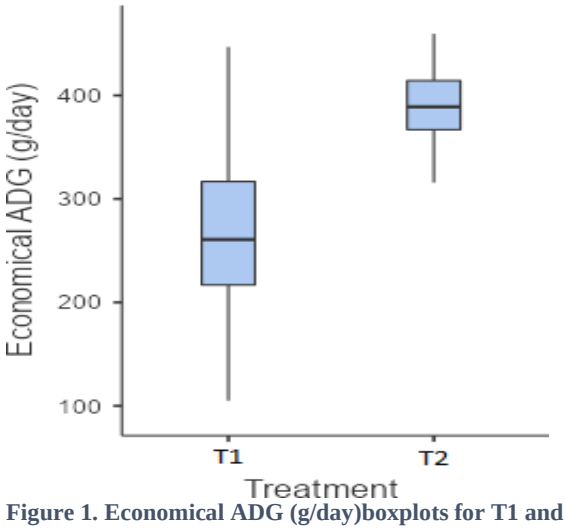

Figure 1. Economical ADG (g/day)boxplots for T1 and T2 pigs.

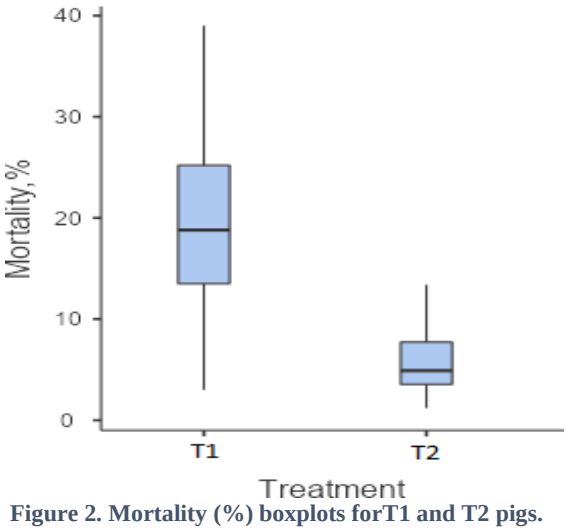

Figure 2. Mortality (%) boxplots forT1 and T2 pigs.

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Evaluation of one versus two doses of an inactivated *Senecavirus A* vaccine in weaned pigs

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Introduction

Senecavirus A (SVA), also known as Seneca Valley virus, is a causative agent of vesicular disease in swine and is grossly indistinguishable from vesicular disease caused by foot-and-mouth disease virus (FMDV). In countries that are FMDV-free, an investigation is required when a vesicular lesion is observed to rule out FMDV. These investigations can impart a substantial economic burden, especially if SVA remains endemic in swine populations. An efficacious vaccine could reduce the number of these investigations and the spread of SVA. The objective of this study was to compare the protective efficacy of one dose versus two doses of an inactivated SVA vaccine in weaned pigs.

Materials and Methods

Forty-eight 3-week-old pigs were purchased from a commercial swine herd and transported to the National Animal Disease Center in Ames, IA. Pigs were divided into four groups (n=12/group): one-dose + sham challenge (Group 1), one dose + challenge (Group 2), two-dose + challenge (Group 3), and sham + challenge (Group 4). One week after acclimation, pigs in Group 3 were vaccinated intramuscularly (IM) with 2 mL of whole-virus inactivated (binary ethylenimine) SVA (SVA/NADC4/2020) and an oil-in-water adjuvant at 12% v/v. Three weeks later, Groups 1-4 were vaccinated IM with 2 mL of either inactivated SVA or sham with adjuvant. Pigs were challenged intranasally with either SVA/NADC4/2020 (10^7 TCID50/mL) or sham two weeks later. Pigs were checked for vesicular lesions daily from 0-10 days post-challenge (dpc) and were scored using a 5-point scale. One point was assigned to each foot with an observed lesion and one point for a lesion on the snout. Pigs were rectal swabbed on 0-3, 5, 7, 10, 14 and 20 dpc. Blood was collected from animals on -36, -16, 0, 3, 5, 7, 14 and 20 dpc. Serum and swabs were tested for SVA nucleic acids by RT-qPCR. Sera was tested by virus neutralization assay using SVA/NADC4/2020 to measure the neutralizing antibody response after vaccination and challenge.

Results

Group 1 served as a vaccine control and these animals did not develop clinical signs or detectable SVA in serum or swabs tested. In Group 2, one animal developed a vesicular lesion at 4 dpc. This group had PCR positive rectal swabs on 1 dpc, but by 2 dpc only 2 animals were positive, and all subsequent samples were negative. None of the animals developed a detectable viremia. In Group 3, one animal developed a vesicular lesion at 9 dpc. In addition, Group 3 had positive rectal swabs on 1 and 2 dpc, but only a few animals were PCR positive on 3 dpc. Only one animal in Group 3 had a detectable viremia, which occurred on 3 dpc. As expected, Group 4 displayed the highest clinical scores and 11/12 animals presented with vesicular lesions during the observation period. This group also displayed the most consistent and extended detection of SVA nucleic acids in rectal swabs. Finally, SVA was detected in the serum of 7/12 animals on 3 and 5 dpc, but rapidly decreased with only two animals having a detectable viremia at 7 dpc.

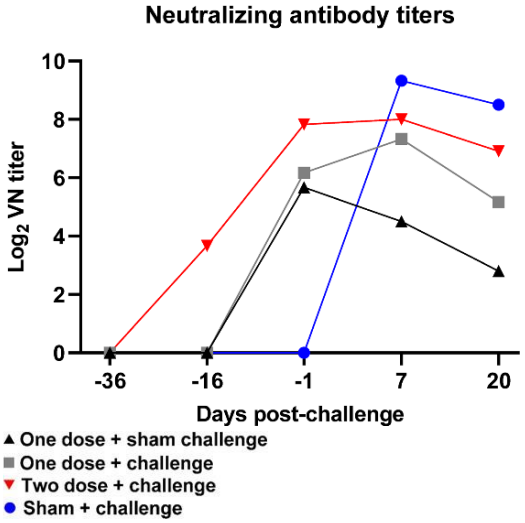

Figure 1. Neutralizing SVA antibody titers. Data points represent the geometric mean of the titer from the virus neutralization assay for all pigs in each group.

In those vaccinated animals, neutralizing titers were observed after one dose. As expected, neutralizing titers were higher in the two-dose group compared to the one-dose group at challenge. Mean titers began to decrease by the time of necropsy.

Discussion and Conclusion

Since the emergence of SVA in multiple countries in 2015, SVA has continued to remain endemic in some areas around the world. This work demonstrated that one dose of an SVA whole virus inactivated vaccine was as effective as a two-dose vaccine in reduction of the development of clinical signs and viral replication after homologous SVA challenge. An effective single-dose inactivated SVA vaccine would be economical and could be easier to apply in the field compared to a two-dose product. In addition, an efficacious SVA vaccine could provide a positive impact on the welfare of swine and control the spread of SVA.

Evaluation of the immune response by the use of OncoTherad® associated with vaccinationagainst *Mycoplasma hyopneumoniae* in piglets.

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Introduction

Mycoplasma hyopneumoniae, the main etiological agentof Swine Enzootic Pneumonia, is a microorganism widely spread in swine production worldwide. Prevention of the infection is of great interest for the production system since the colonization in the lung tissue leads to intense production losses. Commercially available vaccines attenuate the clinical manifestation of the disease and lung lesions but do not prevent the colonization of the bacteria. Therefore, this study aims to associate a commercial injectable vaccine against *M. hyopneumoniae* with OncoTherad®, an innovative immunomodulatory compound recently used as a treatment for Covid-19 (1). Five different protocolswere evaluated aiming to increase the systemic and respiratory tract immune response.

Materials and Methods

Fifteen 21-days old weaned *M. hyopneumoniae*-free piglets were randomly distributed into five groups (G) submitted to different protocols of immunization. G1: commercial single-dose vaccine (Hyogen®); G2: Hyogen® associated with a dose of OncoTherad®; G3: only one dose of OncoTherad®; G4: Hyogen® associated with two doses of OncoTherad®; and G5: control, one dose of sterile saline solution.

On D0, the animals were submitted to the five different immunization protocols. Serum and laryngeal swabs were collected to determine antibody response of mucosal IgA (2) and serum IgG anti-*M.hyo* (IDEXX Laboratories, Inc., USA), and the determination of the concentration of serum inflammatory cytokines (IFN-γ and IL-10) by ELISA test.

Results

The results obtained in the *M. hyo* ELISA tests for IgG and IgA antibodies of serum samples and laryngeal swabs are shown in Table 1.

Table 1. Qualitative representation of antibody response against *M. hyopneumoniae* ELISA results from serum and laryngeal swabs collected from piglets.

	Collection timepoints[1]				
Group	D0	D7	D14	D21	D28
G1	-	-	IgG	IgG	IgG
G2	-	-	IgA	IgG and IgA	IgG and IgA
G3	-	IgA	-		
G4	-	-	-	IgG	IgG
G5	-	-	-	-	-

[1]Weekly (D-1, D7, D14, D21, and D28) blood samples were collected to obtain serum and laryngeal swabs to determine the average antibody response of mucosal IgA and serum IgG.

At least one animal from the group was positive in the ELISA results.

At moments D0, D14, and D28, the group that presented the greatest variation in the concentration of IL-10 between individuals was the G1 group. At D7 the group in which the IL-10 concentration varied the most among the individuals was G3 and at D21 the G2 group presented the highest concentration of IL-10.

Furthermore, there was no statistical difference between the groups according to rectal temperature, physical examination, and application area in D1, D2, and D3.

Discussion and Conclusion

Only the groups that received at least one dose of commercial vaccine against *M. hyopneumoniae* were able to produce serum IgG Ab detectable by ELISA test. It is well known that systemic anti-*M. hyopneumoniae* antibodies are considered to play a minor role in protection against PEP (3, 4).

Our findings, showing the concentration of mucosal IgA was detectable by ELISA test in three experimental groups, where animals from these groups received at least one dose of OncoTherad®. It is known that the mucosal IgA antibody probably participates in the protection and prevention against *M. hyopneumoniae* invasion and adherence (5). Interestingly, animals from G2 presented both IgG and IgA antibodies.

Moreover, vaccinated pigs had a higher number of IL-10-producing cells in their bronchial lymph nodes, which may have an anti-inflammatory effect (6).

Different protocols of immunization of piglets against PEP, associating commercial vaccine with a compound used in human medicine resulted in different humoral and cellular responses. Our findings reflect a milder innate inflammatory response, conferred by a protocol using a commercial vaccine associated with OncoTherad®, which may lead to reduced lung tissue damage.

Acknowledgments

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Evaluation of the performance of two commercial ELISA kits for the detection of PRRSV antibodies in serum of pigs vaccinated with 2 biologicals

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Introduction

ELISA is an important tool for monitoring and surveillance of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) on swine farms (1). Immune responses are often variable between animals and the levels of antibodies detected do not necessarily reflect the virulence of the isolates. It is necessary to promote the knowledge of the benefits of ELISA tests available in the market in order to have the best option according to the need

Objetive

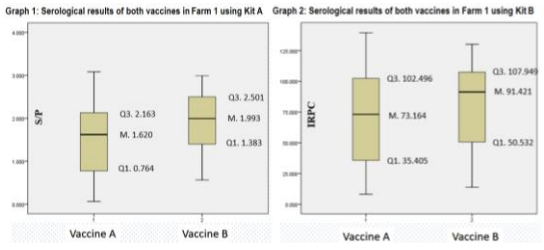
To measure the seroconversion of two vaccines through the determination of antibodies from two commercial kits for the detection of specific antibodies against PRRSV.

Material and Methods

A stratified sampling with statistical significance at 95% of 10 samples per age (3, 6, 9, 12, 15 and 18 weeks) was performed in two PRRSV-positive commercial farms located in Tehuacan Puebla, Mexico. The vaccines were used on the farms with the supplier's criteria for use, as well as the kits used. On each farm, one group per vaccine was immunized (data not shown) and seroconversion in each group was measured with both A and B kits. Vaccine A is a live virus American strain P129 and vaccine B is a live virus ATCC-VR-2332. Kit A, uses a glycoprotein extract (nucleocapsid NP) and Kit B an inactivated whole virus as antigen. The comparative serological analysis was performed with SPSS® v.7 Statistical Package with a T-Student Test.

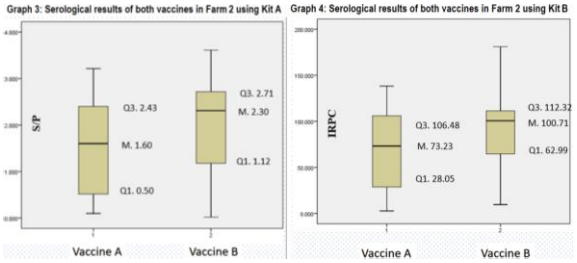
Results and Discussion

Farm 1. The average seroconversion of kit A for the detection of antibodies in both vaccines; is different P<0.05 (P=0.000) while, using kit B there is no significant statistical difference P>0.05 (P=0.121) in the seroconversion of both vaccines. Summarized in Fig.1 and 2.



More negative samples were detected using vaccine A with both kits. Seroconversion using kit A (Fig 1), stability in antibody titer is observed from 9 to 15 weeks with vaccine A however, vaccine B is constant within 9-18 weeks. Using kit B (Fig 2) the antibody titer is stable between 9-18 weeks for vaccine A, while for vaccine B it was stable between 6-18 weeks.

Farm 2. The average seroconversion of kit A and B for antibody detection in both vaccines; is different P<0.05 (P=0.017 and P=0.028 respectively) Summarized in Fig.3 and 4.



A greater number of negative samples were detected using vaccine A with kit B. While kit A detects the same number of negative samples in both vaccines. Seroconversion using kit A (Fig 3), had greater dispersion in the antibody titer with vaccine A, however, the antibody levels of vaccine B are constant between 6 and 15 weeks. Using the B kit (Fig 4) the antibody level is stable between 9 and 15 weeks towards both vaccines.

Due to the nature of the antigen fixed on the plate of an ELISA test, it has been shown that the presence of antibodies to the whole glycoproteins of the antigen is more durable than antibodies generated and detected in nucleocapsid-based tests (2). However, in this study, both kits detected comparable seropositivity to both vaccines/farms of more than 90% in farm 1 and 85% in farm 2.

Conclusion

- Both ELISA kits are able to detect specific PRRSV antibodies after vaccination, although with different levels of sensitivity and specificity.
- Farm 1: Kit A, has better recognition of vaccine antigen B than vaccine A. Kit Befficiently detects the antigens of bothvaccines.
- Farm 2: Both kits detect the antigens of vaccine B but differently from vaccine A.

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Field efficacy and safety study in weaned pigs of a trivalent vaccine mixture (3FLEX) in a 750 sow single-site farrow-to-finish operation

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Introduction

Disease co-infections in this part of the region makes pig farming quite challenging, consequently requiring innovative and strategic immune management approches. 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 this trivalent vaccine mixture under Philippine farm conditions 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 vaccine injection (Table 2). Both groups tested positive serologically for PRRS virus, *M. hyopneumoniae*, and PCV2 using commercial ELISA, tested prior to the start of the study.

Table 1. Vaccination program per pig group aged 21 days (±3d)**.**

GROUP	N	TREATMENT
Yellow	50	Trivalent 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 side of the neck. To measure any performance differences among the treatment groups, ADG and live body weights at market (including body temp.) were recorded and statistically analyzed with 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

TIMING	Flexcombo + 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	FLEX combo + PRRS MLV	3FLEX	*P*-value
Avg. Birth Weight, Kg	1.35	1.33	*0.6762*
Avg. End Nursery Weight, Kg	**25.64**	**26.42**	*0.4894*
Nursery ADG, Kg	**0.366**	**0.377**	*0.4894*
Mortality, head	1	2	
End Finisher Weight, Kg	**121.2**	**122.9**	*0.0064*
Avg. End Finisher Age, day	207	207	
Finisher ADG, Kg	**0.585**	**0.594**	*0.0064*

Discussion and Conclusions

In general, pigs vaccinated with 3FLEX showed a statistically significant growth advantage over their counterparts vaccinated twice concurrently at separate injection sites (FLEXcombo + PRRS MLV). This benefit may be attributed to the reduced levels of iatrogenic stress that pigs typically experience while being handled/restrained during vaccination. There was an obvious significant difference in favor of 3FLEX in terms of higher ADG and finishing weight, while at the same time being compliant with animal welfare protocols.

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Field efficacy comparison of two commercial PCV2 vaccines on three swine farms experiencing different mortality rates in South Korea

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Introduction

Korean swine industry experienced significant losses due to PMWS until 2007. Launching efficacious Porcine Circovirus type 2 (PCV2) mono vaccine resulted in the recovery of performance (1). However, the efficacy of the different PCV2 commercial vaccines may vary according to the farm's production performance. The objective of this study was to compare the efficacy of two commercial PCV2 vaccines in three swine farms experiencing different mortality rates in South Korea.

Material & Method

The field efficacy trial was conducted on three different swine farms sorted by wean-to-finished mortality rate (Table 1). All enrolled farms were currently vaccinating against PCV2.

Table 1. Trial farms

#	Farm	No. of Sow	Average mortality rate after weaning
A	DongYang	250	10%
B	DaeJin	200	6%
C	ShinYoung	200	3%

Before the trial, SERELISA® PCV2 Ab Mono Blocking for use on serum was applied to understand the current infection dynamic. All 3 farms showed seroconversion at the early grower period. A total of 1,063 piglets were included in three trials and vaccinated with two commercial PCV2 vaccines : T1 (Ingelvac CircoFLEX®, Boehringer Ingelheim) or T2 (Suigen® PCV2, Virbac) at ~3 weeks of age , according to label recommendation. To evaluate the performance of the pigs in the two different treatment groups, mortality rate, average days to market, and number of antibiotic injections were recorded . Chi-square analysis was used to make comparisons between the two groups for mortality.

Results

Results are presented on Table 2. The mortality rate in Farm A was significantly reduced in T1 ($P<0.001$). Average days to market and number of antibiotic injections were also lower in T1 at this farm. In Farm B, a numerical advantage in mortality rate was observed in favor of T1.

Table 2. Comparison of performance parameters between treatments on farms A, B and C.

	Mortality rate (%)		Avg days to market		# of AB injection	
#	T1	T2	T1	T2	T1	T2
A	9.3[a]	29.9[b]	179	203	0	107
B	6.8	10.7	198	202	0	0
C	3.0	3.0	162	162	2	5

*T1: Ingelvac CircoFLEX®; T2: Suigen® PCV2

Conclusions and discussion

Ingelvac CircoFLEX ensured better protection and higher performance on the farms experiencing higher mortality associated with PCV2 exposure. When comparing the efficacy results of the two tested vaccines under different mortality scenarios , we observe a tendency of superior protection provided by T1 compared to T2, resulting in lower mortality rate , fewer days to market and less antibiotic injections. The findings of this study are in line with other studies that show that Ingelvac CircoFLEX® is more efficacious compared to other PCV2 vaccines (2, 3). These are reflected in the fact that Ingelvac CircoFLEX® is the preferred vaccine of Korean pork producers because of high mortality in Korea (4).

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Field Efficacy Comparison of Two Commercial Vaccines Against Non-Progressive Atrophic Rhinitis in Taiwan

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Introduction

Bordetella bronchiseptica (Bb) was the primary cause of atrophic rhinitis (AR) causing a mild-to-moderately severe reversible condition called non-progressive AR (NPAR) (1). However, in pigs infected at the age of 4 weeks, regeneration of the turbinals was noted 6 to 8 weeks after infection. When pigs were infected at 3 days of age, this process took five months (2). Thus, sow vaccination is useful in preventing piglet infection. The objective of this study is to compare the field efficacy of two different vaccines indicated for piglet protection against NPAR during the nursery period.

Materials and Methods

The study, conducted from November 2020 until November 2021, included 700 sows from a commercial farrow-to-finish pig farm in southernTaiwan; these were divided in two groups: Group A (RHINISENG®, HIPRA) and Group B (AR vaccine with dl-alpha-tocopherol acetate as adjuvant), vaccinated at 6 and 3 weeks before farrowing, respectively.

Nasal score: over 30 nose samples were randomly selected from nursery pigs (5-12 weeks old) produced from each group and scored based on the European Pharmacopoeia guidelines (3).

Results

Group A and B showed different patterns of nasal lesion score distribution: 92% of group B animals showed nasal lesions, while just 77% were seen in group A (15% reduction). 45.95% of group B animals showed 5-8 grade severity, whereas just 6.45% of group RHINISENG® showed the same severity. (Logistic regression model, $p < 0.001$)(Figure 1). For the average nasal score, group A was 51.3% decreased compared to group B, average 3.9 ± 2.2 to 1. 9± 1.7, with statistical difference.

(Poisson regression model, $p < 0.001$)(Table. 1).

Discussion and Conclusion

Under the conditions of this study, RHINISENG® showed a better field efficacy to protect against non-progressive AR than Vaccine B, with a statistically highly significant difference in nasal lesions.

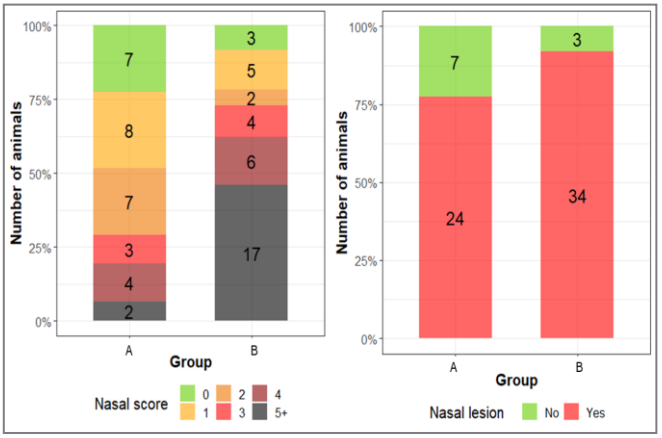

Figure 1. % of animals with a different grade of lesion. % of animals showing lesions or not.

Table 1. % Animals showing nasal lesions, % animals showing grade 5-8 lesions and average nasal score, difference with statistical analysis P value.

	Group A	Group B	Diff	*P-value*
Nasal lesion (% animals)	77%	92%	- 15%	*0.091*
Severity (% animals' grade 5-8)	**6.45%**	**45.95%**	**- 39.5%**	***< 0.001***
Nasal score	**1.9 ±1.7**	**3.9 ±2.2**	**- 1.96**	***< 0.001***

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Field evaluation of the efficacy of an inactivated *Bordetella bronchiseptica* and non-toxic recombinant *Pasteurella multocida* vaccine against atrophic rhinitis in Thai piggery

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Introduction

Porcine atrophic rhinitis (AR) is a prevalent infectious disease of swine herds, caused by toxigenic strains of *Bordetella bronchiseptica* (Bb) and *Pasteurella multocida* type D (PMT) (1,2). In a/this Thai survey, the turbinal atrophy observed was nearly at 80% in both vaccinated and unvaccinated sows (3). The impact of commercial AR vaccines on pig production is of worldwide relevance but the field conditions in Thailand have been limited. Consequently, the goal of this study was to evaluate the efficacy and safety influence of commercial AR vaccine (RHINISENG®, HIPRA) in Thai pig farming.

Materials and Methods

In 2020-2021, a farrow-to-finish unit with 3,000 sows in Nong Bua Lam Phu province, Thailand was conducted on commercial 1,800 sows (Farm 1, Mueng district) and 1,200 sows (Farm 2, Na Tub Kwai), respectively. All pregnant sows on Farm 1 and Farm 2 were vaccinated intramuscularly with RHINISENG® 6 weeks before farrowing and revaccinated 3 weeks later in accordance with the manufacturer's instructions. Side effects in sows and production performance in grower-finisher pigs were recorded and statistically compared before (unvaccinated sow) and after immunizing with RHINISENG® using *t*-test (two sample for means) in SPSS statistical program (version 22.0).

Results

The first results regarding the vaccine safety demonstrated that no adverse reaction following vaccination was reported in all pregnant sows (0%). The main criteria of growth performance index and %loss of pigs from vaccinated sows were significantly improved in pigs from unvaccinated sows including average daily gain (ADG; 802.33 v. 711.86, p=<0.001, Figure 1), feed conversion ratio (FCR; 2.7 v. 2.9, p=0.001), feed cost per gain (FCG; 40.68 v. 41.84, p=0.038) and culling rate (0.53 v. 3.76, p=0.02). The overall pig production in this study is illustrated in Table 1.

Conclusions and Discussion

The current study shows RHINISENG® is safe and achieves high efficacy, with significant nasal lesion reduction and the overall improvement of parameter index in fattening pigs. A preliminary study regarding a framework for comparing the the degree of atrophy of turbinal bones in pigs at slaughter age of RHINISENG® with a vaccine containing inactivated toxigenic strains of Bb, PM and inactivated toxin of PM in this farm had significantly improved (4.67±3.1 v. 6.23±3.6, p<0.039, unpublished). However, we need to further design a field measurement of turbinal atrophy and pneumonia associated with growth rate in grower-finisher pigs in several commercial AR vaccines in large-scale farming. This field study demonstrates that establishing a RHINISENG® vaccination program in sow herds is one of the successful key strategies for the improved control of atrophic rhinitis resulting in significantly better productivity.

Table 1. Production performance of pigs derived from gestating sows before and after immunizing with RHINISENG® in 12 units per group

Index	Before	After	Diff	*P*-value[ab]
Entry weight (kg)	24.57	24.24	-0.33	0.59
No. entry pigs	835	822	-13	0.53
Finishing weight (kg)	107.07	108.7	+1.63	0.29
Finishing age	**26.07**	**24.71**	**-65%**	**<0.001*****
Mortality rate	**2.23**	**0.79**	**-85%**	**<0.001*****
Culling rate	**3.76**	**0.53**	**+90**	**0.02***
FCR	**2.90**	**2.70**	**-0.20**	**0.001****
FCG	**41.84**	**40.68**	**-1.17**	**0.038*****

[ab] P<0.05 indicated statistical differences between group.

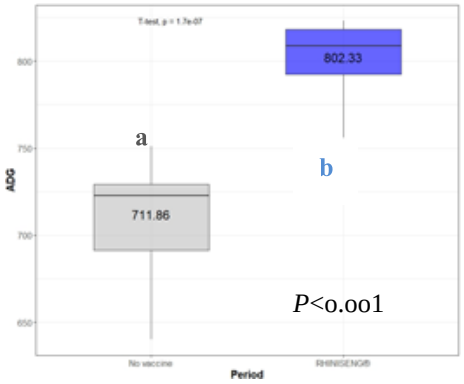

Figure 1. Comparison of ADG of pigs derived from unvaccinated and vaccinated sows

[ab]Different superscript indicated statistical differences between groups (p<0.05)

Acknowledgments

We greatfully acknowledge farm owners for providing the production data and encouragement.

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Field safety of an intradermal recombinant vaccine containing *Mycoplasma hyopneumoniae* strain (Nexhyon)

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Introduction

Mycoplasma hyopneumoniae (*M. hyopneumoniae*, *M.hyo*) and porcine circovirus 2 (PCV2), typically plays a prominent role in the porcine respiratory disease complex (PRDC) that normally circulates in piggeries worldwide (1,2). Until now, PCV2-a-based vaccines have been an essential part of an effective strategy for controlling possible outbreaks and maximizing farm profitability (3,4,5). Following its market launch in the first quarter of 2022, HIPRA Thailand has released the results of a series of studies concerning Mhyosphere® PCV ID, a new intradermal one-shot vaccine containing the recombinant Mhyo^{cpcv2} strain, named Nexhyon, that expresses the PCV2 capsid protein in its cytoplasm and together acts as a single active substance.

Field practitioners have raised concerns about PCV2 vaccine safety and its consequences. Consequently, the purpose of this study was to observe the field safety of Mhyosphere® PCV ID (HIPRA, SPAIN) on commercial swine farms in Thailand.

Materials and Methods

The field safety of Mhyosphere® PCV ID was evaluated in a randomized trial on 11 commercial swine farms in Thailand from December 2021 to early February 2022. Healthy 21 day old piglets were vaccinated with Mhyosphere® PCV ID (0.2 mL, ID). The piglets were observed for adverse effects during or immediately after vaccination and for general health one day before vaccination, at vaccination, 30 min and 4 h after vaccination and daily for 3 days. The injection site was examined by palpating for local reactions at 1 and 4 h after vaccination and daily for 3 days. The frequency of adverse reactions is defined as described in Table 1.

Table 1. Classification index of the adverse reaction.

Index	Adverse reaction
Very common	≥ 1 in 10 pigs treated (≥10%)
Common	≥1 < 10 pigs in 100 pigs treated (≥1% < 10%)
Uncommon	≥1 < 10 pigs in 1,000 pigs treated (≥0.1% < 1%)
Rare	≥1 < 10 pigs in 10,000 pigs treated (≥0.01% < 0.1%)
Very rare	<1 pigs in 10,000 pigs treated (<0.01%)

Results

A total of 24,970 Mhyosphere® PCV ID vaccine doses were administered on eleven swine farms and a complete absence of vaccine-associated side effects in piglets was reported (0%). Evidence of local injection site and systemic reactions on each farm are shown in Table 2.

Table 2. Incidence of adverse reactions in the first phases of clinical trials using ORF2-based vaccines or Mhyosphere® PCV ID

Territory / Region	Farm size No. sows	Before the trials (Farm history)		Mhyosphere® PCV ID vs ORF2-base vaccines	
		PCV2 vaccine	Adverse reactions	Local Side effects	Systemic reaction
North	2,700	ORF2 subunit, IM	Not detectable	0/6,900	0/6,900
North	1,500	ORF2 subunit, IM	Not detectable	0/2,500	0/2,500
North	1,500	ORF2 subunit, ID	Not detectable	0/830	0/830
North	800	ORF2 subunit, IM	Not detectable	0/1,825	0/1,825
North	Wean to finish	ORF2 subunit, IM	Depression	0/590	0/590
East	9,000	PCV2 protein Ag, IM	Un common	0/2,075	0/2,075
East	1,500	ORF2 subunit, IM	Un common	0/2,000	0/2,000
East	300	Killed baculovirus vector, IM	Un common	0/400	0/400
Central	12,500	PCV2 + M.hyo (RTU), IM	Un common	0/1,250	0/1,250
Central	2,000	ORF2 subunit, IM	Un common	0/3,000	0/3,000
Northeast	1,800	PCV2 + M.hyo (RTU), IM	Not detectable	0/3,600	0/3,600
Total				**0/ 24,970**	**0/ 24,970**

Conclusions and Discussion

Preliminary results from this multi-site field safety assessment show the excellent safety profile of a single-dose of Mhyosphere® PCV ID administered intradermally with HIPRADERMIC® in 3-week-old piglets. Additionally, this is the first safety report in Thailand to show that a new intradermal one-shot vaccine based on a recombinant *M. hyo*, called Nexhyon, expressing the PCV2 capsid protein is safe when applied on healthy piglets at 3 weeks of age. On the other hand, large-scale, longitudinal field studies regarding the field safety and efficacy of Mhyosphere® PCV ID are also needed.

Fig. 1. No side effects or clinical symptoms in vaccinated lactating piglets (3 weeks old) were observed in 30 minutes. Picture: HIPRA Thailand

Acknowledgments

The authors wish to thank farm owners for providing the farm information and for their co-operation.

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Field Study Comparing Two Commercial Vaccines Against Non-Progressive Atrophic Rhinitis in Taiwan

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Introduction

Atrophic rhinitis (AR) is a widespread infectious disease of swine characterized by the twisting or shortening of the snout. *Bordetella bronchiseptica* (Bb) was the primary cause of AR causing a mild-to-moderately severe reversible condition called non-progressive AR (NPAR) (1). However, in pigs infected at the age of 4 weeks, regeneration of the turbinals was noted 6 to 8 weeks after infection. When pigs were infected at 3 days of age, this process took five months (2). Thus, sow herd vaccination is useful in preventing piglet infection.

The objective of this study was to compare the efficacy of two different AR vaccines during the nursery period.

Materials and Methods

The study, conducted from August 2019 to April 2020, included 2,000 sows from a commercial farrow-to-finish pig farm in southern Taiwan. The farm was divided in two groups: Group A (Vaccine A, AR vaccine with dl-alpha-tocopherol acetate as adjuvant) and Group B (RHINISENG®, HIPRA), vaccinated at 6 and 3 weeks, respectively, before farrowing.

Nasal score: 36 nose samples by group were randomly selected from nursery pigs (4-10 weeks old) and were scored based on the European Pharmacopoeiaguidelines (3).

Results

The sverage nasal lesion scores of group A and B were 7.8 (between 2-14 points) and 3.0 (between 0-6 points) respectively, reducing lesions with significant difference (Wilcox test *p*-value <0.001) (Fig.1). After analyzing the proportion of nasal lesion severity, 100% of the samples from group A were affected, with 41.7% being severely affected, 41.7% moderately and 16.6% mildly. Whereas, in group B, 83.3% of the samples were concentrated within the range of mild or no lesion, and the other 16.7% were affecteds moderately.

Discussion and Conclusion

After vaccination program introduction, the lesions of nasal turbinals in pigs vaccinated with RHINISENG® were significantly reduced, both in terms of the proportion of animals affected but also the distribution of severity in only 8 months. This study reinforces not only the importance of immunization against NPAR in protecting nursery pigs, but also the efficacy of RHINISENG® as the only registered vaccine that controls non-progressive atrophic rhinitis.

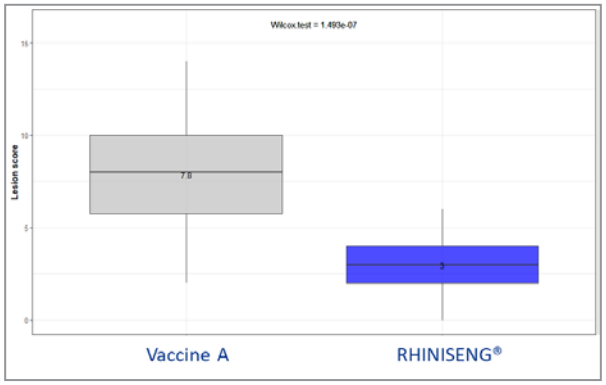

Figure 1. Average nasal lesion score. Average nasal lesion score of group A and B (Wilcox test *p*-value <0.001).

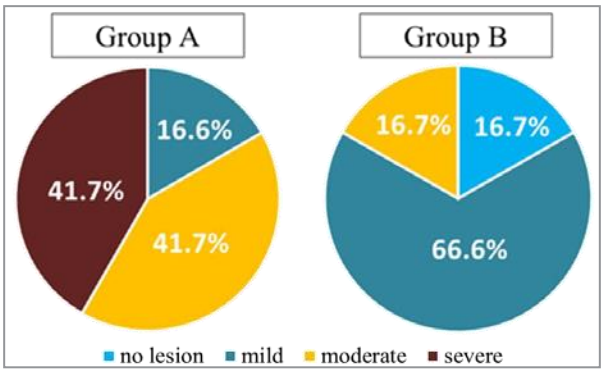

Figure 2. Nasal lesion severity distribution (%animals).

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Immunogenicity of a recombinant vaccine against *Lawsonia intracellularis* in mice model

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Introduction

Pig farming represents an important sector for the Brazilian economy, making relevant the development of inputs that reduce the burden of bacterial infections on swine production. Proliferative Enteropathy (PE) is caused by *Lawsonia intracellularis* and is highly prevalent in swine (1). It is characterized by hemorrhagic diarrhea, decreased growth and reproductive rates of animals (2). The available PE vaccines have protection-related limitations, adverse effects and make it difficult to differentiate between diagnoses from vaccinated and infected animals (3). Reverse vaccinology studies have identified a protein conserved in all known strains of *L. intracellularis*, which has 98% similarity of the 16S-rDNA gene with strains that infect other species. This protein is recognized by infected swine serum, suggesting consistent expression by the *L. intracellularis* bacterium and indicates that this protein has the ability to activate the host immune system (4). Therefore, the objective of the present study was evaluate the ability of *L. intracellularis* recombinant protein, fused with TT-Th carrier molecule (rLiTT), to stimulate specific immune response in mice.

Materials and Methods

The LiTT sequence was inserted into the pet/28a vector. The recombinant plasmid LiTT/pet28a was transformed into *E. coli* BL21 Star™ (DE3) cells and the expression induction was performed with 1 mM IPTG for 4 h at 37 °C. Bacteria were recovered by centrifugation, sonicated, and eluted in 8 M urea. Solubilized recombinant proteins were purified using the ÄKTA Primer (GE Healthcare) system. Subsequently the proteins were analyzed using SDS-PAGE. The concentration of the purified protein was determined using the commercial BCA Protein Assay kit (GE Healthcare). To evaluate rLiTT immunogenicity Balb/c female mice were allocated in three groups and inoculated by subcutaneously injection (200 µl) on days 0 and 21: 1. rLiTT (100 µg) adsorbed in 10% aluminum hydroxide [$Al(OH)_3$; Sigma Aldrich)]; 2. Enterisol® Ileitis commercial vaccine (Boehringer Ingelheim) using 1/20th of a recommended dose (2 ml swine dose) and 10% aluminum hydroxide; 3. saline solution plus 10% aluminum hydroxide. Blood samples were collected by submandibular puncture on days 0, 7, 14, 21, 28, 35 and 42 to evaluate the humoral immune response by indirect ELISA. All protocols were approved by the Ethics Committee on Animal Experimentation (CEEA No. 28134-2019) of the Federal University of Pelotas (UFPel).

Results

The rLiTT expression was evaluated by SDS-PAGE and confirmed by Western blot using a monoclonal anti-His antibody which recognized the recombinant protein, showing a band of 18 kDa size. Mice vaccinated with rLiTT/aluminum hydroxide showed antibodies after the prime vaccination (day 14), the level has increased after the boost and keeping high up to 42 days, presenting title 1.6400. The commercial vaccine group also showed humoral response with significant antibodies since the first dose, obtaining 1.3200 title. The control group, inoculated only with saline buffer (PBS), did not show humoral response (Figure 1).

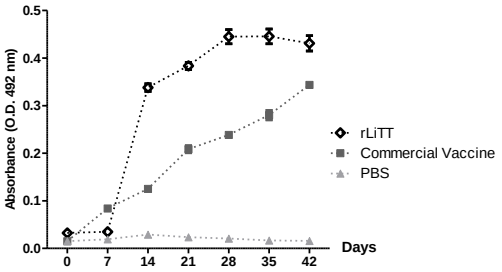

Figure 1. **Evaluation of antibody levels.** Total IgG produced in mice vaccinated with rLiTT and commercial vaccine. Pool of sera were characterized at a single serum dilution (1:100). The mean optical density ($OD_{492\ nm}$) ± standard deviation (bars) from triplicates test is shown.

Conclusions and Discussion

In this study we selected a *L. intracellularis* protein (rLi) fused with TT molecule to be used as antigen to develop an experimental vaccine against PE. The protein rLiTT adsorved in aluminum hydroxide was able to induce humoral imune response in mice, showing IgG dynamics and antibodies title higher than the commercial vaccine. Still, is necessary to characterize the cell response (IL-4, IL-8, IL-17, IFN-y) to determine the potencial of rLiTT to induce protection against PE.

Acknowledgments

Coordination for the Improvement of Higher Education Personnel (CAPES) and National Council of Technological and Scientific Development (CNPq).

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Impact of a single PROGRESSIS vaccination of piglets issued of sows under a PRRS Prime-Boost Strategy vaccination schedule

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Introduction

Dual Technoloy Primer Boost (DTPB) has beenreported as a successful strategy to improve PRRS vaccination efficacy. In addition, piglet vaccination has been proposed as an option to improve results [1,2]. We report our experience using a single shot of PROGRESSIS in piglets with high levels of maternal immunity.

Materials and Methods

An industrial farrow-to-finish Russian farm of about 100000 pigs not vaccinated against PRRS experienced an acute outbreak by the end of May 2019 and was submitted to different vaccination strategies. Since January 2020, all weaned piglets (37 batches) belonged to sows vaccinated with a DTPB scheme, i.e., MLV vaccine at 60 days of pregnancy and PROGRESSIS at 90 days of gestation and remained unvaccinated (T1). In contrast, piglets weaned between March and June 2020 received a single shot of PROGRESSIS at 24-34 days of age (T2, 26 batches). Results were analysed by means of an ANCOVA model including ADG or %mortality during the post-weaning phase as independent variables, mean entrance weight as covariate and treatment and technician as factors.

Results

Our results show that treatment played a significant role in both parameters while mean body weight at entrance and the technician were not significant:

ADG (g/d) results (mean±SD) were: 189±72.9, 354±49.0 for T1 and T2 respectively. α= 0.05, p<0.001.

Mortality (%) results (mean±SD) were: 13.3±8.71, 8.86±5.67 for T1 and T2 respectively. α= 0.05, p=0.005.

Discussion and Conclusion

The introduction of one shot of PROGRESSIS in piglets issued from sows vaccinated according to a DTPB scheme yielded a significant (p<0.001) improvement of ADG and a reduction of mortality compared to piglets left unvaccinated[3,4,7]. It suggests that piglets born in a PRRS endemic farm could benefit for a priming with inactivated vaccine [5,6].

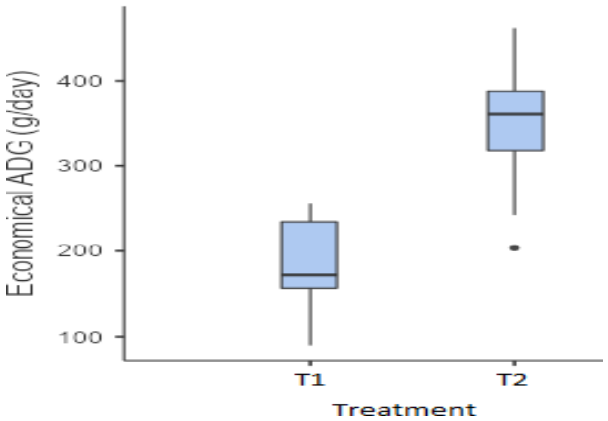

Figure 1. Economical ADG (g/day)boxplots for T1 and T2 pigs

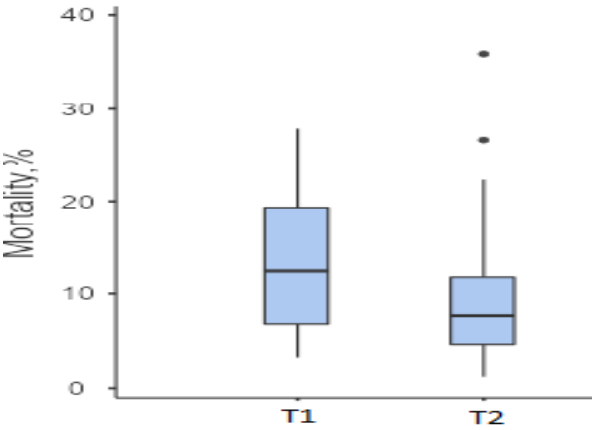

Figure 2. Mortality (%) boxplots for T1 and T2 pigs.

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Improved piglet performance and reduced mortality and antimicrobial use following oral vaccination with a live non-pathogenic *E. coli* F4/F18 vaccine against PWD

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Introduction

Post-weaning *Escherichia coli* (*E. coli*) diarrhea (PWD), also called post-weaning enteric colibacillosis, remains a major cause of economic losses for the pig industry (1,2). PWD typically causes mild to severe watery diarrhea between 5 and 10 days after weaning and is caused primarily by enterotoxigenic *Escherichia coli* (ETEC). The most common adhesins found on ETEC from PWD in pigs are associated with fimbriae F4 (previously called K88) and F18, while the predominant enterotoxins are heat-labile toxin (LT), heat-stable toxin a (STa), and heat-stable toxin b (STb). In addition to F4 and F18, other fimbrial adhesins, such as F5 (K99), F6 (987P), and F7 (F41), have been associated with PWD, but less frequently (3-8). Therapy to combat PWD typically consists of antibiotic treatment. However, emergence of antimicrobial resistance in *E. coli* strains and new EU regulations urge the need for alternative control measures, such as adapted feeding strategies or immunization. Recently, an oral live bivalent *E. coli* F4/F18 vaccine (Coliprotec® F4/F18; Elanco) has become available on the European market, which reduces the impact of PWD provoked by F4-ETEC and F18-ETEC

Materials and Methods

Oral vaccination of suckling piglets using a live bivalent non-pathogenic *E. coli* F4/F18 vaccine was performed in 10 farrow-to-finish sow farms to prevent against PWD due to F4-enterotoxigenic *E. coli* (ETEC) or F18-ETEC. The vaccination strategy was compared to the standard therapeutic approach in each farm, meanwhile collecting data on Average Daily Weight Gain (ADWG), Feed Conversion Rate (FCR), mortality rate and treatment incidence with antimicrobial drugs (TI_{100}) during the post-weaning period.

Results

Vaccine-treated groups demonstrated a significant improvement in FCR (-4.2%), mortality rate (-57.1%) and TI_{100} (-84.9%) as compared to the Control group. The ADWG only marginally and non-significantly improved (+2.3%) in the Vaccine-treated group (Table 1).

Discussion and Conclusion

In conclusion, the present study demonstrated the efficacy of an oral live non-pathogenic *E. coli* F4/F18 vaccine (Coliprotec® F4/F8; Elanco Animal Health) for active immunization of piglets against PWD due to F4-ETEC and F18-ETEC under field conditions. For several economically important performance parameters, such as FCR, mortality rate and TI_{100}, *E. coli* vaccination performed significantly better as compared to the standard therapeutic approach. Therefore, vaccination against PWD due to F4-ETEC or F18-ETEC using an oral live non-pathogenic *E. coli* F4/F18 vaccinated may be considered a good alternative to consolidate post-weaning piglet performance results while meeting the new European requirements concerning prudent use of antimicrobials in intensive pig production.

Table 1. Performance results obtained in Control and Vaccine-treated animals from different field trials using a live, non-pathogenic, oral *E. coli* F4/F18 vaccine in piglets to prevent clinical impact of post-weaning diarrhea due to enterotoxigenic *E. coli* F4 or F18. Significant differences are indicated by their *P*-value. ADWG, average daily weight gain; FCR, feed conversion rate; TI_{100}, treatment incidence per 100 days in production.

Parameter	Control	Vaccine	*P*-value
ADWG (g/day)	348.14	359.29	0.1641
FCR (kg/kg)	1.72	1.66	0.0164
Mortality (%)	5.43	2.33	0.0083
TI_{100} (days)	17.74	2.64	0.0009

Acknowledgments

All Belgian and Dutch swine farmers and veterinarians for collecting and sharing obtained production data for further analysis.

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Improvement of productive parameters in nursery pigs after Non-Progressive Atrophic Rhinitis vaccination

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Introduction

Atrophic Rhinitis (AR) is a disease in pigs causing upper respiratory infection leading to nasal discharge, sneezing, and reducing growth rate. It is caused by the coinfection of *Bordetella bronchiseptica* (Bb, Non-Progressive AR) and *Pasteurella multocida* type D (PmD, Progressive AR)[1].

The aim of this study was to evaluate how vaccination with Rhiniseng® could control respiratory problems in nursery pigs[2] and improve productive parameters[3].

Materials and Methods

A 6,000-pig nursery farm in Casella (Italy), raised pigs showing respiratory problems was studied. *Actinobacillus pleuropneumoniae*, *Glasserella.parasuis* and *Mycoplasma.hyopneumoniae* vaccination but also Florfenicol treatments were implemented in the past with no positive results. Vaccination with Rhiniseng® was given (SPC recommendations) and productive parameters were compared before (Control group - CG) and after it (vaccinated group-VG). A total of 6 and 5 consecutive batches (2,500-3,000 piglets/batch) before and after vaccination were evaluated.

Results

After vaccination, relevant differences were seen at the entry weight (+210 gr, T-Test *p=0.071*) (Figure 1). Better homogeneity at the entrance and end of the period was noticed. Antibiotic treatments were clearly reduced (- 231.06 mg/Kg (T-test, *p=0.12*)) (Figure 2). Mortality reduction by -32% was observed (T-test, *p=0.12*) (Figure 3) and a positive relationship between vaccination and average daily weight gain was clearly seen (R=0.68, *p=0.093*) (Figure 4).

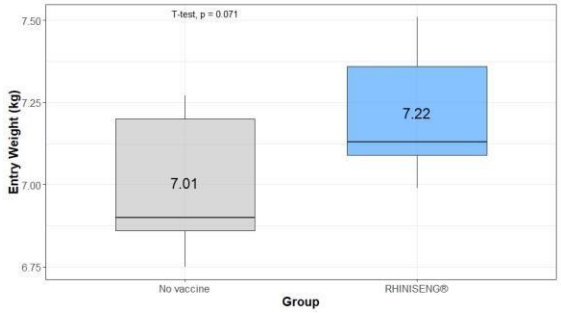

Figure 1. Entry weight at the nursery period before and after vaccination with RHINISENG®

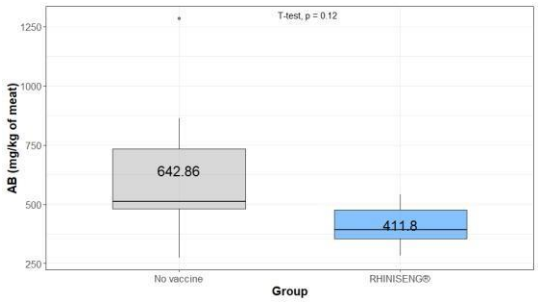

Figure 2. Antibiotic consumption per piglet before and after vaccination with RHINISENG®

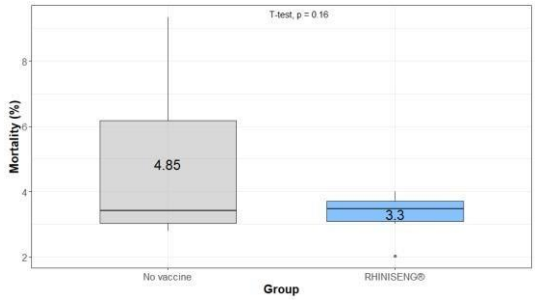

Figure 3. Pig mortality before and after vaccination with RHINISENG®

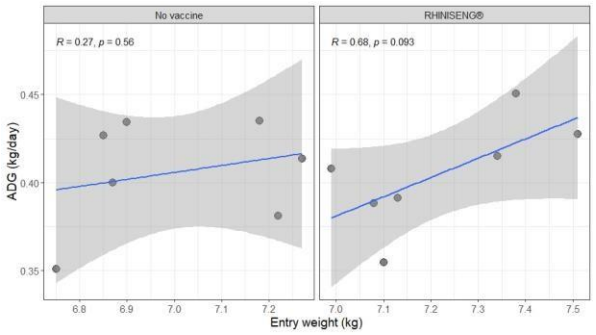

Figure 4. Average daily gain before and after vaccination with RHINISENG®

Discussion and Conclusion

Due to the small nº of batches (n=11), statistical differences were not seen, but a positive tendency could be seen in all the productive parameters studied, improvement in growth performance, reduction in antibiotic treatments, reduction in mortality, and better homogeneity.

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Improving colostrum, neonatal piglet immunity and performance with algal Beta-glucans 1,3

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Introduction

With the trend for hyperprolific sows, it is a challenge to guarantee adequate colostrum supply and intake in the piglets. Piglets which fail to ingest sufficient colostrum have lower survival, show predisposition to diarrhoea and subsequently lower performance (1,2). Beta-glucans are found in the cell walls of algae, yeasts and bacteria. Their efficacy as immunomodulators depend on their size and structure. Unbranched Beta-glucans 1,3 from algal sources have the greatest potential to modulate the immune system (3, 4). A trial was conducted to evaluate the effect of algal Beta-glucans 1,3 in the pre-lactation and lactation phase of sows and its respective effect on the performance and immunological status of piglets in the lactation phase.

Materials and Methods

The study was carried out on a commercial farm, 120 sows were included in the study. The period corresponded to the last 3rd of gestation (day 85 to 115) until weaning (21 days), the diets fed were pre-farrowing and lactation diet, respectively. The sows were split in control and Beta-glucan 1,3 group (200g/t dried algae Beta-glucan 1,3 on top). Number of total and live born piglets, average piglet weight at birth, average piglet weight after colostrum ingestion (18h after farrowing), mortality, number of piglets weaned, average piglet weight at weaning and average feed consumption of the sow were evaluated. Up to 6 hours after delivery, 20 sows/treatment were randomly selected for colostrum sampling (5) and evaluated immunological profiles (IgG, IgA and IgM), 4 days after farrowing, blood was collected from one piglet selected from each litter (20 piglets), to evaluate serum immunological profile (IgG, IgA and IgM). The data was analyzed using the Kolmogorov-Smirnov & Lilliefors tests and Shapiro-Wilk's ($p>0.05$). Normally distributed data were subjected to analysis of variance and Student's t-tests and non-normal quantitative data were compared using the Wilcoxon-Mann-Whitney and Kruskal-Wallis test (6).

Results

Algal Beta-glucans 1,3 supplementation during the last 3rd of gestation did not significantly impact the parameters assessed at birth and weaning. On the other hand, algal Beta-glucans 1,3, lead to a significantly increased colostrum production (Table 1). Additionally, algal Beta-glucans 1,3 promoted better production of IgG, IgA and IgM ($p<0.05$) in the quantity of these immunoglobulins in the colostrum (Figure 1) and, consequently, resulted in higher serum concentration ($p<0.05$) in the piglets at 4 days of age (Figure 2).

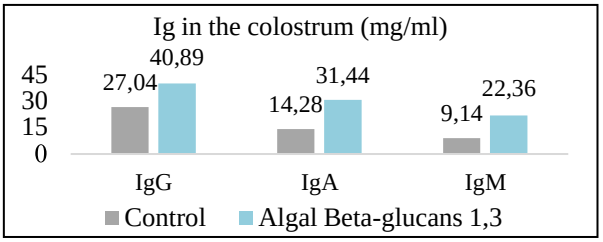
Discussion and Conclusion

The use of algal Beta-glucans 1,3 during the pre-lactation and lactation period in sows promoted the highest colostrum production, and the piglets were able to ingest more colostrum resulting in a greater weight gain. Additionally, algae Beta-glucans 1,3, resulted a higher concentration of IgG, IgA and IgM in the colostrum and improved immunity to the piglets (serum concentration of IgG, IgA and IgM). The investment in the sow´s colostrum production and quality are essential to ensure the immune status in piglets.

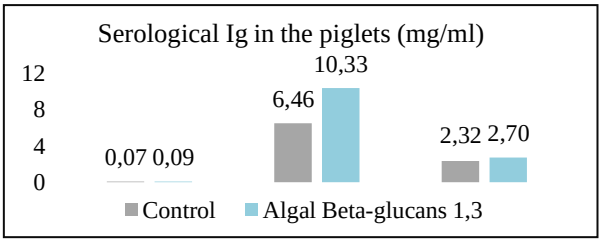
Table 1. Averages of birthweights (Kg), piglet weights at 18h (Kg), weight gains 18h (g), colostrum production 18h (Kg) and colostrum intake 18h (g) in the control and algal Beta-glucans 1,3 groups

	Control	algal Beta-glucans 1,3	CV (%)	p-value
Birthweight	1.342	1.337	15.3	0.9263
18h weight	1.403	1.426	14.9	0.7316
Weight gain 18h	0.068[b]	0.102[a]	69.3	0.0354
Colostrum production	3.476	4.343	41.9	0.0550
Colostrum intake	234.92[b]	294.05[a]	40.1	0.0394

a,b Samples not sharing letters are different according to Student and Wilcoxon-Mann-Whitney test ($p<0.05$); CV = coefficient of variation



Graph 1: Immunoglobulins in the colostrum; $p<0.05$



Graph 2: Immunoglobulin in piglets 4d of age; $p<0.05$

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Innovative oral immunization protocol against *M. hyopneumoniae* results in acute-phase protein responses and reduction in lung tissue damage in challenged piglets.

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Introduction

Mycoplasma hyopneumoniae (M. hyopneumoniae), the main etiological agent of Swine Enzootic Pneumonia, is a microorganism widely spread in swine production worldwide. An oral vaccine against *M. hyopneumoniae*, capable of stimulating the mucosal-associated lymphoid tissue (MALT) was developed using vehicle porous silica and a polymer with specific pH solubility (Mechler-Dreibi et al., 2021). Acute-phase protein responses (APPs) are useful tools to evaluate the efficacy of the vaccine and to monitor the intensity of the infection injuries (Hultén et al., 2003). This study aimed to evaluate the response of a broad profile of acute-phase proteins to the challenge with *M. hyopneumoniae* in piglets immunized with different vaccine protocols.

Materials and Methods

Thirty 21-days old weaned *M. hyopneumoniae*-free piglets were randomly distributed into five groups of six piglets each, as follows: Group 1 (G1): piglets vaccinated with a single-dose commercial vaccine at 24 days of age; Group 2 (G2): piglets that received a single dose of the oral vaccine at 24 days of age; Group 3 (G3): piglets vaccinated with a single-dose commercial vaccine at 24 days of age and with oral vaccine at 55 days of age; Group 4 (G4): piglets that received the oral vaccine at 24 and 55 days of age; Group 5 (G5): control group, with non-vaccinated animals. All piglets were challenged with 5 mL of Friis medium containing 10^6 CCU/mL of *M. hyopneumoniae* (strain 232) at 70 days of age. Blood samples were collected three times: at 24 days of age (D0), one day after the challenge (D49), and one week after the challenge (D56). The serum concentration of total proteins was determined by the biuret method and protein fractionation was performed using the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Results

There was a significant difference in the concentrations of transferrin, α1-antitrypsin, and α1-acid glycoprotein (Fig. 1). There was a significant increase in the concentration of transferrin in the five groups at D56. Serum α1-antitrypsin concentration was significantly higher in G5 at D49, and an increase in the concentration of α1-acid glycoprotein was noted in G2 at D49 and G4 and G5 at D49 and D56. A higher concentration of albumin was observed in group G2 at D56 and a higher concentration of haptoglobin in G2 and G3 at D49. At D56, there was a higher concentration of haptoglobin in G2 and G4.

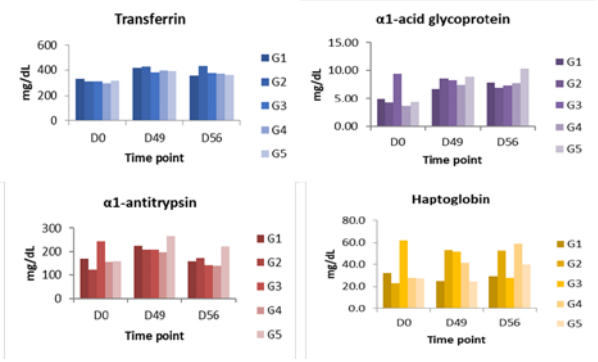

Fig. 1. Mean values of the serum concentration of the proteins transferrin, α1-antitrypsin, α1-acid glycoprotein and haptoglobin.

Discussion and Conclusion

The increase in the serum concentration of the evaluated APPs may be related to the intensity of the immune response caused by the infection and/or vaccineresponse (Rubio and Schmidt, 2014). Transferrinprotein profiles between D49 and D56 were higher in animals vaccinated with the oral vaccine, either alone or combined, than in the other groups, which may indicate a lower intensity of the inflammatory process caused by the challenge. α1-antitrypsin protein increased from D49 to D56 only in the control group, and decreased in G4, possibly indicating lower activity of proteolytic enzymes (Souto, 2019). G2, G4, and G5 had a higher concentration of haptoglobin, which may indicate the occurrence of a greater immune response to the pathogen in these animals since it contributes to the regulation of the immune response to potentially harmful inflammation or infection (Arredouani et al., 2003). Both vaccination protocols resulted in significantly different, positive and negative, acute-phase protein responses, reflecting in a milder innate inflammatory response conferred by vaccine protection, minimizing tissue damage.

Acknowledgements

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Long-Term Field Efficacy Comparison of Two Commercial Vaccines Against Progressive Atrophic Rhinitis in Taiwan

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Introduction

Pasteurella multocida (Pm) is the etiological agent of Progressive Atrophic Rhinitis (PAR) in swine and is of considerable economic impact to the pig-rearing industry throughout the world. PAR is characterized by atrophy of the nasal turbinal bones, which in severe cases can lead to irreversible facial distortion and may negatively affect growth rate and the efficiency of feed conversion (1).

The objective of this study was to compare the field efficacy of commercial vaccines from different vaccination programs on slaughterhouse pigs.

Materials and Methods

The study, conducted from 2018 to 2020, included 2,500 sows from a commercial farrow-to-finish pig farm in southern Taiwan, which were divided in two groups: Group A (Vaccine A, AR vaccine with dl-alpha-tocopherol acetate as adjuvant) and Group B (RHINISENG®, HIPRA), vaccinated at 6 and 3 weeks, respectively, before farrowing. Additionally, piglets from Group A were vaccinated at one week of age with commercial vaccine B (AR vaccine with aluminum hydroxide as adjuvant). 30 nose samples were randomly selected from slaughtered pigs (28 weeks old) from each group and scored based on the European Pharmacopoeia guideline (2).

Group B was sampled twice after continuing vaccination with RHINISENG® at 1 and 2 years after implementation respectively.

Results

Nasal scores from each group of pigs showed different severity proportions: 100% of the samples from Group A were affected, with 37% animals being severely affected, whereas 84% and 83% of animals from group B were affected, with 3% and 0% animals being severely affected. Group B after 2 years, 77% of the animals showed no lesions or mild severity (Fig. 1).

Furthermore, comparing group A and group B 1 and 2 years apart, mean nasal lesion scores were 8.07, 3.52 and 2.67, respectively. Significant reduction in nasal lesion scores between each group was seen (Wilcox test; P-value <0.05) (Fig. 2).

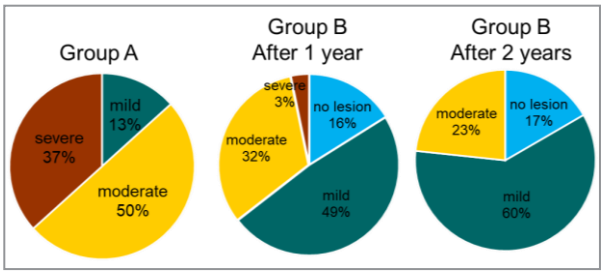

Figure 1. Nasal score proportion of animals by severity. Definition of severity: score 0: no lesion; score 1-4: mild; score 5-8: moderate; score 9-18: severe.

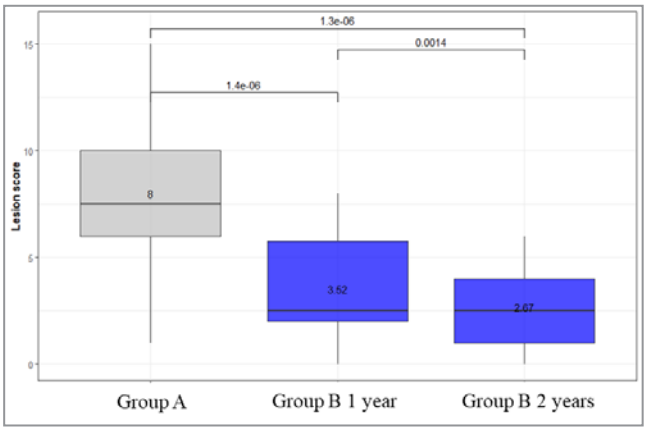

Figure 2. Comparison between Group A and Group B after 1 and 2 years. (Wilcox test; P-value <0.05).

Discussion and Conclusion

Under the conditions of this study, RHINISENG® reveals a better field efficacy to protect sows and piglets against progressive AR than Group A, with a statistically highly significant difference in nasal lesions. Notably, long-term field efficacy can be seen between group B 1 and 2 years, with a 24% reduction in nasal lesion score, which suggests that the more years of protection, the better protection the farm will have.

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Maternally derived antibodies do not interfere with immune response induced in piglets after the administration of a commercial trivalent FMD vaccine

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Introduction

The assessment of the best strategy for vaccination against foot-and-mouth disease (FMD) in piglets born to vaccinated sows with each vaccine applied in the vaccination programs is needed (1). The present work assessed the effect of maternally derived antibodies (MDA) on the immunization of piglets with a commercial trivalent vaccine to determine the optimal age for vaccination.

Materials and Methods

Pregnant sows were vaccinated twice, at 64 and 85 days of gestation, with a regular dose (2 mL) of Bioaftogen®, Biogénesis Bagó (O1 Campos, A24 Cruzeiro, A2001 Argentina vaccine strains). Piglets born from vaccinated and unvaccinated sows were randomly assigned into groups receiving different vaccination schemes (Table 1). Serum antibody levels against each of the vaccine strains were determined by Liquid Phase Blocking ELISA (LPBE) at different times between 0 and 120 days post vaccination (dpv).

Differences antibody titers between animals with and without MDA receiving the same treatment, as well as between animals in different groups, were assessed by the Mann-Whitney test. Comparison of antibody titers within the animals in the same group between two different post vaccination times was performed with the Wilcoxon signed-rank test. The Spearman´s rank correlation test was applied to analyze the relationship between the titers of MDA at the day of vaccination and the antibody titers at 28 dpv.

Table 1. Experimental groups

#	Vaccination Scheme	N	Born from sows
1	1 dose at 2 weeks old	20	Vaccinated
		15	Unvaccinated
2	1 dose at 5 weeks old	10	Vaccinated
		10	Unvaccinated
3	2 doses at 5 and 8 weeks old	10	Vaccinated
		10	Unvaccinated
4	Not Vaccination	10	Vaccinated
		10	Unvaccinated

Results

Sows responded adequately to vaccination during pregnancy, with mean Log_{10} antibody titers > 2.5 after 56 dpv and 91 dpv (corresponding to 1 and 35 days postpartum).

Piglets born from unvaccinated sows showed undetectable antibody titers at 0 DPV, while those born from vaccinated sows exhibited high levels of MDA (mean Log_{10} antibody titers > 2.5). In group 4, MDA levels remained high up to 35 days of age and became undetectable after 82 days of age. All piglets in groups 1, 2 and 3 responded properly to vaccination, mean Log_{10} antibody titers remained above 2.5 up to 120 dpv (Figure 1). No statistically significant differences were observed in antibody titers between animals with or without MDA at different times post vaccination. Also, there were not found significant differences in antibody response at different times post vaccination between animals vaccinated at 2 and 5 weeks old. Moreover, the correlation analysis evidenced no association between the antibody response at 28 dpv and the level of MDA at the time of vaccination.

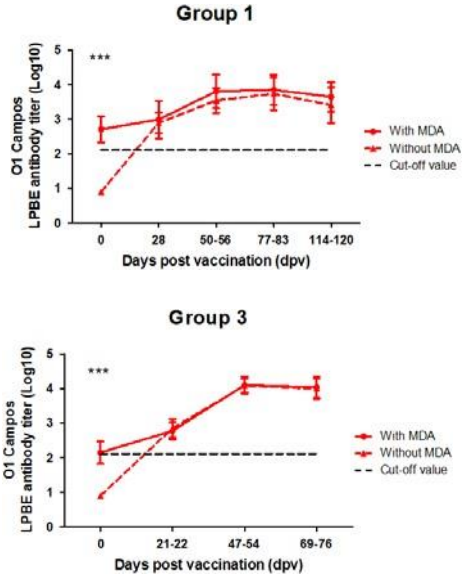

Figure 1. Antibody response against O1 Campos vaccine strain in piglets from groups 1 and 3. *** p<0,001. Cut-off indicates protection value (2.11 log10)

Discussion and Conclusion

The vaccine used in this study induced high and long-lasting antibody response in piglets vaccinated at early age (2 or 5 weeks-old). In our study, MDA did not interfere with the antibody response induced after vaccination as it was shown in previous studies with other vaccine formulations (2,3). Therefore, in high risk areas, administration of this vaccine would be recommended from 2 weeks old.

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Meta-analysis of the efficacy of a PRRS type 2 modified live vaccine against heterologous strains of PRRSV in growing pigs

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Introduction

The extensive heterogeneity of PRRSV presents challenges for the efficacy of vaccines, which are currently based on a single virus strain. The concurrent vaccination with PRRSV-1 and PRRSV-2 modified live vaccines (MLV) may interfere with the efficacy of the PRRSV-2 vaccine (1). Fostera® PRRS (F-PRRS) is a MLV belongs to lineage 8 of PRRSV-2. Its specific attenuation process in a porcine-originated cell line may play a role in its ability to reduce viremia effectively after infection with heterologous PRRSV strains (2). The objectives of this meta-analysis were to evaluate the overall efficacy of F- PRRS in growing pigs compared to unvaccinated pigs against heterologous PRRSV infection, as well as its impact from different ages at vaccination as reported in published studies.

Materials and Methods

A systematic literature search and review followed by meta-analysis were performed, evaluating the efficacy of F-PRRS fulfilled the predefined inclusion criteria. Abstracts, bibliographies, and manuscripts were searched from the CAB and PubMed database between January 5th and 12th, 2022. The primary outcome parameters of interest were average daily gain (ADG) and mortality, while the secondary outcome parameters were macroscopic lung lesions (MLL) score, the magnitude of viremia. Age at vaccination, categorized as vaccinated at day 1, or day 21 or day 28 of age were predefined as co-variates to evaluate their potential impact on the outcome parameters of interest. Data were analyzed using the statistical software CMA version 2.2 (Biostat, Englewood, NJ, USA). Effect size was the risk ratio (RR) for the percentage mortality and the raw mean difference for all other outcome parameters.

Results

A total of 20 published papers fulfilled the predefined inclusion criteria allowing as many as 29 comparisons. 14 articles reported experimental challenge studies, whereas 6 papers summarized results from field studies (environmental infection). PRRSV species used for experimental challenge were either PRRSV-1, PRRSV-2, or concurrent PRRSV-1 & -2. Viruses being present in field studies were either PRRSV-2 or concurrent infection of PRRSV-1 & -2. Vaccinated pigs had an average 38.52 g/d higher daily weight gain and a 65% lower mortality (relative risk = 0.35) compared to non-vaccinates. F-PRRS reduced the maximum macroscopic lung lesion score on average by 16.82% points and the maximum viral load in serum samples by 1.36 log10 PRRSV RNA copies (Table 1).

Table 1. Overall differences in ADG and mortality over the entire evaluation period, the maximum MLL score, and the maximum PRRSV RNA copies (log10) measured in serum samples between unvaccinated pigs and pigs vaccinated with F-PPRS.

Outcome parameters analyzed	n	Mean Difference [95% CI]	P-value
ADG (g/day)	12	+38.53 [+26.06; +50.98]	<0.001
Mortality (RR)	15	0.35 [0.20; 0.59]	<0.001
MLL score (% point)	21	-16.82 [-20.22; -13.43]	<0.001
Maximum PRRSV RNA copies (log10)	29	-1.36 [1.79; -0.93]	<0.001

n = number of comparisons; CI = confidence interval

Discussion and Conclusion

The maximum viral load measured at any time point during the study observation period was significantly reduced in vaccinated pigs compared to unvaccinated pigs (-1.36 log10 PRRSV RNA copies) and the MLL score was reduced by 16.82% points. These findings are consistent with the understanding that the severity of respiratory disease is well correlated with the amount of viral load (3). F-PRRS vaccination led to a 38.52 g/day higher ADG, which can be attributed to the lower severity of respiratory disease, as the decreased ADG with PRRSV infection is mainly due to a loss of appetite and reduced feed intake, caused by high temperature and the respiratory problems (4). The higher ADG and the relevantly reduced mortality (65% reduction) are the economically most important findings as these parameters are considered as key drivers of profitability for producers (5). The data also suggested vaccination at either 1 day or 21 days of age was similarly effective,and the infectious strain(s) used for challenge or being endemic in field studies (PRRSV-1, PRRSV-2, or concurrent PRRSV-1 & -2) did not significantly influence the outcomes. Our findings confirm the efficacy of F-PRRS against heterologous strains of PRRSV in piglets and growing pigs.

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mRNA quantification of intestinal integrity biomarkers after the administration of a single-dose live non-pathogenic *Escherichia coli* oral vaccine.

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Introduction

In terms of mechanisms of the intestinal barriers, there are several components which participate in maintaining intestinal integrity (1). Due to commercial reasons, piglets are weaned when its mucosal immune system is immature. This condition allows determined pathogens to proliferate in the intestine causing postweaning diarrhoea with high morbidity and mortality (2). It is possible to measure intestinal integrity through the evaluation of different biomarkers without using painful methods for the animals. The objective of this study was to evaluate the effect on intestinal integrity biomarkers when using an oral vaccine indicated for postweaning diarrhoea caused by *E. coli*, assessed indirectly by mRNA quantification.

Materials and methods

The study was carried out in 2020 on a 500 sow Spanish commercial pig farm. A total of 30 piglets with mild diarrhea caused by *E. coli* were divided intotwo groups: a control group (CG) and a vaccinated group (VG) with a single-dose live non-pathogenic *E.coli* oral vaccine (vaccinated with 2 ml at 28 days ofage). Fifteen samples of feces per group were obtained from the rectum at preweaning and at 6 and 14 days postweaning. Zonulin 1 (ZON-1) and Calprotectin (CALP) were selected as intestinal integrity biomarkers. A q-PCR was performed using SYBR-Green chemistry. Total RNA was isolated by commercial kit, and reverse transcription was carried out to produce cDNA following previous experiences (3). The quantification was done taking β-actin as endogenous gene and by means of the method correcting to q-PCR efficiency (4).

Results

At 6 days postweaning, CALP was statistically decreased ($p<0.001$) and ZON-1 was increased ($p=0.001$) in the VG. At 14 days postweaning, CALP was statistically increased ($p=0.007$) in the VG.

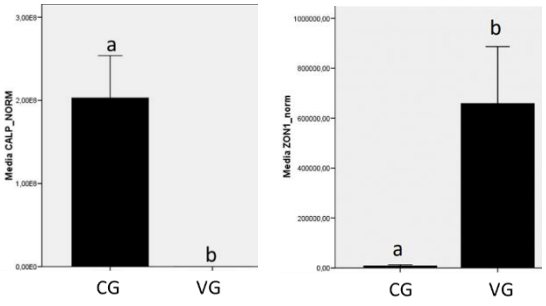

Figure 1. Results for Calprotectin and Zonulin-1 per group at 7 days postweaning. Control group (CG); Vaccinated group (VG) (a, b) Superscripts indicate a significant difference ($p\leq0.05$).

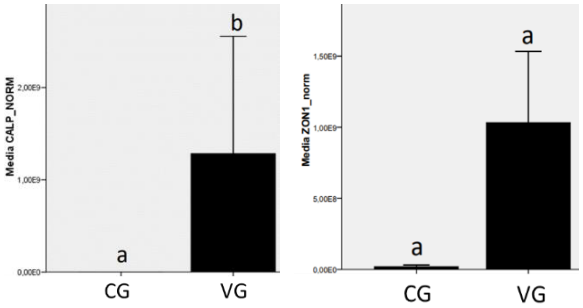

Figure 2. Results forCalprotectin and Zonulin-1 per group at 14 days postweaning. Control group (CG); Vaccinated group (VG) (a, b) Superscripts indicate a significant difference ($p\leq0.05$).

Discussion and conclusion

ZON-1 is the only physiological modulator of intercellular tight junctions (5). As the vaccine strains adhere to the intestines, colonize and considerably replicate, our hypothesis was that it protected against *E.coli* leading to an improved intestinal integrity marked by ZON-1 release. The presence of CALP in feces indicates that there is a migration of neutrophils and monocytes to the intestinal lamina propia (6) and, this happens when there is a local immune stimulation. Our hypothesis was that CALP increased at 14 days postweaning as a possible response to the vaccine strains invasion of the intestine.

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New licensed *Mycoplasma hyopneumoniae*-based vaccine induce systemic and mucosal immunity and prevent the development of Mycoplasmal Pneumonia in pigs

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Introduction

Mycoplasma hyopneumoniae is the causative agent of Porcine Enzootic Pneumonia (EP), an important chronic lung disease that reduces the animal performance and favors co-infections by other pathogens. The control of EP is carried out mostly by the use of inactivated vaccines containing whole bacteria, which might induce antibody and/or cellular (Th1/Th17) immune responses (1). In this study we present the initial immunological characterization of the newest licensed vaccine against *Mycoplasma hyopneumoniae* commercialized in Brazil.

Materials and Methods

The study was conducted using 30 piglets (Agroceres PIC, Brazil) distributed in 3 homogeneous groups with 10 animals each. All animals were assayed and were free of antibodies (IgG) to *M. hyopneumoniae* (serum) and free of *M. hyopneumoniae* genomic DNA (oropharyngeal swab). At 21 days of life (experimental day 0) piglets were inoculated with: a) Safesui Mycoplasma (whole inactivated-bacteria vaccine adjuvanted with Safemune); b) Competitor vaccine; c) Sterile phosphate buffer saline (PBS). A single dose was administered in all piglets. Blood samples and nasal swabs were collected prior to (day 0) and after vaccination to evaluate the presence of antibodies to *M. hyopneumoniae* (systemic IgG and mucosal IgA) by Indirect ELISA. All piglets were then challenged intratracheally at experimental day 26 with 1 x 10^8 *M. hyopneumoniae* (virulent strain) quantified by flow cytometry (2). Seven weeks after the challenge, animals were euthanized and necropsied to assess lung pathology and to quantify the *M. hyopneumoniae* load in tracheal mucus by qPCR. The experiment was approved by the Institutional Committee for Ethical Use of Animals of the University of Passo Fundo (protocol no. 006/2021).

Results

The Safesui Mycoplasma (SM) vaccine and the competitor induced detectable levels of systemic anti *M. hyopneumoniae* IgGs (**Figure 1A**). At the mucosal level, the IgA titers observed in the animals vaccinated with the SM vaccine were slightly higher than those found in the animals vaccinated with the competitor, and significantly ($p<0.05$) higher in comparison to the control group (on experimental days 35 and 70) (**Figure 1B**). At necropsy, we observed a lower lung pathology score in the group immunized with SM in comparison to the control group, as well as to the competitor (**Figure 1C**). The load of *M. hyopneumoniae* in the tracheal mucus was significantly ($P<0.05$) lower in the animals vaccinated with SM compared to control animals (**Figure 1D**).

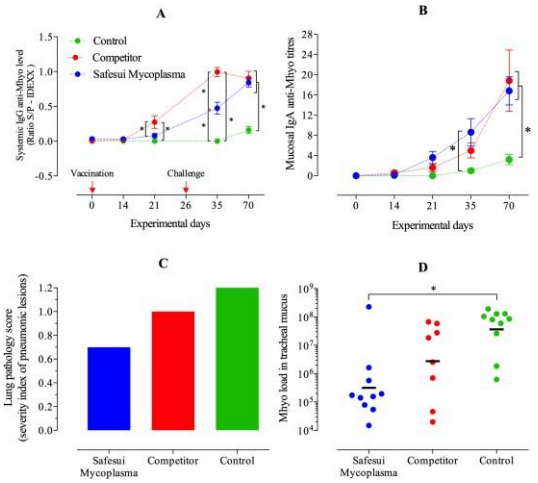

Figure 1. Kinetics of (A) systemic IgG and (B) mucosal IgA anti-*M. hyopneumoniae*. C) Lung pathology score. D) *M. hyopneumoniae* load in tracheal mucus. Statistical differences (One-way or Two-way ANOVA, $p<0.05$) are indicated by asterisk.

Discussion and Conclusion

Complete lung protection against *M. hyopneumoniae* by the use of vaccines has not yet been achieved by the current licensed vaccines. Here, we present the partial immunological characterization of the newest licensed vaccine against *M. hyopneumoniae*, named Safesui Mycoplasma. This vaccine induces a functional antibody response (systemic and mucosal) capable of controlling the development of lung lesionsproduced by *M. hyopneumoniae*.

Acknowledgments

This work was financially supported by Ourofino Animal Health (Cravinhos, SP, Brazil).

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Performance after vaccination with an intramuscular *Lawsonia intracellularis* vaccine at the beginning of fattening in a farm subclinically infected with Lawsonia

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Introduction

Lawsonia intracellularis (LI) is detected in feces in more than 90% of German swine herds with a history of diarrhea (1). The efficacy of an intramuscular *Lawsonia intracellularis* (LI) vaccination has been demonstrated (2). Due to purchase of 30 kg piglets for fattening, in practice, it is not always possible to vaccinate piglets against Lawsonia at young age. In these cases, vaccination at the beginning of fattening is an alternative.

Materials and Methods

The field observation took place in a LI subclinical infected fattening farm in northern Germany with high performance and health level (incoming weight approx. 35,5 kg; live weight at slaughter approx. 133,8 kg). Fattening pigs (only vaccinated against PCV2) showed sporadic diarrhea and 20% were smaller and orally treated with Tylosin. Right after placement more than 50% of the animals showed LI- antibodies and excreted relevant LI amounts(PCR >log GE 6/g feces). Regardless, half of the piglets (appr. 30kg) of 4 consecutive batches were vaccinated with Porcilis® Lawsonia (vacc+ n = 962) right after placement and compared to the parallel unvaccinated groups (vacc- n = 962). The performance parameters of both groups of animals were carefully documented and evaluated by the farmer

Results

LI vaccinated groups showed less diarrhea and reduced LI excretion (weak positive to log GE 4/g feces). Fewer suddenly dead bloated pigs occurred. Only single animals were treated with antibiotics by injection. ADWG increased by 10.2g, FCR improved by 0.1 to 2.83, losses decreased(-1.6%), same with veterinary costs(-.07€/fattening pig; excl. vaccination).

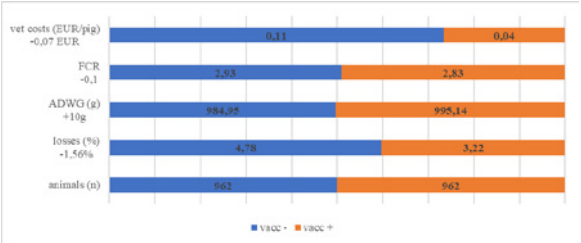

Figure 1 Performance data of fattening pigs with (vacc+) or without (vacc-) Porcilis® Lawsonia vaccination

As a result of the improved biological performance of Porcilis ® Lawsonia vaccinated animals, an economic advantage of 2,84 €/fattening pig (vaccination costs not included) even in the difficult German market situation in the 2nd half of 2020 could be reached.A model calculation shows that this financial advantage increases to 3.30 €/fattening pig in a moderate market situation (vaccination costs not included).

Table 1 Productions costs in Porcilis® Lawsonia vacc+ and vacc- fattener pigs

	vacc -	vacc +	Delta
Feed expenses (€/pig)	63,53	61,74	-1,79
Animal losses (€/pig)	2,72	1,67	-1,05
Lost profit (€/pig)	-0,23	-0,15	0,07
Vet costs (€/pig)	0,11	0,04	-0,07
Total Production costs (€/pig)	**66,13**	**63,29**	**-2,84**

Conclusions and Discussion

Despite pre-existing infection at the time of vaccination at placement of fattening piglets and only minor clinical problems in the fattening unit, Lawsonia vaccinated animals showed improved performance parameters compared to the non-vaccinated animals. This resulted in an economic advantage of 2.82€/fattening pig (better market situation 3.30€) in the vaccinated group.

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Performance and economy of piglets during postweaning period at an organic farm beforeand after using an intramuscular *Lawsonia intracellularis* vaccine

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Introduction

Lawsonia intracellularis (LI) is one of the most important enteral pathogens in pigs (1). Farm prevalence of LI. Infection in Germany is high and is often seen in the post-weaning period but even can happen in the farrowing house (2). Clinical signs usually appear in the fattening period but can also start post-weaning. Disease severetiy ranges from chronical illness with reduction in performance up to severe bloody diarrhoea and acute mortality (2). Due to regulatory standards organic farms are very limited in the use of antibiotics and therefore prophylaxis becomes the only way to prevent clinical outbreaks of the diseases.

Materials and Methods

This field observation was made in an organic farrowing farm in North East Germany. Piglets were vaccinated intradermally against PCV and orally against Lawsonia via drench (oral vacc). However, LI related symptoms like diarrhoea, runts and acute losses were still seen in the nursery period. LI Vaccination was changed to Porcilis® Lawsonia at 24 days of age (i.m. vacc). Performance data from the differently vaccinated groups in the nursery were collected and compared on farm base.

Results

The occurrence of diarrhoea in the nursery was reduced with introducing the intramuscular LI vaccine. Furthermore, animal losses decreased, and group homogeneity improved. Performance was increased by 34 g daily weight gain, a better feed conversion ratio (-0,03) and reduction of mortality from 7.4 to 3.4 %. In total the enhanced performance resulted in a calculated economic benefit of 5,03 €/piglet (vaccination costs excluded).

Table 1. Number of animals and weights in the differently vaccinated groups

	Oral vacc	*i.m. vacc*
Number of animals (n)	10685	2793
Weight at stabling (kg)	9.30	10.2
Weight at slaughter (kg)	31.4	34.0
Daily weight gains (g)	355	389

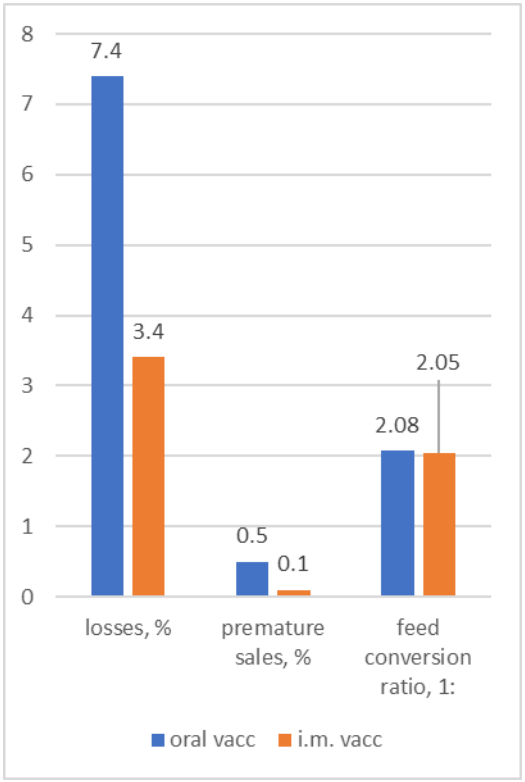

Figure 1: Animals' performance in oral vacc and i.m. vacc group

Conclusions and Discussion

Early onset of severe clinical disorders after weaning may occur in *Lawsonia intracellularis* infected pigs. The limited opportunities in using antibiotics can massively restrict the intervention options and can even become a problem of animal welfare. In this farm changing the vaccination protocol to an intramuscular LI vaccine ensured piglets health and helped to deal with the enteric disease resulting in improved performance and reduced production costs in the nursery period.

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Performance of a *Mycoplasma hyopneumoniae* (*MHP*) serum antibody ELISA for the detection of *MHP* antibody in processing fluids

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Introduction

Serum-based *Mycoplasma hyopneumoniae* (*MHP*) monitoring in breeding herds is constrained by the labor required for collecting blood samples from individual sows and the number of samples required for statistically valid surveillance. Processing fluid (PF), the serosanguineous fluid recovered from tissues collected at the time of piglet processing (3 to 5 days ofage) is an easily collected specimen with high diagnostic utility and potential to serve in sow herd surveillance[1]. The purpose of this study was to evaluate the diagnosticperformance of PF samples for the detection of *MHP* antibody using a commercial ELISA kit.

Materials and Methods

Processing fluid samples (n = 494) were collected from three commercial swine farms. One farm wasconsidered *MHP*-endemic (n = 246 samples) and two farms were considered *MHP*-free (n = 248 samples). Samples were tested at 1:10 dilution using a commercial*MHP* ELISA designed to detect anti-P46 antibody in serum (*M. hyo* Ab test, IDEXX Laboratories, Inc.). Diagnostic specificities and sensitivities for specificELISA sample-to-positive (S/P) cutoffs were estimated by receiver operating characteristic (ROC) analysis.

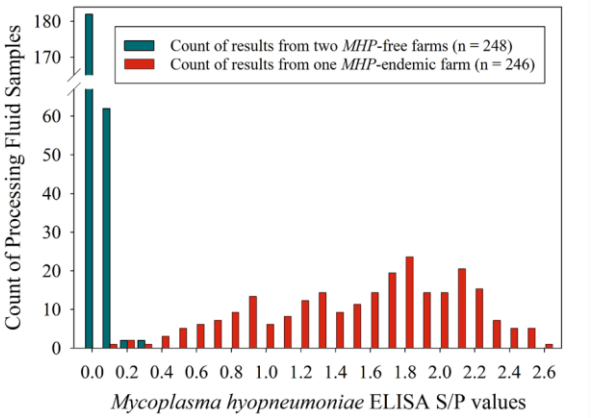
Results

A frequency distribution of the *MHP* ELISA S/P responses by farm status is shown in Figure 1. At a cutoff of S/P ≥ 0.4, the ROC analysis estimated diagnostic specificities and diagnostic sensitivities as 100.0% (95% CI: 100, 100) and 97.6% (95% CI: 95.5, 99.2), respectively. That is, all samples (n = 248) from *MHP*-free farms produced S/P values < 0.4, while 3 of 246 samples from the *MHP*-endemic farm produced S/P values < 0.4 (i.e., 0.387, 0.344, 0.386).

Discussion and Conclusion

The findings in this study are consistent with the report by Boettcher et al. (2010) who described ”excellent” agreement between processing fluids and sow serum samples. Further, our results suggested that processing fluids could be supplemental to, or even replace, sow serum sampling for breeding herd *MHP* antibody surveillance.

Figure 1. Frequency distribution of *Mycoplasma hyopneumoniae* (*MHP*) antibody ELISA* S/P responses by farm *MHP* status.



**M. hyo* Ab test (IDEXX Laboratories Inc., Westbrook, ME, USA).

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Pleuritis impact on swine production and the importance of an effective tool in its reduction

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Introduction

Pleura is a membrane which is responsible to recover the lung on both sides (pleura parietal and visceral). Pleuritis or pleurisy represent the inflammation process of this membrane that mainly occur by secondary infection agents alone or in combination (1). Besides it causes performance reduction, pleurisy leads to a huge economic impact because it increases the chance of partial or total carcass condemnation (2,3). The aim of this study was to evaluate the efficacy of new licensed *Mycoplasma hyopneumoniae*-based (M.hyo) vaccine with a more updated antigen present on the whole market in reduction of secondary infeccions.

Materials and Methods

This trial were conducted in a pig farm in Sao Paulo State - Brazil. A total of 1080 piglets were divided into two groups. One group were composed by 540 piglets that were vaccinated with the standard vaccine used in the farm with a protocol of two shots of 1 mL each one (weaning day and 21 days after), intramuscular (G1). The other group, composed by 540 piglets were vaccinated with the new licensed *Mycoplasma hyopneumoniae*-based vaccine (Ourofino Animal Health®) with a protocol of one shot of 2 mL, intramuscular (G2). Both groups were divided in two weeks of evaluation to reduce some variables present in the farm. A transversal monitoring was done to understand the dynamics of M.hyo circulation on the farm. At the slaughter day (~ 150 days) a total of 176 lungs were evaluated during the slaughter. From this total, 88 lungs were G1 and 88 lungs from G2, both divided into two weeks each one. The method used to analyze all lungs were through the method from 3).

Results

It's possible to see in the table below (Table 1) that G2 piglets had numerically better results than G1 piglets. The difference of prevalence of lesions (%), IPP value, and pleurisy (%) between both groups were,respectively about 6,8%, 5% and 75% better for G2.

Discussion and Conclusion

G2 group had all better results than G1, the diference of pleurisy between both groups were very high. When we thought on pleurisy, its becomes more important, since it is known that this kind of lesion are due to secondary infections (5). In constrast to cases in acute outbreaks, chronic disease is not normally cause by a single agent but by a combination of pathogens involving th primary pathogen and secondary opportunistica pathogens (6). This higher percentual of pleuritis on G1 might be due to a greater M.hyo colonization on respiratory tract (2), which means that may had a lower vaccine coverage, since M.hyo is a primary agent that give opportunity to opportunists pathogen. In this study the new licensed *Mycoplasma hyopneumoniae*-based vaccine (G2) showed better protection than G1.

Table 1. Mean results from percentage (%) of prevalence of lesions, IPP value and Pleuritis from piglets of G1 and G2

Groups	Weeks	N lungs evaluated	Prevalence (%)	IPP	Pleuritis (%)
G1	1st	44	75,00	0,98	4,55
G1	2nd	44	75,00	0,86	4,55
TOTAL G1		**88**	**75,00**	**0,92**	**4,55**
G2	1st	44	81,82	1,05	2,27
G2	2nd	44	56,82	0,68	0,00
Total G2		**88**	**69,32**	**0,87**	**1,14**

G1: standard vaccine used in the farm with a protocol of two shots of 1 mL each one (weaning day and 21 days after), intramuscular. **G2:** new *Mycoplasma hyopneumoniae*-based vaccine (Safesui Mycoplasma - Ourofino Animal Health®) with a protocol of one shot of 2 mL, intramuscular.

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Porcine Parvovirus antibody titers in herds with complete and partial vaccination schedule

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Introduction

Porcine Parvovirus (PPV) is a significant cause of reproductive failure in pigs. Haemagglutination inhibition (HI) is the most frequently used test for detecting and quantifying antibodies to PPV (1). The PPV HI titers of ≥1:512 are usually considered to be indicative of natural exposure (1,2). Naïve animals vaccinated with a killed virus vaccine will commonly develop HI titers up to 1: 512 (1). It is very common to obtain titers of 1:2048 or 1:4096 for naturally infected pig (1–3). During the past 50 years, scientific data on PPV infection in Thailand as well as in south-east Asia in gilts and sows is limited. The objective of the present study was to investigate the current situation of porcine parvovirus infection in relation to vaccination schedules in gilts and sows in Thailand.

Materials and Methods

The evidence of PPV infection among swine herds in Thailand were investigated by analyzing serological data of PPV submitted to HIPRA Diagnos between 2016 and 2020 (n=2410 samples). Antibody titer against PPV infection was determined by using haemagglutination inhibition assay (HI). The occurrence of animals with low PPV antibody titers (i.e., <1:32) were analyzed in association with age group and herds. Serological profiles were collected from six groups of commercial swine herds in Thailand: A, B, C, D, E and miscellaneous. Herds A and B did a partial vaccination schedule (just in gilts). Herd C and E performed a complete vaccination schedule every 4 – 6 months. Herd D vaccinated PPV in gilts only, but occasionally perform whole herd vaccination against PPV when observing some clinical signs. Miscellaneous herds performed different vaccination schedules. The statistical analyses were carried out by using SAS version 9.4.

Results

On average, 84.4% of the pigs had antibody titer against PPV ≥512, indicating a high prevalence of PPV natural infection in swine herds. On the other hand, 5.6% of the pigs had antibody titer against PPV below the protection level (Table 1). This indicates that up to 5.6% of the animals are susceptible to PPV infection. Interestingly, the proportion of pigs susceptible to PPV infection was observed in gilts (9.1%) more than sows (2.0%) and boars (0.2%) (P<0.001). The proportion of pigs susceptible to PPV infection by herds are presented in Table 1.

Table 1 Proportion of susceptible animals (antibody titer against porcine parvovirus < 1:32) by herds

Herds	N	Susceptible pigs (%)
A	314	3.5
B	190	32.1
C	280	2.5
D	793	1.0
E	156	0
Misc.	677	6.9
Total	2410	5.6

Conclusions and Discussion

Based on these results, on average, 84.4% of the pigs in the commercial swine herds in Thailand were naturally infected with a field strain of PPV, while 5.6% of the pigs remain susceptible to PPV infection, differing among herds with different vaccination schedules. The proportion of pigs susceptible to PPV infection was most common in replacement gilts (9.1%).
It needs to be considered, it is not possible to know if the animals with titers ≥1:512 were protected or not before natural exposure. It has been previously reported, that vaccinated and non-vaccinated animals show a marked increase in antibody titers after natural infection[4], so cannot be discarded that in farms with a high proportion of animals with ≥ 1:512, PPV could be causing reproductive disorders.
Therefore, the PPV vaccination schedule and technique should be carefully implemented based on these results where the percentage of susceptible animals are higher in those herds with partial vaccination.

Acknowledgements

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Prevention of the mucosa-related infectious diseases in piglets

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Introduction

The transmissible gastroenteritis virus (TGEV), porcine epidemic diarrhea virus (PEDV), porcine rotavirus (PRV) and enterotoxigenic E. coli (ETEC) as the mucosa-related infectious pathogens of the piglets cause severe diarrhea that leads to tremendous economic loss in the pig industry worldwide (1,2,3). However, the effectiveness of the current methods to control these mucosa-related infectious diseases remains unsatisfactory, indicating there is a need to develop more effective methods for preventing or treating mucosa-related infectious diseases in piglets. Here we investigated if the use of the serum-based product (PAMI serum; PAMI-S) can prevent the piglets from infection of the TGEV, the PEDV, the GAPRV and the ETEC.

Materials and Methods

The PAMI-S to the TGEV, the PEDV, GAPRV and the ETEC was produced from the adult pigs. Then, the viral neutralization (VN) titer to the TGEV, the PEDV and the GAPRV and antigen agglutination (AG) titer to the ETEC were examined as described (4, 5, 6, 7). The PAMI-S was administrated into the newborn piglets within 1 hour after birth via the oral route. The four groups of the piglets (each group includes the PAMI-S-fed and Control serum-fed piglets, respectively) were separated from the sows at two days old and challenged with wild type TGEV (TGEV-wt1), the PEDV-wt1, the GAPRV-wt1 and the ETEC(K88) at three days after birth, respectively. In the PEDV outbreak farm, the PAMI-S was orally administrated into the newborn piglets within 1 hour after birth. Then the clinical symptoms were examined until 28 days old.

Results

As shown in Fig.1, in the piglet group challenged with the TGEV-wt1 and the PEDV-wt1, PAMI-S-fed (Fig.1, lanes 1 and 3) and control serum-fed (Fig.1, lanes 2 and 4) piglets showed 0% and 100% mortality, respectively.

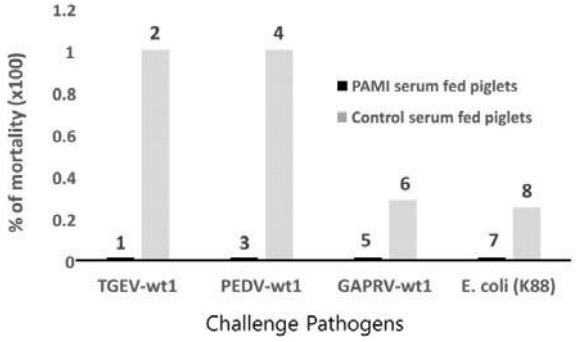

Fig. 1. Percentage of diarrhea in the control or PAMI-S-fed piglets after challenge with the TGEV-wt1, the PEDV-wt1, the GAPRV-wt1 and the E. coli (K88). The number on each bar graph refers to the corresponding lane.

In the piglet group challenged with the GAPRV-wt1, the PAMI-S-fed and control serum-fed piglets showed 0% and 29% mortality (Fig. 1, lanes 5 and 6), respectively. In the piglet group challenged with the ETEC, the PAMI-S-fed and control serum-fed piglets showed 0% and 25% mortality (Fig.1, lanes 7 and 8), respectively.

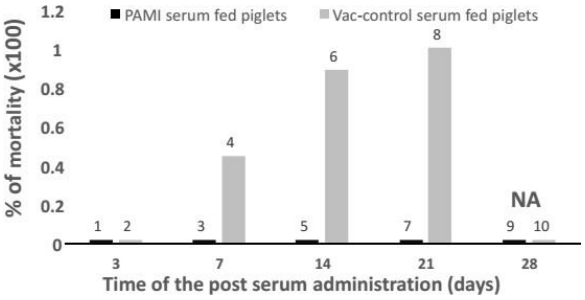

Fig. 2. Death percentage of the PAMI serum-fed piglets (n=28 litters) or vac-control serum-fed piglets (n=9). The number on each bar graph refers to the corresponding lane.

In the PEDV outbreak farm (200 sow units), the PAMI serum-fed piglets did not show any mortality (Fig.2, lanes 1, 3, 5, 7 and 9) until 28 days old. However, the vac-control serum-fed piglets showed 44%, 89% and 100% mortality at 7, 14 and 21 days post infection (Fig.2, lanes 4, 6 and 8).

Discussion and Conclusion

As the way to prevent the infectious diseases in the piglets, the use of the PAMI-S successfully protected the piglets from infection of the TGEV, the PEDV, the GAPRV and the ETEC, respectively, indicating that the PAMI-S harbors protective antibodies with mucosal immunity against the TGEV, the PEDV, the GAPRV and the ETEC, and the protective mucosal immunity of the PAMI-S can be passively transferred to the newborn piglets via an oral route. In the PEDV outbreak farm to which the PAMI-S was applied, newborn piglets were completely protected from the PEDV infection and the further spread of the PEDV infection ceased, indicating that the PAMI-S can be effectively used for preventing mucosa-related infectious diseases in the field. Furthermore, this study suggests that the method for generating the PAMI-S and preventing infectious diseases of the piglets can be extended to other mammals for controlling mucosa-related infectious diseases.

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Relation between IgA producing cells in tissues of piglets supplemented with *Saccharomyces cerevisiae* var. *boulardii* CNCM I-1079 and vaccinated against *Actinobacillus pleuropneumoniae*

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Introduction

The immune system is a very complex structure with defensive functions in all the body regions. Although the immune system appears to be compartmentalized per organ, all compartments are connected across the body notably based on cytokines and chemokines. In this work we have assessed the effect of feeding the live yeast *Saccharomyces cerevisiae boulardii* CNCM I-1079 (LSB; Levucell®SB, Lallemand SAS, France) to sows and their offsprings up until after weaning, on the relation between the IgA producing cells in different organs in piglets after vaccination against *Actinobacillus pleuropneumoniae* (APP).

Materials and Methods

Seventy-two mixed parity sows were supplemented (LSB) or not (CON) with 2×10^9 colony forming units (CFU)/kg of LSB from 1 week before the expected farrowing day until weaning. At weaning, piglets from the same group were allotted to 2 diets: control (CON) and LSB (CON + 2×10^9 CFU/kg and 1×10^9 CFU/kg in phase 1 (18 days post-weaning) and 2 (days 19-42), respectively), resulting in 4 treatments during post-weaning: CONCON, CONLSB, LSBCON, LSBLSB. The piglets were vaccinated against APP (Coglapix, Ceva Santé Animale, France) on days 26 and 49 post-weaning. Samples from lungs, mediastinal lymphnodes and jejunum were taken from 15 pigs/treatment 3 weeks after the second vaccination. Samples were fixed, paraffin embedded and stained by Avidin Biotin Complex (ABC), using a Goat anti-Pig IgA Antibody Affinity Purified (Bethyl Laboratories, USA) as primary antibody incubated overnight, and a Polyclonal Goat Anti-Rabbit Immunoglobulins/Biotinylated (Dako, Denmark) as secondary. Once identified, IgA producing cells were counted by images software analysis under Zeiss Axioskop 40 light microscope (Carl Zeiss, Germany). The count was the average of the cells counted in 10 non-overlapped fields of 10.000 μm^2. Data were analyzed in SPSS Statistics 26 (IBM) by Spearman's correlation analysis, and with Kruskal-Wallis test for k unrelated samples, with treatment as main effect. The experimental unit was the pig. Differences with a *P*-value <0.05 were considered as significant, and *P*-values between 0.05 and 0.1 were considered a trend.

Results

There was a significant and positive correlation between the quantification in the jejunum and the lung (*P<0.01*; r=0.354; Figure 1), and between the lung and the lymph node (*P<0.01*; r=0.378; data not shown). Additionally, differences in IgA producing cells tended to be treatment dependent in the lymph node (CONCON=7.08; CONLSB=5.43, LSBCON=4.55, LSBLSB=3.66; *P=0.059*; data not shown).

Discussion and Conclusion

The correlation in the number of IgA producing cells between jejunum and lung may be explained by the lung-gut axis, demonstrating that, in the pig, gastrointestinal and respiratory tracts share a mucosal immune system. Gut microbiota protects against respiratory infection in mice, and its reduction impairs immune responses (1). Gut microbiota dysbiosis modulates the immune responses of the gastrointestinal tract and those of distal organs like the lung (2). Interestingly, and since LSB was orally administered, we would have expected a stronger effect in the jejunum, but the prioritization of the animals to be protected from the respiratory challenge of the vaccine may have caused a more pronounced response in the lymph node (in the lung region). Given that LSB has been proven to affect gut microbiota of weanling piglets (3), these results suggest that manipulating gut microbiota with probiotics like *Saccharomyces cerevisiae boulardii* CNCM I-1079 could be a strategy to prevent or reduce the severity of lung diseases by increasing mucosal defense.

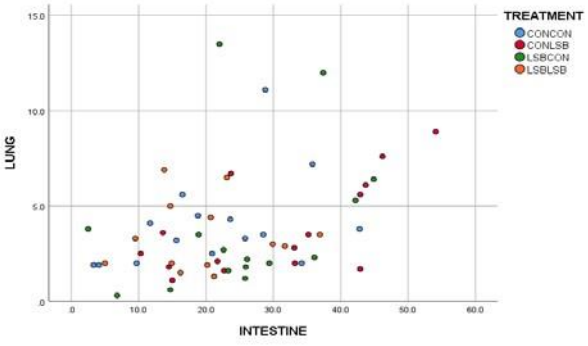

Figure 1 Correlation between IgA producing cells in lung and jejunum (r=0.354; *P<0.01*)

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Repetitive MycoFLEX vaccination results in Antibody seroconversion

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Introduction

Detection of antibodies is a valuable tool to detect presence of disease. However, with the use of concurrent vaccines, the effect of these vaccines on the antibody response should be taken in account withthe interpretation. On a *Mycoplasma hyopneumoniae* (Mhyo) negative breeding farm producing F1 breeding gilts, the vaccination protocol for the F1- gilts changed from single dose Mhyo vaccination (at 21 days of age) to a double dose mycoplasma vaccination (at 21 days of age and a booster at 6 months of age; with MycoFLEX, Boehringer Ingelheim). The involved animals showed seroconversion after the second vaccination, without any clinical symptoms of a Mhyo outbreak. Also, in the herd of origin (grand parent animals) no signs of a changed Mhyo status were observed, and these animals remained seronegative (never vaccinated).
The objective of this study was to assess the effect of multiple dosing of MF and how this will influence the test outcome of commercial Mhyo antibody tests.

Materials and methods

In total 31 pigs were followed longitudinal, with in total three treatment groups of 10: CON (not vaccinated; #10), MF2 (vaccinated at 21 and 112 days age; #11, MF 3 (vaccinated at 21, 112 and 152 days of age; #10). Pigs were bled at the age of 112 days (91 days post first vaccination), 152 (40 days post second vaccination) and 194 days (42 days post third vaccination). Pigs were comingled and group housed together. Serum was submitted to the Dutch Animal Health service (GD Deventer) for Mhyo antibody testing (indirect Elisa and second confirmation by blocking Elisa).

Results

At the age of 112 days (91 days after first vaccination for MF2 and MF3) all pigs remained seronegative for the Indirect Elisa although there was significant different between CON and MF2/3 (-0.02 vs 0.06 p<0.01). This changed after the second vaccination at the age of 152 days (40 days after the second vaccination; CON #0 pos, OD 0.02; MF2 #5 pos, OD 0.44; MF3 #9 pos, OD 0.68; p<0.001) and for the confirmation test (CON #1; MF2 #10, MF 3 #10).

After the third vaccination a further rise in antibodies was seen in the MF3 group (CON OD 0.16; MF2 OD 0.38; MF3 OD 0.83 p <0.001). Box and whisker plots of the Elisa results are shown in Figure 1.

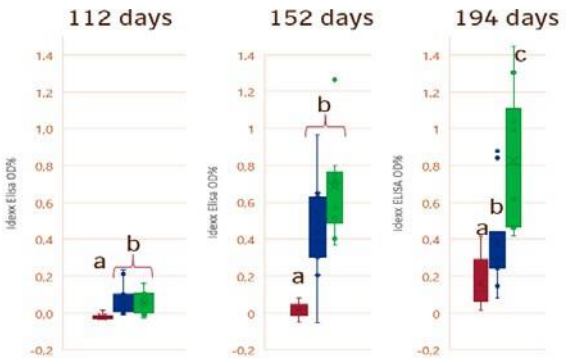

Figure 1 Elisa result's OD% of the 3 treatment groups. CON (red); MF2 (blue) and MF3(green) at the respective age of the animals. Different letters mark a significant difference (P<0.01)

Discussion

MF vaccination led to a small antibody response after the first vaccination. Although this effect was significant, it does not lead to positive samples based on the current cut off specifications. After multiple repetitive doses MF, there is a clear dose response effect, leading to positive samples in as well the standard Elisa as for the confirmation test. This effect must be taken in account when the health status of a Mhyo-free SPF herd is assessed when monitoring multiple MF vaccinated animals. This second vaccination response could be used as a vaccination compliance marker, however further research is needed to validate this on commercial farms with field exposure to Mhyo.

Conclusion

Single administration does not lead to a positive antibody outcome based on the current cut off values off the commercial tests. Multiple administration of MF shows a dose response effect, leading to an increase of antibodies as measured by the OD values and above the cut off values.

Response of IFN-γ and TGF-β as immune stimulation indicators after the administration of a single-dose live non-pathogenic *Escherichia coli* oral vaccine

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Introduction

The intestinal wall must act as a barrier, but it needs to be permeable to allow some substances to enter the epithelium to be absorbed and used by the animal. There are several cytokines which play a crucial role in maintaining intestinal integrity. Proinflammatory cytokines can participate in increasing the intestinal permeability (1), while anti-inflammatory cytokines control the duration and severity of the inflammation (2). The aim of the study was to measure two cytokines (IFN-γ and TGF-β) in feces after the vaccination with a single-dose live non-pathogenic *Escherichia coli (E.coli)* oral vaccine.

Materials and methods

The study was carried out in 2020 on a 500 sow commercial farm located in Spain. A total of 30 weaned piglets with mild diarrhea caused by *E.coli* were divided into two groups, a control group (CG), and a vaccinated group (VG) with a single-dose live non-pathogenic *E.coli* oral vaccine (vaccinated with 2 ml at 28 days of age). Fifteen samples of feces per group were obtained from the rectum at preweaning, 6 and 14 days postweaning. IFN-γ and TGF-β were selected as immune stimulation indicators. Feces were preserved in RNAlater (Life technologies). Total RNA was isolated by commercial kit, and reverse transcription was carried out to produce cDNA following previous experiences (3).

Results

A statistically significant decrease (p=0.008) of mRNA which codifies for IFN-γ was observed in the CG at 6 days postweaning. In the VG, at 6 days postweaning, a significant increase (p=0.043) was observed in IFN-γ.

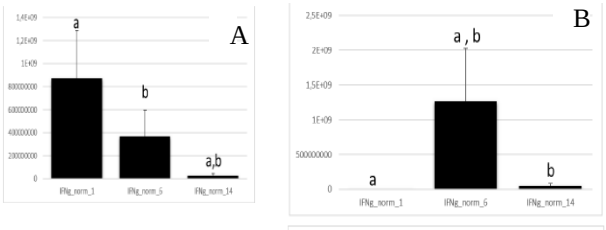

Figure 1. A) Control group (CG) B) Vaccinated group (VG). Quantification of mRNA codifying for IFN-γ over time (preweaning, 6 days and 14 days. postweaning). (a, b) Superscripts indicate a significant difference (p≤0.05).

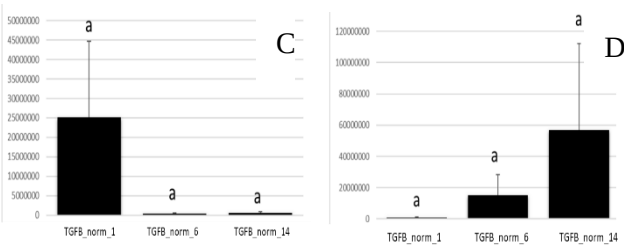

Figure 2. C) Control group (CG) D) Vaccinated group (VG). Quantification of mRNA codifying for TGF-β over time (preweaning, 6 days and 14 days postweaning). (a, b) Superscripts indicate a significant difference (p≤0.05).

Discussion and conclusion

In respect of IFN-γ, the CG experienced a significant decrease which responded to a natural control of the inflammation caused by the postweaning event. The VG had a significant increase of mRNA expression in the second sampling. This proinflammatory cytokine plays crucial roles in modulating inflammation in the gastrointestinal tract (4). IFN-γ was demonstrated to be increased in Enterotoxigenic *E. coli* challenges (5). Our hypothesis was that the vaccine strains were able to temporarily increase a major mRNA expression of IFN-γ after vaccination. TGF-β has an important role in the control of the duration of the inflammatory response by inhibiting the production of proinflammatory cytokines (2). Although there is not any statistically significant increase of this indicator in the VG, the amount of mRNA which codifies for TGF-β increased over time and, it is demonstrated that this cytokine is a precursor of IgA production (6).

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Safety and efficacy of a PCV2 vaccine in field Brazilian conditions

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Introduction

Despite high PCV2 vaccine rate of piglets, the economical importance of subclinical PCV2 infection remains (1). These trials were performed in field Brazilian conditions to assess the safety and efficacy of a PCV2 vaccine based on PCV2d genotype, now recognized as being predominant worldwide (2).

Materials and Methods

One trial was performed in a farrow to finish farm owning 1100 sows in Minas Gerais state (trial A). A total of 1887 piglets were randomly allocated to a tested (T) or control (C) group at weaning from 3 successive batches. In the T group, piglets received a whole inactivated virus PCV2 vaccine (Suigen® PCV2, Virbac) and in the C group a PCV2 reference subunit vaccine (1 mL by IM route in both groups). The allocation was done by litter, so that all piglets from the same litter received the same vaccine.

The other trial (B) was performed in Rio Grande do Sul state according to 2 steps. In the first step, 1531 piglets from 8 different farrowing units were randomly allocated to a tested (T) or control (C) group. The same PCV2 vaccines were administered at weaning as in trial A, but the allocation of piglets was done inside each litter, so that half of piglets within a litter received the T vaccine and the other the C vaccine (by taking into account the sex in groups allocation). Piglets were then moved to a unique nursery site before moving to different fattening units. The pigs could be followed till finishing in 4 fattening units corresponding to a total of 804 included pigs.

In both trials, pigs were individually identified by ear tag at weaning and individually weighed at weaning, at the transition from nursery to fattening and at the latest fattening stage allowed by the farm owner (around 130 days of age in trial A and 170 days at finishing in trial B). In both trials, pigs of both groups were mixed in collective pens according to sex and weight range (during nursery and fattening). Mortality and culling cases were recorded in nursery but could not be followed accurately during fattening, due to distribution of pigs in various units. Statistical analysis was done separately per trial. Means for body weight at weaning, post-weaning and finishing were adjusted (estimated marginal means) by using eventual covariates when appropriate, such as sow parity, piglet sex, weaning date and weight, lactation, nursery and fattening lengths. Comparisons between groups were done by using an analysis of covariance (general linear model: GLM). The mortality rates were compared between groups by the Fisher's exact test. Statistical analyses were performed using R version 4.0.2 (2020-06-22) -- "Taking Off Again"; Copyright (C) 2020, The R Foundation for Statistical Computing. Platform: x86_64-w64-mingw32/x64 (64-bit).

Results

Included piglets were issued from sows whose parity ranged from 1 to 10 in trial A and from 1 to 11 in trial B. No local nor general side effects were noticed after injection of either PCV2 vaccine. Mortality and culling rates (excluding hernia, prolapses and crushing cases) were not significantly different between groups in both trials. The weaning, transition and fattening weights were not significantly different between groups, except a higher transition weight for the T group in trial A (Table 1).

Table 1. Average pig weight from wean to finish and nursery mortality rates

Trial		A		B	
Vaccine		C	T	C	T
Wean weight (kg)	M[1]	6.22	6.22	6.8	6.7
	SE[2]	0.05	0.05	0.04	0.04
Transition weight (kg)	M	16.8[a]	17.1[b]	19.6	19.6
	SE	0.08	0.08	0.1	0.1
Fattening weight (kg)	M	83.0	82.8	119.3	118.8
	SE	0.3	0.3	0.6	0.7
Nursery mortality	(%)	1.9	2.1	0.9	0.8

[1]Estimated marginal mean. [2]Standard error.
[a,b]Values with different superscripts in the same row differ significantly ($p < 0.05$)

Discussion and Conclusion

Safety and efficacy of the tested PCV2 vaccine have been previously reported in Asia either in challenge or field conditions (3,4,5,6). The large scale field trials performed in Brazil corresponded to different management conditions: same (trial A) or multiple (trial B) weaned piglets origin. The later conditions are expected to increase the risk of infectious diseases, due to mixing of animals from different origins. These 2 trials confirmed the safety of the tested PCV2 vaccine and did not show significant differences between the 2 vaccines on growth performances nor on nursery mortality rates.

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Safety and efficacy of a PCV2 vaccine in field Colombian conditions

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Introduction

Despite high PCV2 vaccine rate of piglets, the economical importance of subclinical PCV2 infection remains (1). This trial was performed in field Colombian conditions to assess the safety and efficacy of a PCV2 vaccine based on PCV2d genotype, now recognized as being predominant worldwide (2).

Materials and Methods

The study was performed in a farrow to finish farm located in Antioquia province. This farm owns 180 sows with a 2 week batch management. A total of 168 piglets were randomly allocated to a tested (T) or control (C) group at weaning (19-20 days of age) from 15 litters of the same batch. In the T group, piglets received a whole inactivated virus PCV2 vaccine (Suigen® PCV2, Virbac) and in the C group another PCV2 whole inactivated virus vaccine (1 ml in the T group and 0.5 ml in the C group, by IM route). The allocation of piglets was done inside each litter, so that half of piglets within a litter received the T vaccine and the other the C vaccine (by taking into account the sex in group allocation). Piglets were then moved to the nursery site before moving to the fattening unit. They were allocated in separate pens per vaccine group from wean to finish. Pigs were individually identified by ear tag at weaning and individually weighed at weaning, at the transition from nursery to fattening and at finishing (145-146 days of age). Mortality cases were recorded from wean to finish.

A blood sample was taken on 10 pigs per group at the age of 14 weeks. These 20 individual samples were then analyzed by PCV2 quantitative PCR.

Means for body weight at weaning, post-weaning and finishing were adjusted (estimated marginal means) by using eventual covariates when appropriate, such as sow parity, piglet sex, weaning weight, lactation and nursery lengths. Comparisons between groups were done by using an analysis of covariance (general linear model: GLM). The mortality rates were compared between groups by the Fisher's exact test. Statistical analyses were performed using R version 4.0.2 (2020-06-22) -- "Taking Off Again"; Copyright (C) 2020, The R Foundation for Statistical Computing. Platform: x86_64-w64-mingw32/x64 (64-bit). Significance level was set as $p < 0.05$.

Results

Included piglets were issued from sows whose parity ranged from 3 to 11. No local nor general side effects were noticed after injection of either PCV2 vaccine. Mortality rates were not significantly different between groups. A part of the pigs could not be weighed at finishing, due to departure to the slaughterhouse before weighing (39 pigs in the C group and 37 pigs in the T group).

The weaning, transition and finishing weights were not significantly different between groups (Table 1). In each group, 5 out of 10 sampled pigs had a PCV2 viremia below limit of detection and the remaining 5 pigs had a serum viral load between 3 and 5xlog10 copies/ml.

Table 1. Pig weights (estimated marginal mean ± standard error) and mortality rates per vaccine group

Group	C	T
Weaning weight (kg)	6.0 ± 0.1	6.0 ± 0.1
Transition weight (kg)	23.3 ± 0.4	22.6 ± 0.4
Finishing weight (kg)	98.0 ± 2.0	98.3 ± 1.9
Wean to finish mortality	3.6 %	2.4 %

C: control PCV2 vaccine. T: Suigen® PCV2 vaccine

Discussion and Conclusion

Safety and efficacy of the tested PCV2 vaccine have been previously reported in Asia either in challenge or field conditions (3,4,5,6). This study confirmed its safety in Colombian field conditions and did not show significant differences between the 2 vaccines on growth performances nor on the mortality rates. The serum PCV2 qPCR analyses were positive in half of the pigs sampled per group at 14 weeks of age. However the viral load was below the defined clinical threshold of 6xlog10 copies/ml (7). These findings reflect the field circulation of PCV2 despite vaccination, as PCV2 vaccines do not induce sterilizing immunity, though they decrease dramatically the infection pressure (8).

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Safety and efficacy of PCV2 and *Mycoplasma hyopneumoniae* (M.hyo) vaccines mixed together in the protection against experimental infections.

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Introduction

PCVD (Porcine Circovirus Diseases) and enzootic pneumonia (EP) remain a major health problem in most swine farms (1,2). Different mono-valent or combined vaccines are available on the market. The aim of the study was to assess the safety and efficacy of PCV2a and M.hyo vaccines mixed prior to use (ready-to mix RTM) against PCV2 and M.hyo experimental infections.

Material and Methods

Circovac® (Ceva) and Hyogen® (Ceva), one dose each, were mixed in one vial (DUO) before the administration. In the laboratory safety study, 10 piglets were vaccinated with 2.5ml of DUO and 10 piglets with PBS. Local and systemic reactions were observed for 14 days.

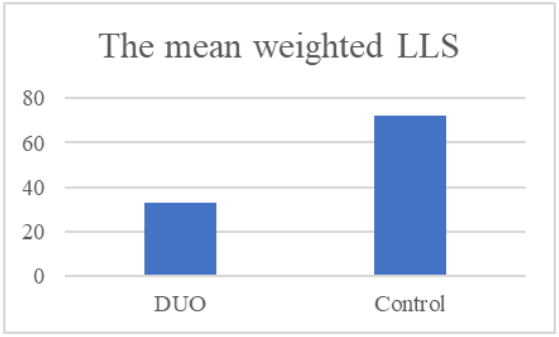
In the efficacy studies piglets were vaccinated with DUO at 3 weeks of age (WOA) or not vaccinated as controls. Animals were infected at 26WOA with either PCV2a isolate or with M.hyo strain. Pigswere necropsied 4 weeks post infections. PCV2 virus loads by qPCR and M. hyo lung lesion (LLS) (European Pharmacopoeia) were measured to assess the efficacy.

Results

Safety: no piglet had a fever and the average body temperature was 40.0 °C in vaccinates and 38.9°C in controls. No significant difference in and ADG (0.32 vs 0.33kg/day vaccinates vs controls) were found.

Efficacy: The mean PCV2 viral loads (log10 copies/ml) in different lymph nodes were 2.5-3.1 for group DUO and 4.3-5.3 for the controls($p<0.05$). The mean weighted LLS were 33.2 for DUO group and 72.3 for the control ($p<0.05$).

Fig 1. Mean weighhted lung lesion scores



Discussion and Conclusion

This study confirmed that those two vaccines mixed together into one dose were safe for piglets. The duration of protection after vaccination for 23weeks was demonstrated against the experimental infection with PCV2 as well as against M.hyo. These vaccines proved to be a safe and highly efficient tool in the protection against PCVD and EP as the RTM product.

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Serology by Flow Cytometry: a smart strategy to assess *Lawsonia intracellularis* circulation in pig farms

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Introduction

Lawsonia intracellularis is the causative agent of Porcine Proliferative Enteropathy, an important worldwide distributed enteric disease of swine (1). Infected animals can be diagnosed by detecting *L. intracellularis* on fecal samples by qPCR and by serological assays such as ELISA, IPMA and Flow Cytometry (FC) that are used to assess the contact of pigs with *L. intracellularis*. We recently described the use of FC to detect anti-*L. intracellularis* IgG in pigs (2). Here, we evaluated the concomitant use of qPCR and FC for the detection of farms positive for *Lawsonia intracellularis*.

Materials and Methods

We evaluated the sensitivity of qPCR and FC to detect the presence of *Lawsonia intracellularis* in conventional farms. A total of 17 farms with frequent episodes of diarrhea during the growth and finishing phase were selected. From these farms, 820 serum and feces samples were transversally and concomitantly collected from pigs with different ages (65 up to 210 days). At least twenty animals per farm or age were included. Faeces samples were diluted to 10% in PBS and used for total genomic DNA extraction (MagaZorb DNA Mini-Prep Kit, Promega, USA). The molecular detection and quantification of *L. intracellularis* was performed by qPCR as previously described (3). Serum samples were used to detect *L. intracellularis* IgG by Flow Cytometry as described previously by our group (2).

Results

All farms (n=17) had at least one animal shedding *L. intracellularis* on faeces, which was detected by qPCR. In parallel, serum samples from the same animals were analyzed by FC and all farms had positive animals. As illustrated in **Figure 1**, the level of positivity at the different moments evaluated varied considerably according to the sample and diagnostic method used. Although the qPCR technique is specific and able to detect a low number of *L. intracellularis* in the faeces, we noticed that the percentage of pigs with shedding *L. intracellularis* in the faeces (*n*=254, 30.97%) was consistently lower than the number of pigs with specific *L. intracellularis* IgG (*n*=617, 75.24%). Although approximately two thirds of the animals were not shedding *L. intracellularis*, the FC results showed that most of them were infected at some point during the growth and finishing phase; and possibly at the time of sampling they had already naturally controlled the infection.

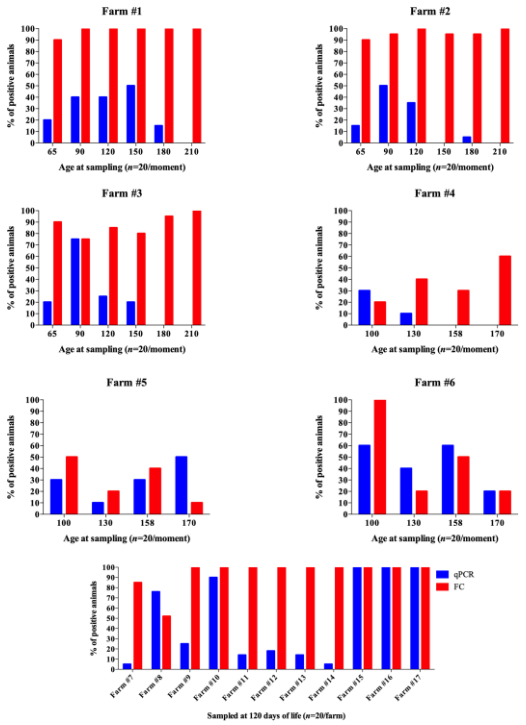

Figure 1. Concomitant detection of *L. intracellularis* by qPCR or specific *L. intracellularis* IgG by Flow Cytometry.

Discussion and Conclusion

Molecular or serological diagnosis of *L. intracellularis* is important for detecting pigs with subclinical infection. Here we demonstrated that FC is a suitable technique for monitoring herds for *L. intracellularis*. Strategically, FC serology can be used primarily to assess the dynamics of infection on the farm. After identifying the time when pigs become positive for the presence of IgG against *L. intracellularis*, fecal samples can be collected (~14 days before the first IgG peak) and qPCR used to understand the infection burden and estimate the economic losses resulting from the infection process.

Acknowledgments

This work was financially supported by MSD.

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Shedding and Transmission of a Lineage One Modified Live PorcineReproductive and Respiratory Syndrome Virus Vaccine

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Abstract:
Porcine reproductive and respiratory syndrome virus (PRRSV) causes annually about $1billion in the US swine industry. PRRSV control has been inadequate because of the genetic variability, lack of broad cross protection, and continuous shedding and transmission of PRRSV vaccine-like strains. Two shedding and transmission studies are summarized to characterize potential shedding and transmission of a recently USDA licensed vaccine. Understanding shedding and transmission may help veterinarians, researchers, and producers make more informed decisions when evaluating modified live (MLV) PRRS vaccine. The two studies are similar but served different goals: Study A is a 21 day study of vaccinated and sentinel pigs observed clinically and diagnostically; the results indicated limited shedding without any transmission event. To further evaluate shedding and transmission, a 70-day study was conducted to define when vaccine induced viremia would cease and if shedding and transmission would be observed. In this study a competitor's vaccine was used to evaluate the difference in the shedding and transmission of vaccine virus. The 21 day study demonstrated Prevacent did not transmit to sentinel pigs. The 70 day study demonstrated Prevacent sheds for 28 days but the trend would indicate up to 50 days is possible. The competitor's MLV vaccine sheds for at least 45 days, but the full extent of transmission could not be determined because all sentinel pigs were infected and removed by day 45. These results demonstrate difference between the vaccineswhich may be important in limiting vaccine like viral shedding.

Introduction:
Porcine reproductive and respiratory syndrome virus (PRRSV) causes annual losses of approximately $1billion in the US swine industry (Holtkamp et al.). To date the controlof PRRSV has been inadequate because of the virus's inherent genetic variability, lack of broad cross protection, and continuous shedding and transmission of PRRSV vaccine-like strains. Genetic evaluations of PRRSV2 ORF 5 into ancestral groups show 9 families or lineages circulating worldwide (Shi et al.). USDA currently requires a label disclaimer that the potential for transmission exists with PRRSV modified live vaccine. Elanco has developed a PRRSV modified live vaccine which is licensed for the control of the respiratory and reproductive phases of PRRSV. The experimental PRRSV modified live vaccines underwent a 21 day study to determine the extent of shedding and transmission of the modified live vaccine strain. Herein is reported a 21 day shedding and transmission study (study A) and a seventy-day shedding and transmission study (study B) to further define the period of transmission and shedding of Prevacent® PRRS.

Methods:
Study A: Twenty 14-day old PRRSV seronegative and reverse transcriptase quantitative PCR (RTqPCR) negative piglets from a PRRSV naïve sow herd were randomly allocated to vaccine or sentinel groups on day -1 of the study. Two pigs from the vaccine group were placed with two pigs from the non-vaccinated sentinel group in one of five 4'x 5' pens in the Biosafety Level (BSL) 2 facility on day -1. On day 0 the vaccine group received 1 ml intramuscular injection of 8.2 log10 TCID50/ml experimental vaccine derived via cell passage from strain PRRSV SD 11-21. Blinding was accomplished through separation of the personnel administering the vaccine from those collecting the following samples. Nasal swabs and serum were collected on day -1, day 3, day 5, day 7, day 10, day 14, day 17, and day 21. Iowa State Veterinary Diagnostic Laboratory performed RT-qPCR, on both specimens. On day 21 all pigs were humanely euthanized and necropsied. The lungs were harvested, and an aseptic sample of bronchial alveolar lavage fluid (BALF) was collected and tested via PRRSV RTqPCR from each pig. In addition, tissues samples consisting of lung, spleen, tonsil, right and left tracheobronchial lymph nodes were collected and tested via RTqPCR for PRRSV. Any positive sample was genetically sequenced using the ORF5 region and compared to the ORF5 region of the experimental vaccine.
The statistical analysis was descriptive only. The case definition for shedding in the vaccinated group was an RTqPCR positive on nasal swab. The case definition of transmission was if a sentinel pig tested RTqPCR positive on nasal swab, serum, and either BALF or tissue.

Study B: Forty 17 to 18 day old weaned PRRSV seronegative and RTqPCR negative from a PRRSV naïve sow herd allotted to two BSL 2 rooms using a randomized complete block design for 4 treatment groups: group 1) Unvaccinated controls (sentinel) placed with vaccine CV (Competitior's vaccine), group 2) Vaccinated with vaccine CV placed together in room 1, group 3) Unvaccinated controls (sentinel) placed with vaccine PREV (Prevacent® PRRS, Elanco AH), group 4) Vaccinated with vaccine PREV placed together in room 2. Each room had 2 pens of 10 pigs (5vaccinated and 5 sentinels per pen) with vaccines separated by room. At placement (day 0) all vaccinated pigs were vaccinated according to label directions. Sentinel piglets received 1 ml of sterile saline as a placebo. Serum and nasal swabs were collected weekly (11 collections) through the study end on day 70 for serological (Idexx PRRSX# Ab) and/or RTqPCR analysis. Oral fluids were collected every two weeks starting on day 0 through the ending day 70 (6 collections). Individual rectal temperature was collected on days 0, 1, 3, 7 and 10 and individual pigs were weighted weekly.

Sentinel pigs were removed from the study when collected samples for PRRSV analysis demonstrated one of the following criteria: RTqPCR positive for two consecutive nasal swab samples or any single serum sample. Removal was within 3 to 4 days, with one exception of 10 days of one pig in group 3.

Results and Discussion:

Study A: Nine out of 10 (90%) of the vaccinated pigs had at least one nasal swab positive during the 21 day study. Only one nasal swab was positive in the sentinel group and no sera tested positive during the 21 day study. No tissues or BALF was RTqPCR positive in the sentinel group at the end of the study necropsy.

All vaccinated pigs were RTqPCR positive in sera (viremic) on at least one sampling day from day 3 to day 21 (Figure 1). All vaccinated pigs were RTqPCR positive via nasal swabs (shedding) on day 21 (Figure 1). The overall average estimated viremic load (standard deviation) of all vaccinated pigs on day 21 was 6.8 x 107 RNA copies/ml (1.8x108 RNA copies/ml).

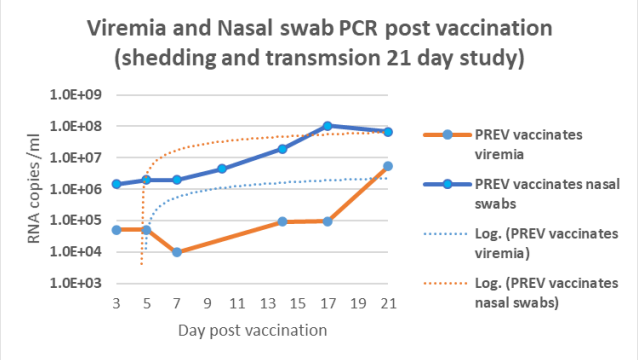

Figure 1. All PREV vaccinates were RTqPCR positive (viremic) during the 21 day study. All were viremic on day 21. Nasal swabs were intermittently positive with 44.4% positive (shedding) on day 21.

All BALF and tissues were RTqPCR positive in the vaccinated group on day 21.

The ORF5 sequence was 99.7 to 100% identical to the experimental vaccine on 60 tissue samples from the vaccinated group.

Although the vaccinated group was viremic and nasal swabs were intermittently RTqPCR positive throughout the study, no evidence of transmission was identified in the sentinel group. No evidence of genetic variation was found in the 21-day study as demonstrated through the high degree of homology found in the ORF5 genetic sequence.

Study B: All vaccinated pigs became viremic. The average viral loads (RNA copies/ml) of serum in the PREV vaccine group were significantly lower ($P<0.05$) compared to CV vaccinated group on study days 14, 21, 28 and 35 (Figure 2).

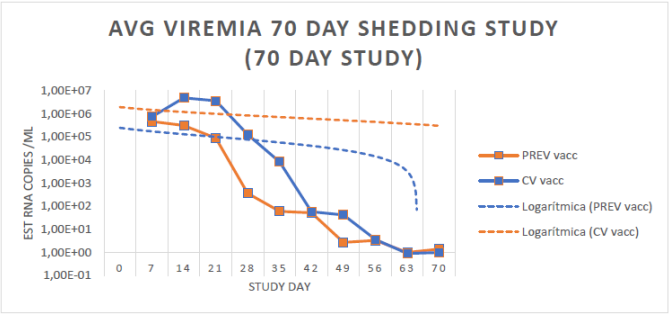

Figure 2 Prev Vaccinated pigs had significantly lower RNA copies/ml compared to the CVvaccinated pigs on days 14, 21, 28, and 35. Logarithmic trend lines were calculated in Excel. The trendlines estimate expected viremia of each vaccinated group.

RTqPCR with nasal swabs were sporadically positive in both vaccinated groups as indicated in Figure 3

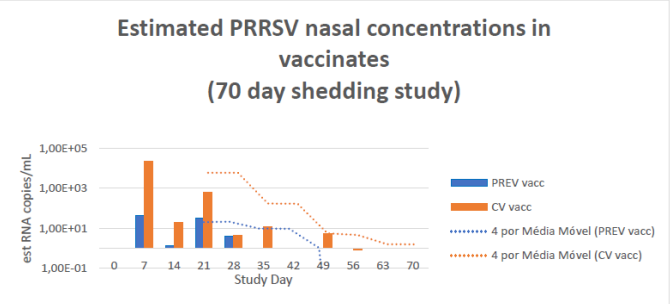

Figure 3 Estimated RNA copies/ml in nasal swabs. The 4 period moving average was calculated in Excel and estimated decay of RNA copies/ml over the 4 period moving average.

The 4 period moving average demonstrates decay of estimated nasal RNA copies/ml of each group. The PREV vaccinated group goes below zero while the CV vaccinated group does not at day 70 (end of study) although at trend line estimates very low levels.

Sentinel pig removal is illustrated in figure 4. Sentinel pigs housed with vaccine PREV or vaccine CV had 90% and 100% removal in each group, respectively. Merging the shedding data (ie nasal swab result) with the transmission data (ie removals) indicates vaccine PREV stopped shedding at around day 42 as no further transmission events were detected and one sentinel remained throughout the 70-day study. Because vaccine CV had all sentinels removed by day 42 no further transmission events could be detected but the shedding data would indicate further transmission events would have been possible. A more robust comparison would have been possible if replacement of sentinel pigs were added upon removal. Field experience and a literature review indicated extended shedding with vaccine CV.

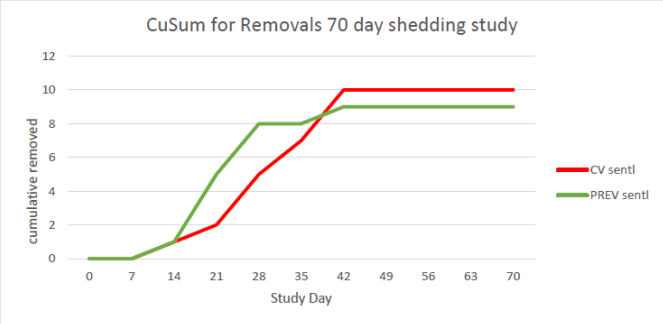

Figure 4 Cumulative sum of removals over the 70 day study. All CV sentinels were all removed by day 42. One Prev sentinel remained until day 70 (end of study).

Figure 5 depicts average estimated RNA copies/ml of pigs removed by day of removal. As time progressed viremia in removed sentinels increase within both groups although the duration and peak appear differences. This is substantiated by the logarithmic trend lines generated for the data. Again, the extent and duration of this observation was truncated because no replacement sentinels were used in the study. The use of replacement sentinels (ie replacement of a sentinel upon removed because of a defined transmission event) would have better defined the observations.

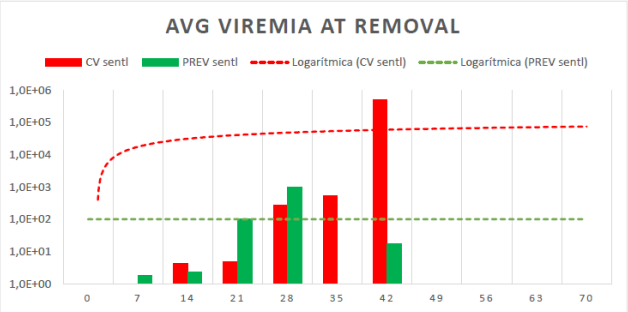

Figure 5 Average estimated RNA copies/ml at removal for each sentinel group. All removed sentinels were removed because of serum RTqPCR positive (viremia).

Although Study A showed minimal transmission, an interesting finding is that all PREV vaccinated pigs were still viremia and shedding at day 21 or the end of the study. Although limited by study design, study B may demonstrate viremia, shedding and transmission differences between two vaccines. Further studies and field observations will be needed to better substantiate these findings.

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Study of synergistic action on intestinal integrity of an oral vaccine against *Escherichia coli* and a β-mannanase added to the feed

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Introduction

The use of vaccines against *E. coli* directly delivered to piglets is one of the most interesting tools to prevent intestinal disorders during nursery and early finishing period. On the other hand, β-mannans are oligosaccharides presents in a wide range of feedstuff, which produce a mimic effect of *E. coli* receptor, resulting in chronic inflammation of intestine and then decrease in performances of piglets. This work describes the results of a trial combining vaccination with a β-mannanase in feed.

Materials and Methods

Four groups of piglets were randomly allotted after weaning, receiving oral vaccination against *E. coli* with Coliprotect® F4/F18 (Elanco Animal Health) (COLI), one group receiving a feed supplemented with Hemicell (Elanco Animal Health), a β-mannanase (HEM), one group with vaccination and Hemicell® HT in feed (COLI+HEM), and one control untreated group (CONT). All piglets were housed in the same barn with the same managements and environmental conditions Samples of feces were obtained at weaning (sampling pretreatment) and subsequently at 9, 23 and 33 days after vaccination or start receiving the feed treatment. The feces were preserved in RNAlater, and freezed after 24 hours in refrigeration. The, total RNA was isolated, cDNA was synthetized using oligo-dT as primer, and qPCR was performed to detect gene expression of Calprotectin (an indicator of inflammatory cell infiltration), Ocludin and Zonulin (proteins of tight-junctions), INF-γ as one of the main proinflammatory cytokines and TGF-β as the main anti-inflammatory cytokine. The housekeeper gene sed was β-actin and the relative quantification was used using the Pfaffal's method (1). The comparison was made by Mann-Whitney's U test, and an analysis of discriminant functions to reduce data.

Results

There was difference in the quantification for all biomarkers in the 2nd, 3rd and 4th samplings. When the discriminant function analysis was performed, the separation of groups is clear at each sampling moment. The Wilks' lambda signification was $p<0.0001$ for all samplings.

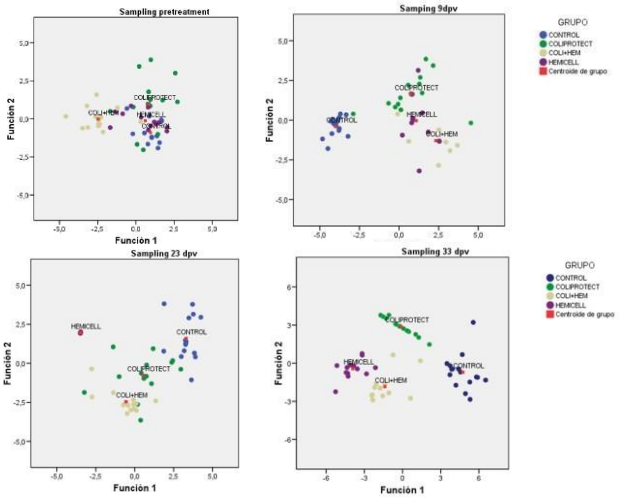
The table 1 shows the discriminant capacity of the functions designed by the statistical software. Generally, all samples in the control group are always correctly assigned to this group. Interestingly, the wrong assignations were made between groups that share some treatment (COLI or HEM with COLI+HEM).

A plot with the canonical distribution of samples is shown in Figure 1.

Table 1. Assignation fo samples to putative groups using the discriminant functions designed.

Sampling		CONT	COLI	COLI+HEM	HEM
9 dpv	CONT	100	0	0	0
	COLI	6.7	73.3	6.7	13.3
	COLI+HEM	0	7.1	92.9	0
	HEM	0	13.3	13.3	73.3
23 dpv	CONT	100	0	0	0
	COLI	20.0	46.7	26.7	6.7
	COLI+HEM	0	6.3	87.5	6.3
	HEM	0	0	0	100
33 dpv	CONT	100	0	0	0
	COLI	0	100	0	0
	COLI+HEM	0	14.3	85.7	0
	HEM	0	0	7.1	92.9

Figure 1. Canonical plot for discriminant functions analysis at each sampling moment



Results evident the separation of the groups along the time which indicate an evident effect of the treatments.

Discussion and Conclusion

The prevention of enteric diseases during nursery, avoiding the use of zinc oxide (banned in June 2022) is going to be based in combinations of tools up to get the best effect. The study of intestinal integrity allows to evaluate the effect of some of these strategies. In this case, we have seen differences in biomarkers as Calprotectin, Occludin and Zonulin, and in IFN-γ. Interestingly, even when the increase of this cytokine is expectable after vaccination, the increase is lower in the group COLI+HEM, which could indicate a reduction of undesirable immune stimulation derived from mannans. Anyway, the effect on all the biomarkers is evident, producing a higher separation of centroids at each subsequent sampling moment. These findings need for confirmation in bigger groups.

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Swine Erysipelas seroconversion failures on South Korean vaccinated farms

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Introduction

Humoral immunity and cell-mediated immunity play a role in host defense against *Erysipelothrix rhusiopathiae* infection (1). The protective role of specific antibodies against Swine Erysipelas (SE) enhanced through vaccination is the key to controlling reproductive disorders in sows such as abortions (2). In South Korea, most of the farms apply live attenuated vaccine against *Swine Erysipelas* (3) commonly combined with Classical Swine Fever live attenuated vaccines (CSF+SE). The efficacy of this type of vaccines to elicit an immune response against SE has not been previously evaluated in field conditions on Korean commercial swine farms due to the lack of an appropriate method. The objective of present study was to verify the efficacy of using live attenuated SE vaccines on Korean farms via serological survey using ELISA test.

Materials and Methods

A total of 600 serum samples from 26 commercial swine farms (size 200 to 1,600 sows) in different provinces of South Korea were tested/studied. The samples were collected from gilts and sows on different farms which had no clinical signs of SE. All farms vaccinated gilts once in acclimation period and sows received a booster in the 3rd week of lactation in every cycle, with 4 different Korean live attenuated Swine Erysipelas vaccines + Classical Swine Fever (NL-11 strain for SE + LOM strain for CSF) following manufacturers' instructions. Up to 25 serum samples were collected on each farm and individual parity were recorded as well. The quantity of antibodies against SE were measured using an indirect ELISA kit (CIVTEST® SUS SE/MR) in a local laboratory. The ELISA results were interpreted as positive when Cut-off IRPC value was > 40. The ability of this kit to detect anti-SE antibodies without bias has been previously reported (4). The correlation between the positivity rate and the sow's parity/antibody titers was analyzed by logisticregression or linear regression with a Turkey Post-hoc test, respectively.

Results

The overall percentage of seropositive animals was 32.67% (196 out of 404 samples) (Figure 1). 24 of the 26 farms had a positivity rate lower than 80% (Table 1). Regarding the analysis by sows' parity, the positivity rate in sows and gilts was 34.48% and 24.04%, respectively, being statistically significant (*P-value* = 0.02) (Figure 1). Regarding antibody titers, the average IRPC value of sows was 34.41, which was significantly higher than 27.78 of gilts (*P-value* = 0.006) (Figure 2).

Table 1. Number of farms based on the range of positive animals and average titers per range

Range of positivity	Average Titers (IRPC)	Nº of farms
0-10	13	5
10-25	24	7
25-50	36	8
50-75	53	4
75-100	69	2

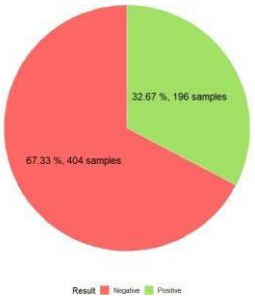

Figure 1. Number and percentage of seropositive animals against SE in farms of South Korea

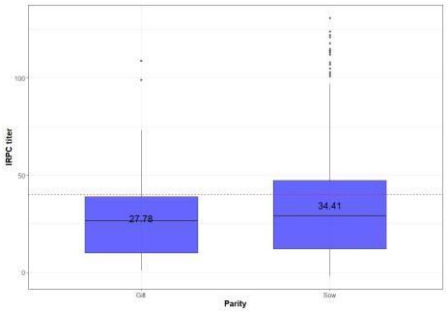

Figure 2. Average antibody titer against Swine Erysipelas as by sows' parity

Discussion and Conclusion

Even though live attenuated vaccines against SE are commonly used in South Korea, these results show that only a small portion of vaccinated animals generated enough antibodies to be considered as protected.Further studies are required to establish what the reasons behind these vaccine failures are in South Korea. Vaccine protocols or/and vaccines used should be revised or changed to those that elicit a higher immune response.

Acknowledgments

The authors wish to thank all the Korean farmers that collaborated in this project.

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Targeting to porcine dendritic cells: a vaccine against porcine circovirus

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ıtroduction

accine development based on DCs-targeting has been udied in several species such as mice, humans, calves, ıd swine (1,2,3,4). Our research group has worked on udying swine DEC205$^+$ DCs in different lymphoid ssues (5). Swine skin has been reported as a tissue with verse populations of DCs, which places it as a suitable ıthway for DCs-targeting (6). Previously, with the oduction of a chimeric mouse x pig anti-porcine EC205 antibody (rAb ZH9F7), it has been possible to udy the effect of targeting in pigs (7,8). The present work med to explore how the DCs-targeting through DEC205$^+$;in DCs works and the effect after targeting the PCV2a ap antigen as a model to evaluate humoral and cellular sponse.

laterials and Methods

/e expressed the rAbZH9F7 joined to PCV2a Cap antigen AbZH9F7_Cap) or eGFP (rAbZH9F7_eGFP). After, an ternalization assay of rAb ZH9F7 by flow cytometry was erformed. The evaluation of the ability of rAb H9F7_Cap to recognize and target DCs in peripheral ssues was carried out by injecting the rAb ZH9F7_Cap or \bZH9F7_eGFP intradermally on the inguinal area from ealthy pigs. After 24 h, skin biopsies were obtained and ıalyzed by confocal microscopy.

loreover, skin migrating cells and lymph node cells were ow cytometry stained to identify different DEC205$^+$-rgeted DCs populations. Finally, to determine the iming of humoral and cellular immune response omoted by the targeting of PCV2 Cap to DCs, pigs were tradermally vaccinated with the rAb ZH9F7_Cap. Three eeks after the first vaccination, a boost was applied. On eek four, a peripheral blood sample was taken to evaluate CV2-specific antibody titers by indirect ELISA and the sponse of IFN-γ-secreting cells by flow cytometry.

esults

gure 1A shows a reduction in the intensity of uorescence DEC205$^+$ cDC1 (blue histograms) and cDC2 ed histograms) from blood, which was triggered by the nding of rAb ZH9F7 to the DEC205 receptor at 37°C in ne 0 (colored) and after 30 min (light-colored). When plied through the skin, rAb ZH9F7 was able to recognize DC1 (Fig. 1B, pink arrows) and cDC2 (Fig. 1B, green rows), evidenced by colocalization with other DCs arkers such as CD172a and CADM1.

/e observed that rAbZH9F7_Cap can recognize and rget blood and skin DCs and found the presence of rgeted DCs in regional lymph nodes (data not shown). hus, the possibility of priming an adaptative immune sponse was almost a fact. To prove it, the evaluation of CV2a Cap-specific antibodies and IFN-g secreting cells in response to Cap was required. Figure 2A shows a higher titer of PCV2a specific IgG in the rAnZH9F7_Cap vaccinated group (blue bars) at week 4 post-immunization, compared with the control group (black bars, non-vaccinated). Moreover, the frequency of double-positive CD4CD8 T cells secreting IFN-γ was also higher in the rAbZH9F7_Cap vaccinated group compared with the control group (figure 2B).

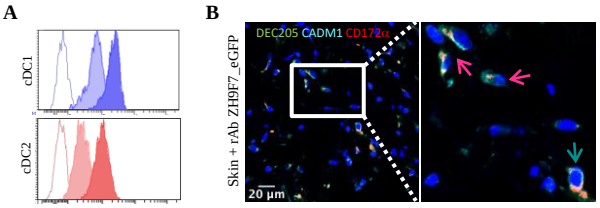

Figure 1. rAb ZH9F7 recognizes cDC1 and cDC2 DEC205$^+$ from blood (A) and skin (B).

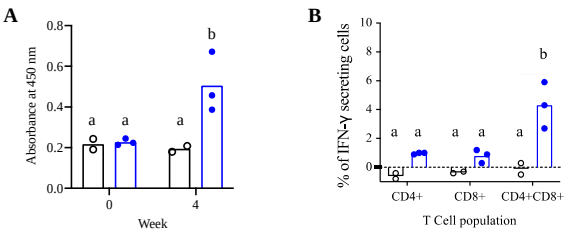

Figure 2. rAb ZH9F7_Cap promotes antibody (A) and IFN-γ responses (B).

Discussion and Conclusion

Porcine skin is a DC-rich tissue able to be targeted. Our results showed that different DEC205$^+$ DCs populations were targeted with rAb ZH9F7_Cap, although the cDC1 population presented a higher percentage, triggering receptor-mediated endocytosis and reaching regional inguinal nodes. Consequently, the priming of the humoral and cellular immune response against PCV2a Cap antigen was achieved, allowing this strategy to remain under scrutiny to be optimized.

Acknowledgments

This work was supported by the Asociación IPVS-México 2014 and self-generated funds.

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The use of a killed PRRS vaccine as a complement to a modified live vaccine to achieve a stable PRRS virus status on farms

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Introduction

On farms that are endemically infected with the PRRS virus, modified live vaccines (MLV) are the first choice for immunological stimulation of animals, so any vaccination programme must include them. PRRS killed vaccines (KV) represent a booster of immunity against the PRRSv and are used as a complement to a previous vaccination with a MLV. This combination of vaccines could have various benefits, namely an increase in neutralizing antibodies and CMI responses (1). On the other hand, a farm with stable PRRSv status is the key to achieving better reproductive parameters, being able to achieve an increase of 1.28 weaned piglets per sow per year if PRRSv stability is maintained for a one-year period (2).

The objective of this study was to assess the use of a KV in a combined protocol together with a MLV, evaluating the concentration of neutralizing antibodies incolostrum from sows and the stabilization of the piglets' PRRSv status on PRRS endemic farms.

Materials and Methods

2 PRRSv2-positive farrow-to-finish farms of 1200 sows each were enrolled in a trial in Thailand. On both farms, piglets were RT-qPCR-positive at weaning, which means that the PRRSv status of both farms was unstable (3). On both farms, the routine vaccination programme for sows was mass vaccination with a PRRS MLV every 4 months. On each farm, the sows were divided into 2 groups: MLV group (vaccination with a MLV every 4 months) and MLV+KV group (vaccination with a MLV every 4 months and a booster dose with a KV (SUIPRAVAC® PRRS, KV, HIPRA, 2 ml dose) 4 weeks before farrowing). Colostrum samples were taken (volume 1 mL from the first front teats of sows) within 1 hour after farrowing from the MLV groups (Farm 1 n=5, Farm 2 n=8) and MLV+KV groups (Farm1 n=5, Farm 2 n=8) to evaluate neutralizing antibodies against EU and US field PRRSv strains in accordance with the guidelines of the Kamphaengsaen Veterinary Diagnostic Center **(**Kasetsart university, Thailand**)**.

Blood and umbilical cords were collected to evaluate the stability of the PRRS in both groups. 80 samples were pooled and a total of 16 pools from 5 animals each (blood + umbilical cords) were analyzed by Rt-qPCR.

Results

The MLV+KV groups had significantly higher titres of neutralizing antibodies against EU field PRRSv than the MLV groups (Table 1).

Additionally, RT-qPCR pools after vaccination of sows with SUIPRAVAC® PRRS on both farms were negative in all the samples that were taken (Table 2).

Table 1. Colostrum neutralizing antibody titres in sows against EU and US PRRSv.

	EU		US	
FARM 1	MLV+KV*	MLV	MLV+KV	MLV
	1:40	<1:20	<1:20	1:80
	1:80	1:40	1:80	1:80
	1:320	1:160	1:160	1:640
	1:640	1:40	1:640	1:80
	1:160	<1:20	<1:20	<1:20
	EU		US	
FARM 2	MLV+KV*	MLV	MLV+KV	MLV
	1:80	1:40	1: 40	1:20
	1:20	<1:20	1:20	1:40
	1:320	1:40	1:80	1:20
	1:320	1:80	1:40	1:40
	1:640	<1:20	1:80	1:20
	1:160	1:160	1:80	<1:20
	1:640	1:80	1:160	1:40
	1:320	1:80	1:80	1:80

*Significantly higher titres of neutralizing antibodies against EU field PRRSv in the MLV+KV group (p-value 0.003)

Table 2. Number of Rt-qPCR-positive pools per group.

FARM 1			
TYPE OF SAMPLE	DAY	MLV	MLV + KV
Umbilical cords	0 days after farrowing	0/1	0/5
Blood	3 weeks of age	1/1 (+) *US strain	0/1
Blood	7 weeks of age	-	0/1
FARM 2			
TYPE OF SAMPLE	DAY	MLV	MLV + KV
Umbilical cords	0 days after farrowing	0/1	0/2
Blood	3 weeks of age	1/1 (+) *US strain	0/2
Blood	6 weeks of age	1/1 (++) **US strain	-

*Ct value: 30-35
**Ct value: 25-30

Discussion and Conclusion

Vaccination protocols that include a MLV every 4 months and a KV at 4 weeks before farrowing raise the concentration of neutralizing antibodies in colostrum and achieve PRRSv stabilization by producing RT-qPCR-negative piglets at weaning.

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Vaccinating piglets with an intramuscular *Lawsonia intracellularis* vaccine effects animals' performance and economy during fattening period

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Introduction

Lawsonia intracellularis (LI) is an intracellular predominantly ileal located bacterium causing subclinical performance depression (1), Porcine Intestinal Adenomatosis (PIA; reduced growth performance, increased fattening time, less homogeneity of the fattening pigs) and Proliferative Hemorrhagic Enteritis (PHE; dark, tarry diarrhea which may result in death). This porcine intestinal pathogen with prevalence ranging from 48 to 100% in different swine producing countries has been identified as one of the main enteric pathogens during fattening of pigs worldwide (2).

Materials and Methods

The observation was made in a closed herd farm (appr. 300 sows, 1500 nursery pigs, 3500 fatteners) in North East Germany. The farm in general showed a good health status. It was negative for PRRSV and clinical symptoms in the respiratory tract were absent. Piglets were vaccinated against edema disease, PCV and M. hyo in the suckling period. LI related symptoms started 4-5 weeks after beginning of the fattening period (severe bloody diarrhea). Individual tylosin injection was insufficient, so that feed medication with tiamulin was necessary in nearly all groups. Nevertheless, appr. 10 % of pigs developed poorly, 2-3 % were severe runts. Diagnostics showed LI seroconversion and high LI loads (PCR >log GE 6/g faeces) in the middle of fattening period. To control the LI symptoms vaccination with the Porcilis® Lawsonia + Porcilis® PCV M Hyo combination (LI vaccine dissolved in PCV M Hyo) was introduced. Performance data from the period without LI vaccination 01.07.-31.12.19 (vacc-) and with LI vaccination 01.04.-30.06.2020 (vacc +) were compared.

Results

With introduction of the LI vaccination the LI related clinical signs strongly decreased. Only sporadically individual treatment with tylosin, but no more group medication was necessary.

Table 1. Number of animals and weights in the differently vaccinated groups

	vacc -	*vacc+*
Number of animals (n)	4021	2034
Weight at stabling (kg)	27.4	27.3
Weight at slaughter (kg)	120.9	126.0

Homogeneity was distinctly enhanced with <1 % runts. Despite of the still present LI load (3.5-5.6 GE LI/g feces) the clinical situation clearly improved. Average daily weight gain (ADWG) increased by 21 g. Animallosses decreased by 0.9 %. Feed conversion ratio was improved by 0.16. Vet costs for gastrointestinal reasons diminished massively due to the reduced antibiotic treatments. Total antibiotic treatment days/pig were 91.8 % lowered in LI vaccinated pigs. In total a benefit of 3.96-4.17 €/pig could be calculated by implementation of Porcilis® Lawsonia vaccination (vaccination costs need to be considered separately).

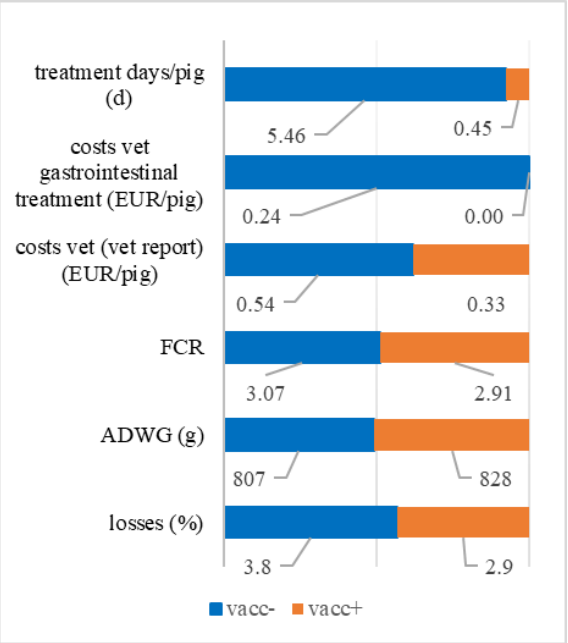

Figure 1: Performance data before (vacc-) and after vaccination with Porcilis® Lawsonia (vacc+)

Conclusions and Discussion

The infection of pigs with *Lawsonia intracellularis* can cause severe clinical disorders in a herd as seen inthis case. Animals have to be treated with antibiotics and economic losses are unavoidable. Vaccination with Porcilis® Lawsonia helped to control clinical symptoms and enhance performance results noticeable. Farmers extra work, manly seen in antibiotic treatments and management of suffering and underperforming pigs was thereby reduced. In conclusion the vaccination ensured healthier pigs and lower production costs.

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MISCELLANEOUS

Application of aggregated histopathology data to assess disease impact in growing pig populations

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Introduction

Histopathology is a longstanding and reliable diagnostic resource, widely used in the global swine industry, where trained pathologists help veterinarians to understand the contribution of various pathogens to clinical signs and disease observed in swine.

However, for the most part, the interpretation from the diagnostician regarding the etiology of lesions detected in tissues is used at one point in time. The submitting veterinarian then makes informed decisions to influence health of swine populations from which samples originated.

There is a tremendous opportunity to use aggregated histopathology data beyond a single point-in-time, characterizing the causal associations between pathogen activity and productivity of swine populations.

The objective of this proof-of-concept observational study was to describe the association between aggregated histopathology data obtained from a swine production system in the USA, and grow-finish mortality.

Materials and Methods

This was a prospective cohort study following 2,568 flows of grow-finish pigs raised in the USA during April 2018-July 2020. Histopathology data and cumulative mortality data was obtained from each flow, and descriptive statistics were implemented using SAS 9.4 software.

The histopathology data consisted of "diagnostic codes" assigned to each case by a diagnostician at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL).[1] In brief, the diagnosticians developed a standardized coding system to report the most likely etiology assigned to tissues submitted for histopathology. Etiology(ies) were assigned based on the professional judgment of diagnosticians considering the case history, macroscopic and microscopic lesions, and ancillary diagnostic tests requested on a case-by-case basis. In cases where the diagnostician did not have enough information to assign a definitive etiology, 'not specified' was assigned. The diagnostic code system allows assigning 1 or more etiologies per accession.

Mortality data was obtained from the digital repository used by the swine production system, and was calculated as a rate of the number of pigs that died during grow-finish divided by the number of pigs placed. Also, the PRRSV health status (negative, endemically, or acutely infected) of source farms was also recorded.

The data (histopathology, health status, and respective mortality) was merged using PROSPER, a digital platform that captures VDL data and production data in an automated fashion using Application Programming Interface. PROC Glimmix from SAS 9.4 was used to compare mortality of grow-finishflows having DX codes as the explanatory variable using binomial distribution.

Results and Discussion

From the 2,568 flows included in the study, the meanand standard deviation of the cumulative mortality observed in the grow-finish population was 9.87±0.04. The most frequent etiologies assigned by diagnosticians were PRRSV, Influenza A, *Glaeserella parasuis* (GPS), and *Streptococcus suis* (*S. suis*).

Overall, the flows having at least one tissue submittedfor histopathology (n=599, 23.3% of observed flows) had increased (1.03% percentage points) mortalitycompared to flows (n=1,969) without tissue submission for histopathology during grow-finish (P<0.005). Mortality was higher for all diagnosticcodes (i.e., all etiologies) compared to flows without diagnostic codes. Results support tissue submission for histopathology was associated with evidence of significant disease expression at the population level, therefore being a proxy of disease activity in grow- finish flows.

Also, mortality was consistently higher (at least 1%) for flows having tissue submission regardless of the PRRSV status of source farms (negative, endemic, or acutely infected). The increase in mortality decreasedas the days post-placement increased. More specifically, the baseline mortality (flows without tissue submission) was 8.1%, and increased to 11.7%,12.3%, 9.5%, and 9.7% when tissues were submitted during early nursery, late nursery, early finishing, andlate finishing, respectively.

Mortality was higher for all diagnostic codes (i.e., all etiologies) compared to flows without diagnostic codes. The mortality was higher when there were multiple etiologies assigned to the flows. For example, mortality of flows having GPS or *S. suis*-only were 10.2% and 10.5%, respectively, and 13.0% and 13.3%when PRRSV was also assigned in addition to GPS and *S. suis* respectively.

Conclusions

This study demonstrates the application of aggregated histopathology data to measure impact on swine health and productivity under field conditions. It expands theutility of histopathology beyond the original point-in-time used to determine pathogen activity in specific animals.

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Assessment of brain activity in swine subjected to water-based foam using electroencephalogram

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Introduction

The swine industry is threatened with foreign animal disease (FAD) outbreaks, such as African Swine Fever. Factors such as global movement and poor animal and feed biosecurity, increase the risk for disease introduction and/ or spread. Depopulation of infected animals is a critical step in controlling FADs. Several methods are currently recommended for depopulation in swine; however, in many cases there are concerns about animal welfare, mental health impacts, cost and logistics. In emergency situations, water-based foam (WBF) is approved by the AVMA for poultry depopulation; however, it is not currently approved for swine depopulation. The aim of this study was to investigate WBF as a depopulation method for swine, by describing time to loss of consciousness and brain death in nursery pigs.

Materials and Methods

Twelve healthy nursery pigs were included in the study. Six subdermal electroencephalogram (EEG) electrodes were placed on each pig as previously described (1) and shown on Fig 1. Individually, each pig was fitted into a sling and a portable, Bluetooth-enabled EEG device (Lifelines Neuro Trackit T4A) was used, which was connected to the electrodes and attached to the pig using a water-resistant carrying bag and a backpack. The sling was put into a plastic container (1.2m L x 1.2 m W x 1 m H) and baseline EEG was collected for five minutes.

Medium-expansion WBF was then applied to fill the container and fully immerse the pig, and EEG data was collected for another 15 minutes. Death was confirmed after removal of the pig via lack of heartbeat. EEG was processed and interpreted by a neurologist and an EEG analyst using Persyst software following the definitions from previous researchers (2): normal (baseline), transitional, high amplitude low frequency (HALF), and isoelectric. Transitional EEG was defined as EEG with amplitude of less than half of that of the pre-intervention EEG, HALF EEG was defined as waveforms of high amplitude and low frequency activity, and isoelectric EEG was defined as electrical activity with an amplitude of < 1/8 (12.25%) of that of normal baseline EEG or EEG with little or no identifiable brain activity (2). Movement artifact was defined as electrical activity originating from extracerebral sources including muscle movements (3). Both transitional and HALF EEG were used to classify an animal as unconscious (2, 4).

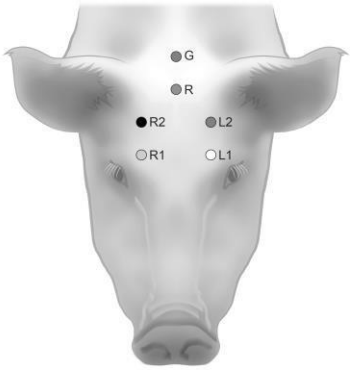

Fig 1. Electrode Placement (3), G=Ground R=Reference

Results

Six males and six female pigs with an average weight of 13.5 ±1.16 kg underwent successful foaming events, which resulted in death. Time to fill the container was consistently 3-4 sec. The average time of movement artifact was 1 min 38 sec ±34 sec. The movement artifact showed a continuous and intermittent component respectively with HALF EEG observed during the final intermittent component of movement artifact for each subject. This was followed by transitional EEG which lasted an average of 1 min 10 sec ±44 sec and was observed immediately after cessation of main movement artifact for most animals. Average time to isoelectric EEG, i.e., brain death, was 2 min 48 sec ±61 sec.

Discussion and Conclusion

This study adds scientific evidence that supports the use of WBF for rapid depopulation of swine. On average, these data show that pigs subjected to depopulation with WBF become unconscious around 1 minute 30 seconds and subsequently brain death occurs by 3 minutes. The precise time in phase where unconsciousness occurs is still a grey area of study, as these changes are gradual and can vary between subjects and species (4).

Acknowledgements

National Pork Board, Ohio Pork Council, USDA.

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Associated factors to the occurrence of *Cystoisospora suis* in Brazilian Pig Farms

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Introduction

Cystoisospora suis is the most frequent parasite of piglets in intensive pig farms. The objective of our study was to assess the associated factors of coccidiosis in industrial pig farms in Brazil.

Materials and Methods

A cross-sectional field study was designed to assess the prevalence of coccidiosis in Brazilian industrial pig herds. We investigated 51 farms from important pig production areas. The sample size gives 95% confidence level to estimate a 5% coccidiosis prevalence with a relative margin error of 6%. Ten random litters per farm were sampled twice on 2nd and 3rd week of life, and feces were examined by flotation. A questionnaire was applied to record 13 variables concerning litter information, farm practices, and management. A multivariable logistic regression model was fit to associate the presence or absence of coccidiosis with management and farm structure factors. The final model was selected using a stepwise procedure.

Results

On the farm level, 82.35% (42/51) farms were positive for *C. suis*, and the average within farm prevalence was 36% (23.47% – 50.77%) 95% confidence interval. Out of the 13 initial variables, five were selected to be offered to the final multivariable model: the presence of diarrhea, parity assistance, type of the floor, room temperature, and the average number of piglets per sow. The final multivariable model retained the room temperature and the presence of diarrhea. The chance of coccidiosis in litters affected by diarrhea is 5.75 higher than the chance of litters without diarrhea. On average, an increase of one degree in the room temperature increased the chance of coccidiosis by 23.2%. Figure 1 and Table 1 show the prevalence and factors associated with coccidiosis in the farms studied.

Table 1. Summary of the final multivariable model fit to predict the prevalence and associated factors with coccidiosis in commercial pigs farms in Brazil.

Variable	Estimate	OR (95% CI)*	Prevalence	P-value
Diarrhea				<0.001
Yes	1.91	6.75 (3.18-15.5)	53.8%	
No	-	1	14.71%	
Room temperature	0.208	1.23 (1.09-1.39)	-	<0.001

* OR: Odds ratio; CI: confidence interval.

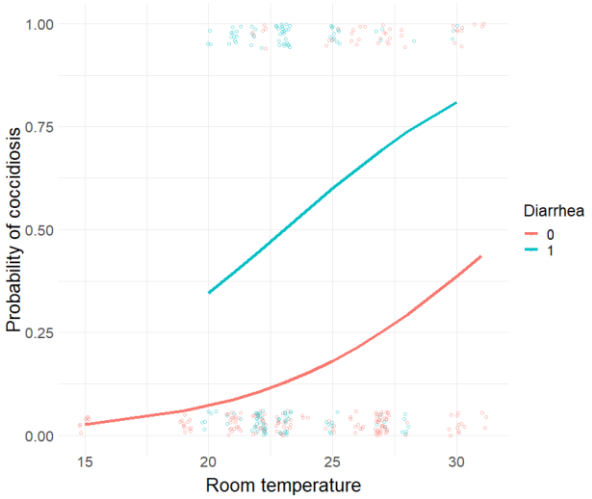

Figure 1. The predicted probability of coccidiosis according to the room temperature (°C) in litters with and without diarrhea. The solid lines are the predicted probability, and the dots are the observed data.

Discussion and Conclusion

A high prevalence observed in our study is similar to other producing countries (1,2). Diarrhea is the primary clinical manifestation of coccidiosis; thus, it is likely both being correlated. Room temperature was positively associated with the chance of coccidiosis and may play a role in the survival and optimal sporulation of the cysts in the environment. The ability of *C. suis* to cause diarrhea in affected piglets is well documented in experimental and field studies as well (3,4). An increase of one degree on the room temperature increased the chance of a litter being positive to coccidiosis by 23.2%, on average. These results corroborate the authors who show that the higher temperature accelerates the sporulation of oocyst in the environment, or increases the sporulation rate, leading to higher exposure levels (5).

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Biosecurity practices on farms in Minas Gerais – Brazil

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Introduction

The prevention and control of health risks in intensive farms include the adoption of biosecurity practices. Biosecurity programs must be designed according to the behavior of the prevalent pathogens in the local conditions and the people involved in the actions' implementation (1,2). Such programs aim to reduce the entry of different pathogens into different farms that can affect the animals' health and consequently, humans' health. In this context, biosecurity has become an indispensable tool to ensure the herds health status, preventing the transmission of pathogenic agents. Therefore, the objective of this study was to verify the adoption of biosecurity practices on full cycle farms in an area of swine production in Minas Gerais (MG) state.

Material and Methods

All commercial swine farms in the municipality of Pará de Minas (MG) were identified, considering only full cycle commercial farms (FC). During the period between January and March 2021, data were collected through a questionnaire containing 120 questions on 16 aspects of biosecurity. These data served as the basis for classifying the farms in terms of biosecurity or health risk, since each question was scored zero (inadequate), five (requires adjustments) or 10 points (adequate), considering statements about the use or application of the practice in focus. Data were tabulated and the score of each answer was added to classify each farm in terms of Biosafety. Descriptive data analysis, Spearman correlation, main components analysis and hierarchical clustering of main components were performed, identifying four clusters. Stepwise regression was performed, and differences were analyzed by ANOVA and Tukey's test. The significance level was $p<0.05$ for all analyses, which were performed with the R softwear (3).

Results

The overall results pointed to a High to Medium-High risk for biosecurity in most farms. The biosecurity score correlated with the number of sows ($r=0.45$, $p<0.05$) and farm productivity ($r=0.50$, $p<0.05$). In general, the farms seem to adequately adopt some biosecurity precautions, such as those related to the management of waste, garbage and pests, and the routine management of animals and semen. Only 24.2% of the farms had a "green belt", but 86.2% had a fence and maintained it, in addition to controlling the gate (69.0%) and having biosecurity signs (41.4%). Among them, some did not register visitors in the guest book (69.0%), did not require a toilet (72.4%) or shower to enter and leave the farm (89.7%), did not define 'clean' and 'dirty' areas (93.1%) and allowed the entry of personal equipment and utensils (65.5%). Only 34.5% of the farms required changing clothes, shoes and hand hygiene and 55.2% provided their own clothes and boots. The care with the receipt of supplements was also inadequate in most farms, except for the frequency of deliveries that needed adjustments in 44.8% of the farms. On average, 61.4% of the farms carried out adequate pest control, at least for rodents (72.4%) and insects (51.7%). For artificial insemination, only 3.9% performed semen sanitary monitoring.

Discussion and Conclusion

Although biosecurity is seen in larger herds and there is an effect on productivity, pig farmers minimize the importance of the biosecurity program, adopting isolated practices without concern for personnel training or the evaluation of such measures. The general classification of the FC farms biosecurity in the municipality was from High Risk to Medium-High risk, involving 92.77% of the matrices, which should put the area on alert, given the great exposure and vulnerability of the farms to potentially devastating pathogens for swine farming, such as PRRS virus (not yet identified in Brazil) or even Senecavirus A, already present in the state of Minas Gerais. However, two important issues are neglected: the use of facilities in an "all in, all out" scheme and the necessary sanitary emptiness for each occupation, especially in the farrowing and nursery facilities. This negligence also occurred in the Brazilian farms studied by Dutra et al.(4), in addition to carelessness with cleaning and disinfection at the nursery facility. Pig farmers in the municipality of Pará de Minas (MG) carry out some isolated measures of external and internal biosecurity practices, which are not part of a biosecurity program and whose effectiveness is unknown. Therefore, commercial farms in this intensive swine industry area are highly exposed and vulnerable to the entry of pathogens.

Acknowledgments

Suidae Health and Production, Algona Iowa 50511 USA

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Characterization of swine oral and fecal microbiota at nursery stage

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Introduction

Microbiota could be defined as the assemblage of microorganisms present in a defined environment (1). The swine fecal microbiota is well established by several researchers (2, 3), but to our knowledge, there is a lack of information about oral fluid microbiota. Therefore, our objectives were to determine the oral fluid microbiota and to compare it to the fecal microbiota of weaned piglets.

Materials and Methods

Fecal and oral fluid samples were collected from 50 healthy weaned piglets (60-70 days old) from five different farms in the state of São Paulo, Brazil. In each farm, animals were randomly chosen from two pens. All samples were evaluated for the determination of their bacterial microbiota by high-throughput sequencing targeting the V4 region of the 16S subunit of the ribosomal RNA coding gene (4). Data were analyzed using R pipelines as previously described (5). The clustered sequences of frequency and total abundance were compared using the Silva rRNA reference database.

Results and Discussion

Feces and oral fluid samples showed no differences in alpha diversity (figure 1) but showed separation of groups in beta diversity (figure 2), which demonstrates their dissimilarity.

Differential abundance in fecal samples were dominated by the phyla *Bacteroidetes* (49.26%) and *Firmicutes* (42.16%), and the most frequent genera were *Prevotella* (29.19%), *Alloprevotella* (6.36%), *Agathobacter* (6.21%), *Subdoligranulum* (5.44%), and *Clostridium sensu stricto* 1 (4.57%). For oral fluid, four phyla were more abundant, namely *Firmicutes* (65.49%), *Bacteroidetes* (12.64%), *Actinobacteriota* (11.24%), and *Fusobacteria* (9.65%), and the most frequent genera were *Subdoligranulum* (13.36%), *Blautia* (10.60%), *Agathobacter* (6.50%), *Faecalibacterium* (5.97%), *Clostridium sensu stricto* 1 (5.84%), *Leptotrichia* (5.45%), *Prevotella* (4.59%), *Fusobacterium* (4.20%), and *Rhotia* (3.66%). The bacterial composition of oral fluid and feces samples had statistically differences (P<0.001) when comparing some genera. Among the most frequent genera (> 1%) in oral fluid samples, eight genera were significantly different from those detected in feces. *Prevotella* showed a decrease in frequency, while the others had increased frequencies in oral fluid samples. Six of the most frequent genera (> 1%) in feces showed significant differences when compared to oral fluid, with two of them presenting increased frequencies (*Prevotella* and the representative of the *Oscillospiraceae* family).

Figure 1. Alfa diversity of oral fluid and fecal samples by Shannon index.

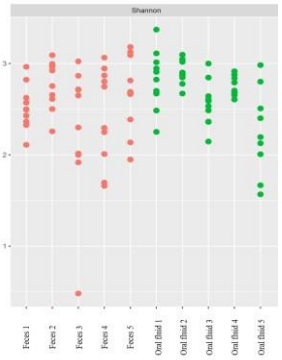
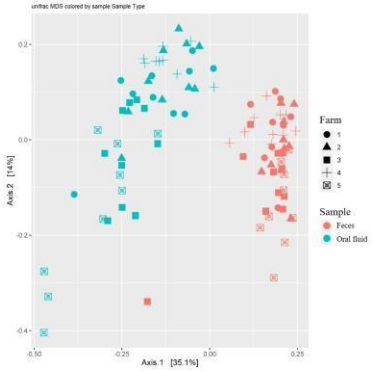


Figure 2. Beta diversity demonstrated by principal components analysis plot from weighted Unifrac analysis of oral fluid and fecal samples.



Conclusion

Fecal samples evaluated in this study showed a composition consistent with other studies (2, 3), with high frequencies of the phyla *Bacteroidetes* and *Firmicutes*. The family *Prevotellaceae* and the genus *Prevotella* were the most abundant. For the first time, we present the results of the bacterial community found in the oral fluid of weaned piglets, with four phyla representing more than 99% of its composition (*Firmicutes*, *Bacteroidetes*, *Actinobacteriota* and *Fusobacteria*), which resuls in greater diversity at the gender level when compared to feces.

Acknowledgments

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Comparison between two methods to evaluate of the water holding capacity in pork

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Introduction

The increase in meat and decrease in fat deposition are characteristics of great importance in the swine industry. In addition to the water holding capacity being greater in muscles that have a greater amount of intramuscular fat (1,2), the genetic improvement radically increased the efficiency in meat production and changed the relationship between protein and water. Because of this change, there is less water retention after a pH drop in improved pigs when compared to a group of pigs without genetic alteration. The aim of this research was to compare two methods for determining the water holding capacity by compression in pork meat (3).

Materials and Methods

Water holding capacity (WHC) was measured using two compression methods. Both for methodology I and for methodology II, two samples of cube meat were made, with a weight of 0.5 grams each, taken from the musculature of a pig, totaling 33 pairs of shoulder samples and 68 pairs of ham, for each methodology. They were weighed on an OHAUS Scout Pro® precision electronic scale. Subsequently, for each two samples, a quantitative round filter paper was used, with medium filtration, white strip, 15 cm in diameter, from the Unifil brand. The papers were properly identified with number, date, muscle and method used.

For methodology I, the two samples were placed on filter paper and between two acrylic plates on which a 10-kilogram weight (kettlebell) was placed for a period of 5 minutes. At the same time, the other two samples were placed on the filter paper and between the acrylic plates, for methodology II. The pressing was done manually for a period of 5 minutes using screw and nut. The areas formed on the filter paper were marked with a ballpoint pen and, using the Image J v. 1.37®, the pressed meat area and the exudate area were accurately calculated, and the results were expressed by the relationship between them. For analysis, the values were entered in the Microsoft® Excel 2010 program, and the average value found in the two samples of each method was considered. The comparison was made using the Pearson's correlation, calculated between the two methods used, both for the ham and for the shoulder (4).

Results

From the statistical analysis, it can be concluded that methods I and II have a relatively moderate and significant correlation, therefore they can to rank the samples in the same way about water retention capacity.

Discussion and Conclusion

When analyzing the correlations of data from method I and method II of the shoulder (Table 1) and the pernil (Table 2) through Pearson's correlation, there is a moderate correlation in both (scores between 0.40 to 0.60, with a perfect ratio gives the result 1 or -1).

Table 1. Correlation values and their statistical significance in parentheses and below (p-value) of the shoulder

Pearson's Correlation Matrix (p-value)	WHC I	WHC II
WHC I	1	0.517325222 (0.0020492)
WHC II	0.517325222 (0.0020492)	1

Table 2. Correlation values and their statistical significance in parentheses and below (p-value) of the pernil

Pearson's Correlation Matrix (p-value)	WHC I	WHC II
WHC I	1	0.636657742 (0.0000000054)
WHC II	0.636657742 (0.0000000054)	1

Analyzing the Proof Values (p), 0.0020492 was obtained for shoulder and 0.0000000054 for ham, that is, both were lower than the significance level of 0.05, indicating that the correlation levels are significant. Even though method I is the gold standard, both can be valid, with method II being less accurate, but more practical, but the correlation could be higher. This value of Person's correlation wasn't high enough to consider the both methods equivalents (5).

Acknowledgments

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Education: support tool for epidemiological surveillance system in wild boar.

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Introduction

Epidemiological surveillance in wild boar has gained special importance in the disease surveillance system around the world (1), especially with the recent outbreaks of African swine fever in Germany (2) and Italy (3), both involving wild boars in the spread of the virus. In Brazil, MAPA's integrated swine disease surveillance plan (4) demonstrates the epidemiological importance of wild boars. In the state of São Paulo, the Secretaria de Agricultura e Abastecimento (SAA) regulated the epidemiological surveillance, transportand destination of wild boar carcasses and crosses withthe publication of SAA Resolution nº 41, of May 29, 2021, to give wide dissemination, sensitize, educate and clarify the Controllers of Invasive Exotic Species (CEEIs) the Coordenadoria de Defesa Agropecuária (CDA) held two circuits of health training events for these central and fundamental actors for the success of the epidemiological surveillance network. The aim of this study is to describe how education focused on One Health approach was essential to attract the interest and participation of CEEIs in the events.

Materials and Methods

The events were organized together with the leaders of the CEEIs, representatives of groups and control teams, associations, municipal political leaders and, above all, the "Aqui tem javali" network, essential for dissemination of the training courses for the target audience. SAA Resolution nº 41/2021 emphasizes in the article 10 the need for training of CEEIs on how to identify the most frequent clinical signs and lesions of the main diseases, especially hemorrhagic (classical swine fever and African swine fever) and vesicular (foot-and-mouth disease) diseases, and how to report suspicions of these diseases, as well as abnormal wild boar mortality, to the Official Veterinary Service (SVO). In addition, the CEEIs are trained on how to collect blood serum samples for laboratory tests and how to send it to CDA. The training was carried out with expositive lectures lasting 4 hours, given by CDA veterinarians. The lecture was prepared based on the SAA resolution nº41/2021 for the presentation of the legal regulation, images of lesions and clinical signs of diseases, videos, technical and scientific content. All events were organized during the period of theCOVID-19 pandemic, respecting prevention protocols and held between September 2021 and January 2022.

Results

Twenty-five courses were held in different regions of São Paulo State, with the participation of 2381CEEIs, with an average of 95 participants per course. The Figure 1 shows a wide geographic distribution in 375 municipalities with at least one trained CEEIs.

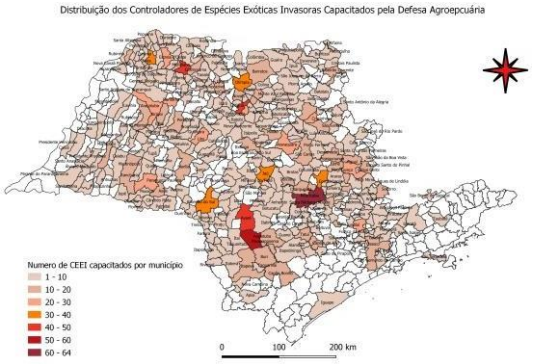

Figure 1. Spatial distribution by municipality of CEEIs trained by the CDA.

Discussion and Conclusion

The events achieved the objective of attracting and delivering technical information to the CEEIs to start the epidemiological surveillance network. The map elaborated made it possible to identify regions that have not yet been covered and to guide the organization of complementary events, however these regions without qualified CEEIs coincide with regions with the absence or little presence of wild boar andtheir crosses. The training, in addition to providing a lot of relevant information for the prevention of diseases with zoonotic potential, also raised awareness of the risks of spreading diseases of economic interest to agribusiness and society as a whole, certainly made the surveillance system more robust and sensitive for detection early disease. The focus on health had a perfect fit with wild boar, as they move freely, modify the environment, interact with domestic pigs, cattle, dogs, and people in handling and consuming the carcasses, this approach was very attractive to CEEIS (5). Complementary studies are needed to evaluate the learning on knowledge and behavior change of trained CEEIs (6).

Acknowledgments

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Associação Manejo Fauna Invasora (AMFI)

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Effect of a biological manure additive in a finishing pig farm in Italy: a preliminary study

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Introduction

The growing interest towards animal welfare and the environmental impact of swine farming has led to the development of several manure treatment products aimed primarily at improving manure quality by reducing gas emissions and substances that have a recognised environmental impact (1,2,3). Among these, biological additives that can be spread in housing facilities may have the secondary benefit of improving pig welfare by reducing ammonia emissions within enclosures (4,5). The present study is aimed at a preliminary evaluation of the potential effects on pig welfare induced by Kopros® usage, an additive containing enzymes and selected bacterial strains that facilitate organic matter mineralisation.

Materials and Methods

Data were collected in a farrow-to-finish farm in Northern Italy, in two different finishing barns. In this farm, a batch of pigs is produced every three weeks, part of them is sold on the market and part is finished *in situ*. A control group (n=1427) and a treatment group (n=1510) of finishers were enrolled for the present study. Each group included 3 separate batches. In the barn housing the treatment group, Kopros® was administered every 28 days. In each group, environmental sampling was carried out on 4 occasions by collecting manure samples that were subsequently subjected to biochemical analyses. Additionally, performance data (i.e. mortality, average daily gain, ADG, and feed converstion ratio, FCR) were collected for each batch. All the parameters were analysed through either parametric or non-parametric two-sample tests to highlight any differences between the control and the treatment group.

Results

Mean performance data and environmental data of the control and treatment groups are reported in Table 1 and Table 2, respectively. Although the statistical analysis did not reveal any significant difference between the two groups for any the examined variables,nitrogen forms, BOD and COD all showed lower mean values in the treatment compared to the control group.

Discussion and Conclusion

Preliminary data on Kopros® usage withing finishing pigs'enclosures showed a tendency to reduce nitrogen forms and organic matter in manure. On the other hand, performance data did not appear to be influenced bythe treatment. However, it must be noted that the limited sample size could have hindered the detection of such effects. Further investigations on a wider sample of batches – possibly from different farms – are therefore needed to confirm the promising results of the biochemical analysis and to possibly highlight any improvements on pigs' welfare and productive performances.

Table 1. Performance data (mean ± Standard Error) of finishing pigs with (Treatment group) or without (Control group) administration of Kopros®.

	Control Group	Treatment Group
Mortality (%)	4.17 ± 0.44	4.28 ± 0.34
ADG (Kg)[1]	0.90 ± 0.01	0.89 ± 0.01
FCR[2]	2.94 ± 0.08	3.03 ± 0.34

[1]Average Daily Gain; [2]Feed Conversion Ratio

Table 2. Environmental data (mean ± Standard Error) in finishing pig enclosures with (Treatment group) or without (Control group) administration of Kopros®.

	Control Group	Treatment Group
Total N[1]	5.25 ± 1.49	4.79 ± 1.33
Nitrate N[2]	60.09 ± 16.01	49.23 ± 13.19
Nitrite N[2]	13.66 ± 3.66	11.19 ± 3.00
Ammoniacal N[3]	0.40 ± 0.02	0.39 ± 0.03
P[2]	15.01 ± 1.97	13.95 ± 3.23
K[2]	3.46 ± 0.28	3.24 ± 0.42
BOD[4]	31.9 ± 2.1	29.8 ± 1.9
COD[4]	93.1 ± 3.9	84.5 ± 5.3

[1]g/Kg; [2]mg/Kg; [3]g/100 g; [4]mg/l x10[3]

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Evaluation of 16S rRNA gene regions V1-V3, V4, and V5-V6 in the respiratorymicrobiome of pigs exposed to *Mycoplasma hyopneumoniae*

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Introduction

Recent studies have explored the composition of sections of the swine respiratory microbiome [1-3]. However, the use of different 16S rRNA gene hypervariable regions in microbiome research can hinder the direct comparison of results, making selection of hypervariable regions a critical decision. Few studies have evaluated the performance of different hypervariable regions in classifying the respiratory microbiome in swine, especially after exposure to pathogens. *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) is a respiratory bacterium causing significant health related problems in pigs by itself [4], as well as facilitating further infections with other respiratory pathogens [4]. Therefore, the objective of this study was to compare the classification performance of 16S rRNA gene regions V1-V3, V4, and V5-V6 in the respiratory microbiome of pigs exposed to *M. hyopneumoniae*.

Materials and methods

Upper (nasal, oropharyngeal, and tonsillar swabs) and lower (laryngeal and bronchial swabs as well as tracheal fluids) respiratory tract samples (n=329) were collected from 30 gilts exposed to *M. hyopneumoniae* using a natural transmission model (University of Minnesota IACUC approved). DNA was extracted from samples using PowerSoil Pro Kit (Qiagen). The detection of *M. hyopneumoniae* in the laryngeal, tracheal, and bronchial samples was evaluated through real-time PCR. Extracted DNA was used for the sequencing of the regions V1-V3, V4, and V5-V6 of the 16S rRNA gene. Quality of reads, performance throughout the Divisive Amplicon Denoising Algorithm (DADA2) pipeline, and classification of the amplicon sequence variants (ASVs) were assessed using mixed effects models.

Results and discussion

The loss of reads throughout DADA2 pipeline was significantly higher for V1-V3, mainly due to the presence of lower quality reads. Classification performance varied significantly by hypervariable region. V1-V3 achieved the highest proportion of ASVs classified at the genus level (86.3-98.5%), which is in agreement with Wang, Liu [5], who documented better discrimination in bacterial classification when V1-V3 was used and suggested that V1-V3 was the best surrogate for full-length 16S rRNA gene in the human airway microbiome.However, the percentage of ASVs classified at the species level using exact matching was greatest when V4 was employed (19.7-72.4%), especially in bronchial swabs. Yang, Wang [6] observed that the regions V4, V5, and V6 allowed the creation of phylogenetic trees with a topology more closely related to that of the full-length 16S rRNA gene. V4 and V5-V6 showed similar accuracy in the detection of *M. hyopneumoniae* in bronchial swabs and trachealfluids (Table 1).

Table 1. Sensitivity and specificity of detection of *Mycoplasma hyopneumoniae* in bronchial swabs and tracheal fluids by hypervariable region.

16S region	Bronchial swabs		Tracheal fluids	
	Se (95% CI)	Sp (95% CI)	Se (95% CI)	Sp (95% CI)
V1-V3	0 (0, 0)[a]	1 (1, 1)[a]	0 (0, 0)[a]	1 (1, 1)[a]
V4	0.44 (0.1, 0.78)[b]	0.86 (0.69, 1)[a]	1 (1, 1)[b]	0.98 (0.96, 1)[a]
V5-V6	0.44 (0.1, 0.78)[b]	1 (1, 1)[a]	0.91 (0.5, 1)[b]	0.99 (0.98, 1)[a]

Proportions in the same column with different superscripts differ significantly at P<0.05. Se: Sensitivity. Sp: Specificity. 95% CI: bootstrap percentile 95% confidence intervals.

Conclusions

Under the conditions of this study, both V4 and V5- V6 showed adequate performance during the bioinformatic processing, while V1-V3 excelled at genus-level classification, and V4 achieved a strong performance at species-level classification and detection of *M. hyopneumoniae*. Therefore, we recommend V4 for the study of the swine respiratory microbiome.

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Initial exploration of MUC5B in swine oral fluid and serum by qPCR

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Introduction

Mucins are large and complex glycoproteins that cover and protect all mucosal surfaces of the body. To date, 22 mucins have been identified with a range of functions depending on their anatomical distribution, e.g., MUC5AC and MUC5B in the airways, MUC2 in the intestine, MUC5B, and MUC7 in the oral cavity (1,2). Mucins defend against infectious agents by capturing and clearing pathogens using a variety of mechanisms, e.g., generating mucosal layers, direct binding, and mediating inflammation (2). Several studies have demonstrated their role in preventing specific diseases, e.g., inhibition of HIV-1 (3), colorectal cancer in mice (4), and limitation of IAV by binding to mucin sialic acid receptors to prevent infection (5). Moreover, mucins and drug interaction are also studied as high mucin levels hamper drug access to the target sites (6).

Despite their importance, mucins are largely under-studied in veterinary medicine. Swine oral fluids are widely used as a diagnostic sample for routine surveillance. Characterization of mucins and the role they play in this matrix has not been studied yet. The purpose of this study was to explore the expression of MUC5B in swine oral fluids using qPCR.

Materials and Methods

Based on data collected from 51 swine oral fluid samples using Next Generation Sequencing (NGS), MUC5B sequence fragments were frequently expressed. Using the NGS and NCBI sequence information (XM_021082487.1), three qPCR primer sets were designed using Primer Express™ Software v3.0.1 (Applied Biosystems). The primers were validated, and an optimal primer set was selected by analyzing the dissociation curves (7500 Fast System v1.5.1, Applied Biosystems) in oral fluids and serum with PowerUp™ SYBR™ Green Master Mix (Thermo Fisher Scientific, Inc., USA). The optimized MUC5B primers were further validated with probe concentrations and master mixes (Thermo Fisher Scientific, Inc., USA).

Thereafter, swine oral fluids (n = 20) and sera (n = 20) collected from commercial production systems were tested with the MUC5B qPCR. All qPCRs were run on the Applied Biosystems™ 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Two oral fluids positive for MUC5B by NGS were used as positive controls. Cq responses were compared among field specimens, with samples producing Cqs ≥ 37 considered negative.

Results

After primer/probe validation experiments, a primer concentration of 500nM and a probe of 100nM were used to perform all MUC5B qPCR assays. No significant differences were observed in MUC5B expression with TaqPath™ qPCR and TaqPath™ 1- Step RT-qPCR master mixes.

Among the oral fluids and sera tested with the MUC5B qPCR, 11 of 20 oral fluids produced Cqs < 37, with Cqs ranging from 34.9 to 36.3, and 19 of 20 sera were < 37 with Cqs ranging from 29.7 to 36 (Figure 1).

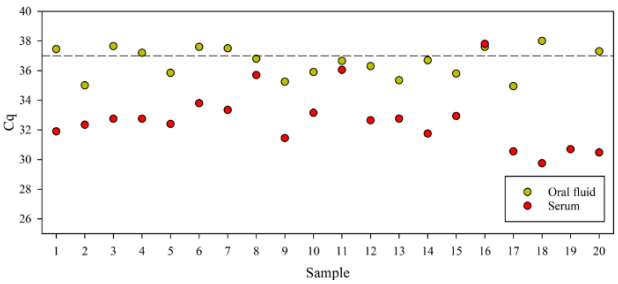

Figure 1. MUC5B Cqs in swine oral fluids (n = 20) and serum (n = 20).

Discussion and Conclusion

The MUC5B qPCR results showed that MUC5B couldbe detected in swine oral fluids and serum, but (unexpectedly) the expression of MUC5B was higher in serum (mean Cq values of 32.7 in serum and 36.5 in oral fluids). This study reports the first attempt to detect MUC5B in oral fluids and serum by qPCR. Unlike swine, the presence of MUC5B in human oral fluids has been widely characterized. MUC5B isknown to be present in healthy individuals and elevated levels of MUC5B are associated with disease(7). Likewise, reports in humans describe the presence of mucins in serum, particularly at higher levels in diseased patients (8). Detection of MUC5B in swine oral fluids and serum denotes a starting point for future studies that could provide useful information on the involvement of this mucin in response to infections.

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L-Carnitine supplementation during gestation increases birthweight of piglets

Lieven Claerhout[1*]; Jens Bach[2]; Lars Kunstmann[3]; Leif Meedom[3]; Wouter Depondt[1]; Natalia Soares[1]
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Introduction

High prolificacy sows produce large litters resulting in an increased number of small piglets (below 700 g). At around 700 g live weight only 50 % of the piglets may survive. A good liver function is crucial for optimal production as various metabolic processes take place. Carnitine stimulates the liver function and optimizes energy production (1,2). The impact of the supplementation of carnitine on the birthweight was investigated in a sow herd with high prolificacy (1200 Danbred LY sows, average 18.7 live born and 2.2 stillborn piglets/ litter).

Material and Methods

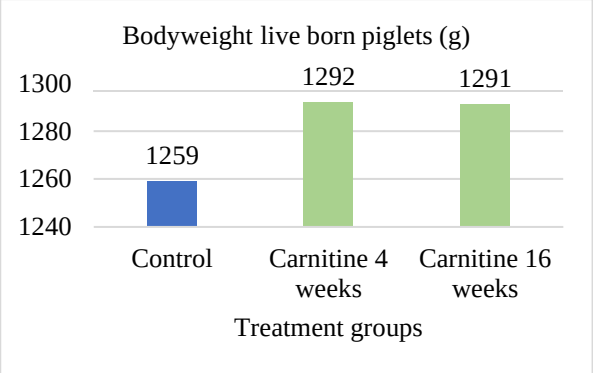
The birthweight was investigated in 3 groups. The control group comprised 41 sows (15% gilts). 2 groups were supplemented with a liquid formulation of L-carnitine, choline, plant extracts and sorbitol (Carnitol-L®). The product was supplemented to a standard liquid feed for sows at 1 ml Carnitol-L® per kg feed during gestation. One group (41 sows) was supplemented for the last 4 weeks of gestation with 120 mg L-Carnitine (4 ml)/ day (17,5 % gilts). The other group (47 sows) received additionally a supplement of 60 mg L-Carnitine (2 ml)/ day for the first 12 weeks of gestation (15 % gilts). Piglets born from sows of the control group and both supplementation groups were weighed at birth by individual weighing in the morning.

Tabel 1. Number of piglets in the control group and the 2 supplemented groups

Number of pigs/ group	Live born	Stillborn
Control	761	118
Carnitine 4 weeks	734	102
Carnitine 16 weeks (12+4)	878	120

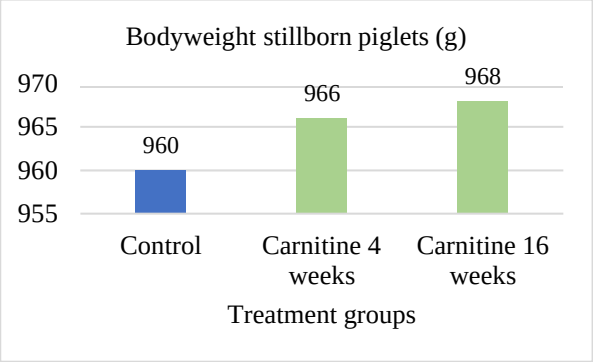
Results

Figure 1. Average bodyweight of the live born piglets in the 3 groups



The p-value of the 4 and 16 weeks supplementation groups versus the control group is 0.05 and 0.04, respectively.

Figure 2. Average bodyweight of the stillborn piglets in the 3 groups



The p-value of the 4 and 16 weeks supplementation groups versus the control group is 0.91 and 0.86, respectively.

Discussion and Conclusion

Supplementation of 120 mg L-Carnitine to sows for the last 4 weeks of gestation increases the live born piglet birth weight significantly with 33 g.

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L-Carnitine supplementation during gestation increases viability and growth of piglets

Lieven Claerhout[1*]; Jens Bach[2]; Lars Kunstmann[3]; Leif Meedom[3]; Wouter Depondt[1]; Natalia Soares[1] *[1]Huvepharma NV, Belgium, [2]Danvet K/S , [3]Huvepharma NV, Denmark,* lieven.claerhout@huvepharma.com

Introduction

A good liver function is crucial for optimal production as various metabolic processes take place. Carnitine stimulates the liver function, optimizes energy production and plays a crucial role in muscle development and glucose metabolism in the pig (1,2). The impact of carnitine supplementation in gestation feed on piglet vitality was investigated in a sow herd with high prolificacy (1200 Danbred LY sows, average 18.7 live born and 2.2 stillborn piglets/ litter).

Material and Methods

Sows were supplemented with a liquid formulation of L-carnitine, choline, plant extracts and sorbitol (Carnitol-L®). The product was supplemented to a standard liquid feed for sows at 1 ml Carnitol-L®, corresponding to 30 mg L-carnitine, per kg feed during gestation. The feeding program was 60 mg L-Carnitine (2 ml)/ day for the first 12 weeks and 120 mg L-Carnitine (4 ml)/ day for the last 4 weeks of gestation. Technical data at birth and during the suckling period of the piglets were investigated and compared for 20 weeks before (1072 sows) and 20 weeks after (1066 sows) the start of the supplementation. Weaned piglets were weighed prior to the supplementation (41 control sows) and following Carnitol-L® supplementation throughout gestation (47 sows).

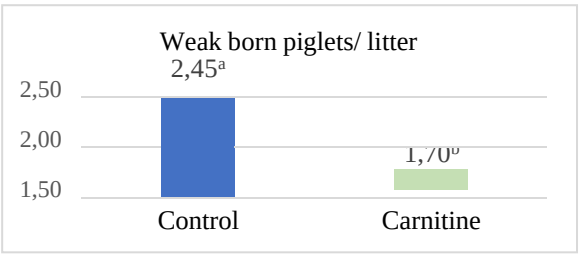
Results

Table 1. Technical parameters of the piglets in the control and supplemented groups

	Control	Carnitine	Difference	p-value
Live born[1]	18,69	18,86	+0,17	0,34
Stillborn[1]	2,36	2,47	+0,11	0,36
Weak born[1]	2,45	1,70	-0,75	0,00001
Weaned[1]	16,10	16,26	+0,16	0,62
Pre-weaning mortality[2]	2,21	1,93	-0,28	0,007
Weaning weight (kg)[3]	7,05	7,25	+0,20	0,007

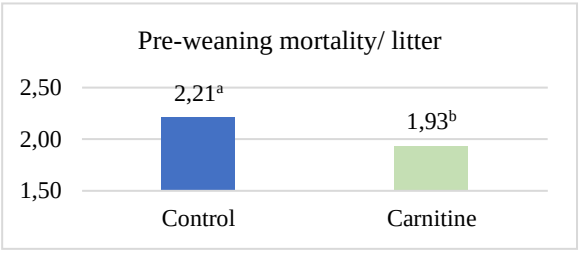
[1] number of piglets per litter; [2] number of piglets per litter, including fostersows; [3] weaning weight (kg) per piglet at 4 weeks of age

Figure 1. Number of weak born piglets per litter in the control and carnitine group



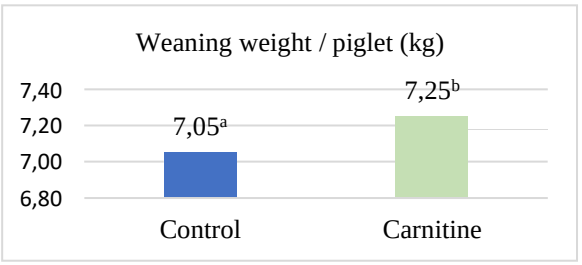
[a,b] Different superscripts indicate significant difference (p 0.00001)

Figure 2. Pre-weaning mortality per litter in the control and carnitine group



[a,b] Different superscripts indicate significant difference (p 0.007)

Figure 3. Weaning weight per piglet (kg) in the control and carnitine group



[a,b] Different superscripts indicate significant difference (p 0.007)

Discussion and Conclusion

Supplementation of L-carnitine in sow feed during gestation results in a significant reduction of the number of weak born piglets, lower pre-weaning mortality rate and higher weight at weaning.

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Measuring behavior following vaccination using an automated camera and drinking water intake

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Introduction

Vaccination of pigs can result in side effects that may impact animal activity. To record pig activities is timeconsuming and can be biased due to the presence of researchers and their interaction with the animals. This study aimed at evaluating the impact of two different vaccination strategies on pig activities using modern camera techniques as well as other variables (i.e. water intake, rectal temperature).

Materials & Methods

A total of 512 24-days old, weaned pigs was equally distributed among 2 study groups accordingly to sow parities. Pigs were vaccinated 8 days after weaning with a combination of PCV2, Mycoplasma and Lawsonia antigens. Group W (n=256) was IM vaccinated with a water-carbomer adjuvant platform (CircoFLEX and MycoFLEX) and with an oral live vaccine via drinking water (Enterisol Ileitis). Group MO (n = 256) was IM vaccinated with a mineral oil adjuvant platform (1 IM injection of 2ml). All vaccines were used according to the label including warming of the MO vaccines. Water intake (Maddalena SPA, Italy) as well as animal activity, expressed as percentage of the changed pixels between two sequential photos (Healthy Climate Monitor, HCM), were continuously monitored by group. Percentage of daily changed pixel observations during the period prior to vaccination was modeled through Auto-Regressive and Moving Average (ARMA) processes to appropriately consider the data's temporal auto-correlation structure of the data. Results from the model were used to predict (forecast) the next 12hrs point values and their confidence limits.

Results

A total of 6 pigs from Group MO showed signs of anaphylactic shock immediate after vaccination which was statistically higher when compared with Group W in which no anaphylactic shocks were observed ($p<0.05$; fisher exact test). Rectal mean temperature of Group MO was significant elevated compared to Group W (40.7°C vs 39.7°C, $p<0.001$; t -test). Results from the ARMA process as well as forecast are shown in Figure 1. The graph for Group W (1B) shows that the model forecasted appropriately since the observed values are mainly within the predicted 95% CL (dark grey areas).

On the other hand, results from Group MO (1A) highlights the fact that pigs did not behave as expected. Mean square difference between expected and observed values overtime differ significantly between groups (p-value < 0.0001).

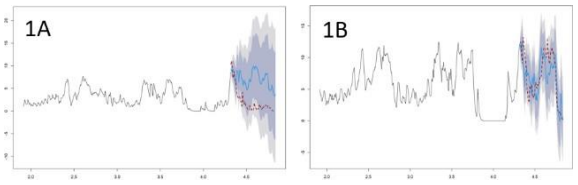

Figure 1 *ARMA and prediction models for animal movement after vaccination event by treatment group*

This is supported by the differences in drinking water intake (Figure 2). Pigs from MO and W groups experienced a drop of 74% (87 ml/kg BW to 21 ml/kg BW) and 6% (84 ml/kg BW to 79 ml/kg BW) respectively during a 24h period after vaccination.
The water intake of Group MO dropped over a 2-day period when compared to group W ($p<0.001$).

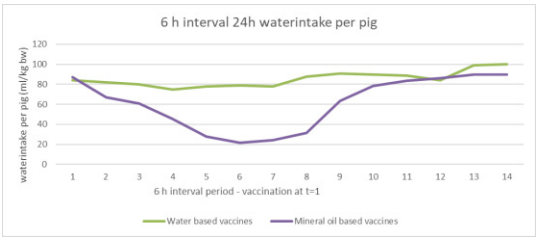

Figure 2 *Drinking water intake (ml/kg BW) per past 24h in intervals of 6h.*

Discussion and conclusions

Results from this study demonstrated a significant impact in pig wellbeing after vaccination with mineral oil adjuvant-based vaccines when compared to those vaccinated with water- carbomer adjuvant-based vaccines. Pigs from Group MO showed a high incidence of anaphylactic shock immediately after vaccination as well as a significant increase of rectal temperature and decrease in water consumption when compared to Group W. The monitoring of animal behavior with objective and noninvasive techniques like camera analysis and drinking water intake monitoring proved to be a useful way of assessing animal welfare.

Microgranulated premixes demonstrate superior homogeneity in the final feed

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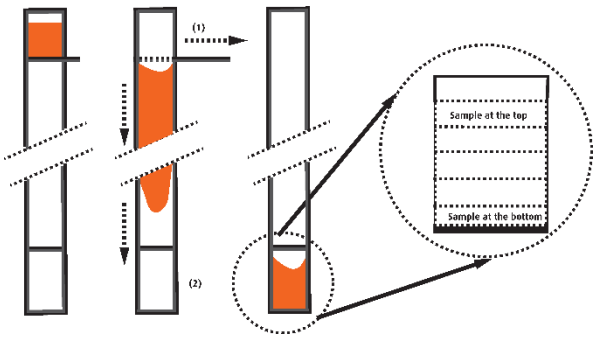
Introduction

During production in the feed mill, transport by trucks and the provision on pig farms, medicated feed is transferred by pipelines over long distances. This is a potential risk for demixing of the veterinary premix. A trial was set up to investigate the impact of the premix formulation on the in-feed homogeneity of the active substance.

Material & Methods

Two veterinary premixes, both containing 100 g tiamulin hydrogen fumarate/ kg, were mixed into a commercial pig mash feed (target: 200 ppm) and evaluated on their demixing potential. The first premix was a powder based formulation. The second premix was microgranulated (Vetmulin®). Microgranulation is a formulation technique providing equally sized and bigger particles and is especially developed for homogeneous and easier mixing into feed. The test was performed at Tecaliman institute in France (1). To mimic the field situations, the medicated feed was dropped in a long PVC tube (5.5 m standard). The shutter on top of the tube was removed to allow the abrupt fall of the medicated feed in the collector pot at the bottom of the tube. The tiamulin concentration in the feed samples taken at the top (C_t) and the bottom (C_b) of the pot were determined by HPLC. For each premix the test was performed 3 times and the average Elutriation Rate (ER) was calculated: $ER=C_t - C_b/ (C_t + C_b / 2) x100$. This rate expresses the demixing potential: the closer to zero, the better the in-feed homogeneity.

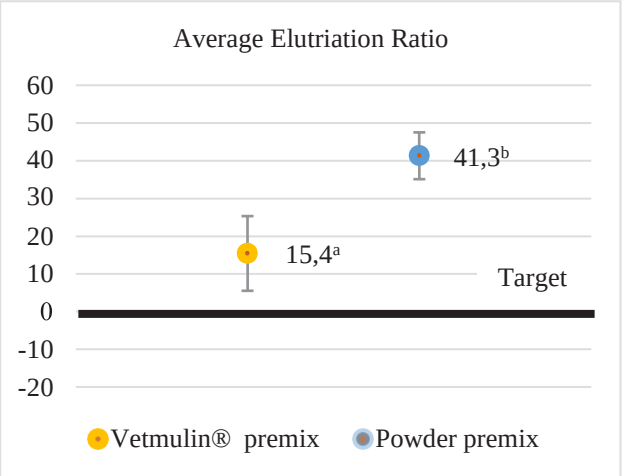
Figure 1. Visualization of the vertical drop and sampling method



Results

The average Elutriation Rate of the powder and the microgranulated premix was 41.3 and 15.4 respectively. This demonstrates a significant difference ($p<0.05$, Student Test) in the demixing potential of a powder based formulation versus a microgranulated formulation.

Figure 2. Average Elutriation index of a microgranulated and powder premix



[a,b] Different superscripts indicate significant difference ($p<0.05$)

Discussion & Conclusion

The formulation of a medicated premix determines the demixing potential and consequently, the homogeneity of the active ingredient in the medicated feed. The equally sized and bigger particles obtained by the microgranulation technology of Vetmulin® premix ensure a superior in-feed homogeneity and result in correct dosing and optimal efficacy (2).

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Occurrence of *Cystoisospora suis* on pig farms in Brazil

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Introduction

Coccidiosis is a parasitic disease caused by the protozoon *Cystoisospora suis*, and piglet coccidiosis is considered the most prevalent gastro-intestinal parasite in intensive piglets farms (1). The most common manifestation is diarrhea, but sub-clinical cases are frequent. In both cases, the parasite damages the intestinal mucosa and impairs intestinal function, therefore, the production parameters decrease, like ADG (Average Daily Gain) and FC (Feed Conversion), with an increase in feed cost. Despite the high efficacy of toltrazuril (TZL) against *Cystoisospora suis* the parasite still seems to be prevalent on pig farms (2). The objective of our study was to assess the prevalence of coccidiosis in industrial pig farms in Brazil.

Materials and Methods

A cross-sectional field trial was designed to assess the prevalence of coccidiosis in Brazilian industrial pig herds. We investigated 50 farms, from eight states (GO=2; MG=9; MS=1; MT=3; PR=7; RS=9; SC=17; SP=2), average size 1.8 k sows (0.16 – 8.3 k), from the most important pig production areas with the sample size giving 95% confidence level to estimate a 5% coccidiosis prevalence with a relative margin error of 6%. Ten random litters per farm were sampled twice on 2nd (7-14 days of age) and 3rd (14- 21 days of age) week of life and feces (minimum 5 piglets per litter) were examined by flotation. A questioner about the farm practices and management was applied in all farms.

Results

On the farm level, 41 (82 %) farms were positive for *C. suis* (of the three farms without toltrazuril treatment, two were n negative). In total, 666 litters were observed in the 50 farms studied for coccidiosis (one farm excluded because of missing information). The overall prevalence of coccidiosis was 32.91% (24.8% – 41.16% 95% confidence interval) on farm level, ranging from 0% to 90% (Figure 1). In total 225 litters (33.8%) were positive at least once and 43 litters (19.1%) were positive in both samplings. All farms implemented protocol based on oral TZL treatment except three farms. On average 5.1 (standard deviation SD: 0.39) litters were positive for oocyst on positive farms. Eight negative farms were w/o diarrhea (5 with and 2 without TZL treatment). The mean TZL treatment age was 3 days (SD: 0.33) (from 2-4 days of age). Only 3 farms were treated on the 2nd day of life or earlier, on the opposite, 8 farms applied a second shot of TZL (end of the first week). Despite the repeated treatment, 5/8 (62.5 %) farms were positive for *C. suis*. 20 farms useddifferent antimicrobial (ATB) protocols in the farrowing house, despite ATB administration, diarrhoea was confirmed on 12 farms.

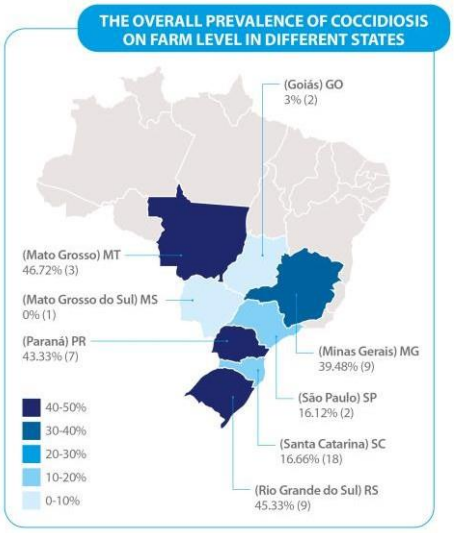

Figure 1. Average percentage of positive litters on farm level on individual states and No. of farms

Discussion and Conclusion

So far, no survey was conducted in Brazil to estimate the prevalence of coccidiosis, and this study gives the current best evidence. Previous case-control studies were focused on diagnostics of *C. suis* together with other pathogens involved in neonatal/pre-weaning diarrhea and performed only in one production state and farms (3, 4). A high prevalence of coccidiosis despite the frequent treatment by TZL was observed in our study, as in other producing countries. 8/50 (16%) farms repeated TZL treatment during the 1st week of life in order to improve the anti-coccidial program.

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Oxidative Stress-Induced Disruption on the Cellular Localization and Expression of Aquaporin 1 Lead to Defected Sodium Sieving During Peritoneal Equilibrium Test: A Porcine Model for Functional Evaluation of Peritoneum

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Introduction

Peritoneal dialysis (PD) is one of the renal replacement therapies. However, complications such as peritoneal fibrosis and ultrafiltration failure during long-term PD were common causes for termination of PD and thus limit its application (1,2). Peritoneal equilibration test (PET) is a common method in clinical to depict overall peritoneum transport characteristics and is an important indication for adjustment on the individual PD prescription (3). On long-term PD patients, increasing of small solutes transport, decreasing of ultrafiltration volume and sodium sieving are the most commonly detected changes upon PET (4). Although thesechanges were considered as relevant indications for peritoneum dysfunction, fibrosis and angiogenesis, the underlying mechanism of these functional changes on peritoneum remains to be elucidated. Previously, we established a novel sodium hypochlorite(NaClO)-induced peritoneal fibrosis porcine model, which helped to bridge rodent model toward pre-clinical human peritoneal fibrosis research (5). In this study, we establish PET in pigs to monitor instant functional changes on peritoneum during the progression of peritoneal fibrosis and also investigate the underlying mechanism for the observed functional changes.

Materials and Methods

For animal experiment, nine five-week-old LYD (mixed breed of Landrace-Yorkshire-Duroc) malepiglets were introduced and randomly divided into three groups as control, 0.1% NaClO and 0.1% NaClO*2 group (n= 3 in each experimental group). In the beginning, all piglets underwent surgery for PD catheter insertion. Later, 0.1% (v/v, 15mM) NaClO or normal saline was injected intraperinatally for the induction of peritoneal fibrosis. Pigs were sacrificed at day 7 to obtain tissue samples for further protein and histological evaluations. PET was performed before and after the induction of peritoneal fibrosis to evaluate instantly peritoneal transport function.

For *in vitro* cell experiments for mechanistic investigation, human mesothelial cell line (MeT-5A), and human umbilical vein endothelial cell line (EA.hy926) were used. Cells were treated with NaClO and ROS detection assay, immunofluorescence staining or western blot were performed to investigate the potential signaling pathway involved.

Results

Increasing small solutes transport and loss of sodium sieving were observed in PET. Besides, decreased protein expression and dis-localization of water channel protein aquaporin 1 (AQP1) with excessive reactive oxygen species (ROS) production on peritoneum were also detected after NaClO injury. Mechanistic investigation from both *in vivo* and *in vitro* data suggested that de-polymerization of cytoskeleton induced by excessive ROS defected intracellular transportion of AQP1 from endoplasmic reticulum to cell membrane, and likely resulted in the disappearance of sodium sieving upon PET.

Discussion and Conclusion

Successful establishment of PET on porcine model created a new possibility to monitor continuously functional changes of peritoneum along with the progression of peritoneal fibrosis and the recovery process upon treatments. This study provided the first evidence supported the hypothesis about how ROS, cytoskeleton and AQP1 was affected inNaClO-induced peritoneal fibrosis porcine model.

Acknowledgments

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Perceptions of veterinarians views of biosecurity measures in Argentinean pig farms

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Introduction

Biosecurity in pig farms is the most effective tool for disease control and prevention. Regarding the availability and credibility of information sources, different studies showed that veterinarians are the source of information in which farmers place greater confidence when animal health and biosecurity are to dealt with (1). Nevertheless, increasing awareness on biosecurity and disease prevention on veterinarians have been suggested as of paramount importance to improve farm biosecurity (2). The objective of this study is to know the perception of biosecurity measures of accredited veterinarians in Argentinean pig farms to design training programs and resources that allow improving the implementation of biosecurity.

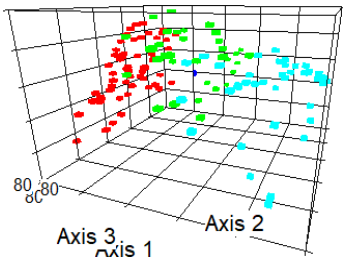
Materials and Methods

During 2021, a survey was carried out among veterinarians accredited by Senasa (*National Service for Health and AgriFood Quality*). The survey consisted of 2 groups of questions, about the level of professional training, production system and farm size, and 74 questions about external and internal biosecurity measures, divided into 9 subsections. The answers were closed, ordered by categories according to an importance scale (null, low, indifferent, medium-high and high). The relative and absolute frequencies of each question were calculated. To explore the existence of veterinarians with different perceptions related with biosecurity measures, a correspondence analysis and a hierarchical clustering analysis (HCA) were performed. The Multi-Response Permutation Procedure Test (MRPP) was used to test the statistical significance of the clusters. The indicator values (IV) are calculated to measure the strength of association of each variable with the different farms groups. All the analyzed was make with PCORD® (3).

Results

In total, 139 surveys were analysed. Between 37.8% and 44.1% of the veterinarians only considered important to establish a quarantine outside the perimeter of the farm. 51% valued carrying out diagnostic tests upon arrival in quarantine, a duration >6 weeks and applying the AI/AO. 67% consider it important that the truck from the slaughterhouse does not go to other farms on the same day, that it is cleaned and disinfected after each trip and that the driver does not come into contact with the farm, but only 49% consider the use of a discharge bay with dirty and clean zones. 69.9% of veterinarians weigh up that staff and visitors change clothes and boots,but only 49% the obligatory shower and 34% hand washing. 35.7% and 46.2% consider important the euthanasia of weak born piglets and animals that do not respond to treatment, respectively, as well as 46.9 and 49.7% the change of needles per litter and per animal house. In the correspondence analysis done with the 139 veterinarians axes 1 and 2 explained 37.6% and 7.82% of the variance explicada between then, respectively. The HCA resulted in the identification of three significant groups (MRPP, $p < 0.0001$) (Fig. 1). Cluster 1: 56 veterinarians, 28,5% had postgraduate courses in swine medicine, work in farms intensive and semi- intensive (mean of 950 sow), cluster 2: 35 veterinarians,14,2% had postgraduate, work in farms intensive and semi-intensive system (mean 620 sows) and clusters 3: 48 veterinarians, 16,6% had postgraduate and work in farms semi-intensive and extensive system (mean 266 sows). The IV, the cluster 1 was characterized for view important 68 measures, while cluster 2, only 1 (exclusive materials in quarantine) and cluster 3 none.

Figure 1. Cluster analysis for veterinarians



References: Cluster 1(red), Cluster2(green), cluster3(blue)

Discussion and Conclusion

The response rate was low, perhaps because the response was voluntary and not face-to-face. Some important measures were undervalued, such as those related to quarantine, discharge bay, showers, hand washing, AI/AO, euthanasia, and needle replacement. There was great variability, between groups and intragroups. We assume that group 1 has greater contactwith sources of technical information, belong to technology exchange groups (2) and in turn advise farms free of pathogens. On the other hand, in group 2 and 3, which advises fewer farms, we observed that theterminological approach of the questions could have influenced the performance of their answers. The resultsshow the gaps in biosafety training that allow improvingthe design of training and sensitivity campaigns adaptedto various production systems, (4) at a time when countries are emphasizing the application of biosafety measures at the national level and for the presence of ASF in the Dominican Republic and Haiti.

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Prevalence of Iron Deficiency Anaemia (IDA): Philippine Results

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Introduction

Iron deficiency Anemia (IDA) is prevalent worldwide given the fact that piglets could not get enough from sow's milk. Negative effects of IDA on total piglet performance have been well studied. Previous study about IDA showed a lower weaning weight by 810 grams (1) and increased piglet mortality. In another study, larger and fast-growing piglets are observed to be more susceptible to iron deficiency (2). The objective of this study is to establish the prevalence of IDA at weaning under Philippine condition.

Materials and Methods

A small, medium, and large piglets (N=299) per litter aged 21 to 30 days were randomly selected from seven (7) commercial swine farms in the Philippines. Blood samples were collected at weaning and were analyzed for Hemoglobin levels using Hemocue® portable analyzer. Piglets were categorized based on the level of hemoglobin following this category: optimum (>11g/dl), sub-anemic (9-11g/dl) and anemic (>9g/dl).

Results

The percentage of piglets under different levels of haemoglobin is summarized in the graph (Table 1). Table 2 shows the prevalence of IDA within the category. Figure 2 shows the level of hemoglobin with the group.

Table 1. Number and Percentage of piglets under different Level of Hemoglobin

Category	Number of Animals	%
Anemic	37	12.37%
Sub-Anemic	83	27.76%
Optimal	179	59.87%
Total	299	100%

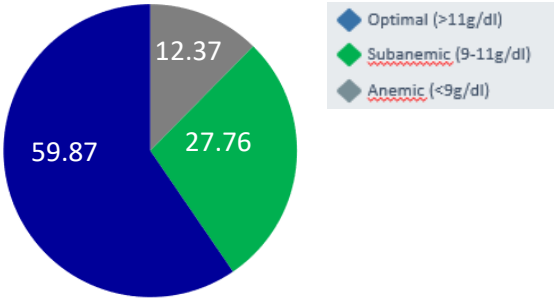

Figure 1: Percentage of piglets under differentlevel of Hemoglobin

Table 1. Number and Percentage of piglets under different Level of Hemoglobin

Category	Anemic	Sub-Anemic	Optimal	Total
Small (hds)	**8** (7%)	**33** (31%)	**66** (62%)	107
Medium (hds)	**13** (14%)	**23** (24%)	**60** (63%)	96
Large (hds)	**16** (17%)	**27** (28%)	**53** (55%)	96

Discussion and Conclusion

The results showed that more than 40% of piglets fall under anemic or sub-anemic level while almost 60% are under optimum level. This data also supported that the larger and fast-growing piglets are more likely to develop anemia deficiency anemia (17%) as compared to small and medium sized piglets.

This survey showed that IDA is prevalent in piglets at weaning under Philippine field condition.

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Prevalence of Iron Deficiency Anemia in Brazilian Piglets

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Introduction

Iron supplementation is a settled management practice in the first days of the piglet's life. Although practically all farms have adopted this practice, iron deficiency anemia (IDA) still challenges swine farms. This study aims to assess the prevalence of IDA in industrial pig farms in Brazil.

Materials and Methods

In total 50 farms were randomly selected and included in the study. All farms use injectable iron supplements. Ten randomly selected litters from different parity-order sows were assessed within each farm. One large, one medium, and one small piglet was sampled within each litter. In total 1983 piglets from 661 batches at weaning (from 17 to 30 DOA) were sampled. The Hb concentration was measured with the Hemocue® Hb 201+ system. Piglets were classified as follows: Hb levels < 90 g/l are anemic, Hb levels ≥ 90 g/l and ≤ 110 g/l are suboptimal, and Hb levels > 110 g/l are optimal. Logistic regression used a stepwise selection for the final model associated management and farm structure factors with piglets' anemia status.

Results

The average Hb level of piglets at weaning was 109,4 g/l, and the percentage of anemic piglets was 9% (Figure 1).

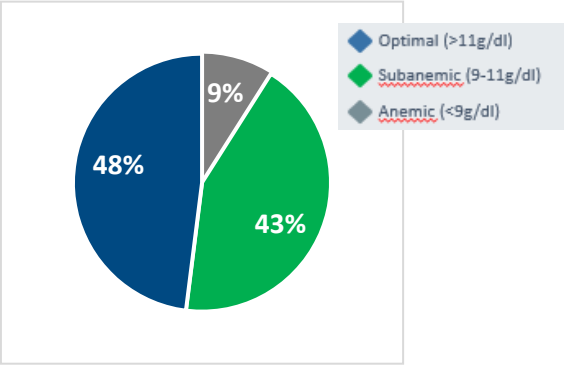

Figure 1. Percentage of anemic, subanemic, and normal animals classified according to Hemoglobin levels

On average, medium and small piglets had less chance of anemia compared to large ones. Also, there is a positive association between the number of piglets/sow and the chance of anemia (Table1).

Table 1. Predicted probability of anemia according to the piglets/sow and the piglet's size.

Variable	Estimate	Odds ratio (95% CI)	Prevalence†
Piglet size:			
Small	-0.57	0.56 (0.56 - 0.72)	48.55% [a]
Medium	-0.47	0.61 (0.48 - 0.79)	50.5% [a]
Large	1	-	60.4% [b]
Piglets/sow	0.3567	1.42 (1.02 - 2)	

†Different letters mean statistical significance ($p<0.001$)

Discussion and Conclusion

We observed anemic and subanemic animals at weaning, even using preventive treatment with iron injection in the first days of life. It was already observed in similar studies (1,2). The low iron body reserves and the low concentration available in sow milk explain piglet anemia. We observed that large piglets are at a greater chance of developing IDA. It is expected that fast- growing piglets, without access to soil, will experience a drop-down in the iron-carrying molecules such as hemoglobin (3), needing a greater supply of iron for the formation of hemoglobin and myoglobin (4). It is complemented by the fact that piglets that share the milk in large litters have a lower individual milk intake and consequently lower iron uptake. This study estimated the prevalence of anemia in suckling piglets from the sampled farms. More studies should be carried out, using a larger number of farms in states with little sampling to estimate the reality of each region.

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Production data and carcass evaluation of piglets weaned at 10 days of age

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Introduction

Due to the expressive increase in the total number of births, some challenges arose in the lactation phase, in which the female's metabolic demand is greater than her physiological capacity. Therefore, an alternative is the use of sows in the maternity ward to meet the high number of piglets. However, the use of lactating mothers leads to a high mortality in piglets and the presence of estrus in the lactation period in some sows (2). Another alternative is early weaning, between 7 and 16 days, which can increase the female's reproductive efficiency and reduce the vertical transmission of diseases. Thus, the present study aimed to evaluate the weaning at 10 days of age of piglets transferred to the nursery, and to evaluate the weight gain of the animals and evaluation of carcass in the slaughterhouse.

Materials and Methods

Piglets from females with Large White X Landrace cross of the DB-25® line, from 2nd to 6th farrowing order, inseminated with semen from a male AGPIC-337 TG ELITE® were used. The treatment was early weaning at 10 days, with the use of milk replacer, and the control was conventional weaning at 21 days. Forty-eight animals were used, divided into two treatments, in 6 randomized blocks, totaling 288 animals. Weights at birth, at 10, 21, 66 and 163 days of age were evaluated. In the slaughterhouse, measurements of hot carcass weight, percentage of lean meat (% MC), backfat thickness (ET) and muscle thickness (EM) were measured. Measurements were performed using the HENNESSEY® pistol. For statistical analysis, the data were submitted to analysis of variance, and the means were compared by the Scott-Knott test, with a significance level of 5%.

Results

According to table 1, there was no difference in weight at birth and at 10 days (P>0.05). From 21 days of age, there was a statistical difference in relation to the average weight of the animals, and the average of the control animals was higher compared to the treatment (P<0.05). According to table 2, there was a statistical difference in relation to muscle depth and carcass weight, and the animals in the control group obtained better results compared to the treatment (P<0.05).

Discussion and Conclusion

Weaning weight has a direct influence on weight at daycare and finishing. Piglets weaned at 21 days show greater growth potential, so maximizing weaning weight is a key point in swine production (3).

Table 1. Averages of animal weights (Kg)

Variable	Control	Treatment	p value
Birth weight	1.42[a]	1.43[a]	0.537
Weight at 10 days	3.52[a]	3.51[a]	0.791
Weight at 21 days	6.42[a]	4.72[b]	0.000
Weight at 66 days	25.88[a]	23.13[b]	0.000
Weight at 163 days	125.53[a]	119.43[b]	0.000

The means followed by the same letter do not differ from each other by the Scott-Knott test, with a 5% probability

Table 2. Means of slaughterhouse data

Variable	Control	Treatment	p value
Muscle depth (mm)	69.37[a]	67.49[b]	0.005
backfat thickness (mm)	11.32[a]	11.06[a]	0.387
Lean meat percentage	60.54[a]	60.43[a]	0.623
Carcass weight (kg)	92.17[a]	87.91[b]	0.000

The means followed by the same letter do not differ from each other by the Scott-Knott test, with a 5% probability

The higher weaning weight generates a positive and lasting effect for the subsequent phases, showing a positive correlation with the final finishing weight (P<0.05) (4). In the present study, weaning at 10 days impaired the performance of piglets in the nursery and finishing phases, and this can be explained by the lower feed intake. The low weight of piglets weaned at 10 days of age influences growth as well as muscle depth, but does not affect carcass lipid deposition and lean meat percentage.

It is concluded that early weaned piglets need a good quality diet, as they are not physiologically prepared for efficient digestion, and consequently, these animals may have a poor performance.

Acknowledgments

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Retrospective analysis of bacterial pathogens associated with influenza A virus (IAV) infections in pigs (2020-2021)

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Introduction

Respiratory diseases represent an important and frequent health challenge in swine worldwide. Most often, respiratory infections are polymicrobial with multiple pathogens aggravating disease outcomes. Respiratory agents can be divided into primary and secondary (or also known as opportunistic) agents (1). Influenza A virus (IAV), for instance, is considered a frequent primary pathogen in swine. IAV infection is commonly followed by opportunistic agents that take advantage of its virulence mechanisms to establish secondary co-infections. Among the secondary agents *Pasteurella multocida*, *Actinobacillus suis*, *Glaesserella parasuis* and *Streptococcus suis* are frequently reported (1,2). Typically, co-infections of IAV and bacteria contribute to a significant enhancement of clinical signs and mortality rate (2). Although IAV and bacterial co-infections cause great economic impact and impairment of animal health, comprehensive descriptive analysis is still scarce and can be used to monitor trends, especially for bacterial pathogens due to the changes on the use of antimicrobials. Diagnostic laboratory cases can provide relevant information of the occurrence of IAV bacterial co-infections. Therefore, the purpose of this study was to perform a two-year (2020-2021) retrospective analysis of diagnostic cases of common bacterial agents associated with IAV co-infections in pigs.

Materials and Methods

Diagnostic data from 3,034 porcine lung samples received at the Minnesota Veterinary Diagnostic Laboratory (MVDL) between January 2020 and December 2021 were analyzed retrospectively to identify co-infections of IAV and bacteria. Lung samples were tested by PCR and the majority of the IAV PCR positive cases were confirmed by isolation using MDCK (Madin Darby canine kidney) cells followed by the observation of cytopathic effects. In addition, subtypes of IAV isolates were determined by PCR. Most of the lung samples were also tested for the presence of bacteria by aerobic culture. In addition, for some cases, infection with *M. hyorhinis* and/or *G. parasuis* was detected by PCR test. Since both pathogens have the capacity to cause systemic infections, specimens from pericardium, peritoneum, pleura, and bronchial swabs were considered. For analytical purposes, we considered all porcine lung samples that were PCR positive for IAV, independently of the result by virus cell isolation. These cases were also tested for other respiratory pathogens (e.g., viruses), but this information was not included in the study, since this was not part of the study objective.

Results

In 2020, 1,647 porcine lungs were received. A total of 263 (16%) samples were IAV PCR positive, 51 (3%) cases were suspect, and 1,333 (80%) cases were negative. From the 263 positive cases, *Bordetella bronchiseptica* (17/263, 6.5%), *G. parasuis* (27/263, 10.3%), *P. multocida* (41/263, 15.6%) and *S. suis* (42/263, 16%) were the bacteria mostly identified in co-infections with IAV. In 2021, a total of 1,387 porcine lungs were received. Out of the 1,387 total samples, 221 (16%) cases were IAV PCR positive, 57 (4%) cases were suspect, and 1,109 (80%) cases were IAV PCR negative. From the 221 (16%) IAV PCR positive cases, *G. parasuis* (19/221, 8.6%) *P. multocida* (20/221, 9%), *P. multocida* with *G. parasuis* (13/221, 5.9%), *S. suis* (26/221, 11.8%) and *S. suis* with *G. parasuis* (10/221, 4.5%) were the most frequent bacteria identified in co-infections with IAV.

A comparison between solely IAV infection cases and IAV bacterial co-infection cases for each year is shown in Table 1.

Table 1. Number of diagnostic cases with solely influenza A virus (IAV) infection and IAV bacterial co-infections for years 2020 and 2021.

	NUMBER OF CASES	
Year	IAV infection only	IAV + bacterial co-infections
2020	75 (28.5%)	183 (67%)
2021	75 (34%)	143 (65%)

Discussion and Conclusion

IAV is an endemic respiratory pathogen of pigs worldwide. Influenza causes damage to the respiratory epithelium lining the airways facilitating interaction with secondary bacterial infections. Our study indicates that bacterial coinfections are prevalent in diagnostic cases of IAV infected pigs. Our study also emphasizes the need for research on microbial coinfections to enhance our understanding of IAV ecology and disease dynamics with other agents. The knowledge obtained by continued research efforts on IAV co-infections will provide useful information to further devise novel, effective approaches for swine disease prevention and control programs.

Acknowledgments

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The impact of injectable combination of toltrazuril and iron on weaning piglet weight – comparative trials

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Introduction

Coccidiosis (*Cystoisospora suis*) and iron deficiency anemia (IDA), when not controlled, can affect animals' performance (1, 2). A combination of gleptoferron and injectable toltrazuril has been shown to improve control of these challenges and increase the weaning weight of piglets (3). The aim of this study was to evaluate the effect of injectable combo product on the weaning weight of piglets on Brazilian commercial farms.

Materials and Methods

Six farms with a history of coccidiosis confirmed and/or diagnosed as positive for *C. suis*. In total 217 litters were selected and randomly distributed into two groups (G1 Group - Forceris® Ceva Sante Animale, France) and G2 Group – Control). In all farms, the control group received 1 mL of Toltrazuril Oral and 2 mL of Iron Dextran Injectable according to farm practice. G1 received 1.5 mL of Toltrazuril and Iron Gleptoferron injectable according to SPC instruction of product. Both groups were treated between 24 and 96 hours of life. The animals were individually identified, and initial (SD 0) and weaning weight were measured. The information on the farms and the number of animals evaluated per farm is shown in Table 1.

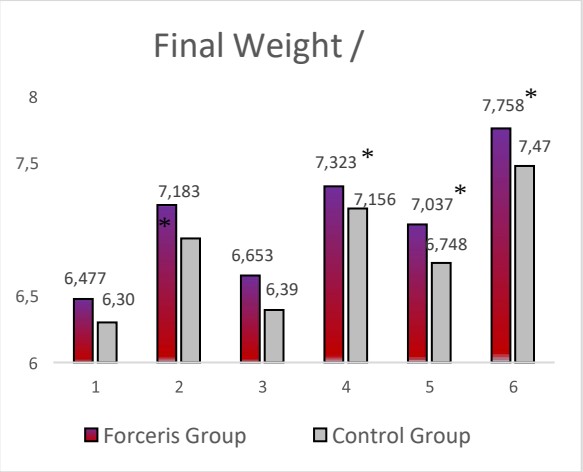
Tabel 1. Number of animals per treatment group in each farm evaluated.

FARM	Location (State)	Sow's number / farm	Forceris	Control
1	RS	700	94	92
2	RS	2000	244	235
3	SC	4200	301	309
4	SC	4100	166	150
5	SC	800	170	127
6	MG	1000	77	70

Results

Initial weights showed no significant difference in all field trials (p>0.05). Final weights are shown in table 2. Farm 1, at 19 DOA G1 = 6.477 Vs G2 = 6.300 (p>0.05), farm 2, at 25 DOA G1 = 7.183 Kg Vs G2 = 6.931 Kg (p< 0.05) and farm 6, at 25 DOA G1 = 7,758 Vs G2 = 7,476 Kg (p>0.05).

Table 2. Average final weight (at weaning) in the six field trials performed with the different toltrazuril and iron protocols.



(*) significant difference (p<0.05)

Discussion and Conclusion

Animal health and the control of recurrent disorders in swine farms have a direct effect on pig performance. As demonstrated in previous studies, the control of *Cystoisospora suis* and anemia in suckling piglets can lead to greater weight gain in piglets. (4,5).

In this study the piglets treated with injectable toltrazuril were heavier at weaning, even when compared with orally treated piglets. The results were consistent amongthe six farms and in four of them the differences were statistically significant, suggesting greater control ofdisorders related of *Cystoisospora suis* and anemia in the maternity phase.

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Use of Statistical Process Control to evaluate the effect of isoquinoline alkaloids supplementation on productive performance of growing-finishing pigs

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Introduction

Statistical process control (SPC) is a statistical method that can be used to evaluate the production variation in swine operations, thus facilitating decision making. However, published data on the use of SPC methods in animal production is still scarce (1). Isoquinoline alkaloids (IQs) are have been used in food animals to modulate stress, improve gut health and growth performance (2,3).

The objective of this study was to evaluate the effect of plant-derived IQs supplementation on production performance of growing-finishing pigs by using SPC.

Material & Methods

The pigs included in the study originated from 20 sow farms in Spain. Historical control (calibration) data was compiled from 2017 to June 2020. During this period, all animals received a standard commercial diet based on barley, wheat, corn, and soybean meal. During the treatment period, which started in July 2020 and lasted until February 2021, all pigs were fed the standard diet supplemented with 1 kg/t feed of a plant-based IQ product (Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany), from day 70 of life until slaughter. A total of 52 batches representing 139,278 pigs were included in the treatment period (SPC Phase II), whereas the data from 866 batches representing 2,111,756 pigs was used as control (SPC Phase I).

Data recorded during both, historical control and treatment periods included feed conversion ratio (FCR), average daily gain (ADG, g/d), average daily feed intake (ADFI, g/d), cost of medicines (Euro/pig), runts (%) and mortality rate (%). All data was first explored to characterize different clusters, based on differences in dietary specifications and health status of the animals.

SPC tools were used to monitor variation in performance parameters and detects if the changes were caused by supplementation with IQs.

Furthermore, boxplot charts were constructed for all parameters to evaluate the dispersion of the data in both, control, and treatment period. Finally, a Student's t-test was performed for all parameters. A p-value ≤ 0.05 was considered statistically significant and a trend was determined at a p-value ≤ 0.10.

Results

FCR and ADG showed a significant ($p \leq 0.05$) improvement after the supplementation with IQs started, whereas ADFI showed values closer to the expected tendency. In addition, mortality and runts % did not show any significant differences between control and treatment periods. However, the cost of medication was significantly reduced during the treatment period with IQs ($p \leq 0.05$).

	CON	IQs
ADG (g/d)	834(80.65)	869(74.81)*
ADFI (kg/d)	2.12(0.25)	2.10(0.24)
FCR	2.62(0.17)	2.48(0.13)*
Mortality (%)	4.19(2.49)	4.16(2.96)
Runts (%)	1.22(0.86)	1.07(1.05)
Cost of medication (€/pig)	1.43(1.05)	0.99(0.72)*

Mean(Standard Deviation) *p<0.05

Discussion & Conclusion

SPC methods were successfully implemented to evaluate the effect of IQs supplementation on growth performance of grow-finish pigs. IQs supplementation in pigs from day 70 of life until slaughter could be a good strategy to improve the efficiency and profitability of the production system.

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Whole-herd risk factors of increased proportion of lightweight pigs at market in a US swine production system

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Introduction

Understanding the factors leading to a higher proportion of lightweight pigs marketed allows improving the efficiency of pork production (1-2). Identifying and measuring the impact of each contributing factor supports decision-makers in developing strategies to reduce economic losses. This study analyzed closeout data of a US swine production company over 18-months, to identify the risk factors associated with the increased proportion of lightweight pigs within each group of marketed pigs (closeout). The data utilized in this study tracked the performance of each batch of pigs from breeding-to-market (BTM).

Materials and Methods

This was a retrospective cohort study including 1.905 closeouts of pigs marketed between 2018-2020, in a production system located in the USA Midwestern region. The performance of each batch of pigs was integrated with the pre-weaning phase information, creating a whole-herd dataset containing information from breeding to marketing. The proportion of pigs marketed with carcass weight below 90.7 kilograms within each batch ofpigs was considered the outcome of this study. The explanatory variables analyzed were: sow farm PRRSV status, PRRSV RT-PCR results and key performanceindicators from the sow farm. Univariate analysis was conducted separately between each predictor collected inthe dataset and the outcome percentage of lightweight pigs, using a Linear Mixed Models on SAS software. This step was conducted to screen the potential factors to be included in the initial multivariable model (p<0.10). The final multivariable model included only parameters significantly associated with the outcome (p<0.05). Multicollinearity was checked as described by Cohen, et al. (1988), and Tukey-test was used for multiple comparisons and to report the marginal means for each predictor on the outcome in the final model.

Results

The average proportion of lightweight pigs (≤ 90.7 kg) for the 1.905 groups of marketed pigs was 16.8% (95% CI 16.3% - 17.2%). The final multivariable model was composed of: (a) average percentage of gilt litters originating the closeout, (b) PRRS status at weaning, (c) average weaning age, (d) season when batches were marketed, (e) PRRS RT-PCR detection during the growing phase, (f) pre-weaning mortality, and (g) average nursery mortality. The variables average nursery stocking weight, interval days to sell the group, and days-on-feed were utilized as covariates. Table 1 describes the differences in the proportion of lightweight pigs between the categories of each risk factor analyzed. Sow farm and nursery sites were utilized as random effects.

Table 1. Average proportion of lightweight pigs in the groups

Risk factor	Categories	Light Pigs %	P-value
Gilt litters % (a)	4.20%	14.7%[a]	0.0008
	19.20%	16.5%[b]	
	24.60%	16.9%[b]	
	36.80%	17.1%[b]	
PRRS status (b)	IA	18.3%[ab]	<0.0001
	IB	18.6%[a]	
	II-vx	15.7%[b]	
	IV	12.6%[b]	
Wean Age (c)	18.6	17.6%[a]	0.0078
	20.8	16.4%[ab]	
	21.9	16.2%[ab]	
	23.5	15.1%[b]	
PRRS PCR (e)	Positive	16.9%[a]	0.0023
	Negative	15.7%[b]	
PWM (f)	13.10%	15.9%[a]	0.0087
	16.40%	15.7%[a]	
	18.80%	15.8%[a]	
	24.80%	17.8%[b]	
Nur. Mort. (g)	1.40%	16.0%[a]	0.0003
	2.80%	15.6%[a]	
	3.90%	15.9%[a]	
	7.40%	17.7%[b]	

abc Different letters represent statistical difference of % of lightweight for the closeouts. PRRS Status (IA – unstable high prevalence; IB – unstable low prevalence; II-vx – stable vaccinated; IV – naïve).

Discussion and Conclusion

Results revealed that sow farm data are highly associated with the downstream proportion of lightweight pigs at market. The lowest proportion of lightweight pigs marketed was observed when the batches originated from groups in the sow farm within the lowest percentage of gilt litters. Factors indicating a worse performance in the sow farm (higher pre-weaning mortality, lower weaning age, circulation of PRRSV in lower to high prevalence), were associated with an increased proportion of lightweight pigs marketed. Groups with PRRSV-positive RT-PCR results in the growing phase, and higher nursery mortality (first 7 weeks after weaning), were also associated with a higher proportion of lightweight pigs. This study demonstrated the importance of identifying and measuring breeding-to-market (whole-herd) risk factors on the final closeout performance, under commercial conditions. These results help supports on-farm disease prevention and control efforts related to carcass performance by providing information that will support decision-makers to reduce the proportion of lightweight pigs and, thus, increased profitability.

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NUTRITION

A case report: using a pre-weaning feeding program in low birthweight piglets can support pre-andpost-weaning performance

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Introduction

The number of piglets born per sow per litter has increased worldwide, which poses the challenge of weaker piglets and greater variation within litters. A multitude of studies have demonstrated that a low birthweight (BW) is one of the highestrisk factors for failure to survive the neonatal period. Low BW piglets show poorer thermoregulatory ability and are less successful in the competition at the udder to achieve early and adequate colostrum intake (1). Under the challenging conditions of normal commercial production, the consequences can be extreme: a 40% pre-weaning mortality among piglets with aBW of <1 kg (2). The piglets with BW higher than 1.2 kg are considered to be viable and can reach the maximum of its production efficiency. The BW of piglets is 1% from its slaughter weight. (3). A case study was done to analyze thepotential of using a supplementary liquid feed inaddition to sow milk for lower BW piglets. It is designed to support smaller piglets to survive and thrive, while supporting good functioning of the digestive system.

Material and method

On a commercial farm in Ecuador 34,820newborn piglets (Topigs) were used followed for 48 weeks (2020-2021), with 1 batch of 725 newborn piglets per week. Within those batches,piglets with a BW <1 kg were separated, being intotal 1,440 piglets or on average 4.14% per batch.Of these piglets, 541 (or 37.6% per batch) died shortly after birth. While the normal practice of this farm was to cull the small and weak piglets with BW <1 kg, in this case study the remaining 899 piglets were given a yoghurt treatment for thefirst 25 days and monitored for performance (Figure 1).

Results

The piglets had an average BW of 764 gram. The average body weight distribution until weaning of piglets receiving the yoghurt treatment is shownin Figure 2.

Figure 1 - Feeding schedule for low birthweight piglets

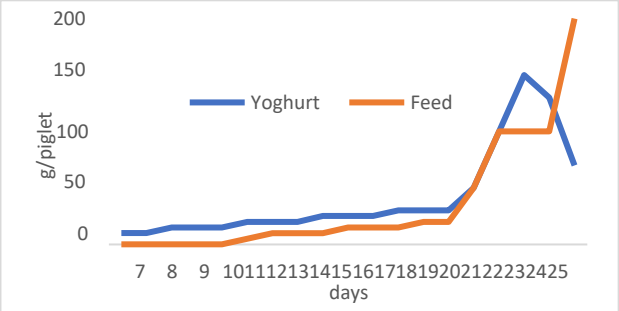
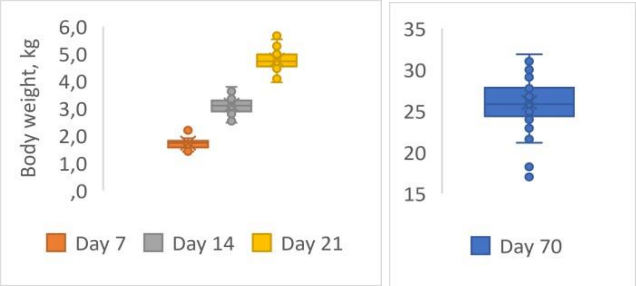


Figure 2 - Average Body Weight distribution of pigletsreceiving yoghurt treatment at



During the pre-weaning period, a total of 599 piglets survived, representing 66.7%. Average weaning weight was 4.76 kg at 23 days, and an average body weight of 26.04 kg was reached at day 70.

Discussion

Using a supplementary liquid feed in low vitality piglets with a BW <1 kg can reduce culling ratesand potentially support those piglets in fulfilling their genetic potential.

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A mix of amino acids and polyphenols reduced inflammation caused by poor hygiene of housing and subsequent metabolic consequences

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Introduction

Recent findings showed that pigs fed diet supplemented with a mix of amino acids (arginine, glutamine, cysteine, valine, leucine and isoleucine) and grape polyphenols (AAP) during the post-weaning period had a better capacity to maintain growth when exposed to an inflammatory challenge caused by poor hygiene of housing conditions during the growing phase (1). These findings were associated with the modulatory effects of amino acids and polyphenols on the inflammatory response and oxidative stress in pigs (1). The present study was then performed to evaluate if these responses were also related to differences in metabolism. Indeed, lower growth performance of pigs observed in poor hygiene of housing conditions is partially explained by metabolic changes caused by inflammation (2).

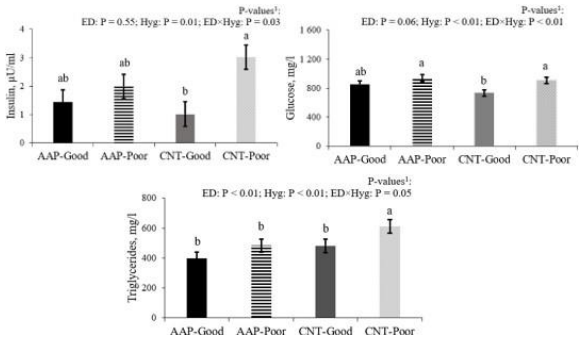
Materials and Methods

Eighty pigs were weaned at 28 days of age and allocated to 2 dietary treatments during 6 weeks post-weaning: a control standard diet supplemented or not (AAP and CNT diets, respectively) with 0.2% of AAP. After 6 weeks, pigs were transferred in a growing unit and housed in good or poor hygiene conditions and fed a standard diet for 3 weeks (challenge period). After an overnight fast, blood was collected at beginning and end of the challenge period to measure plasma concentrations of insulin, glucose, urea and triglycerides. Data were analysed using the MIXED procedure (SAS Institute Inc.) including the effects of experimental diets (AAP and CNT), hygiene conditions (good and poor), and their interaction. Results were considered statistically significant if $P \leq 0.05$.

Results

As already reported (1), contrary to CNT pigs, pigs previously fed AAP during post-weaning were able to maintain their growth rate and to counteract inflammation caused by poor hygiene of housing. Feed intake was not impacted by the experimental factors (1). Our study shows no effect of experimental diets, hygiene conditions and interaction between diets and hygiene conditions at the beginning of the challenge period for any variables ($P>0.05$). At the end of the challenge, plasma urea concentrations were greater in poor than in good hygiene conditions (280 and 238 mg/l, respectively; $P=0.01$). For insulin, glucose and triglycerides plasma concentrations, there was an interaction between the factors ($P<0.05$; Figure 1): plasma concentrations of insulin, glucose and triglycerides were greater in poor than in good hygiene conditions for CNT pigs but not in pigs previously fed AAP ($P<0.05$).

Figure 1. Insulin, glucose, and triglycerides plasma concentrations in AAP and CNT growing pigs housed in good (good) or poor (poor) hygiene of housing conditions. [1,2]



[1]P-values: Probability values for the effect of experimental diets (ED), hygiene conditions (Hyg) and their interaction (ED×Hyg). [2]Diet supplemented or not (AAP and CNT, respectively) with amino acids and grape polyphenols. [a,b] Values with different superscripts differed for Bonferroni test ($P \leq 0.05$).

Discussion and Conclusion

Greater urea concentrations in poor than in good hygiene conditions may be a consequence of reduced protein retention and/or greater protein breakdown associated with lower growth rate (1). Futhermore, greater insulin and glucose concentrations observed only in CNT pigs in poor conditions suggest a hyperinsulinemic state probably caused by a greater inflammation and immune system activation (3). These results are in agreement with the greater haptoglobin and lower vitamine E concentrations observed in poor conditions only for CNT pigs (1). For these pigs, the greater concentrations of triglycerides may be an indicator of a greater lipid mobilization in the fasted state. Our previous results indicated that growth rate and inflammatory status were less affected by poor hygiene conditions in AAP pigs compared to CNT pigs. The present study shows that this is associated with differences in metabolism between AAP and CNT pigs.

Acknowledgments

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A new development in pig growth modelling: Slow growth approaching curve for fatty pigs raised outdoor.

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Introduction

Iberian pig is a fatty pig with high robustness and resistance to disease. The high genetic potential for fat-tissue deposition in seasonal food abundance allow them adapting to change, in nature, to seasonal food shortages. This research was conducted to develop a pig growth model of Iberian (crossbreed 50% Duroc) pig raised outdoor to obtain dry and fresh meat products with high gastronomy value and to predict the economicaly optimum feeding strategy to improve the performance and economic results, and the use of feed, reducing nutrient excretion and the environment impact.

Materials and Methods

After nursery, a total 302 piglets of Iberian x Duroc (50%), 100 days old and 35 kg of body weight (BW), were randomly selected to investigate a pig growth model up to slaughter (365 days old and 160 kg BW). Pigs were placed in outdoor conditions in two batches of 186 and 116 piglets, respectively. A feeding program was designed: 1) **Transition *ad libitum*** (nursery-growth), "potential growth"; 2) **Growth 2.5 %**, access limited to feed at an amount calculated at 2.5 % of their BW, "slow growth"; 3) **Growth 2.0 %**, access limited to feed at an amount calculated at 2.0 % of their BW "slow growth"; 4) **Compensatory growth *ad libitum,*** access *ad libitum* to feed. Diets: **transition feed**, **growth feed**, **fattening feed I**, **fattening feed II**. For both experimental batches individually data were collected overtime: BW (kg), back fat thickness (BF; mm) and loin depth (BL; mm) measured at the P2 position (at the last rib, 4-6 cm from the dorsal midline) using an ultrasound linear probe EXAGO (IMV Imaging, France). The average daily feed intake (ADFI; kg/d) per groups: weighed feed allowance in phases 2 and 3, and estimated feed allowance were calculated with the equation $ME_i = A * \left\{1 - e^{-e^{(-b)*BW^c}}\right\}$, (1) for phase 1 and 4. The differential growth curve, avoiding excessive fat deposition of pigs, described 4 several phases connected to each other, depending on their growth rate, which behave in function of the volume and the composition of lysine-energy ratio of feed consumed at each moment. Phase 1, potential growth under unlimited nutrients conditions, was described by a Gompertz function: $BW\ (AGE) = BW_m * e^{-e^{-k*(AGE\ -AGEPI\ )}}$ (2). Phase 2,3 and 4, differential growth: both "slow" under limited nutrients conditions and following "compensatory", under unlimited nutrients conditions, was described by a Gil, 2019 equation ($BW_i = BW_{i-1} + ADG\ (BW_{i-1}) * (1 + Fn)$), for calculating the ($BW_i$), from instantaneous body weight of previous day (BW_{i-1}) plus a potential weight gains corresponding to that weight (ADG_i) multiplied by a fall of growth rate coefficient (F, %), which was adjusted together with other parameters of Gompertz equation: body weight at maturity (kg) (BW_m), precocity (k), age at maximum gain (d) ($AGEPI$)); since its calculating in function of age was distorted by effect of feed restriction. Therefore, ADG_i was calculated in function of its BW_{i-1}, with first derivative of the Gompertz equation, where the age is function of BW as previously described $\text{GMD}\ (BW_{i-1}) = -k * BW_i * LN\frac{BW_i}{BWm}$ (3)

Results

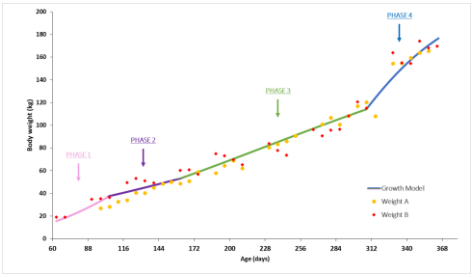
The result of the parameters calculated by optimization and defining the model equations are shown in Table 1.

Table 1. Parameters of the equations of the model.

Parameter		
Body weight at maturity (BWm), kg		218
Precocity (K)		0.00977
Age at maximum gain (AGEPI), d		163
Fall of growth rate in phase 2 (F2), %		-60
Fall of growth rate in phase 3 (F3), %		-46
Fall of growth rate in phase 4 (F4), %		100
Gain of weight, kg		160.97
Total, feed intake, kg		692.2
Feed convertion rate		4.30
Consumption, ME (kcal/kg)		
	A	119229.75
	b	6.76
	c	1.01
Feed restriction calculated phase 2, %		2.47
Feed restriction calculated phase 3, %		2.00

Multiphase differential growth curve calculated by the model and its correlation with the observed weights is illustrated by colors in figure 1.

Fig. 1. Observed and predicted growth curves.



Discussion and Conclusion

The model developed in this study allows predict a slow growth, protein, and fat deposition dynamic to rear Iberian x Duroc pig in outdoor conditions to obtain a traditional pig, which means a high-quality meat to produce fresh and dry meat product in five principal production criteria: a) local genotype, b) old slaughter age, c) heavy slaughter weigh, d) active exercise, and e) natural animal welfare, and built the economically optimum feeding program with precision over time. The implementation of the model improves the efficiency and reduce human labor and environment impact.

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Algorithm-based automatized detection of the effects of diets, rich in functional carbohydrates, on behavioral patterns of intact boars

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Introduction

Current challenges in livestock farming include public and politically demanded improvements in animal welfare (1). Studies suggest that rye, rich in functional carbohydrates, might have a positive impact on the behavior and with this animal welfare in pigs (2,3). However, there is a need for implementing new methods to make this parameter more concrete. AI-supported technology might be a solution in better understanding behavior and make animal welfare measurable. In addition, early detection of behavioral changes could be a contribution to ensuring the health of livestock.

Materials and Methods

The study (two trials) was performed on a farm in two identical barns with six compartments each. Every compartment, housing 12 intact boars, was equipped with a camera. The animals (n=288) were filmed over the entire fattening periods (2 x 16 weeks, changing of barns between trials). During a three-phased feeding, the animals received either wheat- or rye-based diets.

Table 1. Botanical composition of the diets (%)

INGRE-DIENT[2]	FEEDSTUFFS[1]					
	SW	GW	FW	SR	GR	FR
Wheat	**32.0**	**31.0**	**30.0**	**18.0**	**11.0**	**0**
Rye	**5.0**	**10.0**	**15.0**	**40.0**	**50.0**	**70.0**
Barley	20.0	15.0	15.0	15.0	15.0	5.0
Triticale	11.0	12.0	14.0	0.0	0.0	0.0
SBM[3]	7.0	6.0	0.0	7.0	6.0	5.5

[1]SW: starter wheat-based, GW: grower wheat-based, FW: finisher wheat-based, SR: starter rye-based, GR: grower rye-based, FR: finisher rye-based; [2]minor ingredients not listed, [3]soybeanmeal

For the first time, film material of entire fattening periods could be evaluated simultaneously. A software, based on a machine-learning algorithm, determined the activity by recording the position (centroid-based animal activity, CAA) and posture (lying or standing) of every animal twice in a second. In addition, temperature and air quality were measured and the animals were scored according to KTBL-guidelines (4) every two weeks. The effects of the diet on the activity were analyzed by a pairwise comparison based on a Tukey test (5).

Results

With comparable results for air quality, temperature und KTBL-Scorings in both groups/stables the activity of the animals was significantly higher in the group fed wheat-based diets in the first trial. In trial 2, a significant effect of the feed could not be shown statistically.

Table 2. Mean activity of the animals (centroid-based animal activity, CAA).

TRIAL	FEEDING GROUP[1]	
	WHEAT	RYE
1	0.156 ± 0.014^{a}	0.133 ± 0.014^{b}
2	0.123 ± 0.013	0.137 ± 0.013

[1]Superscripts indicate statistically significant differences within main effect ($p \leq 0.05$)

Similar behavioral patterns could be identified in both trials. Among other things, minor differences occur within the average number of animals, which were recognized as standing.

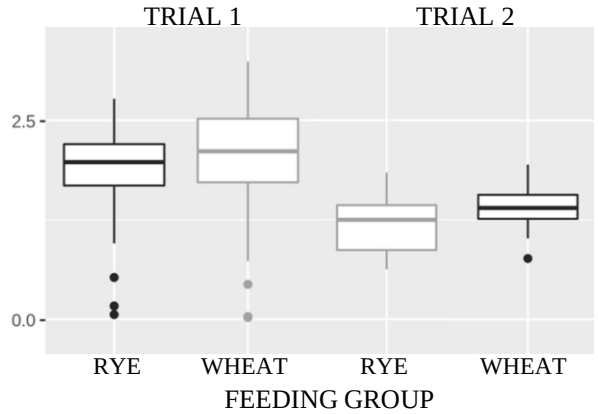

Figure 1. Average number of animals recognized as standing within the compartment.

Discussion and Conclusion

An effect of the rye-based diets on the behaviour is conceivably, but a certain barn-effect cannot be ruled out either. As this is a new and unique method of recording smallest differences over a period of several weeks under practical conditions, further work needs to bone in order to identify additional factors effecting behaviour. Nevertheless it was possible to monitor the activity permantently and accurately providing an detailed insight in the behaviour with potential for early detection of behavioural changes (diseases/tail-biting).

Acknowledgments

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Application of Hemicell HT™ – a β-mannanase enzyme – combined with palm kernel meal in fattening pigs results in similar performance and carcass quality

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Introduction

β-Mannans are strongly anti-nutritive polysaccharide fibres found in most vegetable feed ingredients (1). They belong to the hemicellulose fraction and have a backbone composed entirely of mannose, as in mannans and galactomannans, or of mannose and glucose, as in glucomannans and galacto-glucomannans (2,3). The estimated content of soluble β-mannans in common fattening diets is only 0.20-0.35%, and *in vitro* studies have demonstrated that as little as 0.05% soluble β-mannan in feed can elicit a strong innate immune response (4). This innate response is often referred to as a feed induced immune response or FIIR, which suppresses growth to protect the liver and reserve energy and nutrients for high priority immune functions. Hemicell HT (Elanco) is a β-mannanase enzyme for animal feed that breaks down β-mannans and thereby prevents economic losses from the wasteful immune response to β-mannans. The aim of the study was to compare pig performance and carcass parameters on a control diet and a diet containing 3-6-9% of palm kernel meal (PKM) including Hemicell HT.

Materials and methods

An eighteen-week feeding trial was conducted on a commercial fattening unit with DanBred x Belgian Piétrain pigs starting at 10 weeks of age. Standard three-phase control diets were compared to similar isonutritive diets with 300 g/tonne of Hemicell HT (Elanco) except for following changes:

- ✓ Phase 1 (week 1-4): 3% soy bean meal (SBM) was replaced by PKM
- ✓ Phase 2 (weeks 5-9): 6% SBM was replaced by PKM
- ✓ Phase 3 (weeks 10-18): 9% SBM was placed by PKM.

Standard production and health data were collected including days in fattening, bodyweight at start and slaughter, feed intake, mortality and antibiotic use. Additionally, feed conversion rate and medication cost were calculated from the collected data. Furthermore, pigs were slaughtered on separate slaughter days to reliably collect relevant carcass parameters at slaughterhouse level (Table 1). The data were analysed using JMP 15.0 statistical program.

Results

Overall, performance data did not differ significantly between both trial groups, although there were relevant numeric differences in mortality (27 in control *vs.* 5 in Hemicell HT) and a slightly lower cost of medication in the Hemicell HT group (€ 0.70 *vs.* € 0.75).

Table 1. Overall technical results and carcass data of comparative trial between control diets and diets containing 3-6-9% PKM with addition of Hemicell HT (Elanco). Significant differences are indicated as $P < 0.05$.

	Control	PKM - Hemicell HT	*P*-value
Number of pigs enrolled	300	299	> 0.05
Start weight (kg)	28.70	28.70	> 0.05
Slaughter weight (kg)	130.25	129.40	> 0.05
Days in fattening (d)	126	125	> 0.05
Mortality (# / %)	27 (9.00%)	5 (1.67%)	> 0.05
Cost of medication (€/varken)	0.75	0.70	> 0.05
Corrected hot carcass weight (kg)	105.35	104.45	> 0.05
Lean meat (%)	66.28	66.77	< 0.05
Meat depth (mm)	75.17	73.45	< 0.05
Loin depth (mm)	12.67	11.91	< 0.05
Ham weight (kg)	12.82	12.67	> 0.05

Conclusions and Discussion

The trial demonstrated that Hemicell HT was able to degrade β-mannans in diets with an increasing inclusion of PKM (3-6-9%), and therefore maintain production performance and overall carcass quality. Some of the differences in carcass quality parameters could be explained by the variation in male/female ratio among the treatment groups.

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Application of Hemicell HT™ – a β-mannanase enzyme – in diets with a reduced net energy content results in reduced production costs per kg of carcass weight

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Introduction

β-Mannans are strongly anti-nutritive polysaccharide fibres found in most vegetable feed ingredients (1). They belong to the hemicellulose fraction and have a backbone composed entirely of mannose, as in mannans and galactomannans, or of mannose and glucose, as in glucomannans and galacto- glucomannans (2,3). The estimated content of soluble β-mannans in common fattening diets is only 0.20- 0.35%, and *in vitro* studies have demonstrated that aslittle as 0.05% soluble β-mannan in feed can elicit a strong innate immune response (4). This innate response is often referred to as a feed induced immune response or FIIR, which suppresses growth to protectthe liver and reserve energy and nutrients for high priority immune functions. Hemicell HT (Elanco) is aβ-mannanase enzyme for animal feed that breaks down β-mannans and thereby prevents economic losses from the wasteful immune response to β- mannans. The aim of the study was to compare pig performance and carcass parameters on a control diet and a diet with a 65 kcal NE/kg lower energy contentincluding Hemicell HT.

Materials and methods

An eighteen-week feeding trial was conducted on a commercial fattening unit with DanBred x Belgian Piétrain pigs starting at 10 weeks of age. Standard two-phase control diets were compared to reformulated diets with an energy reduction of 65 kcal NE/kg and inclusion of Hemicell HT (Elanco) at 300 g/tonne. Standard production and health data were collected including days in fattening, bodyweight at start, at 28 days and at slaughter, feed intake, mortality and antibiotic use. Additionally, average daily weight gain(ADWG) and feed conversion rate (FCR) were calculated from the collected data. Furthermore, pigs were slaughtered on separate slaughter days to reliably collect relevant carcass parameters at slaughterhouse level (Table 1). The data were analysed using JMP
15.0 statistical program.

Results

Overall, performance data did not differ significantly between trial groups in both Phase 1 and Phase 2, except for mortality that was significantly higher during Phase 1 in the control group. Carcass quality did only significantly differ for muscle depth (73.58 mm in control *vs.* 75.33 mm in Hemicell HT). Following calculation of feed costs per kg carcass weight and taking into account the cost of enzyme inclusion, Hemicell HT-fed pigs had a € 0.02 lower feed costs as compared to control pig.

Table 1. Performance parameters during the fatteningfor period and carcass data for pigs fed control diets or reformulated diets an energy reduction of 65 kcalNE/kg and inclusion of a beta-mannanase enzyme (Hemicell HT; Elanco) at 300 g/T.

	Control	Hemicell HT
Phase 1		
Number of pigs enrolled	94	94
Mortality (# / %)	3 (3.19%) *	0 (0%) *
Weight d0 (kg)	22.16	22.32
Weight d28 (kg)	40.73	40.01
ADWG (g/d)	648	631
FCR (kg/kg)	2.41	2.42
Phase 2		
Number of pigs enrolled	95	96
Mortality (# / %)	1 (1.05%)	2 (2.10%)
Weight d0 (kg)	42.22	40.07
Weight d99 (kg)	134.55	133.91
ADWG (g/d)	940	943
FCR (kg/kg)	2.75	2.75
Slaughter data		
Number of pigs slaughtered	91	90
Hot carcass weight (kg)	106.55	107.26
Lean meat (%)	62.49	62.89
Backfat thickness (mm)	9.80	9.60
Muscle depth (mm)	73.58 *	75.33 *
Production cost per kg meat		- € 0.02

* indicates significant differences ($P < 0.05$) between both study groups.

Conclusions and Discussion

The trial demonstrated that inclusion of Hemicell HT in reformulated diets with a lower energy content (65 kcal NE/kg) was able to degrade β-mannans in diets and therefore maintain production performance and overall carcass quality. Inclusion of Hemicell HT resulted in an overall reduction in production costs of € 0.02 per kg of carcass weight, which is an additional advantage considering the current feed prices globally.

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Application of Hemicell HT™ – a β-mannanase enzyme – in diets with reduced NE content results in similar production performance in both piglets and fattening pigs

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Introduction

β-Mannans are strongly anti-nutritive polysaccharide fibres found in most vegetable feed ingredients (1). They belong to the hemicellulose fraction and have a backbone composed entirely of mannose, as in mannans and galactomannans, or of mannose and glucose, as in glucomannans and galacto- glucomannans (2,3). The estimated content of soluble β-mannans in common fattening diets is only 0.20- 0.35%, and *in vitro* studies have demonstrated that as little as 0.05% soluble β-mannan in feed can elicit a strong innate immune response (4). This innate response is often referred to as a feed induced immune response or FIIR, which suppresses growth to protect the liver and reserve energy and nutrients for high priority immune functions. Hemicell HT (Elanco) is aβ-mannanase enzyme for animal feed that breaks down β-mannans and thereby prevents economic losses from the wasteful immune response to β- mannans. The aim of the study was to compare pig performance and carcass parameters on a control diet and a diet containing 3-6-9% of palm kernel meal (PKM) including Hemicell HT.

Materials and methods

An analysis of performance data (average daily weight gain (ADWG), average daily feed intake (ADFI), feed converson rate (FCR), % treated animals and mortality) obtained in 3 piglet feed trials and 3 fattening feed trials comparing control diets to alternative diets with reduced net energy content (approx. 63 kcal NE/kgfeed) and supplementation of 300 g/T of a β- mannanase enzyme (Hemicell HT; Elanco) was performed using JMP 15.0 statistical program.

Results

Performance parameters did not significantly differ between treatment groups in both piglet feed trials and fattening feed trials. Nevertheless, there was a clear trend for better health parameters in the Hemicell HT-treated group, including a lower percentage of treated animals (5.31% *vs.* 8.27% in piglets; 6.67% *vs.* 7.14%in fattening pigs) and mortality (0.15% *vs.* 1.05% in piglets; 1.26% *vs.* 4.41% in fattening pigs).

Conclusions and Discussion

The obtained results demonstrated that Hemicell HT was able to degrade β-mannans in diets with a reduced net energy content (approx. 63 kcal NE/kg feed) resulted in similar performance parameters (ADWG, ADFI and FCR). Moreover, improved health parameters could with the numerically better % animals treated with antimicrobial and lower mortalityin the Hemicell HT-treated group.

Table 1. Performance parameters during nursery and fattening period fed a control diet or a reformulated diet with an energy reduction of 65 kcal NE/kg and inclusion of a β-mannanase Hemicell HT (Elanco) at 300 g/T. Statistical analysis performed using JMP 15.0 with ANOVA for all performance parameters. Nosignificance differences ($P < 0.05$) were observedbetween different performance parameters.

Parameter	*Control*	*Hemicell HT*
Piglet trials		
Number of trials	3	3
Trial duration (d)	47,3 ± 0,9	47,3 ± 0,9
ADWG (g/d)	339,2 ± 21,2	342,9 ± 17,3
ADFI (g/d)	551,7 ± 17,3	550,5 ± 23,2
FCR (kg/kg)	1,58 ± 0,10	1,57 ± 0,07
% animals treated	8,27 ± 5,31	5,31 ± 3,09
Mortality (%)	1,05 ± 0,54	0,15 ± 0,15
Fattening trials		
Number of trials	3	3
Trial duration (d)	117,3 ± 9,2	117,0 ± 9,0
ADWG (g/d)	838,5 ± 20,0	850,4 ± 22,2
ADFI (kg/d)	2216,5 ± 75,9	2252,0 ± 44,5
FCR (kg/kg)	2,65 ± 0,08	2,65 ± 0,06
% animals treated	7,14 ± 7,14	6,67 ± 6,67
Mortality (%)	4,41 ± 2,60	1,26 ± 0,64

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Application of spray dried blood plasma as feed additive for the replacement of antibiotics in piglets at the growth and termination phase

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Introduction

Nowadays, there is a demand for the use of antibiotics in the swine sector for better zootechnical and economic parameters. The intensive production systems advocate the use of antibiotics as growth promoters. However, the use of these antimicrobials in swine production has been restricted to meet the worldwide market demand, which increasingly demands quality and safe food (1). Then, it isnecessary to discover new bio-based antimicrobials materials (i.e., probiotics, blood plasma, and natural plant extracts) to replace or decrease the use of antibiotics in swine production (2). One of these natural alternatives recently reported is blood plasma produced by spray drying, which is a concentrated protein ingredient for feed supplementation (3). Moreover, swine diet based on feed without antibiotics and with the presence of natural compounds has been widely studied to ensure the health of the swine herd (4). Therefore, this study evaluated the effect of spray dried blood plasma (SDP) as a feed additive for the replacement of antibiotics in piglets at the growth and termination phase.

Materials and Methods

The treatments were used as strategic pulses of either antibiotics and SDP in the feed of swine during the growth and termination phase. The SDP was added in the animals feed in the concentration of 2% for the growing phase, 1% for the growing-1 phase, and 0.8% for the finishing-1 phase. The experimental design was conducted by 4 dietary treatments with 14 repetitions composed of 3 categories of swine initial body weight at housing. A total of 1,456 swine were selected just after leaving the nursery and were evaluated for 122 days until the slaughter. All zootechnical parameters (feed intake, weight gain, feed conversion, daily weight gain, mortality, and drug interventions) were individually checked in the housing (day 0) and at 40, 70, 84 and 122 days of experiment. The sanitary indexes were evaluated throughout the housing period, with weekly intervals, which were classified as *ante- mortem* analyzes (cough and sneeze count and diarrhea score) and *post-mortem* analyzes (gastric ulcer index and pneumonia index). Data were analyzed using the Statistical Analysis System (version 9.4). (5) The experimental design was randomized blocks, and the experimental unit was the pens. Data were submitted to the Shapiro-Wilk normality test at 5% probability and Analysis of Variance (ANOVA).

Results

The results indicated that the feed with addition of the standard medication protocol (antibiotics) and without the inclusion of SDP had the highest feed intake (289.73 kg) and reached a feed conversion of 2.57 kg feed consumed per kg weight gained. The weight gain during the period of 0-122 days for the treatment with three pulses of SDP with (114.12 kg) and without of antibiotics (114.2 kg) were statistically higher than the control treatment (111.46 kg, without antibiotics and SDP). Furthermore, the antibiotic-based treatment associated with SDP showed a 7.76% increase in weight gain when compared to control group. Furthermore, there was non-significant difference inthe mortality of animals and in the index for pneumonia and ulcer score.

Discussion and Conclusion

In this study, the association between antibiotics and SDP had a positive effect on weight gain and daily weight gain of pigs, compared with the negative control (without antibiotics). On the other hand, the supplementation of piglets with dehydrated blood plasma on the day of birth and during the performance in the nursery phase, found that there was no effect of the treatment with the inclusion of blood plasma on thetotal weight gain (6). Regarding health parameters, the treatments had no influence on the index for pneumonia, stool score, cough, and sneeze index, nor on the gastric ulcer score. However, the treatment with the strategic inclusion of antibiotics had a smaller number of injected medications and a smaller number of medicated animals, with non-significant influenceon the mortality rate regardless of the treatment evaluated.

In conclusion, the application of SDP as feed additive in piglets at the growth and termination phase,associated with antibiotics, favored feed intake and weight gain, being a promising alternative to upgrade the zootechnical parameters in animal production.

Acknowledgments

The authors thanks APC Inc. do Brasil Ltda for the supply of the spray dried blood plasma used in the experiment.

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Correlating blood biomarkers with ileal digestibility and performance in *Lawsonia intracellularis* challenged pigs

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Introduction

Lawsonia intracellularis is a significant cause of intestinal dysbiosis, reduced growth, and reduced nutrient digestibility (1,2,3), however the relationship between digestibility and other metrics in *L. intracellularis* challenged pigs is unclear. Thus, this study aimed to evaluate the relationship between ileal digestibility, growth performance, and blood biomarkers in *L. intracellularis* challenged pigs.

Materials and Methods

Thirty-six *L. intracellularis* negative barrows were assigned to treatment groups as follows (n=12/trt): 1) nonvaccinated, *L. intracellularis* negative (NC); 2) nonvaccinated, *L intracellularis* challenged (PC); and 3) *L. intracellularis* challenged, vaccinated with Enterisol® Ileitis (Boehringer Ingelheim Animal Health, Duluth, GA) via oral drench at 1-week postweaning (VAC). On days post inoculation (dpi) 0 (7 weeks post-weaning) PC and VAC pigs were inoculated with *L. intracellularis*. Serum and fecal samples were collected weekly. At dpi 21, pigs were euthanized for tissue and digesta collection.

Results

The challenge reduced performance, reduced nutrient digestibility, and increased intestinal lesion length in PC pigs compared with NC and VAC pigs. In PC pigs, ileal lesion length was negatively correlated with dry matter (-0.658, P=0.028), nitrogen (-0.706, P=0.015), organic matter (-0.629, P=0.038), and energy AID (- 0.658, P=0.028). Blood serotonin concentrations at dpi14 and 21 negatively correlated with all AID metrics in PC pigs (Table 1). Additionally, serotonin correlated with overall average daily gain and feed conversion ratio in PC pigs (Table 1). In VAC pigs, serotonin at dpi 14, but not 21, was negatively correlated with AID(Table 1). Serotonin did not correlate with overall growth performance in VAC pigs. Blood concentrations of IL-1β at dpi 21 were positively correlated with dry matter (0.672, P=0.023), nitrogen (0.607, P=0.048), organic matter (0.674, P=0.023), and energy AID (0.674, P=0.023) in PC pigs, and tended to correlate with overall ADG (0.570, P=0.053) and FCR (-0.500, P=0.098). Blood IL-1β did not correlate with AID or growth in VAC pigs.

Table 1. Correlation coefficients amongst serum serotonin concentrations, apparent ileal digestibility,and production performance

		Days post inoculation		
		7	14	21
PC	DM[1]	-0.391	-0.845**	-0.673*
	N[2]	-0.455	-0.909***	-0.618*
	OM[3]	-0.400	-0.882***	-0.645*
	GE[4]	-0.391	-0.845**	-0.673*
	ADG[5]	-0.035	-0.357	-0.601*
	FCR[6]	0.084	0.308	0.727*
VAC	DM[1]	-0.335	-0.664*	-0.217
	N[2]	-0.238	-0.504*	-0.385
	OM[3]	-0.336	-0.664*	-0.273
	GE[4]	-0.224	-0.671*	-0.252
	ADG[5]	-0.084	-0.455	0.000
	FCR[6]	0.315	0.440	-0.056

[1]apparent ileal digestibility (AID) of dry matter at dpi 21; [2]AID of nitrogen at dpi 21; [3]AID of organic matter at dpi21, [4]AID of gross energy at dpi 21; Average daily weightgain from dpi 0-19; [2]feed conversion ratio from dpi 0-19; *statistically significant (*P<0.05, **P<0.01, ***P<0.001)

Discussion and Conclusions

These data demonstrate the impact of clinical *L. intracellularis* on AID of nutrients and that these highly correlate with lesion length in unvaccinated, challenged pigs. Additionally, AID had a strong, negative association with serotonin concentrations. Although somewhat surprising, serotonin is associated with diarrhea in human pathologies and may be involved with immune cell function, although this mechanism remains unclear (4). Regardless, these data indicate that blood serotonin concentrations may serveas an early predictor of ileal nutrient digestibility,growth, and feed efficiency during *L. intracellularis* challenge.

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Diets with high protein corn distillers' dried grains, supplemented with algae and clay complex or xylanase, affects organ weight and carcass traits of pigs

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Introduction

The high protein corn distiller's dried grains (HP-DDG) are co-products from the ethanol industry which is used in swine feeding. The digestible nutrients and energy values of HP-DDG, and its effects on performance and carcass traits of pigs, are variable and depend on the feedstuff origin and processing (1). Furthermore, HP-DDG has high content of non-starch polysaccharides (2), which can alter digestibility and carcass parameters of pigs. Therefore, the objective of this study was to evaluate diets with high inclusion levels of HP-DDG, produced in Brazil, supplemented with algae and clay complex or xylanase, on carcass and organ traits of growing and finishing pigs.

Materials and Methods

128 crossbred barrows and gilts (35.7 ± 4.3 kg of body weight (BW) and 73 days old), were distributed in a randomized block design according to BW and gender in groups of four animals per pen. The pigs were fed one of the four following diets: 1) CON: corn-soybean meal basal diet; 2) HP-DDG: diet with 40% (Growing 1), 33% (Growing 2) and 25% (Finishing) inclusion of corn HP-DDG; 3) HP-DDG ACC: HP-DDG diets plus algae and clay complex additive (0.1%); and 4) HP-DDG XYL:HP-DDG diets plus xylanase enzyme (0.01%). At the end of the experimental period (70 days) the animals were euthanized and one animal per pen were selected for carcass evaluation and the measurements on organ parameters (3). The data were analyzed using ANOVA and differences were considered at $p < 0.05$.

Results

Pigs fed CON had greater ($p = 0.01$) hot carcass yield than the pigs submitted to the HP-DDG diets (Table 1). The cold carcass yield of HP-DDG ACC pigs was lower(p = 0.046) than that of CON pigs, and the loin perimeter of HP-DDG animals was lower (p = 0.045) than those of CON and HP-DDG ACC pigs. The HP-DDG animalshad shorter (p = 0.003) small intestine than pigs fed diets containing the fed additives, and shorter (p = 0.038) large intestine than HP-DDG XYL pigs.

Discussion and Conclusion

The inclusion of fiber-rich ingredients in swine diets can reduce carcass yield due to increased gut fil and intestinal mass (4). The greater intestine lengh of HP- DDG ACC and HP-DDG XYL animals, compared to HP-DDG pigs, may increase the digestion and absorption capacity, which can improve pigs growth performance and carcass traits values. These resultssupport the use of xylanase and alga and clay complex when HP-DDG is used, due the improvements in some swine carcass traits and organ parameters.

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Table 1. Carcass and organ parameters of pigs (143d-old) fed a corn soy-bean based diet (CON), or diets with corn HP-DDG, algae and clay complex (HP-DDG ACC) and xylanase (HP-DDG XYL)

	Treatments					
Item	CON	HP-DDG	HP-DDG ACC	HP-DDG XYL	SEM[1]	*p*
Carcass traits						
Hot carcass weight, kg	68.71	66.80	65.65	69.56	1.377	0.093
Hot carcass yield, %	79.10^{a}	77.00^{b}	76.60^{b}	77.06^{b}	0.411	0.010
Cold carcass weight, kg	68.57	65.33	63.05	67.23	1.685	0.081
Cold carcass yield, %	77.95^{a}	75.08^{ab}	74.63^{b}	75.19^{ab}	0.503	0.046
Carcass lenght, cm	90.05	90.48	90.89	94.76	1.618	0.063
Backfat thickness, mm	9.13	12.48	14.29	13.09	1.319	0.130
Loin depth, mm	51.18	55.46	53.21	53.43	3.690	0.306
Lean meat, %	60.69	61.93	63.29	60.76	1.040	0.130
Loin eye are, cm²	38.48	35.21	35.35	34.97	1.705	0.606
Loin perimeter, cm	23.03^{a}	21.82^{b}	22.99^{a}	22.59^{ab}	0.593	0.045
Organ parameters						
Small intestine, m	17.88^{ab}	16.45^{b}	20.08^{a}	18.88^{a}	0.626	0.003
Large intestine, m	4.91^{ab}	4.84^{b}	5.32^{ab}	5.67^{a}	0.417	0.038
Small intestine, %	1.337	1.415	1.633	1.370	0.115	0.097
Large intestine, %	1.661	1.761	1.794	1.719	0.146	0.942
Stomach, %	0.538	0.502	0.487	0.504	0.031	0.809
Liver, %	1.546	1.355	1.358	1.480	0.047	0.071

^{abc} Different letter in the same row differ ($p < 0.05$). [1] Standard error mean.

Does feeder´s color monotony affect feed acceptability and preferences in nursery pigs?

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Introduction

Sensory specific satiety (SSS) is a phenomenon associated with decreased pleasure when consuming a food continuously **[1].** Consequently, animals reduce or stop their food consumption because of the lack of sensory diversity (either texture, color, or odor), thus triggering a decreased hedonism during food intake. Its role is, therefore, to promote a varied consumption of nutrients, taking into account that a varied diet consists of the consumption of foods that differ in at least one sensory property **[2].** Pigs, in a natural environment, consume an extensive variety of foods with its sensory cues associated to meet their nutritional requirements. However, pigs raised in conventional farms do not have the opportunity to search and choose different foods. Therefore, SSS could negatively affect their feed intake and welfare. The objective of this experiment was to explore the effect of feeder´s color variety on feed acceptability and feed preferences of nursery pigs.

Materials and Methods

Thirty-two weaned pigs were allocated in pairs into 16 nursery pens. From the second week after weaning (28 days old, 5.8 ± 0.5 kg) animals were daily exposed during eight consecutive days, to acceptability (n4) and preference tests (n4), in order to assess their SSS for feeder colors. To test feed acceptability, pigs were exposed for 10 min to red or blue feeders after being exposed to the same or different feeder colors for another 10 minutes (red-red, blue-blue, red-blue or blue-red). Subsequently, preference between red and blue feeders was estimated during 10 minutes after a previous exposure for the same time to red or blue feeders. Data was analyzed with an ANOVA procedure by using the statistical software SAS®.

Results

Pigs presented a similar intake of feed when was delivered in red or blue pan feeders during the acceptability test (P=0.648). It was observed an effect of the interaction between the first and second color given **(Figure 1)**, where pigs preferred to consume the feed delivered in different pan feeder colors (P=0.002). When pigs were first given the feed in red feeders, they consume more feed in blue feeders and the other way round**.** When the feed was offered at the same time in different feeder colours, no preferences were observed between red or blue pan feeders (P=0.279). As it is observed in **Figure 2,** no interaction was observed between the color consumed during the preference test and the color previously delivered (P=0.703).

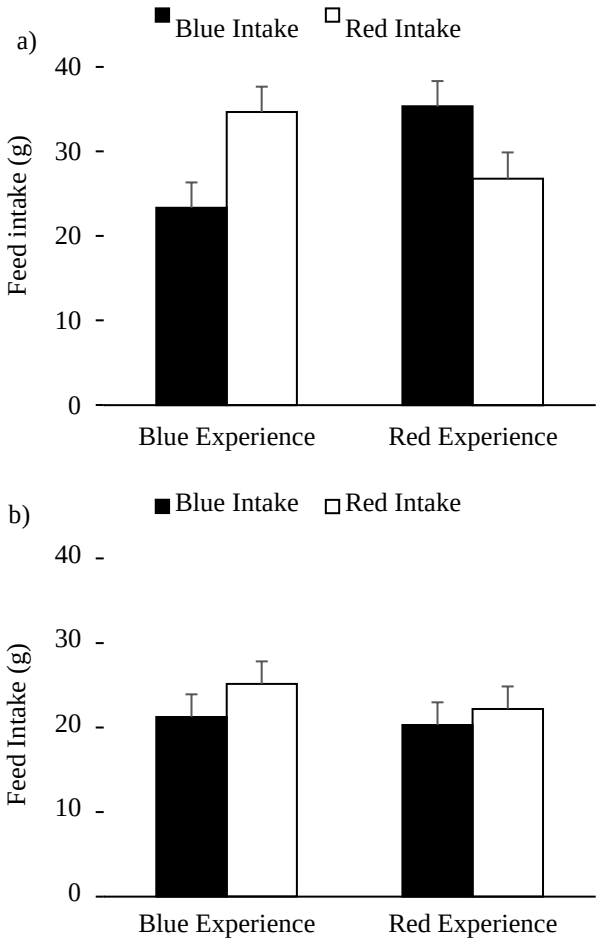

Figure 1. Feed intake (mean ± SEM) of nursery pigs duringa single (a) or simultaneous (b) exposure (acceptability and preference tests respectively) of blue and red feeders during 10 min. Pigs previously had experience for another ten minutes with blue or red feeders.

Discussion and Conclusion

Feeder´s color monotony may decrease the feed acceptability in nursery pigs. Such compromise may be avoided or reversed if pigs are provided with a sensory varied diet. Therefore, swine industries shoulddevelop feeding strategies that promote diet sensory variability, in order to allow animals to express, to some extent, their natural feeding behavior.

Acknowledgments

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Effect of a natural additive based on vegetable extracts of fennel (*foericulum vulgare*), alcaravea (*carum carvi*) and juniper (*juniperus communis*) on weight of lactating piglets.

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Introduction

Sow productivity, through productive performance of her litters, is a key component and optimizing remains amajor challenge for swine production. By increasing milk production, the productive performance of the litteris enhanced. By means of administering natural products that do not metabolically or physically wear out the sow,milk production can be increased and quality improved, resulting in higher piglet weaning weights and increasedpiglet viability (PIC, 2013).

Objetive

To evaluate the positive effect on piglet weaning weights with the administration of a natural additive in the diet of lactating sows.

Material and Methods

The work was carried out in a full-cycle commercial farm located in Queretaro, Mexico. The experimental phase was carried out in two groups, each with 5 Large White x Landrace sows. Both groups were placed in complete maternity units. The additive was administered30 grams per sow per day in two intakes added directlyto the feed. This feed was supplied 5 days before farrowing and then throughout lactation until weaning. The following variables were recorded:

- Piglet weight at day 0, 7, 14 and 21
- Piglet group weight gain/Piglet weight gain.
- Sow condition at weaning.

A statistical test (T-Student Test) was used to compare the average weights of both treatments.

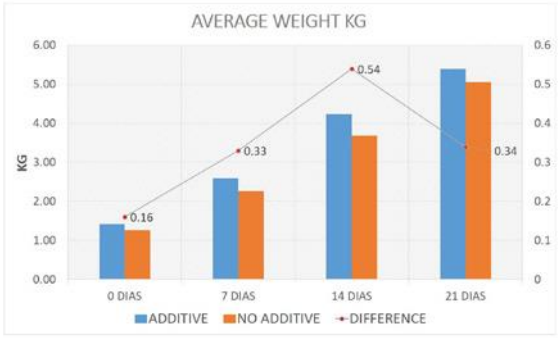
Results and Discussion

The average body condition of treated sows was 3, whilefor untreated sows it was 3.2 Body condition has a positive effect on wean-to-estrus days because the sow does not have the need to mobilize her corporal energy reserves, the sow will be able to come into estrus in lesstime. Wen-Chao Liu, *et al.* 2017 (1), demonstrated the decrease of fat loss with a diet of herbal extracts and optimal body conditions (2) Piglet weights at different ages were variable ($P<0.05$) with an average weight difference of 0.18kg (summarized in Table 1 and Fig 1)while Wen-Chao Liu, et al. 2017 (1) obtained average gain of 0.204Kg ($P<0.05$). lsley *et al.* 2002 (2) reported that the inclusion of an herbal extract in the lactation diet of sows improved the performance of piglets with higherweaning weights. Similar effects were also observed byZhong *et al.* 2011 (3), who reported that supplementation of 0.04% sow phytogenetic additive had a positive effect on litter performance.

An average of 10.2 piglets were weaned at 5.46 kg in the treated group, while 10 pigletswere weaned at 5.06 kg in the untreated group.

Table 1: WEIGHT AND DIFFERENCE BETWEEN BOTH TREATMENTS

WEIGHT	# PIGLETS	TREATMENT	AVERAGE WEIGHT	DIFFERENCE	T-Student
0 Days	51	Additive	1.42	0.16	0.010
	71	No Additive	1.26		
7 Days	51	Additive	2.59	0.33	0.002
	54	No Additive	2.26		
14 Days	51	Additive	4.23	0.54	0.003
	50	No Additive	3.69		
21 Days	51	Additive	5.40	0.34	0.043
	50	No Additive	5.06		



Conclusion

The supplementation with natural additive in the feeding of lactating sows improved 4.7% (0.18kg) on the average weight per piglet with respect to conventional feeding, obtaining 9.18kg of 51 piglets weaned in the treated group.

The results obtained help to consider nutritional alternatives in the feeding of lactating sows to improve the adverse effects that cause low milk production and affect the vital performance of the piglet.

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Effect of *Bacillus* spp. in the performance of sows and their progeny

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Introduction

Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (1). The use of probiotics for human and animal health have been reported in the literature of the past years. In swine, the probiotic effects are diverse and will depend on the strain, dose, and duration of the treatment (2). *Bacillus* species are probiotics known for their immunostimulatory effects and beneficial stimulation of intestinal microbiota, thus enhancing the host's innate and adaptive immunity (3).

The gestation period of the sows is a critical period and there is still some lack of information of the use of probiotics in this phase. Therefore, the aim of this study was to access the effect of *Bacillus* spp. in the performance of sows and their progeny.

Materials and Methods

Sows with parity order ranging from 2 to 8 were assigned to two treatments: Control (95 sows) and *Bacillus* (95 sows), which consisted in the supplementation of Bioplus® 2B containing *Bacillus subtillis* and *Bacillus licheniformis.* Supplementationvia feed occurred daily and started on the first day of pregnancy and persisted until the end of lactation. Performance responses of the sows included: number of piglets born alive, stillborn, and mummified. Performance responses of the progeny included: birth weight, weaning weight at 20th day and weight gain during lactation. Data were evaluated for normality using univariate procedures using SAS (4). After, responses were analyzed using the GLIMMIX procedure. Potential differences were interpreted at 5 and 10% levels. The effects of parity order, litter size, and body condition were tested and maintained in the final model when significant (P<0.10).

Results

Performance results for both sows and piglets are shown in Table 1. The inclusion of *Bacillus* decreased the numbers of mummified (P<0.10) and did not affect the number of piglets born alive. The performance of the piglets was improved by feeding the sows both during gestation and lactation with *Bacillus subtillis* and *Bacillus licheniformis*. Birth weight, weaning weight, and daily weight gain were greater in the piglets that were born from sows supplemented with *Bacillus* (P<0.05).

Discussion and Conclusion

Probiotic inclusion in the gestation and lactation period of sows improved the performance of the piglets born. Probiotics, as *Bacillus*, have the characteristics to maintain gut health and to prevent from intestinal dysbiosis (5). Piglets with healthy gut are more likely to grow and survive during the lactation period. Also,some studies shown that sows fed with *Bacillus*presented colostrum and milk with greater quality (6,7).Therefore, a higher quality milk represents higher piglet body weight in the end of the lactation period.

Table 1. Performance responses of sows supplemented with *Bacillus* during gestation and lactation period and their progeny.

Variables	Control	*Bacillus*	*P*-value[1]
Performance - Sows			
Total born alive, n/litter	13.13	12.91	0.690
Stillborn, n/litter	0.600	0.489	0.423
Mummified, n/litter	0.378	0.236	0.073
Performance - Piglets			
Birth weight alive, kg	1.35	1.44	0.044
Weaning weight at 20 days, kg	5.47	5.85	0.002
Daily weight gain, g/day	194.19	208.50	0.003

[1]Probability of treatment effects.

Based on these results, the mechanisms of the probiotics should be investigated further to better explain the positive results found.

Acknowledgments

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Effect of different application of a multienzyme product on growing and finishing pigs fed corn and soybean-based diets

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Introduction

In Latin America, the most used ingredients in pig diets are corn and soybean meal. In these feed ingredients nutrient digestibility is impaired by high content of non-starch polysaccharide (NSP) and presence of phosphorus (P) in phytic acid form (1). Increasing the levels of digestible amino acid, net energy and digestible P in pig feed may overcome these challenges, but at increased feed cost. On the other hand, the use of phytase and carbohydrase enzymes in corn- and soybean-based diets has the potential of achieving better performance at adequate feed cost. Therefore, the present study aimed to determine the effect on zootechnical performance of growing-finishing pigsfed corn- and soybean-meal diets containing a multienzyme product, including xylanase, β-glucanase, arabinofuranosidase and phytase, either including the enzymes considering a nutritional matrix or including 'on top' of the formulated feed.

Materials and Methods

The experiment was carried out at Bioter's Experimental Facilities, located in Buenos Aires Province, Argentina. A total of 1.080 female and inmunocastrated male pigs (initial body weight [IBW] 31.84 kg, PIC® genetics) were equally distributed in 24 pens (45 animals/pen and 8 pens/treatment), following a completely randomized block design. Treatments were defined as: T1 - control standard diet based on NRC (2012), without multienzyme product; T2 - standard diet with multienzyme product, reformulated according to enzyme nutritional matrix (80 kcal/kg metabolizable energy; 0,023% digestible lysine; 0,008 digestible TSAA; 0,015 digestible threonine; 0,15% AvP and 0,15% Ca); T3 - standard diet with multienzyme product added 'on top', no reformulation. Each pen was equipped with compact floor, nipple drinkers and automatic feeders. Pigs received feed and water *ad libitum* during the whole trial. Prior the trial, animals were fed the same adaptation diet, without multienzyme product inclusion. Multienzyme product tested was Rovabio® Advance Phy T (Adisseo®, France), mainly composed by 1.250 VU of xylanase, 860 VU of β-glucanase and 1.000 FTU of phytase per kilogram of diet. In both T2 and T3, enzyme was included at 100g/ton of feed. Experimental period lasted 80 days (from 74 to 154 days of age) and was divided in 4 phases of 20 ± 1 days each: phase 1 from 74 to 94 days old; phase 2 from 94 to 113; phase 3 from 113 to 133; phase 4 from 133 to 154. Individual body weight, average daily gain (ADG), feed intake (FI) and feed:gain (F:G) were assessed in each phase and for the whole experimental period. Xylanase and phytase recovery analysis were accessed to assure enzyme application and concentration in feed. Data were analyzed using ANOVA followed by Tukey test at 5% of significance level. Pen was considered the experimental unit.

Results

Results for the whole period are presented in Table 1. No significant effects were observed in final weight, ADG and FI for growing-finishing pigs fed standard corn-soybean based diet (T1) or the treatments receiving diets reformulated with Rovabio® Advance Phy T full matrix (T2) or added on top (T3). When multienzyme product was added 'on top' (T3), an improvement in F:G (P<0,05) was observed compared to reformulated diet with nutritional matrix (T2). However, no difference was observed between 'on top' and control groups.

Table 1. Performance results for growing-finishing pigs during full experimental period.

	Initial weight (kg)	Final weight (kg)	ADG (kg/day)	FI (kg/day)	F:G
T1	31.84	108.14	0.954	2.493	2.61^{ab}
T2	31.84	109.96	0.977	2.600	2.66^{b}
T3	31.84	109.71	0.973	2.484	2.55^{a}
		NS	NS	NS	*

NS= not significant difference; *different letters represent significant difference (P<0.05).

No significant differences were observed among treatments at phases 1, 2 and 3 for any performance variable. Nevertheless, in phase 4, animals fed standard corn and soybean meal diets reformulated with multienzyme matrix (T2) presented higher average daily gain (P<0.05), compared to control group (T1).

Discussion and Conclusion

Rovabio® Advance Phy was efficient in maintaining pigs' performance when used in feed formulation and assuming content of metabolizable energy, crude protein, digestible aminoacids, available phosphorus and calcium levels. Rovabio® Advance Phy was also efficient in improving average daily gain in full matrix application, leading to additional 120g/day during pig finishing phase, and compared to standard diet. These results show the potential of taking the multienzyme full nutrient matrix when feed formulating swine feed based on corn and soybean meal, as well as the potential of the multienzyme product to optimize pig performance and feeding costs.

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Effect of pellet quality on late finishing pigs performance

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Introduction

The use of pellet diets can improve the performance of pigs, being related to a higher average daily gain (ADG) and a better feed conversion (FC) (1, 2). However,it is necessary that the pellets remain intact and form a minimum of fines, which are a portion that disintegrates from the initial shape (3). In the qualitative technique of Pellets Durability Index (PDI), the amount of fine granules in pelleted is negatively correlated with the PDI (4). The objective of this work is to correlate the observed quality of the pellets with the performance of finishing pigs.

Materials and Methods

A total of 1200 piglets were housed in a farm with a standard structure for the region (Santa Catarina, Brazil). Immunocastrated boars and females were housed with initial body weight (BW) (117.5 ± 5.3 kg), distributed in 24 split-sex pens, 50 piglets per pen at 0.87 m^2/pig of stocking density in three experimental replications. Pens were subjective allotted in one of three pellet quality treatments: T1 (High quality pellets with a high level of integrability and without the presence of fine granules), T2 (Medium quality pellets, with the presence of up to 50% of fine granules) and T3 (Low quality pellets with more than 75% of fine granules not integral to the pellet). A mixed model was used with subjective class of pellet quality and sex as fixed effect and experimental replication as random effect, to report LSmeans of variables ADFI, ADG and FCR was used as covariate Initial BW in 117.5 kg (7), software Statistical Analysis System (SAS®).

Results

The pigs fed with a higher percentage of fine granules or less pellet integrability had lower feed intake and weight gain. Pellets graded in terms of quality as T1 and T2 did not negatively impact performance, however T3 demonstrated a significant loss in feed intake, 172 g of average daily feed intake (ADFI) and 151 g of average daily gain (ADG) comparing high quality T1 vs. lower quality T3 in swine aged 154 to 161 days, Table 1. In FCR, the difference between the three treatments is clear, highlighting the comparison between T1 and T3, the advantage of using high quality pellets reflected in 181 g of feed intake less for 1 kg of BW gain, (Table 1).

Discussion and Conclusion

The results demonstrate that the poor quality of pellets can affect the field performance of pigs. Corroborating results that observed competitive advantages for pelleting in performance that resulted in 5% higher daily weight gain, 7% lower feed conversion and increased dry matter digestibility from 5 to 8% (5).The beneficial results for the quality of the pellets or the use of the processing can be linked to several benefits already described in the literature such as: less waste, better palatability, reduction of the segregation of ingredients, decrease in selectivity by animals, decrease in feed intake time and increasing the digestibility of the diet (6).

Table 1. Performance of pigs using pellets of different integrability levels with higher, medium and low percentage of fine granules, aged between 154 and 161 days.

Item [1]	Treatments [2]			p value [3]
	T1	T2	T3	
Initial BW, kg	118.3	116.6	117.7	0.44
Final BW, kg	127.7	126.2	126.3	0.37
ADFI, kg	3.307 [b]	3.500 [a]	3.135 [c]	<.0001
ADG, kg	1.394 [a]	1.347 [a]	1.243 [b]	0.002
FCR, kg	2.426 [b]	2.609 [a]	2.607 [a]	0.03

[1]Item evaluated: ADFI - Average Daily Feed Intake; ADG - Average Daily Gain and FCR - Feed Conversion Ratio. [2] Pellets quality: T1 - Highest; T2 - Medium and T3 – Lowest. [3] Superscripts indicate statistically significant differences within pellet quality class ($p \leq 0.05$)

It is possible to conclude that the industry must be attentive not only to the implementation of new technologies, but must have expertise in the execution of processes. With this work we can analyze the relationship between the quality of feed processing and performance, the significant difference in the comparative treatments (T1 vs. T3), with advantages for T1 as higher ADFI and ADG. The use of pellets in swine nutrition can generate good results in the field, but the use of low quality pellets can worsen the results.

Acknowledgments

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Effect of the inclusion of ENERAT (Glycogenic precursor) in the daily diet during the lactation in sows of 1st farrowing.

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Introduction

The sows every day have a greater demand for the improvement of their production indexes, in the maternity area we can highlight in particular: the average weight of the weaned piglet and the Days from Weaning to the 1st Service. This requirement results in greater physical wear and is mainly reflected in 1st parity sows, so supplementation with glugenic precursors (ENERFAT) presents an opportunity for productive improvement.

Materials and Methods

This work was developed in La Joya Farm, located in Tenextepec, Puebla, Mexico. The farm has an average population of 3,000 sows and has a budget of 142 farrowings per week.

For the evaluation of Average Weight at Weaning, 4 large groups were worked on, each group made up of sows from 4 different weeks. Control of weeks 22-25, Treated (Enerfat) from weeks 26 -29.

For the evaluation of Days from Weaning to 1st Service, we worked with 4 large groups, each group made up of sows from 3 different weeks. Control of weeks 26-28, Treated (Enerfat) from weeks 29 -32

As of week 26, all 1st parity sows were added 10 grams of ENERFAT to the 1st meal of the day until weaning.

The evaluation of results will be done against previous groups with the information generated in PigKnows.

Results

The piglets weaned from the treated sows had 0.170 kg (2.6%) more than the piglets from untreated sows.

Table 1. difference in that of piglets at weaning between the group treated with ENERFAT and the control group.

Treatments	Number of piglets	Average weight kg	Difference kg	Piglet price, USD /Kg	Kg Profit per weight difference, USD
4 group/ Control	1,040	6,640	0.170	5.0	$884.00
4 group/ Enerfat	1,040	6,470			

The return to heat (days) after weaning in the sows treated (75) with ENERFAT was 5.9 days on average, while the control sows (75) was 9.7 days on average, the difference was 3.8 days between both groups, which means a total of 285 extra DNP in total between both groups.

Table 2. Difference in Days from weaning to 1st service between the group treated with ENERFAT and the control group

Treatments	Number of treated sows	Days from weaning to 1st service	Difference, days	Total days of the group	Opportunity value per day USD	Total opportunity value DNP reduction, USD
3 control	75	9.7	3.8	285	$ 4.10	$ 1, 169
3 ENERFAT	75	5.9				

With a sale price of $5.00 USD, they generated an income of $884 USD, the cost of the treatment in the 92 sows was $92 USD having a return on investment of 8.6 times.

Table 3. Return of investment in that of piglets at weaning between the group treated with ENERFAT and the control group

Sows #	Costo f inclusión, USD	Total cost of tratments, USD	Difference, USD	Return on investment
92	$1.00	$92.00	$792.00	8.6

In La Joya farm there is an average production of 30 weaned piglets / female / year, which means that each DNP the sow can produce 0.082 piglets, with an average cost per weaned piglet of $50 USD, a DNP has an opportunity value of $4.10 USD.

Table 4. Return of investment in that treatment in Days from weaning to 1st service between the group treated with ENERFAT and the control group

Treated sows	Cost of inclusion, by gilth, USD	Total cost, USD	Benefit, USD	Return on investment
75	$1.00	$75.00	$ 1,094.00	15

Discussion and Conclusion

The use of glycogen precursors[1] (ENERFAT) as a daily supplemental energy source during lactation of gilts generated a higher weight of weaned piglets and had fewer days between weaning and 1st service. Generating a return on investment of 8.6 times on investment for the weight at weaning and 15 of return on investment in relation to days from weaning to 1st Service.The inclusion of ENERFAT (glucogenic precursor) as an additional source of energy improves the weight at weaning of piglets, as well as reduces the days from weaning to 1st service, so this nutritional strategy can be implemented to generate savings in the growth of pigs , as well as improvement in productivityin female births year of the farm

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Effect of zinc on growth performance and health of low and normal birth weight piglets after weaning

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Introduction

Diarrhea is common in weaned piglets. Feed supplementation with pharmacological levels of zinc oxide (ZnO) is one of the strategies used to prevent such disturbances. A total ban on the usage of therapeutic doses of ZnO will be implemented in the European Union from June 2022; hence, the evaluation of different sources of Zinc (Zn) that can be as effective at lower inclusion is essential and challenging. The objective of this trial was to verify the effect of replacing $ZnSO_4$ with a potentiated source of Zn at EU authorized level on performance and health in low and normal birth weight piglets after weaning.

Materials and Methods

A total of 64 piglets with low and normal birth weight (LBW = 0.92 kg and NBW = 1.37 kg, respectively) were weaned at 25 days of age (LBW: 6.29 kg; NBW: 7.78 kg) and allotted to 4 treatments arranged in a 2x2 factorial design into 8 replicates per treatment (2 piglets). Treatments consisted of two birth weights (LBW and NBW) and two zinc sources supplemented at 120 mg/kg: a $ZnSO_4$ and a potentiated Zn source (pZn; HiZox®, Animine, France). Diets were based on barley, soybean meal, corn and wheat and contained 750 FTU of phytase. The experiment lasted 21 days. Piglets were weighted individually at the start of the experiment and on days (d) 7, 9, 14, and 21; feed intake was recorded during the whole experiment. Fecal score (1 = hard feces, 5= watery feces; 3 is the diarrhea cutoff) was evaluated daily.

Results

Overall, piglets remained healthy, and the average fecal score was below 3. During the period between d4 and d9 (acute phase), there was an increase on fecal score. No effect of the treatments (P>0.05) on the fecal score was observed for each period; however, piglets fed pZn treatment had lower score than those fed $ZnSO_4$ (Figure 1).

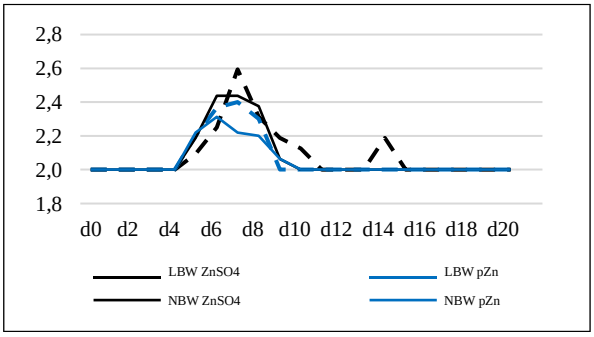

Figure 1. Effect of zinc supplementation on the fecal score of post weaning piglets (based on a scale of 5 points: 1 hard feces – 5 watery feces).

The Zn source did not affect the feed intake of the animals, but the pZn treatment tended to increase the BW at d14 post-weaning (Table 1). Furthermore, the pZn improved in 3% the G:F ratio early post-weaning and in the whole period of the trial (P<0.05).

Table 1. Effect of zinc supplementation on the performance of post-weaning piglets.

	$ZnSO_4$		pZn		*P*-value	
	LBW	NBW	LBW	NBW	Diet	BW
BW d0, kg	6.36[a]	7.84[b]	6.34[a]	7.82[b]	0.93	<0.05
0-14d						
BW, kg	8.18[a]	9.98[b]	8.80[a]	10.6[b]	0.09	<0.05
ADG, g/d	139	165	168	194	0.13	0.16
FI, g/d	198[a]	233[b]	203[a]	239[b]	0.70	0.01
G:F	0.69[a]	0.67[a]	0.82[b]	0.80[b]	0.02	0.75
0-21d						
BW, kg	11.1[a]	13.2[b]	11.7[a]	13.7[b]	0.20	<0.05
ADG, g/d	234	261	251	278	0.32	0.10
FI, g/d	328[a]	376[b]	334[a]	382[b]	0.72	0.01
G:F	0.71[a]	0.69[a]	0.75[b]	0.73[b]	0.04	0.26

Discussion and Conclusion

No data are reported on the effect of replacing $ZnSO_4$ with the pZnO at authorized level on pig performance. Our data shown that piglets fed with pZnO had a better feed efficiency than those supplemented with $ZnSO_4$. This observation is partially explained by the lower fecal score of animals fed pZnO, and may be related to the better digestive function due to a positive effect on the gut health status of these pigs. In our previous study testing this potentiated Zn source for weaned piglets challenged with ETEC (Trevisi et al., 2014), we observed that the pZn at 300 mg/kg showed betterweight gain than NC and similar to ZnO at 3000 mg/kg.Similar outcomes were observed also by Wang et al. (2018) the potentiated Zn source at a dose of 220 mg/kgincreased the ADG compared to the negative control, being equivalent to ZnO at 3000 mg/kg. Furthermore, the number of diarrhea days was lower for pZn (1.3) compared to negative control (4.0) and ZnO at 3000 mg/kg (3.0). The better modulation of the intestinal microbiota profile (Vahjen et al., 2016) and improved intestinal morphology (Peng et al., 2019) as well as the reduction of the oxidative status of piglets can contribute to explain the efficacy of pZn in replacing ZnO at pharmacological.

To conclude, piglets fed a potentiated source of ZnO perform better when compared with $ZnSO_4$ and may have an improved gut health.

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Effects of a mixture silage on finishing pigs' intestinal microflora populations

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Introduction

Currently the European Union has set ambitious targets concerning the reduction of microbial resistance. To achieve this, reduction of antibiotic useis necessary as well as development of alternative farm animal feeding strategies, which can improve animal health status. Today, some agro-industrial by-products that are often considered waste products andpollutants could be used as bioactive animal feedingredients.

In this dietary experiment a mixture silage which was constituted by olive mill waster water, grapepomace and deproteinized cheese whey was used in finishing pig diets in order to investigate their intestinal microflora populations

Materials and Methods

All experimental procedures were in accordance with the National guidelines for animal trials (1).

18 crossbreed finishing pigs 120 days old were individually ear-tagged and allocated to 3 treatments (control-0%, 5% and 10% inclusion of silage). The experimental period lasted 60 days (from the start of the fattening phase until slaughter at 160 days). Microbiological analysis of intestinal digesta was performed in fresh digesta samples from jejunum and caecum that were collected during slaughter (6 animals per treatment).

Total aerobic and anaerobic bacteria counts were determined using standard plate count method. For *Enterobacteriaceae*, *Enterococci*, *Bifidobacteria* enumeration and isolation MacConkey agar, Kanamycin aesculin azide (KAA) agar and Transoligosaccharide propionate agar medium (TOS) supplemented with glacial acetic acid and mupirocin were used respectively. Typical colonies from an appropriate dilution were counted and were expressedas Log_{10} cfu / 1 g of digesta. In addition, typical colonies grown on media were identified by Bruker MALDI Biotyper (Bruker Daltonics) and their mass spectra were processed using the MALDI Biotyper 3.0 software package (Bruker, Leipzig, Germany).

The collected data was subjected to one-way ANOVA, using the IBM SPSS Statistics v. 20.0 Statistical Package (SPSS Inc., Chicago, IL, USA). Data homogeneity was tested using Levene's test. Significance was set at 5% ($P<0.05$).

Results

Table 1 presents the effects of the dietary silage on the intestinal bacterial populations. In the ileum digesta itwas noted that: *Enterobacteriaceae* were lower($P<0.001$) in treatment 10%, compared to the other two treatments; *Enterococci* where lower ($P<0.001$) in the 10% treatment, intermediate in the 5% treatment and higher in controls. *Lactobacilli* were higher ($P<0.001$) in both experimental treatments compared to the controls. Aerobes, anaerobes and *Bifidobacteria*did not differ significantly ($P\geq0.05$). Furthermore, in the cecum digesta it was found that: Aerobes were higher ($P<0.05$) in the 10% treatment compared to controls; *Enterococci* were lower ($P<0.001$) in the 5%,treatment, moderate in the 10% treatment and higher in the controls; anaerobes, *Enterobacteriaceae*, *Lactobacilli* and *Bifidobacteria* did not differ significantly ($P\geq0.05$).

Table 1. Effect of silage on finishing pigs intestinal microflora populations

	Silage inclusion				
	0%	5%	10%	SEM	P
Ileum, Log cfu/g					
Aerobes	9.097	8.773	8.164	0.1678	0.060
Anaerobes	8.802	8.879	8.553	0.1111	0.484
Enterobacteriaceae	4.964 ^{b}	5.146 ^{b}	3.865 ^{a}	0.1713	<0.001
Enterococci	5.967 ^{c}	4.590 ^{b}	3.469 ^{a}	0.2728	<0.001
Lactobacilli	6.674 ^{a}	8.166 ^{b}	9.008 ^{b}	0.2686	<0.001
Bifidobacteria	6.048	5.821	5.915	0.1055	0.703
Cecum, Log cfu/g					
Aerobes	9.194 ^{ab}	8.966 ^{a}	9.484 ^{b}	0.0865	0.038
Anaerobes	9.247	8.905	9.017	0.0947	0.344
Enterobacteriaceae	5.376	5.460	4.873	0.1251	0.112
Enterococci	7.006 ^{c}	3.750 ^{a}	4.743 ^{b}	0.3408	<0.001
Lactobacilli	8.749	9.641	9.459	0.1782	0.090
Bifidobacteria	6.023	6.279	6.093	0.1073	0.632

^{a,b,c} Values with no common superscript differ significantly ($P\leq0.05$).

Conclusions and Discussion

Our results are in accordance with previous studies reporting that the *Lactobacillus* populations were increased due to the presence of high amounts of lactose in the feed (2). It has also been reported that the dietary use of grape pomace inhibits the growth of *Enterobacteriaceae* species in the gut (3).

In conclution it seems that agro-industrial by- products with bioactive compounds can be used in finishing pigs' diets, affecting the gut microbial populations, thus improving the health status of the finishing pigs.

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Effects of maternal plane of nutrition on gastrointestinal morpho physiology in different birth weight piglets

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Introduction

There is evidence that litter birth and weaning weights may be improved by feeding an increased amount of feed during late gestation (90 days onwards), practice known as bump feeding, which enhances nutrient availability for fast-growing fetuses (1). We have recently shown that increased feed allowance during late gestation supported a higher nutrient availability for the offspring of hyperprolific sows (2). However, it does not reduce the proportion of low birthweight (LW) piglets, which are still a challenge in commercial farms. Growth performance of LW pigs is not always improved by additional nutrient provision (3), which suggests that physiological disturbances may impair optimal response of LBW to nutrient availability compared to high birth weight (HW) pigs. In the present study, it was hypothesized that LBW piglets would show impaired development and physiology, which would beattenuated by a bump feeding plane of nutrition.

Materials and Methods

A total of 15 out of a population of 135 gestating sows (Landrace females × York-shire boars; DB Brazil, Patos de Minas, MG, Brazil) with a BW of 225.2 ± 19.8 kg were included in the trial during parities 3 and 4. They were randomly placed on trial over 3 blocks using body weight as selection criteria, and were assigned to one of three planes of nutrition during parities 3 and 4, as follows: Req – plane designed to meet requirements of prolific sows (2.3 kg per day from day 1 to 21; 1.8 kg per day from day 22 to 75; 2.3 kg per day from day 76 to farrowing); Bump – plane designed as the Req, with increased feed intake during late gestation (3.0 kg per day from day 91 to farrowing); and Maintenance – plane designed to closely meet maintenance requirements of sows (1.8 kg per day from day 1 to farrowing). All treatments were fed the same gestation diet. After farrowing, 15 pairs of littermate male piglets (HBW and LBW) were euthanized immediately after birth and samples from the small intestine and liver were collected for histomorphometrical and enzyme activity analyses. Data were analyzed using ANOVA, differences between means were determined using the Tukey post-hoc test and considered significant at $P \leq 0.05$. The piglet was considered the experimental unit for all data analyzed

Results

The main results are summarized in Table 1. Plane of nutrition affected liver weight and hepatocyte area, which were smaller in the Maintenance group (P<0.05). Even though the small intestine morphology was not affected by maternal plane of nutrition, birthweight influenced villus width, which was lower in LBW animals (P<0.05). Piglets from sows in the Maintenance group showed lower lactase levels in the duodenum and lower glucose levels (P<0.05).

Discussion and Conclusion

Taken together, the present study sheds light on the independent negative effects of maternal nutrient restriction during gestation and low birth weight on offspring measurements, apparently through impaired hepatic and intestinal development and functionality. Furthermore, a maternal plane of nutrition close to maintenance requirements during gestation negatively affected lactase activity in the offspring and glucose blood content in LBW pigs. Finally, increased nutrient allowance in late gestation may be required to support accelerated gastrointestinal development in HBW piglets.

Table 1. Effects of plane of nutrition during gestation on liver morphology, lactase activity and plasma glucose levels

Parameter	Req	Bump	Maint
Liver weight, g	32 ± 10[a]	32± 10[a]	26± 10[b]
Hepatocyte area, µm	228 ± 16[a]	218± 16[ab]	208± 16[b]
Lactase, U/mg	343 ± 65[ab]	446± 65[a]	207± 65[b]
Glucose, mmol/L	51 ± 4[a]	52 ± 4[a]	43 ± 4[b]

[ab] Within a row, LSMeans with different superscripts differ (P<0.05).

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DB Genética Suínaq, Fapemig, CNPq and Capes

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Efficiency of a Protein Energy Supplement and an Acidifier on the intestinal health of nursery piglets

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Introduction

The transition between weaning and the introduction of solid feed in piglets is considered a critical period, as it often results in stress, temporary fasting and low feed intake. Consequently, piglets are predisposed to poor digestion and absorption, colonization by intestinal pathogens, poor performance and diarrhea. In order to minimize these impacts, recent studies have highlighted that the use of acidifiers and energy and protein sources via drinking water constitute important post-weaning strategies with the objective of stimulating food consumption, improving animal performance and reducing mortality and morbidity at this stage. Therefore, the present study aimed to evaluate the efficiency of the use of a Protein Energy Supplement (PES) and an Acidifier used continuously via drinking water on morphometric and morphological intestinal parameters and microbiota of piglets in the nursery phase.

Materials and Methods

Thirty-two piglets from a commercial farm were evaluated. The average of initial weight was 6.250 ± 0.351 kg and the animals were weaned at 27-days-old. A completely randomized design was used and the animals were divided into three treatments: T1 (n=8) Control, without inclusion; T2 (n=16) PES; T3 (n=8) PES + Acidifier.

The PES was composed by energy and protein source, symbiotic, organic acids and palatability additives. It was used in drinking water (0.2% solution in the 1st and on the 2nd day ad libitum administered via automatic drinker + 150 mL of 3% solution administered in the auxiliary trough on the 1st day). The Acidifier was a mixture of citric (13 g/kg), ascorbic (450 mg/kg), phosphoric acids (773,5 g/kg) and monosodium phosphate (400,000 mg/kg) used in drinking water and administered through drinking fountains from the 1st to the 35th day of housing (initial pH of water: 7.5 to 8.0; final pH of water: 4.0).

The Feces were collected on the 35th day and formed a pool for metagenomics analyze. On the 36th day, all animals were sacrificed in a slaughterhouse and the jejunum was collected for morphological and morphometric evaluation. Through the histological findings, the intestinal impact factor (FII) was calculated, according to Kraieski (1). To calculate the intestinal absorption index (IAI), the methodology proposed by Kisieinski (2) was used. All results were analyzed using descriptive statistics (mean and coefficient of variation), analysis of variance (ANOVA) and Duncan's Test as a Post Roc, at a significance level of 0.05%.

Results

Table 1 shows the results related to histomorphometric evaluations. It was observed that animals in groups T2 and T3 had superior performance for all parameters evaluated ($p<0.05$). These results reflected in the intestinal absorption index, which was higher in both groups. In the morphological evaluation of the jejunum,it was evidenced that the animals in the control group had a higher incidence of histological changes (edema, inflammatory infiltrate, congestion and desquamation of the epithelium), which had a negative impact on intestinal integrity. In the metagenomic analysis, the presence of a greater proportion of microorganisms with probiotic function was verified in the animals of the groups treated with PES and with the Acidifier (T2 and T3), such as *Pediococcus pentosaceus* (T1 - 22.94%, T2 - 18 .37%) *Lactobacillus* spp. (T1 - 4.97%; T2 - 12.3%) and *Streptococcus macedonicus* (T1 - 24.54%; T2 - 25.83%). In T1, the highest prevalences were *Clostridium* spp. (22.68%) and *Methanobrevibacter boviskoreani* (12.23%).

Table 1 - Effect of experimental diets on intestinal morphometry in nursery piglets.

Variables	T1	T2	T3	P Value
VH (µm)	0,254[a]	0,479[b]	0,537[c]	<0,0001
CD (µm)	0,062[a]	0,052[b]	0,049[b]	<0,0001
VW (µm)	0,146[a]	0,137[b]	0,116[c]	<0,0001
WT (µm)	0,730[a]	0,787[b]	0,793[b]	0,0490
V/C	4,050[a]	10,017[b]	11,250[c]	<0,0001
IIF	22,25	7,00	5,25	-
IAI	2,820	6,541	8,598	-

VH: villus height; CD: crypts depth; VW: villus width; WT: Wall thickness; V/C: Villus/crypt relationship; IIF: Intestinal Impact Factor; IAI: Intestinal Absorption Index. *Means on the same line followed by the same letters do not differ statistically from each other ($p>0.05$).

Discussion and Conclusion

There was a positive impact of both products on all variables related to gut health. It is noteworthy that the combined use of SEP and Acidifier promoted evenbetter results. The benefit of the use of acidifiers and protein energy supplementation on intestinal health hasalready been reported by other studies (3,4). However, the effects of the combined use of these two product categories are unprecedented and very promising for swine farming.

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Evaluation of a homeopathic natural additive added to the diet and its effects on the performance and occurrence of diarrhea of newly-weaned piglets

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Introduction

In order to improve performance and health, antibiotics have been used as growth performance in animal production, however, their misuse can cause bacterial resistance (1,2). The use of homeopathy in animal science has already obtained positive results on animal health, even so, it has not been fully clarified (3,4). Thus, the objective of this study was to evaluate a homeopathic product, replacing an antimicrobial growth performance in the diets of piglets, on performance and occurrence of diarrhea.

Materials and Methods

The experiment involved 126 animals, with an initial weight of 5,62 ± 1,16 kg, which were allotted to six treatments in a completely randomized block design with seven replicates and 3 piglets per experimental unit. The treatments were: T1 - Negative control (without any additive); T2 - Positive control (120 mg/kg of chlorohydroxyquinoline); T3 - 4,5 kg/ton of Homeosuis Crecheplus® in the feed; T4 - 6,0 kg/ton of Homeosuis Crecheplus® in the feed; T5 - 7,5 kg/ton Homeosuis Crecheplus® in the feed; T6 - 9,0 kg/ton Homeosuis Crecheplus® in the feed. In order to achieve the average daily gain (ADG), average daily feed intake (ADFI), and feed conversion rate (FCR), the weight of piglets was registered on days 1, 7, 21and 35 of the study. Also, the occurrence of diarrhea was recorded daily during the experiment. The diarrhea data was divided into three periods: 1 to 7, 1 to 21 and1 to 35 days of study. Performance data were analyzed using general linear models considering T1, T3, T4, T5and T6. The positive control was compared with each treatment using Dunnett's test (p<0.05). The percentage of occurrence of diarrhea (OD) was compared using the Chi-square test, considering p<0.05.

Results

ADFI was statistically lower for animals from T6 than for T2 (1 to 7 d), and for animals from T3 than T2 (1 to 21 d) (p<0,05). The Body Weight (BW 7 d) of the animals declined linearly as the levels of homeopathic in the diet were increased. Regarding the OD, the first level of homeopathic product (T3) showed better results, similar to antimicrobial treatment (T2) (Table 1).

Discussion and Conclusion

The lower ADFI (1 to 7 d) in T6 negatively influenced the body weight (7 d). This event may have been caused by a change in the palatability of the feed, since it did not occur with the positive or negative control (5).

Table 1 Mean of performance and occurrence of diarrhea (%) of weaned piglets supplemented with homeopathic product

	TREATMENTS					
ITEM	T1	T2	T3	T4	T5	T6
d 1 to 7						
BW†	7,24	7,30	6,41	6,47	6,55	6,14
ADG (kg)	0,15	0,18	0,13	0,14	0,17	0,14
ADFI (kg)	0,18	0,20	0,18	0,17	0,18	0,16*
FCR	1,16	1,13	1,33	1,20	1,19	1,31
OD (%)	6,52^{b}	6,38^{b}	4,35^{b}	10,42^{a}	9,09^{ab}	9,09^{ab}
d 1 to 21						
BW	12,56	12,25	11,60	11,53	11,50	11,17
ADG (kg)	0,30	0,30	0,27	0,29	0,29	0,29
ADFI (kg)	0,38	0,40	0,34**	0,38	0,37	0,36
FCR	1,27	1,40	1,41	1,33	1,33	1,30
OD (%)	3,01^{b}	3,82^{b}	2,31^{b}	5,11^{a}	4,62^{ab}	3,79^{b}
d 1 to 35						
BW	21,58	21,85	21,11	20,28	20,27	20,31
ADG (kg)	0,44	0,45	0,44	0,42	0,43	0,43
ADFI (kg)	0,64	0,66	0,62	0,62	0,62	0,61
FCR	1,45	1,47	1,47	1,49	1,46	1,42
OD (%)	1,79^{b}	2,23^{ab}	1,79^{b}	2,99^{a}	3,13^{a}	2,26^{ab}

*Significant difference by Dunnett's test (p<0,05) - T2 *vs.* T6. **Significant difference by Dunnett's test (p<0,05) - T2 *vs.* T3. ^{a,b}Different letters within a row are statistically different by Chi-square test (p<0.05). †Linear effect (p = 0,0089) of the homeopathic levels in the diet on the BW 7 d (y= -0.1080x + 7,1461; R^2=0,65).

The results suggest that the homeopathic can replace the antimicrobial in the diet of weaned piglets,however, in lower levels. Further researches are neededto know the influence of the product on the palatability of the feed and the consequences in the weaned piglets performance.

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Feed additives supplemented to suckling piglets via drench and milk replacer improved daily weight gain

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Introduction

Newborn piglets are challenged due to missing immunity until first colostrum intake, high energy demand and intestinal disorders because of pathogen load (1). A targeted support of suckling piglets with dietary feed supplements (DFS) based on colostrum, energy, pre- and probiotics leads to a higher performance until weaning (2). Concepts based on controlled oral application and additional voluntary intake of DFS are interesting concerning work management and an effective support for the animals. Therefore, the objective of the present study was to evaluate the combination of two products for suckling piglets via oral application (paste) and voluntary intake (powder).

Materials and Methods

268 suckling piglets (Duroc) averaging 1.74kg of birth weight, from 32 sows (ø parity 2.45) were observed in a commercial sow farm in Spain. Treated piglets (n=123, $\bar{x}$=1.50kg of birth weight) received 0.5 g/ day of a powder product mixed into milk replacer until day five and following with prestarter day six until 15. The product is based on immunoglobulins, probiotics, yeast cell walls and egg powder (Bimulac® Pre - Biochem Zusatzstoffe GmbH, Lohne, Germany). Low birth weight piglets (< 1kg of birth weight) were supplemented additionally with 2 ml of a paste product based on immunoglobulins, probiotics, medium chain triglycerides, vitamins (A, E, C and B_{12}) and organic minerals (Zn, Mn, Cu and Se) (Piglet Protector® - Biochem Zusatzstoffe GmbH, Lohne, Germany) orally after first colostrum intake, and with a second dose within 24 hours. Control piglets (n=145, $\bar{x}$=1.94kg of birth weight) were not treated. Individual birth and weaning weight were documented. Data of daily weight gain were subjected to statistical analysis using a non-parametric test (Mann-Whitney-U, SPSS Vers. 24).

Results

The treated piglets presented numerically higher average body weight at weaning than the control piglets (6.87kg vs. 6.45kg, respectively), and higher daily weight gain than the control group (0.195kg; SD ±0.038 vs. 0.179kg SD± 0.045; p=0.006) (Figure 1).

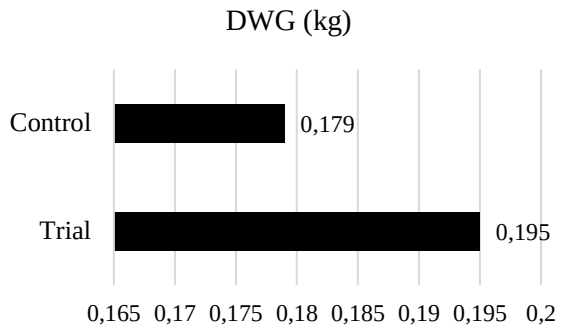

Figure 1. Daily weight gain (DWG) of piglets supplemented with Bimulac® Pre and Piglet Protector® compared to control group (p=0.006).

Discussion and Conclusion

The present study showed that the combined support from the feed additives (Piglet Protector® and Bimulac® Pre) can be one alternative to increase viability and performance of piglets, according to the improvement of almost 10% in daily weight gain in suckling piglets.

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Field Brazilian study of an injectable minerals formulation on lactational catabolism and reproductive performances of high prolific sows

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Introduction

An increased systemic oxidative stress is described during late gestation and lactation, due to high energy demand, particularly in highly prolific sows, which can impair fertility (1). Among trace minerals, selenium and copper are known as antioxidants (1, 2). This study was done to assess the effects of an injectable mineral formulation on catabolism of hyperprolific sows during lactation and reproductive performances.

Materials and Methods

The selected commercial farm was located in the state of Ceara (Brazil) and owned 2500 sows of a commercial genetic line (Topigs TN70). A total of 244 females were randomly allocated to 4 groups according to body weight, backfat thickness (ultrasound measure: Preg-Tone, Renco®), body condition score (Caliper measure) and parity class (gilts/P1/P2-P3/P4-P6 sows). The gilts were included on the first day of a flushing period of 15 days before expected estrus. The sows were included at weaning. All females received 2 intramuscular injections (5 mL each) of either the tested or a control product, respectively at inclusion and at 100 days of pregnancy. The tested product contains phosphorus, potassium, magnesium, copper and selenium (Fosfosal®, Virbac) and the control product was a saline serum (Table 1).

Table 1. Treatments group design

	1st injection: C	1st injection: F
2nd injection: C	Group CC	Group FC
2nd injection: F	Group CF	Group FF

F: Fosfosal®. C: Control product (saline serum).

Body weight, backfat thickness and body condition were measured per sow at inclusion, at 100 days of pregnancy, at farrowing and subsequent weaning. Estrus was detected after the 1st and 2nd weanings. The body protein and lipid losses between farrowing and weaning were estimated from body weight and backfat thickness at these 2 stages, according to previously reported formulas (3). Piglets issued from each sow were counted and weighed per litter at birth, 48h after birth and at weaning. Numerical data were compared between groups by analysis of variance followed by post hoc Tukey test when applicable, and categorical data by the Chi-square test (statistical significance level: $p < 0.05$).

Results

Among the 244 included females, 70 were gilts and 174 were sows (no significant differences of mean parities between groups). No general nor local side effects were noticed after injection of the tested product or saline serum. A total of 14 gilts and 13 sows were excluded after the 1st injection due to either late estrus detection or return to estrus. The gilts late estrus rate was numerically lower in the FF group than in the CC group but the difference was not statistically significant. Backfat thickness and body condition score were not significantly different between groups. However mean total body weight loss and estimated protein loss between farrowing and weaning were significantly lower in the FF group than in the CC group, the estimated mean lipid loss being numerically lower in the FF group than in the CC group ($p=0.064$). The mean numbers of born alive and weaned piglets per litter were not different between groups. The mean litter weight at weaning was numerically higher in the FF group than in the CC group (+3.7 kg). The mean wean to estrus delay after the 2 injections was significantly lower for sows having received at least 1 injection of the tested product at the previous weaning (FC or FF) than for sows injected twice the saline serum (Table 2).

Table 2. Metabolism and reproductive mean criteria

Group	CC	CF	FC	FF
Gilts late estrus (%)	22.2	30.0	17.6	6.7
Weight loss (kg)[1]	44.6[a]	42.1[ab]	39.7[ab]	36.3[b]
Weight loss (%)[1]	17.0[a]	16.2[ab]	15.5[ab]	14.0[b]
Protein loss (kg)[1]	7.2[a]	6.8[ab]	6.4[ab]	5.8[b]
Lipid loss (kg)[1]	13.9	13.0	12.6	11.9
Born alive[2]	14.7	14.8	14.6	14.9
Weaned[2]	12.2	12.2	12.1	12.2
Weaning weight (kg)[2]	81.2	81.6	83.1	84.9
Wean to estrus delay (d) [3]	6.5[a]	5.9[ab]	4.0[b]	4.0[b]

[1]Between farrowing and weaning. [2]Per litter. [3]At study end.
[a,b]Values with different superscripts in the same row differ significantly ($p < 0.05$)

Discussion and Conclusion

This study indicates a beneficial effect of the tested product decreasing weight loss during lactation as well as reducing the return to estrus interval after weaning, especially when it was injected twice during the sow´s cycle (at weaning and before farrowing). Further investigations are necessary to clarify how this product is involved in reducing the impact of oxidative stress.

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Growth performance and lysine intake of 25 – 40 kg growing pigs using precision feeding strategies: A systematic review

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Introduction

New studies have been published over the years suggesting the use of precision feeding as a new approach to determine pigs' requirements, reducing the waste of nutrients provided. Therefore, the main aspect of precision feeding strategies is to provide to individual (1) or group (2) of pigs more flexible diets, reducing the nutrient concentration over time. However, there has been little discussion on the impacts of precision feeding on growth performance of pigs, especially during the first phase of growth in which pigs still do not have the maximal feed intake and growth potential. Therefore, the research aimed to determine and summarize how precision feeding impacts growth performance and lysine of pigs averaging 25 to 40 kg.

Materials and Methods

A total of 45 studies were retrieved from the search, however, after the deletion of articles that did not fulfill the criteria for inclusion, only six studies (published between 2016 and 2019) remained to perform the systematic review. The online database "Web of Science" was used to search relevant publications of growth performance and lysine intake of growing pigs using precision feeding strategies. The keywords used to perform each search were: ("swine" or "pig" or "pigs") and ("precision feeding" or "precision nutrition") and ("conventional phase feeding"). Once all publications have been collected; only those that met the following criteria were maintained: a) in vivo studies using pigs comparing precision feeding strategy with conventional phased feeding; b) published in English; c) reported growth performance results, average daily gain (ADG; g/day); average daily feed intake (ADFI; g/day); feed to gain or feed conversion efficiency or gain to feed ratio; d) report sample variance (SD or SEM); sample size (n); age; sex of pigs and duration of the study. All recorded feed conversion metrics were converted to feed efficiency (EF) in order to be compared across experiments. The Review Manager 5 (RevMan) software was used to conduct the meta-analysis and perform the forest plots.

Results

The forest plot is shown in Figure 1 with the results of the parameters analyzed in the meta-analysis, considering the mean differences for each variable and 95% confidence intervals. The ADFI was increased by 126,9 g for pigs in precision feeding strategy, without changes on ADG. The EF slightly reduced (by 0.01%) for precision fed pigs, however, the most remarkable result is the 2.82 lysine intake reduction for precision fed pigs.

Discussion and Conclusion

Given that the findings about precision feeding are based on a limited number of studies, some changes in these results are expected over the next years. Although the precision-fed pigs increased ADFI, it is important to highlight the adequate nutrient composition of the diets provided. Reduced nutrient composition of diets is correlated with lower nutritional costs once more expensive ingredients are replaced by less expensive ingredients (e.g., corn). Therefore, the reduction of SIDLys intake evidenced for the precision-fed pigs is of great interest for pig producers, especially because moreAAs also may be reduced with SID Lys.

We can conclude that the precision feeding strategy can reduce the use of expensive ingredients such as soybean meal and synthetic amino acids without impacting the ADG of pigs.

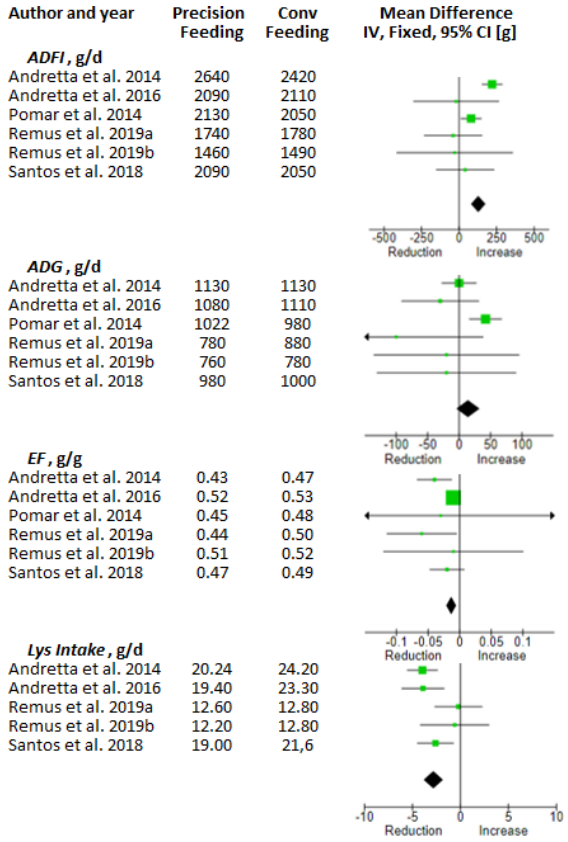

Figure 1. Forest plot of the means and confidenceinterval of precision feeding effects on ADFI, ADG, EFand lysine intake of pigs.

Acknowledgments

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Gut microbiota of weaned piglets fed a novel silage produced by olive oil, winery, and cheese wastes

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Introduction

The increasing future demand for livestock products, driven by global population growth and consequent urbanization, will impose an increasing shortage of available feed resources. The efficient andsustainable livestock development should include the reduction of the wastage and the enlargement of the feed resource base. Such tasks can be achieved through development of novel animal feeds,particularly those not competing with human foods.

In this trial, a novel silage from olive, winery, and cheese waste by-products was fed to 34 day old weaned piglets in a commercial farm in Greece in order to investigate their intestinal microflora populations.

Materials and Methods

All experimental procedures were in accordance with the National guidelines for animal trials (1).

45 crossbreed piglets were individually ear-taggedand allocated to 3 treatments (control, 5% and 10% inclusion of silage). The experimental period lasted 40 days (from days 34 to 74 of age). Microbiological analysis of intestinal digesta was performed in fresh digesta samples from jejunum and caecum that were collected during slaughter (6 animals per treatment).

Total aerobic and anaerobic bacteria counts were determined using standard plate count method. For *Enterobacteriaceae*, *Enterococci*, *Bifidobacteria* enumeration and isolation MacConkey agar, Kanamycin aesculin azide (KAA) agar and Transoligosaccharide propionate agar medium (TOS) supplemented with glacial acetic acid and mupirocin were used respectively. Typical colonies from an appropriate dilution were counted and were expressed as Log cfu / 1 g wet weight sample. In addition, typical colonies grown on media were identified by Bruker MALDI Biotyper (Bruker Daltonics) and their mass spectra were processed using the MALDI Biotyper 3.0 software package (Bruker, Leipzig, Germany).

The collected data was subjected to one-way ANOVA, using the IBM SPSS Statistics v. 20.0 Statistical Package (SPSS Inc., Chicago, IL, USA). Data homogeneity was tested using Levene's test. Significance was set at 5% ($P<0.05$).

Results

Intestinal microflora populations were affected bythe dietary use of silage (Table 1). In the ileum digestait was noted that anaerobic bacteria, counted in PCA, were increased ($P\leq0.05$) in treatment 10%, compared to treatment 5%, whereas *Lactobacilli* ($P\leq0.001$) were increased in treatments 0% and 10% compared to treatment 5%. In the caecum digesta, anaerobic bacteria were lower ($P\leq0.001$) in treatments 5% and 10% compared to the control treatment 0%, while *Lactobacilli* were lowest ($P\leq0.001$) in treatment 10%,intermediate in treatment 5% and highest in treatment 0%. The other evaluated microflora populations (Aerobic bacteria count, Enterobacteriaceae, *Enterococci*, *Bifidobacteria*) did not differ between the three treatments.

Table 1. Effect of silage on piglet intestinal microflora populations

	Silage inclusion				
	0%	5%	10%	SEM	P
Ileum, Log cfu/g					
Aerobes	5.83	6.47	6.44	0.19	0.30
Anaerobes	7.14^{ab}	6.31^{a}	7.60^{b}	0.20	0.02
Enterobacteriaceae	5.15	4.26	5.35	0.33	0.39
Enterococci	0.88	0.00	2.10	0.46	0.16
Lactobacilli	7.00^{b}	6.09^{a}	7.56^{b}	0.18	≤0.001
Bifidobacteria	2.99	0.62	1.15	0.43	0.05
Cecum, Log cfu/g					
Aerobes	8.90	8.01	7.81	0.32	0.37
Anaerobes	10.17^{b}	8.08^{a}	7.36^{a}	0.35	≤0.001
Enterobacteriaceae	5.01	4.17	5.46	0.32	0.25
Enterococci	2.33	0.00	1.98	0.52	0.13
Lactobacilli	10.88^{c}	9.79^{b}	8.12^{a}	0.29	≤0.001
Bifidobacteria	3.53	1.43	2.27	0.44	0.14

[a,b,c] Values with no common superscript differ significantly ($P\leq0.05$).

Conclusions and Discussion

To our knowledge, silage created with olive oil wastewater, grape pomace and deproteinized cheese whey has never been used in pig diets. In this experimental trial, gut microbial populations were affected in weaned piglets, that were fed with 5% or 10% of the tested novel silage.

Based on these results, the use of silage from by- products of the Greek agro-industry sector had an impact on anaerobic bacteria, both in ileum and cecum,as well as in *Lactobacilli* counts, thereby affecting thegut microbial populations of weaned piglets.

Acknowledgments

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Hemicell HT - a new beta-mannanase enzyme - combined with an *E. coli* F4/F18 vaccination retains post-weaned piglet performance in the presence of challenging protein sources

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Introduction

β-Mannans are strongly anti-nutritive polysaccharide fibres found in most vegetable feed ingredients (1). They belong to the hemicellulose fraction and have a backbone composed entirely of mannose, as in mannans and galactomannans, or of mannose and glucose, as in glucomannans and galacto-glucomannans (2,3). The estimated content of soluble β-mannans in common fattening diets is only 0.20-0.35%, and *in vitro* studies have demonstrated that as little as 0.05% soluble β-mannan in feed can elicit a strong innate immune response (4). This innate response is often referred to as a feed induced immune response or FIIR, which suppresses growth to protect the liver and reserve energy and nutrients for high priority immune functions. Hemicell HT (Elanco) is a β-mannanase enzyme for animal feed that breaks down β-mannans and thereby prevents economic losses from the wasteful immune response to β-mannans. The objective was to compare piglet performance and antibiotic use between a Control group, fed a conventional 3-phase diet, and an Enzyme treated group, fed an adapted 3-phase diet including a β-mannanase enzyme (Hemicell™ HT; Elanco).

Materials & methods

A seven weeks feeding trial was conducted with 896 piglets – vaccinated with an *E. coli* F4/F18 vaccine (Coliprotec F4F18; Elanco) in two rotations of 448 piglets in 32 replicate pens of 14 pigs. Two different 3-phase diets were compared: a standard 3-phase control diet and an adapted 3-phase diet including a β-mannanase enzyme included at 300 g/tonne. The following adaptations were made:

- ✓ Phase-1 (weeks 1-2): 1.14% potato protein concentrate and 1.00% Forcital (extruded soya product) were replaced with soybean meal.
- ✓ Phase-2 (weeks 3-4): 0.46% potato protein concentrate and 0.68% Forcital were replaced with soybean meal.
- ✓ Phase-3 (weeks 5-7): β-mannanase was formulated to replace 63 kcal/kg NE.

Standard piglet performance parameters (ADWG, ADFI, FCR) and antibiotic use were recorded. All data analyses were performed using R version 3.6.3 (R Core Team, 2020).

Results

Throughout the trial and within each phase, ADWG, ADFI and FCR were not significantly different ($P > 0.05$) between Control and Enzyme group. Mortality was significantly ($P < 0.001$) lower (-0.90 %) in the Enzyme treated group. Antimicrobial use was significantly ($P < 0.01$) lower (-56%) in the Enzyme treated group as compared to the Control group.

Table 1. Overall trials results including initial body weight (BW), final BW, average daily feed intake (ADFI), average daily weight gain (ADWG), feed conversion rate (FCR), mortality and antimicrobial use (expressed as number of individual injections). The Control group was fed a standard 3-phase diet, whereas the Enzyme treated group received an adapted diet where expensive proteins sources were partially replaced by soybean meal in Phase-1 and -2, and net energy was reduced by 63 kcal/kg in Phase 3. Statistical data analysis was performed using R version 3.6.3 (R Core Team, 2020).

	Control	Enzyme	*P*-value
Initial BW, kg	4.955	4.943	0.427
Final BW, kg	21.682	21.206	0.223
ADFI, kg/d	0.533	0.535	0.468
ADWG, kg	0.341	0.332	0.174
FCR, kg/kg	1.584	1.604	0.414
Mortality, %	1.79	0.89	< 0.001
Antimicrobial use (# injections)	123	54	< 0.01

Discussion & Conclusions

Inclusion of a β-mannanase to nursery diets with an adapted formulation by replacing expensive protein sources by soybean meal in the first two phases or reducing the NE content by 63 kcal/kg in the third phase, resulted in similar piglet performance post-weaning with reduced mortality and less antimicrobials used.

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Influence of the continuous acidification of drinking water on the blood parameters of weaning piglets

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Introduction

The weaning period represents one of the most challenging phases in pig farming as it imposes environmental, nutritional, and social stress (1).

Access to good quality water is one of the many factors that require attention during weaning. Acidification with blends of organic and inorganic acids throughcontinuous dosing systems has become an excellentalternative to improve water quality. It ensures the supply of drinking water with an optimal hydrogenic potential (pH), favors digestive functions, allows the reduction of antibiotic use, and controls enteric challenges. However, little is known about the systemic effects of continuous acidification in swine. Therefore, the aim of this study was to evaluate the influence of continuous drinking water acidification on blood parameters of weaning piglets.

Materials and Methods

The experiment was carried out in a commercial farm located on Linha Guará, Quatro Pontes, Paraná, Brazil. A total of 1080 female piglets (Landrace x Large White),with average initial body weight of 6.81 ± 0.29 kg,were allotted to one of three treatments in a randomized block design, witheight replicates and 45 animals per pen. Treatments were as follows: control treatment (CT); drinking water with 5.31 pH (pH5); and drinking water with 3.40 pH (pH3). To achieve and maintain such pH levels, the product pHPerfect Acid® was used. It is composed of ascorbic, citric, and phosphoric acid, monosodium phosphate, nucleotides, and flavor enhancers.

Twenty-four animals from each treatment were subjected to blood collection via cranial vena cava.Samples were sent to the laboratory where they were processed for plasma and serum obtention and further analysis of alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose, urea, and total protein (TP) concentrations. The data obtained were submitted to the Tukey test at 5% probability levelusing the SAS University Edition statistical program (2).

Results

ALT and AST levels were lower ($p < 0.05$) in pigs receiving pH3 (Table 1). TP levels were higher ($p < 0.05$) in pigs from CT. Glucose and urea were not influenced by the treatments ($p > 0.05$).

Discussion and Conclusion

It is possible that the lowest water pH (3.40) provided by the acidifier prevented liver and tissue damages in the weaning pigs, as ALT and AST plasma concentrations were reduced. When organ cells are injured or degraded by factors such as inflammation both these intracellular enzymes are released, leading to an increase in their concentrations in the blood (3).

The values found for ALT and AST in the present studyare close to the reference values for weaning pigs 31 to 58 IU/L and 32 to 84 IU/L, respectively (4).

Table 1. Influence of the acidification of drinking water on the blood parameters of weaning piglets

Items	Treatments			$\bar{x}$	SEM	p
	CT	pH5	pH3			
ALT, UI/L	88,0[a]	84,1[a]	24,7[b]	65,65	4,86	<0,001
AST, UI/L	89,0[a]	87,2[a]	32,7[b]	69,69	5,85	<0,001
GLU, mg/dL	89,7	93,4	89,0	90,6	2,48	0,758
URE, mg/dL	14,2	12,8	16,5	14,5	0,86	0,225
TP, g/dL	5,6[a]	4,8[b]	5,0[b]	5,1	0,10	0,004

CT: control treatment; pH5: drinking water with 5.31 pH; pH3: drinking water with 3.40 pH; $\bar{x}$: mean; SEM: standard error of the mean; p: significance level; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GLU: glucose; URE: urea; TP: total protein.

Lower TP values were obtained for piglets that received acidified water. This reinforces the idea that the acidifier may have reduced inflammation in the piglets, given that TP in serum mainly comprise albumin and globulins, the latter related to the immune system and response to inflammatory processes(5).

It is concluded that the acidification at pH 3.40 of the drinking water of weaning piglets with a blend of organic and inorganic acids, acid salts and nucleotides positively influenced the blood biochemical parameters of the animals.

Acknowledgments

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Long term effect of feeding spray dried plasma during the nursery on subsequent performance and health status to market weight

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Introduction

The benefits of feeding spray dried plasma (SDP) during the post-weaning period are well known (1). However, there are limited studies about the long-term impact of feeding SDP to nursery pigs on subsequent performance and health status of grow-finish (GF) pigs (2). This study aimed to determine the effects of various quantities of SDP provided during the nursery phase on subsequent performance and health of GF pigs to market weight.

Materials and Methods

300 PIC pigs weaned at 22d of age and 5.81 ± 0.04 kg BW were allotted to 5 treatments in separate sex pens (12 pens/treatment; 5 pigs/pen). Treatments representedthe inclusion of different SDP levels used in 4 nursery feed phases (pre-starter I and II, d22-29 and d29-36; starter I and II, d36-43 and d43-64 of age). Treatments by respective nursery phases and level of SDP in the diet were: T1) Control without SDP; T2) 3, 2, 0 and 0% SDP; T3) 5, 3, 1 and 0% SDP; T4) 7, 5, 3 and 0% SDP; T5) 7, 5, 3 and 1.5% SDP, respectively representing 0, 86, 165, 311 and 600 g of SDP consumed/pig. Thereafter, the nursery pen was maintained and moved to the GF facility where the pigs were fed common diets by phase to market weight. Performance data were evaluated per phase and the index of pneumonia (IP) was obtained at slaughter. Regression analysis using the covariance of initial BW was done considering the effects of sex, block, wean batch, and the average cumulative grams of SDP consumed per pig. Non-normal pneumonia index data was compared by Kruskal-Wallis's test.

Results

Table 1. Nursery to finish performance of nursery pigs fed different grams of SDP per pig (values in kg).

Parameter	SDP intake, g/pig					P value
Nursery	0	86	165	311	600	
ADFI	0.582	0.628	0.619	0.612	0.610	C[1]
ADG	0.396	0.430	0.427	0.415	0.416	C[2]
FCR	1.469	1.460	1.453	1.473	1.463	ns
FBW	22.45	23.88	23.77	23.23	23.29	C[2]
Grow-finish						
ADFI	2.295	2.304	2.330	2.410	2.386	L[3]
ADG	0.972	0.969	0.982	1.001	0.991	ns
FCR	2.360	2.379	2.372	2.407	2.406	ns
FBW	117.2	118.5	119.7	120.9	120.2	ns
Overall nursery-finish						
ADFI	1.770	1.800	1.811	1.861	1.816	Q[4]
ADG	0.798	0.808	0.815	0.824	0.819	ns
FCR	2.216	2.233	2.220	2.255	2.215	ns

[1] Cubic response to SDP intake (P = 0.08). [2] Cubic response toSDP intake (P < 0.05). [3] Linear response to SDP intake (P <0.05). 4 Quadratic response to SDP intake (P = 0.08). ns=not significant (P>0.10).

Table 2. Index of pneumonia (IP) in lungs of finishing pigs at slaughter.

Parameter	SDP intake, g/pig					P-value
	0	86	165	311	600	
IP	2.20[b]	0.87[a]	0.63[a]	0.57[a]	0.75[a]	0.01

IP - the frequency of the lung lesions of each pig in each category was recorded in an index from 0 – 6, with 0 being absent of lesions and 6 100% lesions. [a.b] different letters indicate a significant difference by Kruskal-Wallis's test.

Discussion and Conclusion

Total wean to finish culling+mortality was (4.7%) and did not differ among treatment groups. Table 1 shows that SDP increased (P<0.05) ADG and FBW and tended(P = 0.08) to increase ADFI in a positive cubic responseto SDP intake per pig while in the nursery. These subsequent positive effects of SDP intake linearly increased (P < 0.05) ADFI of pigs during the grow- finish phase and tended (P = 0.08) to increase ADFI over the entire nursery to finish period. Increasing level of SDP fed during the nursery numerically and linerarly increased (P=0.13) final BW at slaughter with a maximun increase of FBW for pigs fed 311 g SDP per pig during the nursery. Remarkedly, all SDP treatments provided during the nursery phase reduced the index of pneumonia lesions in lungs of pigs at slaughter weight compared to the control treatment group (Table 2). The reduced IP index is in agreement with other research indicating reduced severity of respiratory diseases in pigs and other species fed diets with SDP (3). This potential modulation of immunity from feeding SDP could be linked to the better GF performance and reduced pneumonia score at slaughter. There are few studies evaluating the effects of SDP fed in the nursery and its impact on subsequent phases of production.However, our results agree with others (2) that verified challenged pigs fed SDP during the nursery phase had improved immune response, survival, growth performance, and carcass traits of GF pigs, while also showing a synergic effect with a combined Mhyo-PCV2 vaccine. In conclusion, feeding SDP to nursery pigs demonstrated extensive benefits through the GF phase by improving performance and health of pigs at market weight.

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Performance of post-weaning piglets fed with autolyzed yeast

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Introduction

The post-weaning period of pigs is often associated with nutritional imbalances, due to changes that occur in the animal's organism. Usually related to the digestive and immune system and the fact that they are not fully developed, these changes are often responsible for intestinal inflammation and diarrhea, consequently leading to poor animal performance at this stage (1). Yeast-based ingredients are often used, as they reduce the deleterious effects of this nutritional transition (3). Processed yeast can make components present in its structure more available, such as mannan oligosaccharides and ß-glucans (4). These components have been shown to have the ability to improve the intestinal health of piglets, increase feed digestibility (5), balance the microbiota, and stimulate the immune system (2). Thus, the objective of this study was to observe the performance of post-weaned piglets fed with autolyzed yeast (AY).

Materials and Methods

The trial was carried out in the nursery facility of a commercial pig farm, For this, 1600 weaned piglets (hybrid Topigs x DB), aged 24±4 days, were distributed in a randomized block design, with 2 treatments and 16 replications of 50 animals each. The treatments were, Control (farm standard diet) and AY (farm standard diet formulated with LysCell®, from ICC Brazil Company). The diets were corn and soybean meal-based, isoproteic and isoenergetic. The treatment with AY had different inclusion on the phases: pre-starter 1 and 2 (20 kg/MT), initial 1 (10 kg/MT) and initial 2 (5 kg/MT). The experimental period lasted 42 days, and feed intake was measured daily using an automatic feeding system software. The animals were weighted at 12 and 42 days. Body weight (BW, kg), daily body weight gain (DBWG, kg), daily feed intake (DFI, kg) and feed conversion ratio (FCR) were calculated. Data were analyzed using the SAS Statistical Analysis System software, version 9.4, at Tukey text level of significance of 5% (SAS Inc., Cary, NC, USA).

Results

The results obtained are shown in Table 1. The piglets receiving diets with AY improved FCR (P<0.05) from 13 to 42 days and for the total period.

Discussion and Conclusion

The AY treatment influenced and optimized the performance of the animals, based on FCR improvement. Even though BWG was not statistically improved, numerically the piglets receiving diets with AY gained an daily average of +4% versus control group.

Table 1. Performance results of piglets

			SEM	P
	Control	Autolyzed Yeast		%
BW d1, kg	7.372	7.249	0.201	0.765
BW d12, kg	9.729	9.815	0.236	0.859
BW d42, kg	27.866	28.562	0.472	0.470
1-12 days				
DFI, (kg)	0.246	0.248	3	0.750
DBWG,(kg)	0.197	0.214	7	0.219
FCR	1.339	1.199	0.065	0.286
13-42 days				
DFI,(kg)	0.916	0.907	1	0.723
DBWG,(kg)	0.605	0.625	9	0.266
FCR,	1.520[b]	1.451[a]	0.016	0.026
1-42 days				
DFI, (kg)	0.724	0.719	10	0.762
DBWG,(kg)	0.488	0.507	7	0.183
FCR	1.489[b]	1.416[a]	0.013	0.003

[(a,b)] Superscripts indicate statistically significant differences within the main effect ($p < 0.05$).

Hu et al. (2014) also observed that the use of yeast-based components can impact growth parameters and also beneficially influence the performance of post-weaning piglets, reducing FCR , which is similar to what we found in this work.

Through these results, we can conclude that the inclusion of AY in the diet of post-weaning pigs can improve FCR.

Acknowledgments

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Probiotic roles of *Lactobacillus salivarius* on the modulation of swine gut microbiota

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Introduction

Probiotics is live microorganism that introducebeneficial functions to gut microbial communities, intestinal epithelium cells, and the immune system. Gut microbiota is a population of microorganisms such as bacteria or fungi that colonizes the intestines. The gut microbiome plays an essential role in individual's healthstatus (1,2). In swine, the use of Generally Recognized as Safe (GRAS) Lactobacilli as probiotics has beenincreasing due to their ability to improve growth performance and prevent gastrointestinal infection (3). *Lactobacillus salivarius* is a well-characterized bacteriocin producer. It has been frequently isolated from human, porcine and avian gastrointestinal tracts (GIT), and other sources, which have their ability to modulate gut microbiota and produce bacteriocins (4). Theobjective of this study was to determine the effect of isolated *L. salivarius* probiotic supplements on weaning pig gut microbiota using *16s rRNA* metagenomic analysis.

Materials and Methods

The marked weaning pigs were randomly divided into two groups (n = 10, for each group). The treatments were fed with creep feeding and supplemented with 10 ml per day of probiotic *Lactobacillus salivarius* (LactoA) and without probiotic culture (Control group). Fecal samples were collected on marked pigs at day 0, 6, 12, 18, 24 and 30 after feeding a probiotic.

The microbial genomic DNA from collected fecal samples were extracted using QIAamp PowerFecal Pro DNA Kit. The *16S rRNA* library was constructed using universal *16S rRNA* gene primers and ligate with sequencing adapters. The NGS library was sequenced using MiSeq600 platform. Raw sequences were categorized and processed using DADA2 pipeline. Amplicon sequence variant (ASV) at genus level was determined to identify the difference between gut bacterial compositions among groups.

Results

From fecal microbiome analysis, the Figure 1 showed that the observed number of ASVs, Chao1 richness, Shannon diversity, and PD whole tree of LactoA group was significantly higher than that in control group (p=0.0057, p=0.0068, p=0.0016 and p=0.0018, respectively) (**Figure 1**). The data suggested that the bacterial abundance of weaning pig supplemented with LactoA was higher than control group. At genus level, *Lactobacillus* sp. was predominant in both groups from day 12 to 30. As expected, supplemented LactoA increased *Lactobacillus* sp. in day 30 (**Figure 2**). Specifically, in day 18, the relative abundance of *Terrisporobacter* and *Clostridium sensu stricto 1* was decreased, but *Blautia*, and *Prevotella* were enhanced in supplemented with LactoA.

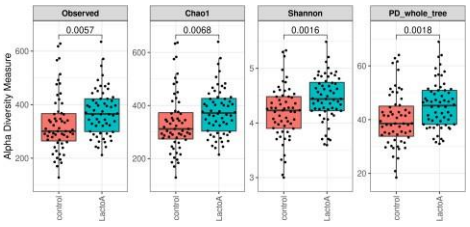

Figure 1. Alpha diversity of weaning pigs suplemented with (LactoA) and without (Control) *L. salivarius* LactoA. Box plot with bar representing the mean from three sequencing replicates.

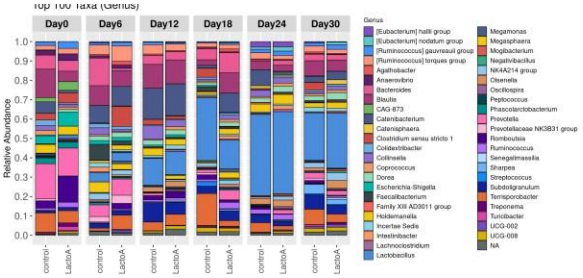

Figure 2. Gut microbiota analysis from feces of weaning pig supplemented with LactoA and control without LactoA as determined by the relative abundance of bacterial diversity at genus.

Discussion and Conclusion

Among several probiotics, *L. salivarius* strains are a well established probiotic with multiple applications inanimal health, particularly to reduce the colonization of gastrointestinal pathogens (4). In this study, the isolated *L. salivarius* (LactoA) was used as a supplement probiotic to weaning pigs and the results show the alteration of the bacterial abundance in genera of *Terrisporobacter*, *Clostridium sensu stricto 1*, *Blautia*, and *Prevotella*. In addition, *Terrisporobacter* is an opportunistic pathogen, which can cause intestinal inflammation (5). *Blautia* and *Prevotella* on the other hand are the predominant genera across the large intestine of pigs associated with health status.

In conclusion, our study demonstrtaed that LactoA supplementation decreased the opportunistic pathogen strains, *Terrisporobacter* and *Clostridium sensu stricto 1*, and increased the benificial bacteria genus, *Blautia* and *Prevotella*.

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Probiotic supplementation increases piglet resilience to a realistic Deoxynivalenol contamination of the diet: a DNA microarray transcriptomics and 1H-NMR metabolomics investigation.

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Introduction

Low-dose contamination by deoxynivalenol (DON) is a real feed safety issue. Pigs are particularly sensitive to this mycotoxin that disrupts normal cell functions. Some probiotics have demonstrated ability to tackle various factors threatening pig intestine health. We analysed on a tiered-approach the health effects of probiotics supplementation to piglets facing a subclinical DON challenge.

Materials and Methods

This study was designed to analyse on a tiered-approach the health effects of feed supplementation with a *Saccharomyces cerevisiae* strain against a background of subclinical challenge of the piglet with DON. First, a whole-transcriptome analysis was performed *ex-vivo* on jejunal explants to decipher the early response of the pig intestine to DON challenge after administration of *S. cerevisiae* boulardii strain CNCM I-1079. Then, an *in vivo* trial was conducted to investigate the intestinal and systemic effects of the mycotoxin, and the probiotic yeast supplementation.

Results

Compared to the control condition, no differentially expressed gene (DE) was observed after *ex-vivo* exposure of the intestine to yeast only. By contrast, 3619 probes - corresponding to 2771 genes - were differentially expressed following exposure to 10µM DON, and 32 signaling pathways were identified from the Ingeniuty Pathway Analysis software processing of the set of differentially expressed genes. When the intestinal tissue was treated with S. cerevisiae boulardii prior to DON exposure, the number of DE genes decreased by half (1718 probes corresponding to 1384 genes). Prototypical inflammation signaling pathways triggered by DON, including NF-κB and p38 MAPK, were reversed, although the yeast demonstrated limited efficacy toward some other pathways. *S. cerevisiae* boulardii also restored the lipid metabolism signaling pathway, and reversed the down-regulation of the antioxidant action of vitamin C signaling pathway.
For the *in vivo* study, no clinical signs, nor significant modifications of the blood biochemistry parameters were detected in piglets exposed to DON at 3 mg/Kg of feed. However, histological changes were observed in the piglets' jejunum, liver and kidney samples. Also, 1H-NMR metabolomic profiling of plasma and liver samples revealed that the metabolism of amino acids and 2-oxocarboxylic acids were altered. Dietary supplementation with *S. cerevisiae* boulardii clearly reduced the DON-induced histological lesions and restored the plasma metabolic profile. By contrast, the effect of yeast supplementation on the liver metabolome remained marginal.

Discussion and Conclusion

DON is one of the most notorious mycotoxins affecting pig health. It is responsible for the highest percentage of positive samples in the feed supply chain, and the largest ranges of contamination. Although the strict enforcement of current regulations on maximum levels permitted in feed and feedstuffs has solved the problemof the acute toxicity of DON and other mycotoxins, residual subclinical doses can still have a significant impact on animal health and productivity, especially that of young animals. The aim of the present study wasto investigate the effects of supplementation of a *S. cerevisiae* boulardii strain in feed as a strategy to alleviate the subclinical effects of exposure to DON in piglets.
Application of the yeast significantly reduced the global impact of DON on the intestine transcriptome. Most signaling pathways linked to inflammation and immunity triggered by DON were reversed by the S. *cerevisiae* boulardii treatment. Probiotic supplementation also reduced the burden of the global DON-induced oxidative stress in intestinal tissue and restored the lipid homeostasis. Although neither clinical signs nor significant modifications of the classical blood biochemistry were detected, exposure of piglets to DON was seen to induce histological alterations in organs which play a key role in the mycotoxin metabolism. Indications of alteration in protein metabolism were also provided for two different biological matrices. Supplementation with the yeast strain clearly prevented tissue lesions in piglets exposed to DON. Moreover, no significant metabolic modification was found in the plasma, and continuing alteration of the liver metabolic profile was limited. Taken together, these observations strongly suggest that *S. cerevisiae* boulardii supplementation can increase piglet resilience to a subclinical DON challenge.

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Supplementing a bacterial xylanase in lactating diets improves the body condition of the sows and the performance of the piglets

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Introduction

The lactation phase is critical for sows and, consequently, to piglets´ productive performance. During this period, the sow feed intake capacity can be insufficient to meet its nutritional requirements. Facing this scenario, strategies to improve the nutritional value of diets, such as the supplementation of exogenous enzymes in lactating diets, are highly demanded. A bacterial xylanase can improve nutrient digestibility and energy use of diets by both releasing encapsulated nutrients and reducing digesta viscosity (1), which is a major issue in diets with high fiber content as wheat-barley-based diets. Therefore, the objective of the present study was to evaluate the effects of a bacterial xylanase supplemented in lactating wheat-barley-based diets on the body condition of sows and the performance of piglets.

Materials and Methods

Twenty-four final gestation sows (233.5 kg average body weight) of the same genetic were allotted in two treatments in a completely randomized design with 12 replicates each from 14d before farrowing until weaning (19d post farrowing). The experimental diets were both wheat-barley-based and consisted of: T1 (Control), without xylanase supplementation and T2 (Xylanase) with the supplementation of a *Bacillus subtilis* xylanase at 100 g/t (Belfeed/Jefo) on top. Diets were formulated to meet sow´s requirements (2). The sows' body condition was evaluated at the beginning of the trial and at weaning by individually weighing animals and assessing backfat thickness (BFT) at P2. Feed intake (FI), BFT variation and body weight loss (BWL) were also assessed. The piglets were individually weighed at birth and at weaning and their average daily gain (ADG) were calculated. Data were analyzed using mixed model of SPSS and a t-student test was performed at a level of 5% significance.

Results

Body weight loss and BFT variation were higher for the Control treatment group compared to the Xylanase treatment group ($P<0.05$). No difference for FI of sows was observed (Table 1). No differences for body weight (BW) of piglets at birth nor at weaning were observed ($P>0.05$); however, the ADG of piglets from sows of the Xylanase treatment group were higher than those from sows of the Control group ($P<0.05$) (Table 2).

Discussion and Conclusion

Supplementing lactating sow diets with a bacterial xylanase promoted better body condition by reducing BFT variation and BWL. Additionally, the ADG of piglets from sows fed diets supplemented with the bacterial xylanase was 11% greater than those from the sows of the Control group. A possible explanation for this is that the addition of the bacterial xylanase in lactation sow diet improved nutrient digestibility and energy utilization in sows, which may have led to improvements on their body condition and milk quantity/quality, having positive effects in their litter. A bacterial xylanase might be able to be active in the whole small intestine of sows because its optimal pH is 5-9 (3). Such characteristic enables this enzyme to be active during most part of digesta transit through gastrointestinal tract effectively improving nutrient digestibility (1).

Table 1. Body condition of lactating sows fed diets supplemented or not with a bacterial xylanase

Item	BWL, kg	BFT variation, %	FI, kg/d
Control	32	21.5	4.85
Xylanase[1]	23	7.8	4.94
SEM[2]	3.0	4.73	0.116
P-value	0.023	0.042	0.500

BWL=body weight loss, BFT=back fat thickness, FI=feed intake.
[1]Jefo (origin *Bacillus subtilis*).
[2]Standard error of the mean.

Table 2. Piglet performance from lactating sows fed diets supplemented or not with a bacterial xylanase

Item	BW birth, kg	BW weaning, kg	ADG, g/d
Control	1.51	5.74	211.0
Xylanase	1.50	6.22	234.5
SEM[1]	0.100	0.148	12.24
P-value	0.930	0.552	0.023

BW=body weight, ADG=average daily gain.
[1] Jefo (*Bacillus subtilis* origin)
[2] Standard error of the mean.

In conclusion, the bacterial xylanase can be used as a strategy to improve the sows body condition and uniformity during the lactation period, also having positive effects on piglets´ performance.

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The effect of inclusion of a specific mix of encapsulated fatty acids in sow's diets on the quality of IgG and weaned piglet performance

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Introduction

The widespread use of hyper-prolific sow lines has provoked an increase in numbers born but at the same time a reduction in average birth weight which is highly correlated with pre waning mortality (1). Piglets are born with a naïve immunological status and depend on obtaining maternal antibodies via the immunoglobulins in the sow's colostrum (2). Colostrum intake is positively correlated with daily gain post weaning (3).

Materials and Methods

The control (C) sows received their normal gestation and lactation diets. The treatment (T) group received an integration of the specific encapsulated fatty acid mix (ColfaPig Devenish Nutrition Ltd, UK) at the rate of 2.8kg/ton for the last 56 days of gestation and 1.0kg/ton in the lactation diet. Colostrum samples were taken within three hours of farrowing from 82 Cand 93 T sows. The quantity of IgG present was estimated using a digital refractometer (Atago PAL-1) and classified into four categories (4). Approximately 200 piglets from each treatment were individually identified and weighed at weaning and again at 33 days. Individual daily live weight gain (DLWG) was measured. A Chi-square test was used to analyze the IgG categories between the treatments. The DLWG data was analyzed by GLM ANOVA using SAS 9.4.

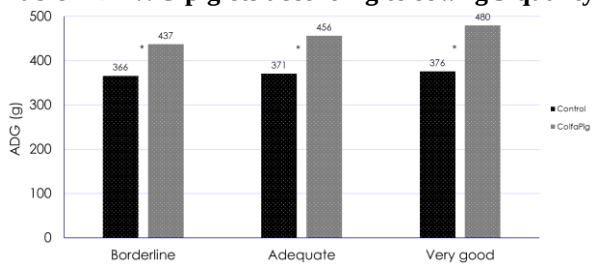
Results

The four categories of the IgG results were split into two groups, "poor" and "borderline" were classified as unacceptable, whilst "adequate" and "very good" were classified as acceptable. The T group tended (P=0.06) to have higher IgG values.

The T piglets grew significantly faster than the C group (P<0.001). Further subdivisions of the piglets depending on the parity of their mothers showed significant differences in DLWG(P>0,0,05) over the first four parities.

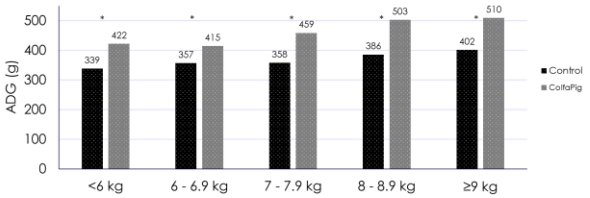
The DLWG was further analyzed depending on the IgG quality of the dams. As the IgG improved, so did the DLWG, but significant differences (P<0.05) were observed between the treatments for each IgG category.

Table 1 DLWG piglets according to sow IgG quality



Finally, the DLWG was analyzed depending on the individual weaning weight. Heavier pigs grew better in both treatments, but significant differences (P<0.05) were observed in each weight category between the treatments. The lightest T piglets grew fastest than the heaviest C piglets.

Table 2 DLWG piglets according to weaning weight



Discussion and Conclusion

Lighter birth weight pigs have been shown to have a lower duodenal mucosal height (5). Many publications indicate the role of fatty acids in increasing villa height. Colfapig has been shown to significantly improve birth weights, weaning weights and subsequently DLWG post weaning (6).

The quantity and quality of IgG intake over the first twenty-four hours will provide more protection, alleviate the stress of weaning, and enable higher DLWG in the weaner phase. Increased weaning weights will have a profound effect on lifetime performance of the pig (7). The use of a digital refractometer provides a reliable pen side method for assessing the sow's colostrum and can be used as a predictor of post weaning piglet growth rate. This could lead to differentiated feeding regimes for lighter weight pigs.

The use of the encapsulated fatty acid mix in sow nutrition will improve the quantity and quality of IgG in colostrum and stimulate faster weight gain post weaning.

Acknowledgments

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The influence of different sucrose solution concentrations on time budget of exploratoryand social behaviors in nursery pigs

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Introduction

Pigs innately detect and prefer sweet compounds **[1],** increasing the pleasure they perceive as the inclusion of carbohydrates such as sucrose increases **[2].** This could increase motivation and competition for obtaining such resources, generating agonist behaviors in animals that receive sweet additives. In addition, animals could also modify the time budget that they allocate to other behaviors during the consumption period of palatable resources. Theobjective of the present experiment was to quantify and relate the time budget of exploratory, feeding andsocial behaviors to different concentrations of sucrosesolutions delivered to nursery pigs.

Materials and Methods

A total of 24 nursery pigs were allocated in 12 pens (2 pigs/pen) and daily exposed (10 min), during 7 consecutive days, to different sucrose solutions (0.5; 1;2; 4; 8; 16 and 32%). Animals were video-recorded inorder to determine the time budget they spent during the consumption tests on exploratory (locomotion, environment exploration, feeder exploration, feeder rooting), and social behaviors (near the feeder, away from the feeder, agonistic near the feeder and agonistic away from the feeder). The effect of sucrose inclusion on the measured behaviors was analyzed (SAS®).

Results

In relation to exploratory behaviors (Figure 1a), sucrose concentration affected locomotion (P=0.029),observing the highest values with the lowest concentrations. Sucrose concentration also had an effect on environment exploration (P<0.01) observing the highest values at intermatiate concentrations. Finally, feeder exploration (P=0.012) and feeder rooting (P<0.001) were affected by sucrose concentration, observing the higher values at the highest and lower concentrations respectively.

In relation to social behaviors (Figure 1b), sucrose concentration had an effect on social behaviors near (P=0.001) and away from the feeder (P<0.001), observing at the higher sucrose concentrations fewer social interactions near the feeder but higher social interactions away from it. Agonistic behaviors near the feeder or away from the feeder were also affectedby sucrose concentrations (P = 0.004 and P = 0.005 respectively), where at the higher sucrose concentrations pigs presented more agonistic behaviors near the feeder but less agonistic behaviorsaway from it.

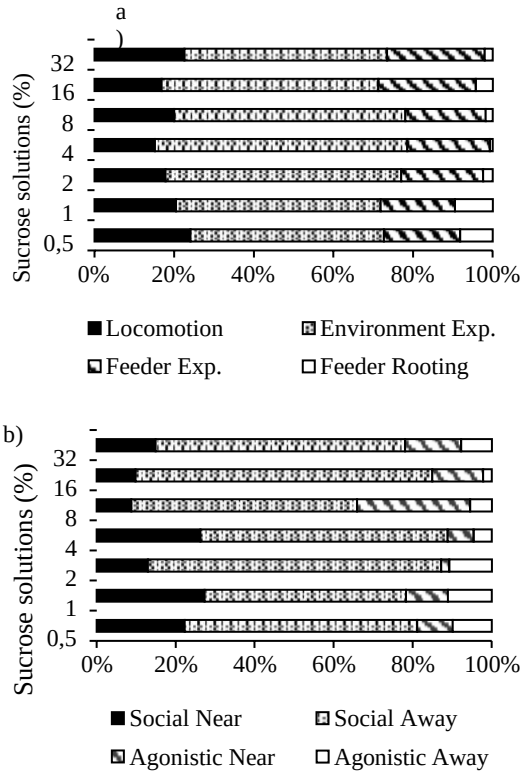

Figure 1. Time budget of exploratory (a) and social (b) behaviors (expressed as a percentage of behaviors analyzed)in nursery pigs' pairs that were exposed to different sucrose concentrations in water solutions during 10 min.

Discussion and Conclusion

Pigs presented more locomotion and feeder rooting when the reward was low probably because of the lossof interest in the solution, and presented more exploration (environment and feeder) when the reward increased probably due to high motivation to seek for such resources. Pigs presented more social interactions away from the feeder and agonistic behaviors near the feeder when the reward was high, probably because they concentrate agonistic behaviorsnear the nutrient source. The perceive reward (palatability due to energy concentration) may modifytime budget for exploratory and social behaviors in pigs.

Acknowledgments

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Use Kinetio Technology to manage your protein sources to control piglet feed costs

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Introduction

Nutrition of weaned piglets is challenging. Weaning stress and the abrupt transition from sow milk to feed reduces feed intake and digestion of piglet feed. The sub optimal protein digestion and absorption of feed often results in gut health problems. In a previous trial we showed that Kinetio Technology can be used to replace pharmaceutical levels of ZnO in piglet diets without increasing scours and without decreasing technical and economic performance (1). One of the good and fast degradable protein sources used in this Kinetio piglet diet was spray dried blood plasma (2). The use of this kind of excellent protein sources comes with a financial cost. The objective of current trail was to test the hypothesis whether Kinetio Technology can be used to formulate piglet diets without spray dried blood plasma while maintaining performance.

Material and Methods

In total 576 piglets (192 piglets per treatment and gender balanced) were allocated to three treatments, Control, Kinetio with spray dried blood plasma (Kinetio + SDBP) and Kinetio without SDBP (Kinetio - SDBP) at an age of 26 days. Piglets were housed in 36 pens, 16 piglets per pen and 12 pens per treatment. Piglet received the dietary treatment from weaning till 42 days postweaning. Corn, wheat, and soya bean meal were used inthe control diets. Feed budget was 1.5 kg phase 1 diet, 5kg phase 2 diet and 25 kg phase 3 diet. SDBP was used in phase 1 in the Control diet and Kinetio + SDBP. In phase 3, all piglet received the same piglet diet. The control diet was a standard commercial piglet diet. All diets were isocaloric. Piglet's weight and feed usage was measured at weaning, at days 14 and 42.

Results

Daily gain, daily feed intake and feed efficiency throughout the study were significantly higher in piglets receiving diets using Kinetio Technology (Table 1). The same observation was made for final body weight ($P<0.05$). Additionally, this trial showed the cost/kg gain was significantly lower for piglets receiving diets using Kinetio Technology without plasma, compared to the control group. When applying Kinetio Technology, there was no added value of plasma compared to vegetable protein sources on final performance results.

Conclusions

Kinetio Technology can be used to improve pig performance and can be used to manage piglet feed cost without compromising pig performance.

Table 1. Technical and economical traits in the first14 and 42 days, respectively.

		CONTROL	Kinetio + SDBP	Kinetio – SDBP
Day 0-14 performance				
Feed intake	g/d	174[b]	200[a]	190[a]
Daily gain	g/d	115[b]	175[a]	160[a]
Feed conversion rate		1.51[b]	1.14[a]	1.19[a]
Day 0-42 Performance and Economics				
Feed intake	g/d	525[b]	590[a]	580[a]
Daily gain	g/d	360[b]	422[a]	414[a]
Feed conversion rate		1.45[b]	1.40[a]	1.40[a]
Final body weight	kg	21.6[b]	24.2[a]	24.1[a]
Feed cost per kg gain	€	0.51[b]	0.51[a]	0.49[a]
Margin over feed cost	€/piglet	18.8[b]	20.8[a]	21.1[a]

Superscripts indicate statistically significant differencesbetween treatments ($p \leq 0.05$)

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Use of a supplemental source of injectable energy for piglets (21 days old) improved viability

Introduction

The increase in the number of piglets born per farrowing reduced the uniformity of litters and, consequently, increased the frequency of low-birth-weight animals. These piglets have greater need of energy reserves due to the increased competition for colostrum, milk, heat sources and space. The association of these factors reduce animal welfare levels, chance of survival and performance rate of piglets (1,2). Thus, this evaluated the effects of supplementation with energy sources (MOV® -Vallée S/A Brazil*) via intramuscular on weight daily gain and mortality rate of piglets.

Material and Methods

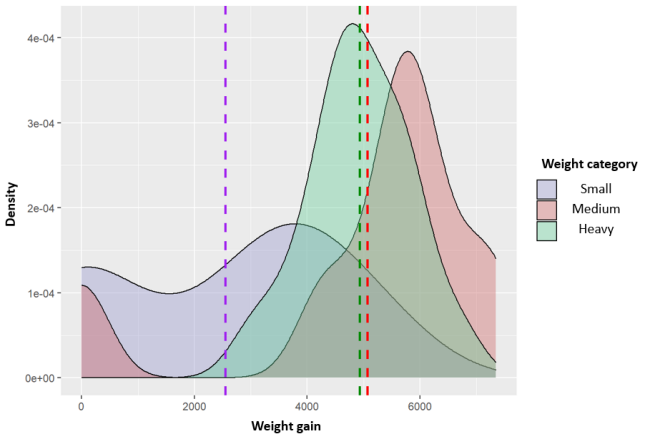
The field trial was carried out in a Brazilian commercial farrow-to-finish operation.

A total of fifty-four (54) piglets from the same farm (located in the Southern Brazil) and genetic were evaluated for the weight gain and mortality rate. Nine piglets from each litter (N=9) were distributed in three groups according to the born weight: light (up to 0.910 kg; 9 piglets), medium (1.100 to 1.335 kg; 9 piglets) and heavy (1.400 to 1.427 kg; 9 piglets) in the control (T1; N=27 piglets) and supplementation (T2 – use of 2 mL MOV® via IM; N=27 piglets) treatments. Each sow selected for the study had 14±2 milking piglets. Each 100 mL MOV® contains iron dextran (11,1111) ml; copper chloride dihydrate (0,00444 g), sodium glutamate monobasic monohydrate (1,03333 g), arginine hydrochloride (0,56667 g), lysine hydrochloride (1,11111 g), threonine (0,55556 g), valine (0,46667 g), nicotinamide (1,66667 g), choline chloride (2,22222 g). All piglets were distributed according to a randomized block design. Data was statistically analyzed through statistical model considering the effects of birth weight and treatment corrected for variables of interest through analysis of covariance using the R software. The litter was used as experimental unit.

Results

Piglets treated with MOV® were heavier at the weaning (on average 1,307 kg). There was a significant effect of birth weight group (P<0.01) on the weight gain using MOV®. The light, medium and heavy group of piglets were on average 411 g, 1,335 kg, 1,055 kg, respectively, heavier than the control group. The mortality was significant lower in the treatment group (0 vs 15%; P < 0.05), especially in the light (80%) and medium (20%) weight groups.

Figure 1- Distribution of weight gain (mean) at 21 days of treated and untreated piglets in each weight group



Conclusions and Discussion

The use of MOV® improved weight gain and mortality rate of piglets up to 21 days old. In the gastrointestinal tract of neonates, amino acid oxidation occurs as the main energy fuel for enterocytes (3) can MOV® helped to the strongest effects on mortality rate were found in piglets up to 1.335 kg. Supplemental sources of energy can help piglets to cope is daily challenges, improve survival rate, litters uniformity, welfare and health. The MOV® supplementation at this stage proved to be an important tool to improve the performance of piglets in the farrowing. Further studies evaluating MOV®-supplemented piglets from birth to slaughter are necessary to identify its effects on intestinal integrity, health and carcass quality.

Acknowledgments

Not applicable in these sections.

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Use of Prebiotic Additive in pregnant/lactating sows as an alternative to improve birth weight and weaning weight

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Introduction

Birth weight and weaning weight are important indicators for the future performance of animals, whether productive or reproductive. In this way, you can use nutritional strategies to increase these indicators.

Materials and Methods

The experiment was carried out on a commercial farm with 3800 sows of their own genetics (Landrace x Large White), which is located in the city of Tehuacán in the state of Puebla, Mexico. For the experiment, 500 sows (parturition order 1-6) were used in each of the treatments. Treatment 1 (T1) sows that were fed during pregnancy and lactation with a blend of organic acids + Mannooligosaccharide and treatment 2 (T2) sows from the negative control group. Sows from T1 received the basal diet from the farm plus the addition of 1kg/ton of the additive from 100 days of gestation and throughout the lactation period (23 days). The T2 sows received only the basal feed from the farm. All piglets were individually weighed at birth and at weaning. After birth, piglets were equalized among sows of the same treatment. The variables evaluated were birth weight, maternity weight gain, maternity mortality and weaning weight. The experimental design was completely randomized, in a 2X2 factorial arrangement (two prepartum diets and two post-partum diets). Data were submitted to analysis of variance at 5% probability of error and, when significant, Duncan's mean test was applied, also at 5% probability.

Results

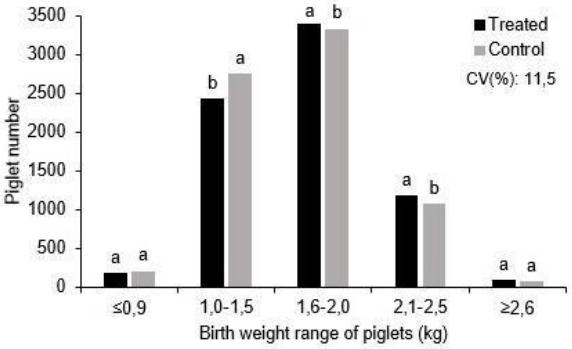
In graph 1 we can see the weight of piglets at birth of each treatment distributed by weight range. In Graph 2 we can see the weight of piglets at weaning of each treatment distributed by weight range.

Conclusions and Discussion

Piglets from Treatment 1 (T1) had a mean birth weight (1,698a) that was statistically higher than Treatment 2 (1,665b). Analyzing Graph 1, we have that the piglets born from treatment 1, showed a reduction (P<0.05) in the number of piglets in the lighter weight classes (1.0-2.0) and, in the same way, presented a higher numberof piglets (P<0.05) in the heavy range (2.1-2.5). This indicator is very important because as we increase the average birth weight, we improve its viability and vigor, which consequently directly impacts the performance of maternity. This statement corroborates the results obtained in this work, even though there was no difference in maternity mortality when comparing T1 (4.80%) against T2 (5.94%), it was observed that the weight gain of piglets in the maternity ward of T1 (217g/day) was statistically higher (P<0.05) to the weight gain of T2 piglets (193g/day).

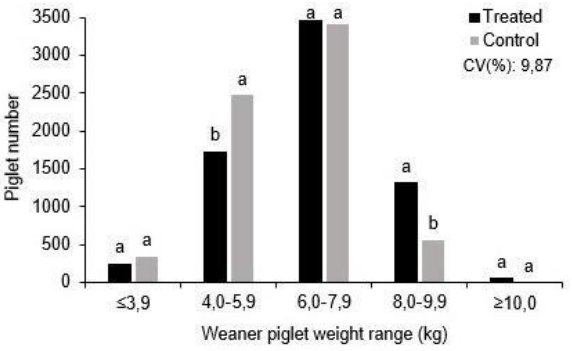
Analyzing Graph 2, a greater number of weaned piglets with greater weight at T1 is evidenced. There was a statistical difference (P<0.05) in the average weaning weight between the treatments, with T1 (6.679kg) weaning piglets on average 590 gramsheavier than T2 (6.090). T1 has the lowest number ofanimals in the light weaning range (4.0-5.9kg) and the highest number of weaned piglets in the lightest range (8.0-9.9kg). The experiment indicated that treatmentwith the prebiotic additive during pregnancy and lactation resulted in piglets with a higher average birthweight, a reduction in the number of low-birth-weightpiglets and, with this, a better maternity performance of the piglets was also observed. of Treatment 1 consequently reflecting in heavier piglets at weaning.

Graph 1. Weight of piglets at birth of each treatment distributed by weight range**.**



[1] [a,b] means followed by distinct letters on the line indicate difference by Duncan test (P <0.05).

Graph 2. Weight of piglets at weaning of each treatment distributed by weight range.



[1] [a,b] means followed by distinct letters on the line indicate difference by Duncan test (P <0.05).

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Vitamin-mineral supplementation via water improves the performance of piglets post-weaning

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Introduction

The management practices that follow weaning are sources of stress in the nursery period, compromising the homeostasis of the organism, making the piglet more susceptible to damages and loss of performance (1). Therefore, the higher productivity is accompanied by greater vulnerability to oxidation, increasing the demand for antioxidant components (2). For this reason, the objective was to investigate the effects of vitamin supplementation, via water, for piglets after weaning on water and feed consumption, performance, redox status and behavior.

Materials and Methods

A total of 90 piglets (45 castrated males and 45 females) weaned at 24 days of age (BW of 7.626) were evaluated for a nursery phase (42 days). Water and feed were offered *ad libitum*. The experiment consisted in completely randomized block design with three treatments and 10 repetitions of 3 animals each: control group (CON; receiving regular water throughout the experimental period), 5D (vitamin-mineral supplementation via water during the first five days after weaning and for another five days in the transition from starter I to initial II – 23rd to 28th experimental day, totaling ten days of supplementation) and 10D (vitamin-mineral supplementation via water during the first ten days post-weaning). Piglets were weighed at the beginning and at the end of experimental period. Feed and water consumption, performance, redox status and animal behavior were analyzed for the nursery period. Variables were subjected to analysis of variance and differences between treatments were evaluated by Tukey's test.

Results

The treatments influenced water consumption, with the 10D group being responsible for increasing water consumption by more than 1.3 L per piglet/d. Supplemented piglets also showed lower MDA values at 10 days of nursery, with a better redox status. Furthermore, the 10D group also showed the best feed consumption behavior, staying longer in the feeder.

Discussion and Conclusion

The post-weaning period is a phase that the animals take time to adapt and start drinking water (3). A higher water consumption provide a beneficial effect, favoring feed consumption and improving piglet performance (4). Then, the supplemented piglets had a superior performance, reaching 1.2 kg heavier ate the end of nursery. The improvement in performance and/or feed efficiency may be due to the greater water consuption, since better hydration favors the structure and functioning of the digestive system, improving the absorption and transport of nutrients (4). The first week of nursery is characterized by an increase in MDA concentration (7). In agreement with our findings, other authors reported that piglets weaned and supplemented with vitamins and minerals (5,6). We showed the antioxidant potential of supplementation and allowing a higher value of MDA in the first ten days post-weaning in control group.

Table 1. Evaluation of vitamin-mineral supplementation on the water and feed consumption, performance, redox status and behavior in the nursery period.

Variables	CON	5D	10D	P[1]
DWC	4.194[b]	5.138[a]	5.540[a]	0,0053
ADFI	0.755	0.741	0.768	0,6277
Final BW	27.939	27.937	29.190	0,1786
MDA	3.94[b]	1,28[a]	1.39[a]	0,0189
TEat	0.28[b]	0.40[b]	0.69[a]	0,0459

[1]Obtained by analysis of variance and evaluated by Tukey's test (p<0,05); DWC: daily water consumption (L/d); ADFI: average daily feed intake (kg/d); Final BW: final body weight at nursery (kg); MDA (Malondialdehyde in µmol/mg of protein); TEat %: time eating ration in percentage.

Thus, treating piglets with a vitamin-mineral supplement via water for 10 consecutive days after weaning or for five days after weaning and another five days in the transition of diets was beneficial for the redox status, feed and water intake and overall performance.

Acknowledgments

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PRODUCTION & INNOVATION

Artificial Intelligence and Slaughtered Pigs: a promising affair

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Introduction

Animal protein demand has been increasing over the last decades, stimulating meat industry to achieve higher efficiency levels (1). In this respect, the slaughterhouse is worldwide recognized as a suitable checkpoint to monitor herd management, animal welfare and health status (2). However, the huge number of slaughtered pigs along with the high speed of the slaughter-chain make challenging, if not impossible, the systematic collection of data.

Artificial intelligence (AI)-based technologies could offer valuable tools to solve that issue, thus improving the efficiency of veterinary inspection. In particular, so called "convolutional neural networks" (CNNs) appear suitable to solve highly repetitive tasks and to consistently analyze large amounts of data, such as those collected by veterinarians during postmortem inspection in high-throughput slaughterhouses (3).

We summarize herein the investigations carried out over the past three years (4,5), which aimed to detect and score respiratory lesions on digital images by means of AI-based methods.

Materials and Methods

1) Training CNNs to detect and score pleurisy. To this aim, the inner surface of 5902 porcine half-carcasses (pigs 9–11 months of age; 150–180 kg) was photographed under routine field conditions. Two skilled veterinarians scored pleurisy following a previously published method (6). The same veterinarians annotated all the above pictures using a dedicated open-source image annotation tool (namely, "labelme") (7).

2) Training CNNs to detect and score pneumonia. To this aim, a total of 7564 lungs were photographed and annotated by three skilled veterinarians, using "labelme" annotation tool. The presence of pneumonia, if any, was scored as a percentage of the entire lung surface.

In both cases, annotated pictures were given to the CNNs, to train and to test their ability. The CNNs' performances were compared with veterinarians' scores (gold standard) and assessed in terms of accuracy, Intersection over Union (IoU), sensitivity and specificity.

In addition, pneumonia scores provided by the CNN were compared with traditional scoring systems (i.e. Madec and Christensen methods) (8,9), which were performed by the same veterinarians along the slaughter chain. Data were analyzed by means of a multiple-linear regression model.

Results

1) *Training CNNs* to detect and score pleurisy. The overall accuracy of the trained CNN was 85.5%. The accuracy was higher for healthy half-carcasses (96%) and for severe lesions (between 84 and 92%). Likewise,
specificity ranged from 78% to 100%, being extremely high in severely affected pigs.

1) Training CNNs to detect and score pneumonia. Specificity (99.38%) and sensitivity (ranging between 81.25% and 100%) proved to be always very high. In particular, the CNN was able to detect all "large" lesions, i.e., affecting >2% of the entire lung surface. Average values of IoU ranged between 0.78 and 0.97.

The comparison between traditional scoring systems and CNN outputs showed a correlation coefficient of 0.83.

Discussion and Conclusion

Overall, the present investigations indicate that AI- based methods could provide a fast and cheap tool to systematically record lesions in slaughtered pigs, thus supplying an enormous amount of useful data to all stakeholders in the pig industry, without interfering withthe slaughter chain routine. Support to preventive medicine in pig rearing, detection of biosecurety flaws in herds and reduction of economic losses are among the possible vantages. In the next future, CNNs could offerinteresting, effective and cost-efficient alternatives in the field of food safety as well, supporting veterinariansand optimizing the management of human resources, especially in high-throughput slaughterhouses. As a matter of fact, we consider that we are very close to theumpteenth, epochal revolution in the field of veterinary medicine. Veterinarians should be able to face such challenges, using new technologies, to improve their professional activity (4,5).

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Artificial intelligence as a new method of assessing enzootic pneumonia and atrophic rhinitis lesions

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Introduction

The assessment of pulmonary lesions compatible with Enzootic Pneumonia (EP) and Atrophic Rhinitis (AR) at the slaughterhouse is one of the reference techniques for assessing the incidence of these diseases on pig farms and the effectiveness of vaccines against *Mycoplasma hyopneumonia* (*Mhyo*), *Bordetella bronchiseptica* (Bb) and *Pasteurella multocida* type D (PmD).

Until now, monitoring the grade of pulmonary lesions caused by *Mhyo* and snout lesions requires highly skilled technicians to check the pulmonary and snout score at the slaughterhouse. The process is slow and time-consuming and, even if carried out by skilled personnel, the disparity in the pulmonary assessments carried out by different technicians shows that this is a subjective process and that it is therefore very difficult to produce a systematic record of lesion scoring data.[1]

Artificial intelligence (AI) is a discipline that aims to develop intelligent agents, i.e. machines, that can perceive the environment and take action to maximize their success with regard to a defined target.

The aim of this poster is to explain the development process used to train an artificial intelligence-based system, in order to automatically score EP and AR lesions in slaughtered pigs and to compare the AI system of assessment with the experts' assessment.

Materials and Methods

Artificial Intelligence Diagnos (AI Diagnos) is a fully automated diagnostic system developed by HIPRA, that uses AI to simplify, objectify, and facilitate the whole process of slaughterhouse assessment. Operation, detection and classification processes, output of results and practical applications were needed to develop this system.

The system was trained using over 11,000 images inspected and photographed by a smartphone camera under slaughter conditions. These images were evaluated by the system and corrected for learning by five different experts in *Mhyo* and AR from all over the world, using a modified Madec scoring system for EP lesions and European Pharmacopea guidelines for AR lesions.[2,3]

The first step was the detection of objects, which is a smart image recognition technique which allows objects within an image to be identified and located. AI Diagnos has two detectors per model: the detector of focus,which identifies the lung or the nasal septum, and the detector of areas of interest (Image 1), which identifies the various areas of the lung or the nasal septum.

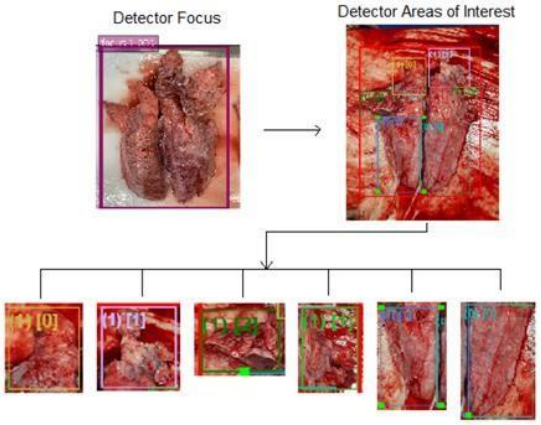

Image 1- Processing of the Mycoplasma model.

In order to create our models, we trained the system to be able to classify the lesion grade of the various areas of interest, according to the set of images previously analyzed by the same system during training. The model takes every image for analysis and shows the lesion grade for each section.

Results

After the system had been trained with over 11,000 images that were first evaluated by the specialist and then by the system, the result was that the system currently has over 85% direct accuracy by system- evaluator, which means that the system and the evaluator both gave the same score, and over 96% if weconsider an error per lobe rate of ±1.

Discussion and Conclusion

AI Diagnos is a new and innovative system for the evaluation of lesions caused by EP and AR with clear advantages such as automation, as it only requires photos to be taken and uploaded into the system and saves time in the individual assessments. Furthermore, AI Diagnos is a fully objective process, where the subjectivity of the evaluators is eliminated, and the images are always evaluated according to the same set of criteria, with a high accuracy system-evaluator, thus AI Diagnos is a systematic service for standardization of data from slaughter lungs and snout inspections.

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Assessment of adenosine triphosphate (ATP) bioluminescence tests to use in hygienogram for evaluation of cleaning protocol and methicillin-resistant Staphylococcus aureus (MRSA)detection in Argentinean pig farms.

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Introduction

Optimal hygiene management is an essential tool to limit the spread of pathogens and antimicrobial resistant bacteria. The most common method is a visual inspection, but 1/3 of "very clean" sites reveal a large number of colonies [1]. Microbiological swabs for aerobic total viable counts (TVC), are routinely used to effectively assess hygiene, but are impractical. The measurement of adenosine triphosphate (ATP) test providing information on the level of organic dirt and microbiological contamination[2]. In pigs, most studies are related to the colonizer or infections of Methicillin Resistance *Staphylococcus aureus* (MRSA) in the nasal cavity of pigs, veterinarians, farm and slaughter workers, however few of them in the environment contamination of the facilities. The objective of this study was to analyse comparative the utility of ATP *vs* TCV test to detect the bacterial contamination as well as their effectiveness assessment tool to determine cleanliness (hygienogram) and MRSA detection post-cleaning pens in pig farms.

Materials and Methods

The 8 commercial pig farms (from 500 to 5000 sows) located in Argentina, were visited once and were take 224 swab samples from 2 randomly chosen facilities after cleaned that comprised the farrow rooms (F) and grower-finisher (GF) units. In each pen: 2 pre-moistened swab samples taken from floor (concrete and plastic), 2 from feeder (stainless steel) and 2 from wall. Each sample were taken by wiping the area of 100 cm^2 horizontally and vertically. Two swab was taken for TVC, two for ATP Tests and in addition 5 swab was taken for MRSA culture. All samples were stored (4 to 7 °C) and processed within 48 h. For TVC test, serial dilutions were made with buffered peptone water, deep seeding and incubation at 35ºC for 48 hours. The ATP test was make, by shaking, and the amount of emitted light was measured by a luminometer Higyena® tech in relative light units (RLUs). For MRSA detection, the samples were pre-enriched in into an enrichment tryptic soy broth containing 6.5% NaCl and seeded in medium CROM Agar™ MRSA. To verify the relationships between the ATP test and TVC they were analyzed Spearman correlation coefficient. To obtain of the validity of the ATP test about sensitivity (SE), specificity (SPE) and cut-off values, the data were computed and visualized by Receiver Operating Characteristic (ROC) curves. Therefore, TVC variable was categorized and assigned 1 to values < median, and 0 to larger values. We carry out the same procedure with quartile 1 and 3. The optimal cut-point on the ROC curves was identified as the point with maximum SE and SPE. The area under the curve (AUC) was calculated (a perfect test AUC= 1, an uninformative test AUC=0.5). Statistical analyses were performed within the R environment[3-4]

Results

A significant correlation (concrete in GF: r = 0.55; stainless steel (feeder): r = 0.54, plastic (F): r = 0.47) was found between the ATP and TVC. Assessing the effectiveness threshold values were established:

Table 1: The optimal cut-points on the ROC in con crete (wall and floor of GF).

	ATP (URLs/100cm²)	SPE-SE % cut-point. AUC	TVC (Log10 cfu/cm²)
Very good	≤ 390	72.7/93.3 AUC:83.85%.	< 1,48 (Q1)
good	<1010	50/93,8 AUC: 77.34%.	< 1,93 (Median) >
bad	>1010		
very bad	>1430	44,4/95,7. AUC:78.6%.	> 3,09 (Q3)

Table 2: The optimal cut-points on the ROC in plastic (wall and floor F)

	ATP (URL/100cm2)	SPE-SE % cut-point. AUC	TVC (Log10 cfu/cm2)
good	< 380	50/93,8. AUC:83.98%.	< 1,93 (Median) >
bad	> 380		
very bad	> 584	25/91,7. AUC: 68.95%	> 2,89 (Q3)

Table 3: The optimal cut-points on the ROC instainless steel (feeders)

	ATP (URL/100cm2)	SPE-SE % cut-point. AUC	TVC (Log10 cfu/cm2)
good	< 2840	25/93,8. AUC:80.86%.	< 2,55 (Median)>
bad	> 2840		

No sample positive for (MRSA) was found.

Discussion and Conclusion

According with previous investigation (1,2), ATP test can be used as a monitoring tool for rapid assessment ofsurface cleanliness of facilities in pig farms. The stainless steel feeders have larger values of URL and TVC values than plastic and concrete and should be a critical point for effectively cleaning (2). The absence ofpositive samples for MRSA reinforces the idea that thispathogen is not an environmental contaminant and the facilities are not an important point of transmission.

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Benefit of Vaccination Against Porcine Circovirus Type-2 (PCV2) in a Non-Vaccinated farm in Malaysia

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Introduction

Porcine circovirus Type-2 (PCV2) is the smallest known DNA virus affecting pig health and farm performance. Pig infected with PCV2 virus but do not vaccinate had 2.75 times higher chance to die during the production period [1]. Mortality due to PCV2 can be 10 to 40% in the non-vaccinated pig farm [2]. Almost all farms in Malaysia had practiced PCV2 vaccination in piglets. However, there are still few farms that choose not to vaccinate piglets against PCV2. Production performance such as mortality rate can be used to monitor vaccine efficacy [3]. Therefore, the objective of the present study is to show the benefit of PCV2 vaccination by measuring the mortality pattern, in a before and after study model, performed in a farm that have not used PCV2 vaccine.

Materials and Methods

The trial was conducted in a farrow-to-finish farm with 200 sows in Malaysia. Before the trial, the only piglet vaccination program performed in the farm was *Mycoplasma Hyopneumoniae* (Ingelvac MycoFLEX®) and Classical Swine Fever (Pestiffa), both from Boehringer Ingelheim. While no PCV2 vaccination program has being done in the farm over the decade. Serology sampling revealed that the farm is endemic with CSF and PRRS. During the experimental period (6 months), a PCV2 vaccine (Ingelvac CircoFLEX®, Boehringer Ingelheim) was added to the piglet vaccination program, being administered together with Ingelvac MycoFLEX® at the age of 3 weeks. After the experimental period, the PCV2 vaccination was stopped. The mortality percentage for each stage of porker production: nursery (1-2 months old), grower (2-3 months old), and finisher (>3 months old) was recorded, and the overall mortality pattern was plotted.

Results

The mortality for nursery, grower, and finisher was reduced by 44.6%, 46.8%, and 40.2% respectively when compared to the non-vaccinated period (Table1). The overall mortality dropped from an average of 2.3% to 1.2% when all different age groups were vaccinated against PCV2 and increased back to 2.5% when the vaccination was discontinued again (Graph 1).

Conclusion and Discussion

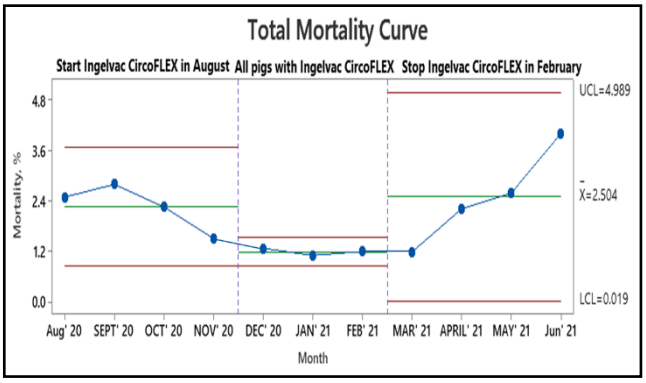
PCV2 is endemic in Malaysia and in this trial, piglet vaccination with Ingelvac CircoFLEX® supported to reduce mortality in every stage of porker production thus reducing the overall mortality. Although some other endemic pathogens in the farm could also contribute to the mortality percentage, we can see from Graph 1, that the overall mortality was reduced when all the pigs in porker production were vaccinated against PCV2 and started to increase back when the vaccination was stopped for monitoring purposes.

Table 1. Mortality rate (%) in different porker stages before and after the implementation of PCV2 vaccination.

	No PCV2 vaccination	Ingelvac CircoFLEX®	dif.	p-value*
Nursery	4.5	2.5	-44.6	*0.298*
Grower	6.6	3.5	-46.8	*0.020*
Finisher	0.9	0.6	-40.2	*0.024*
Total	2.8	1.2	-57.1	*0.026*

*Data was tested using Mann-Whitney test at 95% confidence level, using Minitab Statistical Software

Graph 1. Total mortality pattern before and after the implementation of PCV2 vaccination.



The data suggested that despite a complex farm environment, Ingelvac CircoFLEX® has demonstrated its efficacy, helping to reduce porker mortality. Antibiotic usage was not monitored, but farmer commented that during the trial period pigs looked healthier and had lesser complications.

Acknowledgments

The authors would like to thank Agritech Enterprise Sdn Bhd and the farm for their cooperation.

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Birth weight relation with prolificity, pre-weaning mortality and average daily gain in lactation (ADGl)

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Introduction

Pig production is in a state of permanent improvement and evolution. Antibiotic reduction or "Zero ZnO" law, is forcing the swine industry to adapt to new realities. An increase in prolificacy implies a reduction in birth weight (1,2) and a lower birth weight implies a higher mortality and a lower average daily gain (ADG) in lactation, in nursery and during the fattening period (3). In this scenario, we set out to discover how much birth weight is lost by increasing the litter size, and the impact of this birth weight upon pre-weaning mortality and average daily gain in lactation (ADG_l).

Materials and Methods

Individual data of 3483 piglets of the L241 line, DNA Genetics F1, were recorded in a commercial sow farm. Piglets were identified and weighed at birth and at weaning at an average of 22 days. Statistical analyses were carried out using the SPSS v.26 program and performed and interpreted according to Petri and Watson's recommendations (4). Intensity of the association between quantitative variables was quantified estimating the statistical correlation, and prediction of values of a quantitative variable from another quantitative variable was performed by linear regression. Comparison between groups of qualitative and quantitative variables was performed using the Chi square test and ANOVA respectively.
Correlation between prolificacy and birth weight was analyzed, and a regression line was established between them. Then, piglets born were placed into nine groups according to birth weight (**Table 1)** and the relationship of these groups with regards to pre-weaning mortality and ADG_l was analyzed.

Results

In this study, it was found that for every extra piglet total born in a litter, the birth weight for each individual decreased by 25 grams.

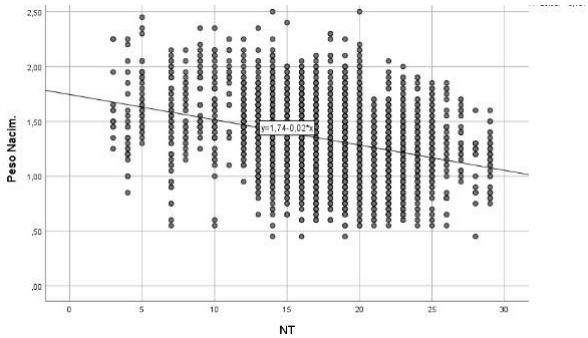

Figure 1. Birth weight and total born regression line.

With an average pre-weaning mortality in sow L241 of 10.4%, we find a highly significant difference ($p<0.05$) in piglet mortality according to their birth weight range.

Table 1. Pre weaning mortality according birth weight.

Tabla cruzada Mortalidad-pesosnac

			recodinterpesosnac									
			0,45-0,65 kg	0,66-0,86 kg	0,87-1,07 kg	1,08-1,28 kg	1,29-1,49 kg	1,50-1,70 kg	1,71-1,91 kg	1,92-2,12 kg	2,13-2,45 kg	Total
Mortalidad	Vivo	Recuento	34a	201b	382c	679c, d	741c, d	670d	279e	102a	32b, c, e	3120
		% dentro de recodinterpesosnac	48,6%	74,2%	86,0%	91,0%	91,7%	93,8%	95,5%	98,1%	94,1%	89,6%
	Baja	Recuento	36a	70b	62c	67c, d	67c, d	44d	13e	2a	2b, c, d	363
		% dentro de recodinterpesosnac	51,4%	25,8%	14,0%	9,0%	8,3%	6,2%	4,5%	1,9%	5,9%	10,4%
Total		Recuento	70	271	444	746	808	714	292	104	34	3483
		% dentro de recodinterpesosnac	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%

In addition, highly significant differences ($F=155.713$; $p<0.001$) were found between the weight of the deceased individuals and the weight of the piglets that did not die.

Finally, highly significant differences were observed ($F=46.431$: $p<0.001$) in ADG_l between groups with a birth weight below 1.08kg and those above.

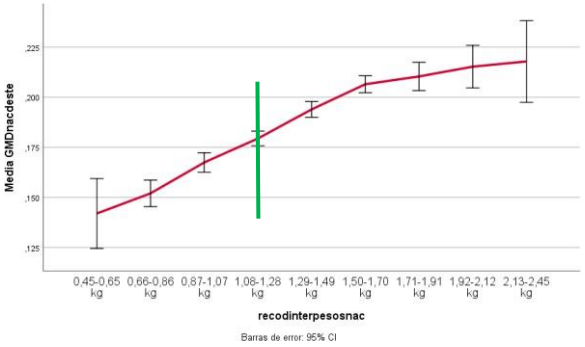

Figure 2. Relationship between ADG_l and Birth weight.

Conclusions

Birth weight is a determining factor in the productive results in our farms.
The results obtained confirm that by increasing the number of total piglets born in a litter, the birth weight of each of its individuals decreases.
Birth weight is directly and significantly related to pre-weaning mortality and average daily gain in lactation (ADG), lower birth weight, higher mortality and lower ADG.
Therefore, working both at a genetic level and at the management level to obtain better quality and weight of piglets at birth has a direct impact upon improved production results.

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Bromhexine efficacy to decrease the nasal mucus viscosity and pulmonary in swine

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Introduction

The main respiratory infectious agents of swine are enzootic in most farms, and some of them are part of the normal microbiota of the respiratory tract. However, the occurrence of disease is variable, being influenced by the presence, to a greater or lesser extent, of environmental and management risk factors that predispose animals to infections (1, 2), in addition to presenting clinically or subclinical. As a result, we have severe respiratory distress, resulting in decreased performance and increased mortality, worsening food efficiency, increased spending on medicines, increase in production costs, in addition to condemnation of carcasses in the slaughterhouse. The bromhexine, which alters the structures of secretions bronchial tubes due to fiber fragmentation mucopolysaccharides, providing the reduction of viscosity and improving the respiratory process. Aiming at reducing the clinical signs of the animals and mitigating the aforementioned losses, a study was carried out to evaluate the effectiveness of Bromhexine to reduce nasal and pulmonary mucus secretion in weaned piglets.

Materials and Methods

The experiment was carried out in the nursery phase. 36 piglets were used in the experiment, divided into 3 treatments, 4 animals per group with 3 replications. Group A was designated as a control, Group B treated with Bromhexine orally (1 mg/kg) for 3 consecutive days and Group C with Bromhexine via spray at a concentration of 7.5% for 3 consecutive days. Tracheobronchial lavages were performed on dayszero, 2 and 4 of the experiment for all groups. All tracheopulmonary lavage samples were evaluated for viscosity measurement. The statistical program StatGraphics 18 was used, performed for ANOVA statistical analysis with a significance level of $p < 0.05$. To determine the density of the samples, a pycnometer was used. With the use of water as a known solution and with the standard formula, it was possible to determine the density of tracheobronchial washes, using the Ostwald Viscometer to determine the viscosity.

Results

Viscosity results of tracheobronchial washes, as well as the average weights of the animals used in the experiment, are expressed in Table 1. In Graph 1, we can observe the dispersion of the results of viscosity according to the different groups.

Conclusions and Discussion

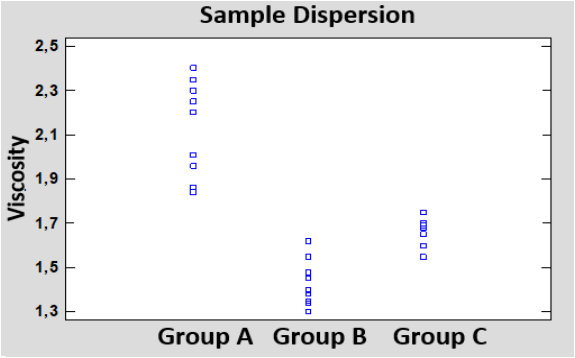
The results of animals in Group A (control negative) had the highest viscosity index. In contrast, the animals in Groups B and C (treatments with Bromhexine) showed indices of lower viscosity, that is, the expected effects of the product, especially with regard to the effect mucus fluidification, wereevidenced. Bromhexine as an agent mucolytic that alters the structures of bronchial secretions by fragmentation of mucopolysaccharide fibers, provides the viscosity reduction, which was evidenced in this work. Regarding the application routes, in Group B (drinking water) and in Group C (spray) there was no evidence of statistical difference, the which allows it to be used by different ways of application.

Table 1. Indicators according to experimental treatments.

Indicators	Treatments		
	A	B	C
Numbers of Animals	12	12	12
Weight (kg)	15,808	15,867	15,875
Viscosity*	2,177b	1,427a	1,677a
P value	0,1299	0,007	0,0356
Coefficient of variation (%)	9,39	7,33	3,88

A. Negative control; B. Bromhexine Oral (1mg/kg); C. Bromhexine Spray (7.5%).
[a,b] means followed by distinct letters on the line indicate difference statistics ($P<0.05$).
* Density values are expressed in mPa.s – Millipascal/ second.

Graph 1. dispersion of the results of viscosity according to the different groups*.



A. Negative control; B. Bromhexine Oral (1mg/kg); C. Bromhexine Spray (7.5%).
* Density values are expressed in mPa.s – Millipascal/ second.

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Carcass quality in pigs vaccinated with Porcilis Ileitis

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Introduction

Ileitis or Proliferative Enteropathy is caused by an obligate intracellular bacterium, *Lawsonia intracellularis*, and manifests clinically in several ways. The objective of this study was to evaluate the quality of pork meat carcasses in pigs vaccinated with Porcilis Ileitis Vs unvaccinated as a complement to the evaluation carried out during the growth of the pigs and with Ileitis challenge.

Materials and methods

The trial was conducted on a farm with 2100 sows located in the region of Antioquia (Colombia) with a history of Ileitis, diagnosed by Elisa (Svanovir® L. intracellularis/Ileitis-Ab) and clinical signs associated with intestinal hemorrhagic syndrome at the end of the fattening. For this study, 4085 piglets were randomly selected at the beginning of the fattening phase with an average age of 75.3 days of life, which were randomly distributed in two treatments, 1942 piglets vaccinated with Porcilis Ileitis (Treatment 1) with 25 replicates and 2143 unvaccinated piglets (Treatment 2) with 30 replicates. The Porcilis Ileitis group was vaccinated at 21 days of age (weaning), while the control group was not vaccinated. The environmental and management conditions were the same for both groups. The 1942 piglets vaccinated with Porcilis Ileitis were distributed in 25 groups or experimental units and the 2143 non-vaccinated piglets were distributed in 30 groups or experimental units. All pigs had ad libitum access to feed and water throughout the trial. Diets were formulated to be identical in all treatments, the % Crude Protein (PC) of the diets was 15.50%, 15.02% and 16.50% for the grower, fattening and finishing feeds, respectively. The fattening feed was medicated with 200 ppm tiamulin, 600 ppm chlortetracycline, 82.5 ppm methylene disalicylate bacitracin and 80 ppm halquinol.

1415 carcasses of the Porcilis Ileitis treatment were evaluated, corresponding to 74% of the pigs in the trial, and control treatment information was obtained from 1373 carcasses corresponding to 65% of the pigs in the trial. Hot carcass weight and backfat were analyzed using multiple linear regression models (1). All the analysis was performed in R software (2).

Results

The group vaccinated with Porcilis Ileitis obtained better carcass quality compared to the non-vaccinated pigs (summary in table 1).

Table 1. Carcass Evaluation

Parameter	Vaccinated group (PI)	Control group
N° Carcasses	1415	1373
Final Age	147,4	150,61
Back fat	13,58	14,74
Lean meat	56,37	55,36
Hot carcasses weight (Kg)	95,18	95,86

For dorsal fat, it was observed that the carcasses of the treatment control showed a tendency to a higher proportion of animals with higher values of back fat. These results indicate a positive association between these two variables, when carcass weight increases thereis a tendency to increase back fat. With 95% confidence,it is expected that for each kilogram of increase in hot carcass weight, the average dorsal fat increase between
0.14 and 0.18 mm. It is expected that the animals of the treatment Porcilis Ileitis present on average -1.05 mm of dorsal fat at the same weight in hot carcass ($P<0.0001$).

Conclusion and Discussion

In addition to the field results obtained in the present study, the quality of the carcasses was evaluated, where better results in backfat and carcass yield could be seenin the pigs treated with Porcilis Ileitis vs. the control or non-vaccinated group. The hypothesis is raised that this result may be related to an optimization of the nutrients and microelements of the diet, to have a better intestinal integrity, generating a greater benefit to meat processors

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Consolidating production, management and health data from all phases of production to assess the causes of wean-to-finish mortality

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Introduction

Multiple factors affect swine wean-to-finish (W2F) mortality, creating a problematic scenario when the goal is to identify and measure the major factors impacting pig survivability (1). At the same time, insightful data on most of the factors that affect swine performance, such as sow farm productivity parameters, closeout data of growing pig lots, health status, management, and diagnostic data, are commonly collected under commercial conditions but disconnected. The objective of this study was to develop an automated framework to capture and merge multiple data streams from one swine production system to identify and measure the major risk factors impacting swine wean-to-finish mortality.

Materials and Methods

The unit of analysis for this retrospective study were groups of growing pigs originated from a U.S swine production system, stocked in wean-to-finish barns or nurseries followed by finishers. Closeout mortality for the marketed groups was the outcome (referred to as W2F mortality). The growing phase performance and health/diagnostic information were linked to productivity and health data from the source breeding herds, using SAS software to generate a final master table (2). Univariate analysis was conducted for each variable on W2F mortality to reveal trends ($P < 0.10$), and then a multivariable analysis was executed using a linear mixed model and log-normal distributionfor W2F mortality having source as a random variable and explanatory variables holding $P<0.05$.

Results and Discussion

SAS algorithms were built to import, manage, and analyze data from 2,721 closeouts marketed between 2018 and 2020. The geometric mean W2F mortality of the study population was 9.7% (95% C.I. 9.5% - 9.8%). Overall, the lower the source sow farm productivity, the higher the downstream W2F mortality of the groups (Table 1). Closeouts originating from sow farms with higher neonatal losses average categories and highest pre-weaning mortality (PWM), had the highest W2F mortality compared to the other categories. Closeouts originating from weaned batches with the highest proportion of gilt litters and lowest average parity had the highest W2F mortality downstream. For sow farm and growing phase health status, higher mortality was observed in the presence of porcine reproductive and respiratory syndrome virus (PRRSV). For closeouts originated from sow farms with PRRSV-epidemic status (Status IA), higher W2F mortality was observed compared to other statuses. Likewise, higher mortality was observed in groups with positive PRRSV RT-PCR in the growing phase, where groups with Ct values below 27, and when a positive RT-PCR result occurred early in the growing phase (nursery phase) experienced the highest W2F mortality for these groups.

Table 1: Average W2F mortality of the groups

Risk factor	Category	W2F mort.
Litters parity (avg.)	2.6	10.0%[a]
	3.4	8.65%[b]
	3.7	9.19%[b]
	4.6	9.08%[b]
Gilt litters (%)	5.70%	9.06%[a]
	19.20%	9.31%[a]
	24.60%	9.09%[a]
	36.80%	9.87%[b]
PRRS status	IA	12.9%[a]
	IB	9.22%[b]
	II-vx	9.27%[b]
	IV	9.76%[b]
Weaning Age (days)	18.6	9.73%[a]
	20.8	9.25%[ab]
	21.9	8.93%[b]
	23.5	9.45%[a]
Neonatal losses (%)	7.10%	9.09%[a]
	8.40%	9.41%[ab]
	9.30%	9.08%[a]
	11.80%	9.93%[b]
PRRSV RT-PCR	Positive	10.0%[a]
	Negative	9.17%[b]
Pre-wean mortality (%)	13.10%	8.83%[a]
	16.40%	9.32%[b]
	18.80%	9.13%[ab]
	24.80%	9.95%[c]
PRRSV RT-PCR Ct.	≤ 27	10.9%[a]
	≥ 27	9.27%[b]
	Negative	9.16%[b]
PRRSV RT-PCR date	Nursery	10.9%[a]
	Finisher	9.64%[b]
	Negative	9.13%[c]

abc Different letters represent statistical difference. PRRS Status IA – high prevalence; IB – low prevalence; II-vx – vaccinated; IV – naïve.

Conclusion

Multiscale data consolidation within a swine production system provides the capability to collectively analyze previously dispersed and underutilized field data. This study identified the drivers of swine W2F mortality under the conditions of this company, but the results may vary over time or across other production systems.Therefore, measuring the true effect of different risk factors requires ongoing and on-farm analysis under field conditions. The pattern of increased W2F mortality in sow farms with lower productivity and PRRS presence was observed in previous studies in different conditions (1,3-4). The model developed in this study supports decision-makers to constantly measure the risk factors` effect on W2F mortality, and thus, to strategically allocate resources or interventions.

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Correlation between cough alarms indicated by a sound-based monitoring technology and diagnostic monitoring in growing pigs

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV), influenza A virus in swine (IAV-S), and *Mycoplasma hyopneumoniae* (Mhyo) are the main respiratory pathogens negatively affecting swine during their growing-finishing period. SoundTalks® is a sound monitoring technology that detects, aggregates, and analyzes the sound of respiratory distress (coughs) in swine using artificial intelligence by providing a green-yellow-red alerting scheme to producers. It is reported that this technology provides early warnings (yellow alarms) 2-5 days before clinical signs become evident for care givers[1]. However, further research is needed to compare these alarms to the traditional 'gold standard' of diagnostics via oral fluids[2]. Therefore, the objective of this study was to correlate the SoundTalks® alarms (yellow/red) with pathogen detection via oral fluids (OF) in a grow-to-finish commercial facility.

Materials and Methods

Three-week old piglets from a PRRSV positive source were placed at two sites: East and West (each site contained 4 rooms of 2100 pigs) and monitored for the entire wean-to-finish period Variance in age between rooms within a site was approximately 2 weeks, Variance in age between sites was approximately 3-4 weeks. Each air space (room) was equipped with 4, systematically spatially located SoundTalks® monitors (sampling zones) and one OF sample was collected[2] under each monitor weekly throughout the 24 weeks grow out for routine surveillance of PRRSV, IAV-S, Mhyo, and porcine epidemic diarrhea virus (PEDV). Extra sampling (clinical sampling) was also performed based on alarms (at day 2 of a yellow alarm or day 1 of a red alarm) for all sampling zones within the same air space. For all diagnostic samples, sampling zone, monitor color as well as pig´s age at the time of sampling were documented. OF were shipped to ISU-VDL for diagnostic analysis by qPCR. Results were analyzed firstly using a chi-square test of independence to examine the relation between SoundTalks® alarms and PCR result. Secondly, to predict the strength of this potential association, an ordinal logistic regression was used to predict the odds of having a yellow or red alarm given the PCR result.

Results

A total of 844 OF samples (698 routine and 146 clinical OF surveillance samples respectively) were analyzed for the study. There was a significant relationship between the different alarm colors and the PCR results (i.e., Positive or Negative) for PRRSV, IAV, and Mhyo ($\chi^2_{PRRSV} = 15.6$, p-value < 0.001; $\chi^2_{IAV} = 60.9$, p-value < 0.001; $\chi^2_{Mhyo} = 77.9$, p-value < 0.001).

Upon further analysis of the data, it was determined that the number of PRRS-positive diagnostics was likely increased due to a PRRS MLV vaccination occurring during the first week of July for both sites. As a result, PRRSV was excluded from further analysis.

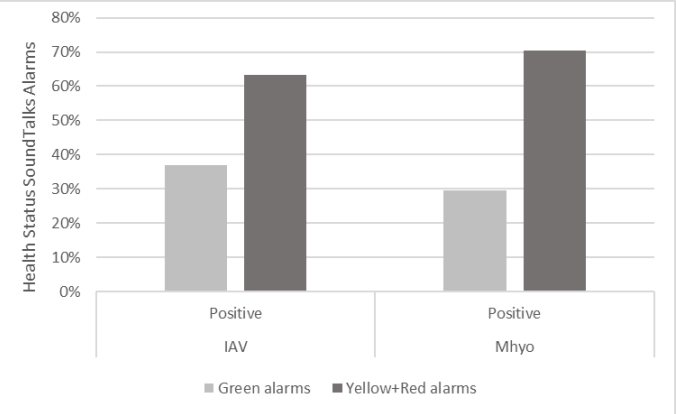

Fig 1. Proportion of SoundTalks® alarms colors during the IAV and Mhyo outbreak periods (positive days).

A proportional odds logistic regression was considered to regress the SoundTalks® alarm colors (i.e., green, yellow + red) onto the PCR results (i.e., Negative and Positive), weeks of age and farm site. As shown in Fig 1. for a positive result for IAV, the odds of having a red and yellow alarm (i.e., an alarm color potentially indicating a problem) were 4.1 times higher than for a green alarm (i.e., an alarm color indicating no problem). In addition, for an increase of one week of age the odds of having red or yellow alarms were 10% higher compared to a green alarm. On the other hand, for a positive result for Mhyo (Fig 1), the odds of having a red and yellow alarm were 3.1 times higher than for a green alarm, and for an increase of one week age the odds of having a red or yellow alarm is 6% higher compared to a green alarm. Site was not statistically significant neither for IAV nor for Mhyo.

Conclusions and Discussion

Results from this study demonstrated that, in the case of 2 of the major swine respiratory pathogens, SoundTalks® yellow and red alarms are reliable as a diagnostic tool under commercial conditions. The odds of having a red and yellow alarm with positive OF results were higher for IAV than for Mhyo and increased with the age of the piglets. Further studies are needed to evaluate the effect of PRRS positive OF results considering field vs vaccine strain.

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Could extra virgin olive oil modify boar semen qualities?

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Introduction

Boars' spermatozoa have got a high percentage of polyunsaturated fatty acid and low percentage of cholesterol in its cytoplasmic membranes. These biochemical characteristics make them highly susceptible to lipid peroxidation, which could affect some seminal characteristics like total motility (TM), progressive motility (PM) and sperm conservation. Arauco variety extra virgin olive oil (EVOO) has got antioxidant components like tocopherols, carotenoids, and phenolic compounds (PC). Other vegetable oils lack PC and among those present at the EVOO have special interest those that have got an ortho-diphenolic group (1, 2). PC have shown to have an antioxidant activity equal to or more than other known natural antioxidants like vitamin E, vitamin C and lutein (3). EVOO also contains triglycerides and fatty acids, such as palmitic and stearic acid (saturated), linoleic and α-linolenic acids (PUFAs), and oleic acid (MUFA), which represents between 56-84% of the total fatty acids in EVOO (1, 2, 3). The aim of this study was to determine whether oral supplementation with EVOO could increase semen quality.

Materials and Methods

Eight mature boars of defined genetics were used, housed under controlled environmental conditions in an boar stud (Córdoba, Argentina). They were randomly distributed into two equal groups. The treated group (TRT) was supplemented orally each day with 5% v/w of EVOO on top of the feed ration for 7 weeks. Boars of the control group (CON) did not receive any supplementation. The semen quality was determined through microscopic analyses performed by the computerized semen analysis system (AndroVision®, CASA system from Minitube), once a week for a total of 19 weeks; 6 weeks prior to treatment (PRE), 7 during it (INTRA) and 6 after the end of treatment (POS), from April to July. The parameters evaluated were % of total motility (TM), % of progressive motility (PM) and % of morphological abnormalities (MA). CASA system detected the following sperm abnormalities: proximal cytoplasmic droplet (PCD), distal cytoplasmic droplet (DCD) and bent tail (BT).
Parameters of both groups were compared. Data were analyzed by repeated measures of ANOVA over time using the GLM procedure of SAS.

Results

Significant dispersion of TM was found in ejaculates of the CON group in the POS period, ranging between 73.32-95.19% (Table 1). However, in the TRT group, in the same period, there was a less dispersion and TM was more concentrated around the mean, ranging between 86.49-96.09%. The PM of the ejaculates in the TRT group POS treatment was ranged between 69-80.85%; while none of the ejaculates reached 80% in the CON group. Significant DCD and BT levels were not obtained in any period or group. However, the PCD in the CON group during the PRE and POS periods ranged between 2-11%. The same parameter in the TRT group was maintained in both periods between 0 and 7%(Table 1).

Discussion and Conclusion

Oral supplementation with EVOO improves several boar semen parameters, although not significantly. Our results agree with those observed previously (4, 5) who did not obtain significant differences in the TM between its experimental groups. On the other hand, PM in boar ejaculates were significantly improved after oral supplementation with grape marc (6).
It is possibly, with a higher number of animals or in a period of the year during which a decrease in semen quality is commonly observed, significant differences between the groups could be obtained.

Table 1. Means and SE of different evaluated seminal parameters

	CON		TRT	
%	PRE	POST	PRE	POST
TM	90,43+/-0,83	90,47+/-1,25	91,29+/-0,67	91,64+/-0,55
PM	67,65+/-2,08	64,96+/-2,70	65,88+/-2,21	70,26+/-1,44
PCD	4,91+/-0,47	5,31+/-0,54	2,87+/-0,49	2,34+/-0,49
DCD	0,75+/-0,12	1+/-0,23	2,54+/-0,53	2,86+/-0,54
BT	0,54+/-0,13	0,81+/-0,16	0,91+/-0,11	1+/-0,18

Acknowledgments

Workers and Veterinarians of the Swine Artificial Insemination Center. Genetics company Topigs Norvins Argentina.

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Early diagnostic capability of a sound-based monitoring technology compared to oral fluids surveillance ingrowing pigs

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Introduction

Swine respiratory diseases, including porcine respiratoryand reproductive syndrome virus (PRRSV), influenza A virus in swine (IAV-S), and *Mycoplasma hyopneumoniae*(Mhyo), have negative health, welfare and economic consequences.[1] Pen-based oral fluid (OF) sampling could be considered the standard approach for respiratory pathogens surveillance in swine populations[2] however frequency and number of samples continues to be a burden that may underestimate the presence of thesepathogens in the farm. SoundTalks® (ST) is a cloud-based sensor technology that monitors 24/7 the sound emitted from pigs[2]. Using artificial intelligence based on an algorithm for finishing pigs, this technology generates early warnings (yellow/red LED alerts) for producers to intervene prior clinical signs of respiratory distress episodes are evident for care givers. However further research is needed to understand the advantages of this technology compared to an intensive OF surveillance program in the field. Therefore, the objective of this studywas to determine the timing of cough episodes´ diagnostics based on ST monitor alerts compared to an intensive OF surveillance program in swine finishing populations.

Materials and Methods

This clinical case originated from 2 wean-to-finish sites (4 rooms/site, 8,400 head spaces/site), monitored for respiratory episodes 24/7 by 4 systematically spatially located ST monitor/room and by OF sampling under each ST monitor on a weekly basis or immediately after a yellow/ red alert. For the purpose of the study, cough episodes were identified as such if the monitors were redor yellow for 5 consecutive days or more without a breakof 5 or more green days. If 5 or more consecutive green days were observed, the previous episode was consideredtermed. Within each episode, OF were assessed to determine if there was lead time between the time of ST alarm (yellow or red) and when the diagnostics identifieda positive result. Variables such us daily ST monitor color, sampling zone, cough episode, lead-time between diagnostic techniques, PCR results as well as pig´s age atthe time of sampling (nursery vs finishing phase) were consolidated for the analysis.

Results

844 OF samples were analyzed and 114 were positive forMhyo, 91 for IAV, and 450 for PRRSV. PRRS-positive diagnostics was likely increased due to a PRRS MLV vaccination occurring 1 week prior the study for both sites. As a result, PRRSV was excluded from further analysis. In all rooms, a total of 25 cough episodes (mean=3.1, max=5, min=2) were identified based on ST daily monitoring for the entire wean-to-finish period.

Fig 1 represent daily ST alarm status/room, OF results for IAV or Mhyo and number of cough episodes for 1 of the 8 rooms in the farm

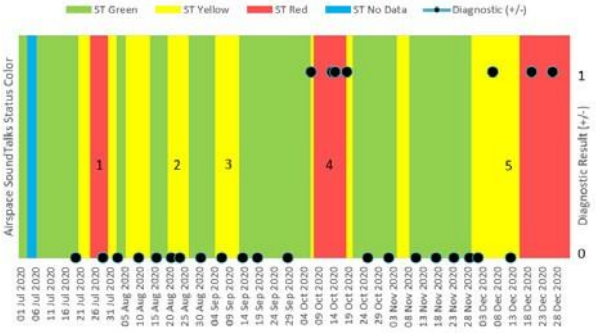

Fig 1. Weekly diagnostic results (pos=1, neg=0), daily SoundTalks (ST) alarm by color and number of cough episodes identified in room5.

Results from the study demonstrated that 13/25 of cough episodes detected by ST happened during the nursery phase (11 weeks on feed or younger). From those identified during the finishing period (12/25), ST detected 4.8 days sooner, as an average, than OF sampling the outbreaks events. When considering each of the pathogens individually, ST was capable to detect IAV outbreaks 5.1 days earlier and, in the case of Mhyo, 1.5 days earlier than intensive OF surveillance program in the field.

Conclusions and Discussion

Results from this study demonstrated the importance of continuous and real-time monitoring of the swinegrowing population due to the high incidence of cough episodes in commercial barns. SoundTalks® technology is a sound monitoring system available for producers that allows them to identify earlier clinical signs of respiratorydistress. Under the conditions of this study, SoundTalks®detected cough episodes 4.8 days earlier (max 5.1- min 1.5) than intensive OF sampling monitoring for IAV and Mhyo agreeing with previous literature when compared on observation of clinical signs. As a follow up to the early diagnostic capability of this technology, furtherstudies are needed to understand the impact of early interventions trigger by SoundTalks® that aimed at reducing production losses due to the presence of respiratory pathogens in swine growing populations.

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Effect of a phytobiotic blend on yield and carcass quality of finishing pigs subjected to transport stress.

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Introduction

In Mexico, it is estimated that 6 out of 1,000 pigs are lost due to deaths associated with ship process. The prevalence of lower quality carcass related to stress represents 50% of the analyzed carcass, reflected in economic losses (1). Pig transportation has been associated with increased oxidative stress in the muscle tissue and triggers autophagy (2). Grupo Nutec has developed a phytobiotic blend based on flavonoids (AXION®) to mitigate some problems related to the peripartum in lactating sows, by reducing the dangerous effects of oxidative stress. The flavonoids of this product are characterized to reduce the reactive oxygen and nitrogen species while increasing antioxidants enzymes by activating the Nfr2 system; besides promoting neurogenesis, neuronal differentiation, and improving dopamine, norepinephrine, and serotonin secretion (3). Therefore, the objective of this study was to evaluate the effect of dietary supplementation of AXION® on yield and carcass quality of finishing pigs subjected to transport stress.

Materials and Methods

154 d- Forty-eight barrows (Topigs TN70 X PIC PB 410) were randomly distributed in one of the treatments: 1) Control (Basal diet) y 2): AXION® (Basal diet + AXION® 2 kg/ton of feed). Pigs were transported to the slaughterhouse (Qro, Mexico). Carcasses were weighted and after 45 min the pH was measured. After 12 h carcasses were measured again. Data were analyzed by a one-way ANOVA model followed by a T-test (JMP).

Results

Growth performance and carcass yield means are shown in Table 1. No differences were observed among treatments for the growth performance; carcass yield was improved with supplementation of AXION® ($p \leq 0.05$).

Discussion and Conclusion

The use of flavonoids has been tested in pigs at different periods showing improvements in the appetite, feed intake, fed conversion ratio, and nutrient digestibility, also diminishing noxious gases and oxidative stress by increasing antioxidants, which is reflected in improvements in carcass quality (4, 5 and 6).

In the present study, pigs supplemented with AXION® had greater daily feed intake (0.130 kg) than control, and greater average daily gain (0.10 kg) than the control treatment, however, due to the short period of the trial we did not observe significant differences.

Differences in the carcass yield may be associated with fluid losses by fasting, urine, excessive sweating, and increased respiratory rate related to stress (7).

The results in this study suggest that differences in carcass yield may be related to an effect on stress reduction in animals supplemented with AXION®. The same effect was observed in pH measurement, which is closer to the values referred to a normal carcass, while without supplementation (control) the initial pH would be considered likely to be a Dark, Firm and Dry meat (7).

Table 1. Effect of dietary supplementation of AXION® on yield and carcass quality of finishing pigssubjected to transport stress.

	Control	*AXION®*	*SEM*	*SIG.*
Initial weight (kg)	124.16	123.07	8.44	0.97
Final weight (kg)	130.93	131.18	9.11	0.92
ADG	0.97	1.06	0.23	0.14
Feed efficiency	0.40	0.37	0.08	0.38
Hot carcass (kg)	109.84	111.83	2.45	0.01
Hot yield carcass %	82.76	83.41	0.80	0.01
Cold carcass (kg)	108.12	110.27	2.62	0.01
% Drip loss (12 h)	1.58	1.40	0.45	0.20
Initial pH	6.61	6.45	0.25	0.048

These results are the beginning of this research about the effect of AXION® improving growth performance, carcass yield and meat quality.

Acknowledgments

Municipal slaughterhouse "TIF 412" (Qro, Mexico), experimental farm IIIA, Rafael Romero and Salvador Helgueros for their support in this project.

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Effect of pig synthetic pheromones and positive handling of pregnant sows on piglet performance

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Introduction

Piglets use odor cues for identifying the mother and teat position as early as 12 hours after birth (1). The olfactory senses aid in recognition of pen mates (2) and discrimination between different littermates (3). Application of a synthetic maternal pheromone increased exploratory behavior and reduced agonistic behavior among weaned pigs (4). Previous studies did not show a statistically significant effect of synthetic pheromone on live weight, growth rate or feed conversion efficiency.

This study investigated the effect of a synthetic pheromone on the performance of nursery pigs thatwere weaned from sows previously positively handled (scratching, music) in the farrowing room.

Materials and Methods

In the farrowing houses, auditory enrichment (music from a radio) was provided to treated sows (T-sows) daily from 0600 to 1800 h until the end of lactation. Until the day of farrowing, T-sows were additionally subjected, for 15 s per day per sow, to continuous back scratching by one member of farm staff. From both treated (T) and control (C) sows, piglets (T; n = 299; C; n = 715) were included in the second study in the nurseries. The groups were represented as TT, TC, CT and CC, with the first letter referring to the group allocation of sows of the first experiment and the second letter referring to the piglets in the nursery. Weaned piglets of groups TT and CT received treatment with synthetic pig appeasing pheromone blocks (Secure Pig®, Signs®, France) for a period of 6 weeks. Nurseries with groups TC and CC received no treatment. The average daily gain (ADG) and feed intake were measured at group level. Piglets were weighed using a scale at weaning and at 39 days after entry into the nurseries. Feed silos were emptied at the end of 6 weeks (study period) and feed remaining from the silos was weighed to calculate the group feed intake. The data were analysed using SPSS software (SPSS version 25®; IBM, Armonk, NY, USA). Growth means and mortality were compared between the T and the C groups for sows and separately for the piglets. Additionally growth means and mortality were compared for all 4 groups. A p-value of 0.05 was considered significant for all analyses. Tests with multiple groups were adjusted for all pairwise comparisons using the Bonferroni correction.

Results

Treated piglets from treated sow groups (TT) and control piglets from treated sow (TC) groups had significantly ($p<0.05$) better ADG and final weight when compared to treated piglets from control sow groups (CT) and control piglets from control sow groups (CC). Significant difference ($p<0.05$) was observed in the final weight (TC 16.77 kg vs TT 17.33 kg) and ADG (TC 282.65 g/day vs TT 299.49 g/day) of treated and control piglets when sows were treated. No significance was observed in the final weight between treated (16.68kg) and control piglets (16.86 kg) when they came from control sows (Table 1). Piglets form treated sows (ignoring the securepig ® treatment) had better performance: ADG +15.54 g/day, FCR -0.65 and mortality -1.23 %.

Table 1. Combined effect of positive handling in maternities and pheromones in nurseries on production parameters

	CC	CT	TC	TT
WEANING WEIGHT (KG)	$5,74_{a}$	$5,74_{a}$	$5,56_{b}$	$5,69_{a,b}$
END WEIGHT (KG)	$16,86_{a,b}$	$16,68_{a}$	$17,26_{b,c}$	$17,39_{c}$
GROWTH (G/DAY)	$284,98_{a}$	$280,31_{a}$	$299,10_{b}$	$299,84_{b}$

Note: Values in the same row and sub-table not sharing the same subscript are significantly different at $p< 0.05$

Discussion and Conclusion

Sows subjected to the combined effect of music and back scratching had better piglet survival rates in comparison to the sows without these treatments. The peri-parturient period is a critical time for piglet survival and needs positive intervention for better welfare and productivity. Regardless of whether the piglets were treated, improvements were observed in the final weight and ADG of piglets when sows were treated. In conclusion, for overall performance, exposure of piglets to the synthetic pheromone and positive handling of their sows during gestation had positive effects on pig weight gain and feed efficiency in the nursery phase.

Acknowledgments

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Effects of using olive, winery, and cheese waste by-products in the diet of weaned piglets ontheir performance parameters

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Introduction

During the last decades, there was an increased worldwide interest for new innovative feeds with a minimum environmental footprint, while keeping a high level of animal health, welfare, and productivity. For thisreason, many researchers have tested the use of agro- industry by-products in farm animal diets.

Our trial was performed in a commercial pig farm in Greece using a silage which contained a special mixture of olive, winery, and cheese waste by-products from the Greek agro-industry sector. The silage was added in weaned piglets' diets to investigate possible effects on growth performance and feed efficiency.

Materials and Methods

All experimental procedures were in accordance withthe National guidelines for animal trials.

The experimental silage was prepared using corngrains supplemented with grape pomace, deproteinizedcheese and olive mill wastewater at a relative ratio of20/20/60. The silage was vacuum-packed and fermentedfor 30 days, after inoculation with *Lactobacillus buchneri*.45 crossbred (1/4 Large White × 1/4 Landrace × 1/2 Duroc) weaned piglets were individually ear-tagged and randomly allocated to 3 treatments. The experimentcommenced at 34 days of age and ended at 74 days of age.The diets were in meal form and formulated according to standard recommendations. Group A was the controltreatment (no added silage), while in groups B and C theexperimental silage was included at 5% and 10%accordingly. Access to feed and drinking water was *ad libitum*.

The collected data was subjected to one-way ANOVA. Significance level was set at 5% ($P<0.05$). Data homogeneity was tested using Levene's test.

Results

Table 1 presents the effect of the dietarysupplementation on the weaned piglets performance parameters. Final body weight of the piglets did not differ($P>0.05$) between the treatments. It was noted that body weight gain was lower for treatment Group B ($P\leq0.05$) compared to the other two treatments during period 22-40days, however the weight gain for the overall period (34-74 days) did not differ significantly ($P>0.05$) between thethree treatments. Feed intake and feed conversion ratio were within the expected ranges for the commercial pig farm that housed the experimental trial.

Table 1. Effect of the dietary supplementation ofsilage on performance parameters of weaned piglets.

	Silage inclusion				
	0%	5%	10%	SEM	P
Live weight, kg					
Day of age: 34	8.30	8.32	8.40	0.18	0.98
Day of age: 56	17.46	18.51	18.37	0.40	0.53
Day of age: 74	26.47	26.38	27.64	0.49	0.52
Weight gain, kg					
Day of age: 34	9.16	10.18	9.97	0.29	0.32
Day of age: 56	9.01^{ab}	7.87^{a}	9.27^{b}	0.24	0.04
Day of age: 74	18.16	18.06	19.24	0.39	0.40
Feed intake per pig (kg)					
Day of age: 34	14.54	15.97	16.09	-	-
Day of age: 56	16.86	14.10	15.12	-	-
Day of age: 74	31.39	30.07	31.21	-	-
Feed conversion ratio					
Day of age: 34	1.59	1.57	1.61	-	-
Day of age: 56	1.87	1.79	1.63	-	-
Day of age: 74	1.73	1.67	1.62	-	-

^{a,b} Mean values with different superscripts differ significantly ($P\leq0.05$).

Discussion and Conclusions

Huge quantities of vegetable and animal-origin wastes and by-products of agro-food processing industries are available throughout the world, and in many cases represent a serious environmental hazard.It is known that grape pomace and olive mill wastewater contain high levels of polyphenols. Polyphenolic compounds from olive mill wastewater have improved the growth performance of weaned piglets (2). In addition, deproteinized cheese whey is important for weaned piglet diets due to its high concentration of lactose (3).

Based on the results of this feeding trial, the silagecreated with these three by-products of the Greekagro-industry sector can be used in weaned piglet diets without any negative effects on their performance.

Acknowledgments

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Effects on meat quality characteristics of 74 days old piglets fed by products rich in polyphenols, beneficial bacteria, and antimicrobial peptides

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Introduction

During the last decades, farmers are facing multiple challenges concerning their productions systems. One of the most important issues is the constantly increasing cost of the feed. On the other hand, consumers nowadays show a preference for healthy food with advanced nutritional properties. Thus, researchers try to find economically and innovative animal feeds which could lead to the production of functional foods.
In this trial, meat quality parameters were measured in weaned piglets which were fed a silage with by-products (olive mill waste water, deproteinized cheese whey and grape pomace) of the Greek agro-industry sector. These by-products are rich in proteins, polyphenols and beneficial (lactic acid) bacteria.

Materials and Methods

All experimental procedures were in accordance with the National guidelines for animal trials (1).
A total of 45 crossbreed piglets (1/4 Large White × 1/4 Landrace × 1/2 Duroc), 34 days-old were, used in a dietary experiment. The pigs were allocated into three groups, 0%, 5% and 10% inclusion rate of silage. The whole experiment lasted 40 days. Meat cuts from the shoulder, ham and the pancetta were collected after slaughter from 6 animals per treatment. Chemical analysis (moisture, protein, lipid, collagen, and ash) antioxidants parameters (TBARS, protein carbonyls, catalase, and glutathione), total phenols and pH were measured for the different meat cuts (ham, pancetta, shoulder).
The collected data was subjected to one-way ANOVA, using the IBM SPSS Statistics v. 20.0 Statistical Package (SPSS Inc., Chicago, IL, USA). Data homogeneity was tested using Levene's test. Significance was set at 5% ($P<0.05$).

Results

Fat, protein, collagen, and moisture did not differ significantly between the three treatments for all meat cuts ($P>0.05$). Shoulder and belly meat ash content was increased in treatment 10% ($P<0.05$). Antioxidant parameters and pH of different meat cuts were affected (Table 1). TBARS in ham and pancetta meat were reduced ($P<0.05$) in the supplemented treatments. Total phenols were increased significantly in pancetta and shoulder meat cuts with the inclusion of the silage. pH values of ham, pancetta and shoulder meat did not differ significantly between the groups ($P>0.05$).

Conclusions and Discussion

It has previously been reported that use of grape pomace in weaned piglets could increase the total antioxidant capacity (TAC) levels of the meat (2). Moreover, the major phenolic compounds of the olive mill wastewater (oleuropein, hydroxytyrosol, tyrosol and elenolic) have been previously tested as natural antioxidants (3).

Table 1. Effects of dietary supplementation on total phenols, TBARS and pH content of ham, pancetta, and shoulder meat cuts.

	Silage inclusion				
	0%	5%	10%	SEM	P
Ham meat					
Total phenols[1]	1.75	2.24	1.96	0.09	0.08
TBARS[2]	0.12^{b}	0.08^{ab}	0.07^{a}	0.01	0.05
Pancetta meat					
Total phenols[1]	1.55^{a}	2.15^{b}	1.79^{ab}	0.10	0.02
TBARS[2]	0.08^{b}	0.05^{ab}	0.04^{a}	0.01	0.02
Protein carbonyls[3]	19.5	15.4	12.8	-	-
Catalase[4]	56	59	66	-	-
Glutathione[5]	0.20	0.18	0.17	-	-
pH	5.52	5.50	5.54	0.01	0.40
Shoulder meat					
Total phenols[1]	0.86^{a}	1.79^{ab}	2.32^{b}	0.25	0.02
TBARS[2]	0.09	0.05	0.05	0.01	0.40
Protein carbonyls[3]	18.6	13.4	11.8	-	-
Catalase[4]	52	58	64	-	-
Glutathione[5]	0.22	0.17	0.16	-	-
pH	5.57	5.66	5.6	0.05	0.79

^{a,b} Mean values with different superscripts differ significantly ($P<0.05$). [1]Total phenols (g/L); [2]TBARS (mg MDA / kg) [3]Protein carbonyls (nmol/mg); [4]Catalase (U/mg protein); [5]Glutathione (nmol/mg protein)

Based on the results of this feeding trial, administration of silage which contains by-products from the Greek agro-industry sector rich in bioactive compounds, enhanced antioxidants mechanisms and altogether the meat quality of weaned piglets.

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Evaluation of efficacy of Ingelvac® PRRS MLV and 3FLEX® in wean-to-finish pigs in a commercial farm in Malaysia

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) remains endemic in Malaysia and causes devastating economic consequences in domestic herds (1). PRRSV not only restricts the reproductive performance of sows and boars, it also causes respiratory disease in wean-to-finish pigs leading to the herds highly susceptible to secondary diseases (2). The objective of this study was to evaluate the efficacy of Ingelvac® PRRS MLV and 3FLEX® (PRRS MLV/PCV2/ *Mycoplasma hyopneumoniae*) in wean-to-finish pigs.

Materials and Methods

The trial was conducted in a PRRS-endemic, farrow-to-finish farm with 500 sows in Malaysia. Sows were routinely vaccinated with Ingelvac® PRRS MLV but not in piglets. Upon trial, two-week old piglets were vaccinated with Ingelvac® PRRS MLV (2ml IM) and the mortality rate at each stage were recorded. After 5 months, significant improvement was observed in mortality rate especially in 4-6 months old finisher. Therefore, farmer decided to try out 3FLEX® (2ml IM) in three-week old piglets. Mortality rate were recorded for all production stages from April 2020 to June 2021. Statistical analysis was carried out for the average mortality of 2-4 months old grower and 4-6 months old finisher using Chi-Square (Microsoft Excel Spreadsheet).

Results and Discussion

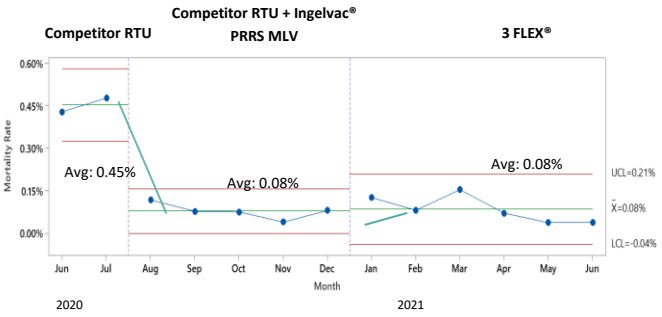
There was reduction in mortality rate in the grower stage (numerically) and finisher stage (statistically) after implementing Ingelvac® PRRS MLV in piglets. 47.6% and 82.2% improvement in mortality rate were observed in 2-4 months old group and 4-6 months old group respectively. Furthermore, in conjunction with hot and humid weather in Malaysia, installation of fans completed in 4-6 months old finisher house in August 2020, synergically decrease the mortality rate in finisher. After the implementation of 3FLEX®, positive comments were received from farmer with the benefits of saving labour work and time, as well as mortality rate in all stages were maintained. In March 2021, farmers decided to stop vaccinating Ingelvac® PRRS MLV in piglets due to cost constraint. After withdrawing PRRS vaccination, surging of mortality rate up to 0.35% were observed in 2-4 months old pigs, proving the importance of PRRS vaccination in piglets.

Table 1. Average mortality rate (%) from April 2020 to June 2021

	Competitor RTU	Competitor RTU + Ingelvac® PRRS MLV	3 FLEX®
2-4 months old	0.42	0.22	0.23
4-6 months old	0.45[a]	0.08	0.08[b]

°*Superscripts indicate statistically significant differences ($p \leq 0.05$).*

Figure 1. Monthly mortality rate in 4-6 months old pigs (%)



Conclusions

Ingelvac® PRRS MLV has proven to significantly reduce mortality rate in the finisher stage. Moreover, 3FLEX® provided better alternatives as single injection to minimize stress and disease spreading, as well as less labour work is required. In addition, environmental parameters should not be overlooked in trials as it has a direct impact onto the farm performance.

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Evaluation of production performance following the implementation of 3FLEX® in a commercial farm in Malaysia

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is an economically important swine diseases that affects commercial swine production worldwide including Malaysia [1]. PRRS is characterised by late term reproductive failure in sows and gilts, and respiratory problem in wean-to-finish pigs leading to devastating economic consequences in domestic herds. The objective of this study was to evaluate the production performance of wean-to-finish pigs following a vaccination scheme of 3FLEX® (PRRS MLV/PCV2/ *Mycoplasma hyopneumoniae*).

Materials and Methods

The trial was conducted in a well-managed, farrow-to-finish farm with 800 sows in Malaysia. Piglets were routinely vaccinated with Ingelvac CircoFLEX® (1ml IM) prior to weaning. The farm has been producing PRRSV negative weaners for quite some time. However, PRRSV seroconversion was observed in the grower-finisher, suggesting the field PRRSV exposure. Therefore, farmer decided to implement 3FLEX® vaccination in porkers to provide immunity against PCV2, PRRS and *Mycoplasma*. 3FLEX® (2ml IM) was administered in three-week old piglets and the production performance at each stage were recorded. Parameters observed include percentage of sick weaners, mortality rate, final body weight to market and Modified Madec lung scoring (Continuous scoring system: 0-4) [2] that involve relative weight of each lobe. While the Madec lung scoring was 2-dimensional and imprecise because it ignored the three-dimensional nature of the lesions. Statistical analysis was done using Histogram and Kruskal-Wallis Test (Minitab 20).

Results and Discussion

There was significant reduction in percentage of sick weaners, from 13.56% to 9.91% after implementing 3FLEX® in piglets. In addition, 14.3%, 8.2% and 18.4% improvement in mortality rate were observed in weaners, starters and finishers group respectively. Furthermore, 3FLEX® group required fewer day, 176 days to achieve higher average final weight which was 117.1kg as compared to CircoFLEX mono group required 189 days to achieve 115.3kg average final weight. Besides that, there was noticeable decrease in average Modified Madec lung scoring after the implementation of 3FLEX® which indicated vaccination has effectively reduced the lung lesions of the finisher as observed at the slaughterhouse.

Table 1. Sick weaners (%) before and after implementing 3FLEX®

Group	Sick Weaner (%)
Pre-3FLEX®	13.56
Post-3FLEX®	9.91

Table 2. Mortality rate (%) of weaners, starters, finishers before and after implementing 3FLEX®

Group	Weaner	Starter	Finisher
Pre-3FLEX®	0.70	0.61	0.76
Post-3FLEX®	0.60	0.56	0.62

Figure 1. Histogram of finisher body weight (kg) achieved by 3FLEX® and CircoFLEX mono groups in different days

Table 3. Average Modified Madec Lung Scoring by 3FLEX® and CircoFLEX mono groups

	Pre-3FLEX®			Post-3FLEX®
	Aug 2020	Oct 2020	Dec 2020	April 2021
Average Modified Madec Lung Scoring (max score 4)	1.28[a]	1.87[b]	1.81[b]	0.99[c]

Conclusions

3FLEX® has proven to reduce sick weaners and mortality rate in all production stages. Moreover, piglets vaccinated with 3FLEX® able to perform better in termof reaching market size earlier with heavier final weight.Hence, cost saving in feed able to turn into profits to thefarm. Besides that, implementation of 3FLEX® also significantly decrease the lung lesions of finishers.

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Fecal bacterial diversity of pigs during fattening with different carcass composition at slaughter

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Introduction

Body composition is of crucial importance for the marketing of pork (1), as fattened pigs are paid in central Europe based on lean meat content. Therefore, aim of pig fattening is to achieve a certain body weight (BW) with a high muscle and low fat percentage in the shortest possible time. However, pigs differ in their performance potential and thus in their nutrient requirements. Characterization of intestinal microbiota is of great interest due to its seeming impact on growth, feed efficiency and pig carcass quality (2). The aim of the study was to evaluate retrospectively, whether pigs with carcass composition at slaughter show differences in fecal microbiota diversity already during the fattening period.

Materials and Methods

The study was performed in a conventional large group-housing barn (n=300 pigs) with sorting gates and automatic individual body weight recording. For animal identification in sorting gates and at slaughterhouse, all pigs received transponder ear tags. Feed and water was offered *ad libitum*. At four different times during the fattening period (26., 29., 31., 34. week of life (WL)), 40 fecal samples were collected. The pigs were slaughtered at Ø BW of 120 kg. The animals were retrospectively divided into four groups according to their BW at 34. WL and lean meat content (LMC) evaluated at slaughter: "light fat" (BW <108.3 kg and LMC <61.2%), "light lean" (BW <108.3 kg and LMC >61.2%), "heavy fat" (BW >108.3 kg and LMC <61.2%), "heavy lean" (BW >108.3 kg and LMC >61.2%)).

Microbiota was analyzed in fecal samples (16S rRNA gene amplification within the hypervariable region V4, sequencing with Illumina MiSeq platform). Alpha diversity indices were measured in R (version 4.1.2) with the R-package "phyloseq" (version 1.36.0). Means of alpha diversity indices were first checked for normality by analyzing the model residuals with the Shapiro-Wilk normality test, before multiple and pairwise comparisons were conducted. Statements of statistical significance were based upon p-values < 0.05.

Results

Up to 29 weeks of age, bacterial richness and evenness was the lowest in pigs classified at slaughter as "light fat" (see Table 1). Pigs that were later classified as "heavy fat" showed lowest bacterial richness and evenness latest at an age of 31 weeks of life.

Table 1. Alpha diversity indices (means)

Index	light fat	light lean	heavy fat	heavy lean	p-value
26. WL	n=7	n=14	n=9	n=5	
Observed	205	243	242	241	0.036
Shannon	3.21	3.5	3.45	3.44	0.291
29. WL	n=3	n=11	n=16	n=5	
Observed	200^{ab}	262^{a}	229^{b}	246^{ab}	0.003
Shannon	3.02^{b}	3.77^{a}	3.40^{b}	3.52^{ab}	0.004
31. WL	n=5	n=12	n=12	n=9	
Observed	270	267	248	257	0.250
Shannon	3.91	3.88	3.63	3.76	0.109
34. WL	n=10	n=9	n=11	n=7	
Observed	270	255	259	266	0.204
Shannon	3.83	3.76	3.72	3.78	0.822

[a,b] Different superscripts indicate statistically significant differences at $p \leq 0.05$
[WL] means week of life

Discussion and Conclusion

Results suggested that a link exists between intestinal bacterial diversity and carcass composition in pigs. Pigs that tend to be fattier generally seem to have a lower bacterial diversity, with a breakdown by body weight also revealing differences over time. However, no conclusion can be drawn as to whether the differences in bacterial diversity are causally involved in the development of carcass composition or whether diversity is rather influenced, like carcass composition itself, by other, identical factors.

The prediction of carcass composition at slaughter via ultrasound examination (backfat to M. longissimus dorsi diameter ratio) was better the later the examinations were performed (3). However, when measured early, the prediction with this method was the best in "light fat" pigs (3). Similar to these observations, bacterial diversity in the feces of pigs later classified as "light fat" appeared to differ from the other groups early in the fattening period. Later, however, differences were less clearly. Together with ultrasound examinations (3), fecal microbiota could be another indicator of the performance potential of fattening pigs. To fatten pigs more efficient, a more individualized classification of fattening pigs need to be established. Early detection of the rather slow-growing and fat-tending animals represents a great potential to establish a resource-efficient and thus sustainable feeding for these animals.

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Heating of piglets: comparison between incandescent light bulbs and infrared FIR panels

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Introduction

The temperature in the farrowing unit is a relevant parameter that greatly affects the welfare of piglets and the related potential risk of mortality (1,2).
The lamps commonly used in farrowing crates work with an incandescent light bulb, which emits radialheat and light. However, some studies suggest that far-infrared radiation (FIR) panels that produce non-ionizing electromagnetic radiation may be more electric-efficient, safe, and may lead to more effective heating of animals compared to the incandescent light bulb (3,4). This is relevant because adequate heating is one of the key factors for decreasing hypothermia and crushing mortality in the first 72 hrs of life (5,6).
The present study aimed to compare the performance of these different heating devices in pig farming, exploring the differences in mortality rates between pig litters exposed to traditional lamps and FIR panels during weaning (7).

Materials and Methods

A total of 175 sows from three different batches were selected for this study from the same pig farm. Half of the sows were housed in farrowing crates heated with an infrared panel, while the other half were housed in crates with an incandescent light bulb heating system. For all the fostering units the room temperature remained in a range between 23 and 23.5°C and cross- fostering was adopted no later than 72h after birth within the same heating system group of study. The collected performance data were: piglets' weight (at birth and weaning), stillborn, mortality, causes of death, sows parity, cross-fostering.
Relationships between mortality rates in litters and the heating device used in the crate were investigated through a mixed logistic regression, including mean weight at birth as a covariate and the batch as a random intercept.

Results

The 175 sows included in the study produced a total of 2371 suckling piglets (13.5 ± 0.2 ES piglets/sow), 189 were then culled, 108 died by crushing, and 185 by other pathological causes, leading to 1997 weaned piglets. Mortality within-litters differed significantly depending on the heating system (X^2 =11.4; p=0.0007). Piglets exposed to traditional lamps during weaning had almost twice the odds of dying compared to pigletsexposed to FIR panels (OR: 1.76; 95%IC: 1.26 – 2.45; mortality: 9.1% vs 5.4%). Litters housed in crates heated with FIR panels suffered a lower amount of deaths by both crushing and by other pathological causes and had therefore a higher percentage of weaned pigs (88.3% vs 83.1%). Additionally, mortality was influenced by weight at birth, with litters withhigher mean weight showing lower mortality rates (X^2 =4.6; p=0.032).

Discussion and Conclusion

Our results show that the FIR panel heating system significantly reduces piglets mortality due to crushing and other pathological causes. To the best of our knowledge, this is the first study on the application of FIR panels in the pig farming industry.
As the number of weaned animals per litter is determinant for the farm economic balance, a lower mortality rate during weaning allows the pig industry to produce more animals and consequently achieve more efficient and profitable husbandry (8). Our findings on the positive relationship between weight at birth and the survival of piglets are consistent with prior research (9,10) and highlight another important point concerning the economical balance and management in the swine industry. In light of the promising results highlighted, the FIR technology shows an interesting potential in the swine industry, and could also be useful in other livestock productions in the future. However, the comparison betweentraditional lamps and FIR will be further tested in otherpig farms in order to consolidate the promising results obtained from this initial research. Moreover, further investigations at the individual level rather than litter level are underway to assess whether FIR panels have also a positive impact on the growth rates of piglets.

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Immunization against gonadotropin-releasing factor (IM) in gilts harvested at 24 weeks of age: effects of second immunization timings on pork fat quality and its fatty acid profile.

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Introduction

Immunization against gonadotropin-releasing factor (IM) in market gilts results in predictable secondary effects on growth performance and carcass composition. Feed intake has been shown to be higher after the second week of 2nd application of immunological product (V2), resulting in fastergrowth and greater carcass fat deposition (1). This research aimed to evaluate the effects of three timings of immunization and two feeding programs on fatty acid profile of gilts.

Materials and Methods

A total 480 cross-bred commercial gilts (PIC Genetics) with 12 weeks of age (84 ± 1 day) were distributed in a randomized block design, 4 × 2 factorial arrangement, comprising two feeding programs [*ad libitum* (AL) and restrict (RF)] and three immunization timings of V2 (Table 1). Gilts in one group (T1) remained as an untreated control and three groups (T2, T3 and T4) received two doses of Vivax® with V2 timing respectively at 4 weeks (T2), 6 weeks (T3) or 8 weeks (T4) prior to harvest at 24 weeks of age. They were housed in 96 pens, 5 animals per pen and 24 pens (replications) per treatment. The gilts were fed with a corn and soya bean-based diets, formulated to meet the Brazilian Nutritional Requirements (2). At 24 weeks of age the gilts were harvested and backfat samples (100g) taken from the neck (collar, C3-C4, 6 samples per treatment), vacuum packed and frozen stored at -20º C. The fatty acid methyl esters of the neck fat samples were analyzed (n=48) according to the American Oil Chemists Society (3). Subsequently, total saturated, monounsaturated, and polyunsaturated fatty acids were calculated (n=6 per treatment). Additionally, the thrombogenic index (IT), defined as the relationship between the pro-thrombogenetic (saturated) and the anti-thrombogenetic fatty acids (MU-FAs, PUFAs – n6 and PUFAs – n3) was calculated. The results were submitted to ANOVA, and compared by Tukey's Test.

Results

No interaction effects between the factors as well as no differences were found among feeding programs. Significant ($p<0.05$) findings were observed for timing of V2, where T2 and T4 presented higher ratio of n − 6/n − 3 compared to T1 and T3, that showed, respectively, a reduction of -0.6% and 2.4% for this trait. There is a trend ($p>0.10$) towards saturated fattyacids decreasing according to immunization time, compared to the control group (T1), with percentage values of 1.5%, 5.4% and 5.6% for T2, T3 and T4, respectively. Similarly, IT decreased by 2.0%, 7.9% and 7.9% for treatments T2, T3 and T4, respectively, compared to T1 group ($p<0.10$).

Discussion and Conclusion

It is known that fat composition is affected by immunization against GnRF in gilts (4). T2 group had the better n−6/n−3 ratio, but still far from the recommended 4:1. This elevated ratio might have been caused by the nutritional program of the gilts based on corn and soybean meal, which have higher concentrations of n-6. Differently from other studies that found an increase of saturated fatty acids in IM females (4,5), our results presented a decreasing trend ($p>0,01$). On the other hand, the lower content of saturated fatty acids in the fat of IM gilts may be beneficial to health by preventing cardiovascular problems such as coronary heart disease (6). According to these findings in our study, IT, being an important parameter to human health, was improved in IM market gilts harvested at 24 weeks of age.

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Table 1. Fatty acid profile (g/100g) and thrombogenic index of control and immunized gilts submitted to restricted and *ad libitum* regimen.

	Treatments				Feeding		CV	p-value	
	T1	T2	T3	T4	AL	RF	(%)	Treat	Feeding
Saturated (g/100g)	33.8a	33.3ab	32.0b	31.9b	32.8	32.7	6.2	0.0663	0.9575
Monounsaturated (g/100g)	39.3	39.6	39.7	39.8	39.6	39.6	4.1	0.8518	0.9578
n – 3 (g/100g)	1.4	1.443	1.534	1.508	1.4	1.4	7.9	0.1540	0.8307
n – 6 (g/100g)	23.3	23.5	24.4	24.5	23.9	24.0	8.0	0.4274	0.8797
Polyunsaturated (g/100g)	24.7	25.0	25.9	26.0	25.3	25.4	8.1	0.3920	0.9758
n − 6/n − 3	16.2ab	16.3a	15.9b	16.3a	16.1	16.2	2.4	0.0229	0.2512
Thrombogenic index	0,93a	0,91ab	0,85b	0,85b	0,89	0,89	9,3	0,0603	0,9713

[a,b] - groups with different letters within the same row are statistically different at $p\leq0.05$ and $p\leq 0.10$ a trend. AL: *ad libitum*; RF: restricted feeding;

Immunocastration (IM) in market gilts harvested at 26 weeks of age: effects of feeding programs and second immunization timings on pork fat quality and its fatty acid profile.

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Introduction

Immunocastration (IM) is widely used in commercial pig production, particularly in male animals, but recent studies have evaluated this technology in market gilts production up to heavier harvest weights (1). The IM in gilts results in growth performance improvement related to increased feed intake and body fat deposition (1,2). Thus, this work aimed at evaluating the effects of the feeding regimens (restricted and *ad libitum*) and timings of the second immunization (V2) on fat quality and fatty acid profile of gilts harvest at 26 weeks of age.

Materials and Methods

Four-hundred and eighty cross-bred commercial gilts (PIC Genetics), with 12 weeks of age (84 ± 1 day) were submitted to a randomized block design, 4 × 2 factorial arrangement, comprising two feeding programs [*ad libitum* (AL) and restrict (RF)] and four immunization timings. T1 group remained as an untreated control and three other groups (T2, T3 and T4) received the second immunization against gonadotropin-releasing factor (Vivax®) at three different timings (V2) before harvest at 26 weeks of age: 4, 6 and 8 weeks. Gilts were distributed in 96 pens, 5 animals/pen with 24 replications per treatment. The feeds were corn and soya bean-based ingredients and were formulated to meet the Brazilian Nutritional Requirements (3). At 26 weeks of age, the gilts were harvested and backfat samples (100g) were taken from the neck (collar, C3-C4, 6 samples per treatment), vacuum packed and frozen storaged at -20º C. The fatty acid methyl esters of neck fat samples were analyzed (n=48) by American Oil Chemists Society (4). Subsequently, total saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA) were calculated (n=6 per treatment). The results were submitted to ANOVA, and the means were compared by Tukey's; differences were declared significant at $p<0.05$ and a trend at $p<0.10$.

Results

No interactions effects ($p\geq0.05$) were observed between the feeding program and timing of immunization, and no differences ($p\geq0.05$) were found between the immunization timing (Table 1). However, gilts submitted to restricted feeding program presented 4.9% more saturated fatty acids ($p<0.05$) compared with animal fed with *ad libitum* regimen. On the other hand, there was a decrease of -7.2, -8.2, -7.5 and -6.6% in polyunsaturated, n - 3, n - 6 and PUFA/SFA fatty acids, respectively for females submitted to a restriction program ($p<0.05$).

Discussion and Conclusion

IM increased feed consumption in gilts after the second week of V2 (1) and, as consequence, the highest feed intake increases the proportion of unsaturated fatty acid deposition. However, this finding was not observed in this study, showing that the IM do not affect this trait, regardless of the feeding regimen practiced. Our findings were consistent with the reported studies showed restricted feeding in finishing pigs decreases the carcass fat content (6), changing the fatty acids profiles (2). We also observed the gilts raised under restricted feeding conditions had a higher amount of SFA and lower amount of PUFA. The lower total PUFA, n – 3, n – 6 contents detected in fat from RF could lead to a better storage stability and flavor of the pieces due to their lower susceptibility to oxidation spoilage (7). Moreover, different V2 timings in heavy gilts prior to harvest at 26 weeks of age do not have any negative consequences on pork fat quality and fatty acid profile.

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Table 1. Fatty acid profile (g/100g) of control and immunized gilts submitted to restricted and *ad libitum* regimens.

	Treatments				Feeding		CV	p-value	
	T1	T2	T3	T4	AL	RF	(%)	Treat	Feeding
Saturated (g/100g)	33.98	33.73	34.60	33.50	33.12b	34.77a	5.9	0.4029	0.0033
Monounsaturated (g/100g)	39.84	39.30	39.58	38.99	39.28	39.62	2.8	0.4673	0.2912
Polyunsaturated (g/100g)	23.99	24.54	23.52	25.11	25.18a	23.36b	8.5	0.1154	0.0010
n – 3 (g/100g)	1.26	1.28	1.23	1.31	1.33a	1.22b	8.7	0.1434	0.0004
n – 6 (g/100g)	22.73	23.26	22.29	23.63	23.86a	22.06b	8.7	0.1736	0.0009
PUFA/SFA	0.71	0.73	0.68	0.75	0.76a	0.67b	8.9	0.3935	0.0031

[a,b] - groups with different letters within the same row are statistically different at $p\leq0.05$. AL: *ad libitum*; RF: restricted feeding; CV: coefficient of variation.

Improvement of farm performance after whole herd vaccination with type-2 PRRS modified live vaccine

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Introduction

PRRS is known to cause significant production and economical loses to pig farmers all over the world and the lost is the highest when both reproductive and respiratory symptoms are present.[2] PRRSV was shown to be present in 94% of the Malaysia pig farms[1]. Adoption of mass vaccination to protect sow herds against PRRSV is common, but PRRS vaccination in piglets is still not a common practice. Unlike PCV2, benefits of PRRS vaccination in piglet might not be always obvious at first sight. The objective of this trial is to monitor farm improvement by mortality record, serum PCR detection and serology changes after whole herd vaccination with Ingelvac® PRRS MLV.

Materials and Methods

The trial was conducted in a PRRS endemic farrow-to-finished farm with 250 sows in Malaysia. The farm has been using Ingelvac® PRRS MLV only in sows for half a year and was able to achieve a stable PRRS status by producing and weaning PRRS negative piglets. No PRRS vaccine was given in piglets. Recently, Type-1 and Type-2 PRRSV was found in 4 weeks old and sick piglets. Ingelvac® PRRS MLV piglet vaccination was implemented in 2 weeks old piglets over a period of 6 months. Monthly mortality percentage for piglets (1 month old), nursery (1-2month old) and porker (>2 months old) were collected. Whole herd serology data was also collected at the end of trial to compare to serology status a few years back. Serum of 4 weeks old piglet were also pooled and tested for PRRSV by PCR test.

Results

After starting Ingelvac® PRRS MLV vaccination in piglets, an improvement in reduction of porker mortality percentage at all stages was documented. The mortality was reduced by 7.4%, 19.6% and 57.3% in piglet, nursery, and porker (>2 months old) respectively (Table1). With a whole herd vaccination protocol, the overall mortality decreased from 6.73% to 3.74%, that accounted to approximately 44% improvement. Whole farm PRRS s/p value and deviation were lowered when compared to before. Other than that, pooled serum sample for 4 weeks old pigs were negative compared to before piglet vaccination.

Conclusion and Discussion

In this field trial, whole herd PRRS vaccination with Ingelvac® PRRS MLV was able to reduce porker mortality in farm due to PRRSV infection.

The largest improvement was seen in porker at > 2 months old, where the mortality was reduced by more than 50% (statistically significant) resulting in twice as many pigs to market. And after the implementation of whole herd vaccination, the farm became PRRS stable again by weaning PRRS negative piglets.

Table 1. Porker mortality percentage

	PRRS Vaccination in Sow only	PRRS Vaccination in Whole herd	Diff.%	p- value*
Piglet	10.14	9.39	-7.4	0.405
Nursery	10.51	8.46	-19.6	0.405
Porker	3.34	1.43	-57.3	0.014
Total	6.73	3.74	-44.3	0.033

*Data was tested using Mann-Whitney at 95% confidence interval by Minitab Statistical Software

Table 2. Porker pooled serum PRRS PCR before and after Ingelvac® PRRS MLV piglet vaccination.

Age	Sow	Whole Herd
1 week	-ve	-ve
4 week	+ve; Type 1 PRRSV	-ve
Sick pig	+ve; Type 1 & 2 PRRSV	-ve

Implementing a whole herd vaccination protocol (vaccinating sows and piglets) resulted in significant reduction in mortality

Acknowledgments

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Influence of mefepronic acid on gilts' litter growth, a control-case

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Introduction

The implementation of hyperprolific gilts and sows has resulted in a decrease in the body weight of piglets at birth, and subsequently at weaning. Any strategy that helps to improve the litter growth is appreciated by farmers, leading with small piglets and litters with high weight variability. The fibrates improve on the hepatic metabolism is critical to have a correct lipidic metabolism, meeting then the requirements of breeder and theoretically improving the growth of litter, especially in gilts which are still growing. We have investigated the growth of piglets from gilts after administration of 2-Methyl-2-phenoxypropanoic acid (MPA).

Materials and Methods

Two hundred gilts were involved in this trial, being 100 treated into 24 hours after farrow with an IM injection of Liverfine® (Fatro Ibérica, Spain), but only data from 192 were recovered. The gilts were randomly allotted, and were used all the gilts farrowing into two consecutive weeks to avoid environmental factors. Two different genetic lines were evaluated; being 68 gilts from genetic A and 124 from genetic B. All piglets were weighted twice, at 24 hours after birth and at weaning. The average growth per litter and animal, and the coefficient of variation were calculated. Since not all the litters were the same days in lactation at weaning, the weight was normalized at 21st day of life. Also, it was recorded mortality during lactation and weaned piglets per litter.

The comparison of data was performed using Student 's t test, considering significant a p-value<0.05. It was researched the influence of litter size by GLM multivariate.

Results

Finally, 2,629 piglets were individually weighted at 24 hours of life and 2,336 at weaning. The values for performance appear in table 1 and 2

Table 1. Performances of weighting at 24 hours

GEN	GROUP	P-24h	BW1	LW1	CV1
A	CONTROL	13,8	1,47	20,26	13,73
	TREATED	13,76	1,36	18,67	14,27
	p-value	NS	NS	NS	NS
B	CONTROL	13,74	1,5	20,56	13,97
	TREATED	13,56	1,52	20,66	14,63
	p-value	NS	NS	NS	NS

Where: P-24h= piglets per litter at 24 hours, WB1= body weight at 24 hours of life, LW1= weight of litter at 24 hours of life, CV1= coefficient of variation for weights at 24 hours of life

Table 2. Performances of weighting at weaning

GEN	GROUP	BW2	LW2	CV2	LW_21D	WP
A	CONTROL	5,57	61,48	6,9	58,61	12
	TREATED	5,52	59,86	6,73	57,24	11,79
	p-value	NS	NS	NS	NS	NS
B	CONTROL	5,56	61,7	7,83	59,39	12,13
	TREATED	5,7	65,36	7,54	63,16	12,5
	p-value	NS	**0.066**	NS	**0.048**	NS

Where: WB2= body weight at weaning, LW2= weight of litter at weaning, CV2= coefficient of variation for weights at weaning, WP=weaned piglets.

There was not significant difference neither for growth in genetic A ($ADG_{Control}$= 0.151±0.006 Kg/d versus $ADG_{Treated}$= 0.154±0.004 Kg/d) nor for mortality ($M_{Control}$= 13.38±2.28 versus $M_{Treated}$= 12.42±2.16. However, there was significant difference in genetic B for growth ($ADG_{Control}$= 0.151±0.003 Kg/d versus $ADG_{Treated}$= 0.161±0.003 Kg/d, p=0.048) and a trend for mortality ($M_{Control}$= 12.51±1.34 versus $M_{Treated}$= 9.66±1.26; p=0.072). There was an influence of the litter size, even when the size of animals at 24 hours was not different in the control and treated groups. For the GLM multivariate litters with 13, 14 and 15 piglets were included (96.5% of the litters). There was a different among litter sizes for control and treated in genotype A for mortality (p=0.012 and p=0.027, respectively), and (p=0.030) for treated group in genotype B. There was a linear regression among mortality and litter size at 24 hs for control and treated groups (p=0.018 and p=0.021), and for control but not for treated in genotype B (p=0.027). This suggest that the mortality was independent from litter size at 24 hours for the treated gilts in genotype B. The difference in the average individual weight increase per piglet among smallest and biggest litters was 0.491 Kg and 0.554 Kg forcontrol and treated groups in genotype A and 0.326 and 0.237 Kg for both groups in genotype B.

Discussion and Conclusion

The current hyperprolific genetic lines produces smaller piglets at birth, with a higher weight variation. The mefreponic acid, improve the usage of fat and muscle during the lactation in gilts (data not shown in this communication), and in this trial has demonstrated higher weight of litter at 21 days in genetic line B comparing treated to control gilts. The difference arises up to 3.77 Kg more per litter. This effect could be the results of a better fat metabolism and then a higher availability of nutrients, or even due to a higher quantity of fat in the milk, but this term has not been investigated. The shortest difference in weight gain for the treated gilts in Genotype A suggest that the mefreponic acid reduced the influence of the litter size at 24 hours.

There is no literature available on the subject, so further research is needed.

Introduction to an Index Measuring overall Grow-Finishing Efficiency in Swine Production

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Introduction

As we all know, wean-to-finish pigs are the most prominent motivator to keep the farms for the farmers. Farmers always want to cost less and get more meat in order to generate more profits. However, there is not a sole indicator such as ADG, FCR, etc, can reflect the true profitability. For instance, higher ADG is often correlated with higher feed and higher FCR. Therefore, we propose a new and comprehensive indicator called "FSA Index" to solve the dilemma.

Materials and Methods

We define "FSA Index" mentioned before as below:FSA Index=SR * ADG / FCR

FSA Index: The index considers **F**eed conversion ratio, **S**urvival ratio, and **A**verage daily gain.

SR: Survival Ratio of a batch of wean-to-finish pigs;

ADG: Average daily gain(g/d); i.e., ADG= weight gain per pig(kg)/feeding days*1000

FCR: feed conversion ratio; i.e., FCR= total feed amount consumed per pig/ weight gain per pig

The higher the indicator, the greater growing efficiency the batch of pigs has got. That is to say, under a high FSA, ADG and FCR can reach a balance between good weight-gain speed and reasonable feed consumption, therefore reducing feed costs.

We collected cost and production data of 113 batches of finisher barns in Sichuan province, China, from the same company during year 2021, and calculated the FSA Index to demonstrate the effectiveness of the indicator. The quality criteria of analyzed data are defined as follows:

5 kg ≤ average initial weight ≤ 10kg, 20days ≤ average weaning age ≤ 40days, 101kg ≤ average marketed weight≤ 150kg，159days ≤ on-feeding days ≤ 232days, and survival ratio ≥ 90%.

The data of FSA and weight-gained feed costs are analyzed as shown in Figure 1.

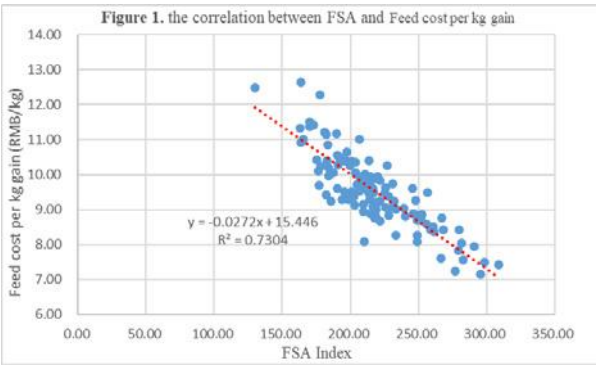

Figure1. Correlation between FSA and Feed cost per kg gain. Feed cost per kg gain (RMB/kg)= total feed cost per marketed pig (RMB)/ weight gain from weaning to market (kg)

Results

As shown in Figure 1, there is a significantly negative correlation between FSA and feed cost per kg gain ($P < 0.01$ and R^2=0.7304). In other words, FSA had a statistically significant impact on feed cost per kg gain, and was able to interpret 73% variation of feed cost per kg gain.

Discussion and Conclusion

This indicator mainly illustrates the growing trend of a batch of finishing pigs and is fairly enough to rank the growing efficiency of different grow-finishing batches with various indices(i.e., ADG, FCR, and SR).

FSA is an easy way for us financial analysts to compare performance of various batches and help management to monitor performance. For example, if FSA of a certain batch is substantially low, it most likely indicates that pigs experienced health challenges,including spread of severe disease. Meanwhile, under different ingredients and all else equal, nutritionists may need to adjust their formulations. Moreover, FSA is able to assist us in genetic selection and to discover sub-health condition in grow-finishing herds.

It's time to abandon averages. Who cares about number of sows on the unit? Put Pigs per Sow per Year into the last century's mistakes bin

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Introduction

The pig industry must move forward and reduce variation wherever possible. Using average numbers must be consigned to history, this change is long overdue. Instead, change the farm's KPI to an output based concept, such as kilograms marketed, away from pigs per sow per year (PSY) or defining herd size based on sow inventory. Instead, a pig flow plan based on batch farrowing is proposed which results in reduced variation in production across the year.

Materials and Methods

To rationalise a method of monitoring pig production to reduce the variation in the system the merit of different industry KPIs was reviewed. In indoor systems the consistent feature of the farm is the number of farrowing places. If the farm is to adopt all-in/all-out systems, the number of farrowing places and their layout throughout the farm is generally constant over a multiyear programme. Outdoor production can move arcs.

The sow herd inventory is rejected as a KPI because the reproductive performance varies over the year – seasonal infertility. To produce consistent performance a sow herd is bigger in the hot summer than the cold winter months. The finishing herd inventory was also rejected as a KPI for the same seasonal effects in growth performance. In the hot summer months finishing pigs will reduce their feed intake and thus growth rates. Therefore, there are more pigs on the farm and thus more space is required at the end of summer; sometimes even having finishing buildings empty in winter. Instead, farms were re-organised along their farrowing place inventory and various batching systems were adopted to maximise the possible performance of the farm. A farm plan was designed and then the farm was expected to farm this plan. With this model it is the number of batch farrowing places filled at weaning that matters. The actual number of farrowing sows must be equal or more (not less) than the places available. This conceptof MINIMUM is an important feature.

There are five obvious pig flow models which farms can adopt (noting that 3-week weaning is illegal within the EU): 1 week batching with either 3 or 4 week weaning. Therefore, thereare 4 or 5 equal groups of weaning sows respectively.

2 week batching with 3 week weaning requiring two equal groups ofweaned sows.

3 week batching with 4 week weaning requiring two equal groups ofweaned sows.

4 week batching with 3 week weaning requires one (all) of thefarrowing places, and

5 week batching with 4 week weaning which requires one (all) of thefarrowing places.

With some imagination, producers have modified this concept into other plans which fitted their particular circumstances better.

Results

The move to batching pig flow resulted in an increase in kg produced over the annual period and reduced the costof production on farms where it was adopted. The farm production was more disciplined with consistent results in all sectors of the farm from gilts, breeding, gestation, farrowing and lactation, through nursery to finishing. Teamwork was easier across the farm as the element of surprise was removed, each unit of the farm working together instead of separately.

Compliance with welfare legislation can be standardised because production does not bounce between over- and under-stocking. Purchase of feed, medicines, genetics, and bedding becomes more uniform and easier to monitor in real time.

Statistical parameters of 10 farms adopting pig flow modelling – pre-adoption and post-adoption.

The results looks at the variation from the pig flow plan.

	No plan			Pig flow adopted		
Area	**μ**	**σ**	**σ²**	**μ**	**σ**	**σ²**
Breeding	97	38	1478	103	4.3	18
Farrowing	98	39	1523	108	5.7	32
Weaning	95	30	904	99	7.5	56

The major difference is the lack of variation in the system and therefore less surprises. With the pig flow model farm output was achieved more consistently.

Two lessons were learnt early on:

1. The first day of the batch is the day after weaning. Monthly accounting should be discarded it has nothing to do with pig production. Records should follow the pigs not just a calendar day.
2. Production is based on reaching MINIMUM targets not just averages or maximums, which are what traditional stock-people bonuses are based on.

Discussion and conclusion

The move to pig flow modelling creates different paradigms for the farm teams. Problems can arise if farm bonuses remain based on the previous key indicator parameters (KPI's).

Examples where current KPI's should be discarded in terms of their actual impact on profitability:

- PSY – this is too vague and open to cheating.
- Sow herd size –The number of sows on the farm is not relevant as it is determined by the requirement to achieve the minimum weaning farrowing place batch on batch. Sows are cheaper than empty farrowing places.
- Farrowing rate % is irrelevant, as this average number does not describe the requirement to fill the farrowing house 100% of the time. It is the variation that must be accounted for.
- Pre-weaning mortality is not relevant. Rather, the number of quality pigs at weaning determines the number of pigs finished. A higher pre-weaning mortality may be a good thing!

Further reading:

Muirhead, M. and Alexander, T. Editor Carr, J. (2013). **Managing Pig Health**. 5M Publishing. ISBN 978-0-9555011-5-9

Carr, J., Chen, S-H., Connor, J, Kirkwood, R. and Segalés, J. (2018). **Pig Health**. CRC Press. ISBN 13:978-1498704727 and ISBN 10: 1498704727.

Multi-suckle common creep: a novel production model for nursing piglets

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Introduction

One of multiple selection indices driving the improved productivity and efficiency of pork production is litter size (1), with the unintended consequence of increased piglet mortality and the necessity of implementing complex management strategies to reduce it.

Multi-suckle common creep (MSCC; also known as open creep or open farrowing) is a production model that allows free access of piglets to multiple sows and a common creep area. Limited field trial data has indicated a reduction in pre-weaning mortality and increased weaning weights in MSCC conditions. However, the effect of MSCC on production parameters and the transmission of common bacterial agents among piglets has not been fully investigated. Therefore, the objectives of this study were to evaluate the production parameters and detection of *Mycoplasma hyorhinis* in piglets reared with MSCC.

Materials and Methods

The study was conducted on a 5,000-sow farm in the Midwest United States in the initial stages of a porcine reproductive and respiratory syndrome virus (PRRSv) outbreak. Sows in one weekly farrowing group were randomly allocated into conventional (CONV) or MSCC treatments, balanced by parity. Within each of four rooms of 48 farrowing stalls, three groups of eight sows were allocated to MSCC and three groups of 8 sows were allocated into CONV. At birth, pigs were individually weighed and identified with electronic ear tags. All piglet mortalities and individual antibiotic treatment injections were recorded. Pigs were individually weighed at weaning and assigned to nursery pens by treatment group. Samples were collected from sows and piglets from pre-farrow to weaning. Forty-eight sows and 96 piglets were randomly selected for sampling among treatment groups and parity. In sows, vaginal samples were collected pre-farrow and oropharyngeal and udder swabs were collected at farrowing, mid lactation and weaning. Piglet tonsillar and nasal swabs were collected at birth, mid-lactation and weaning. Piglet samples were tested for detection of *M. hyorhinis* by real-time PCR. Nursery phase mortalities and ending weights were recorded. R Studio was utilized for data analyses, including t and Wilcoxon rank sum tests (4).

Results

Pre-weaning mortality (8.8% MSCC; 8.1% CONV) and weaning weight (6.6 kg MSCC; 6.8 kg CONV) were similar between treatment groups. A statistical difference in the number of piglets treated with injectable antibiotic from birth to weaning was observed (60 MSCC; 108 CONV, $P< .001$). Nursery mortality was elevated with the onset of PRRS. A significant difference in nursery mortality was observed between the treatment groups (MSCC 23.89%, CONV 31.99%, $P<.001$). No difference in prevalence of *M. hyorhinis* at birth, mid-lactation or weaning was observed in nasal and tonsillar swabs (51.1% vs 44.4% nasal swab positive, $P=.67$, 60.0% vs 53.3% tonsillar, $P=.68$). However, *M. hyorhinis* prevalence increased significantly from birth and mid-lactation to weaning in both treatment groups ($P<.001$). A statistical difference in the median nasal swab *M. hyorhinis* Ct value at weaning was observed (25.29 MSCC, 33.69 CONV, P=.004). *Mycoplasma hyorhinis* was not detected in pre-farrow vaginal swabs and mid-lactation oropharyngeal swabs with PCR, with low prevalence in pre-farrow and weaning oropharyngeal swabs (3/48, 2/46) respectively.

Discussion and Conclusions

A novel production model for piglet nursing was implemented on a limited scale to evaluate its efficacy. Initial results in field trials indicated a reduction in pre-weaning mortality and increased weaning weights in pigs raised using MSCC. However, a difference in pre-weaning mortality and weaning weights in piglets reared in MSCC vs conventional conditions was not observed in this study. The significant difference in nursery mortality observed in the nursery phase in MSCC pigs compared to conventional pigs is of interest and merits further investigation.

The prevalence of *M. hyorhinis* in piglets did not differ at various ages in the lactation period between treatment groups. Nevertheless, the Ct value was significantly lower in the MSCC treatment group than in the CONV treatment group.

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Performance, crude protein digestibility and economic analysis of acid protease supplementation in the swine diets

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Introduction

The use of enzymes in swine diets has been a reality as a tool to increase digestibility for dacades. Phytases and carbohydrases present consistent results, however, proteases still present divergent results (1). Thus, the objective of this study was to evaluate an acid protease (EC 3.4.23.18) added in the diet of growing and finishing pigs, on performance, crude protein digestibility and economic viability.

Materials and Methods

One hundred and thirty piglets at 63±1 days of age were housed in a randomized block design (depending on initial weight and sex), with five treatments and nine replications. The experimental unit consisted of a pen with three animals. The experimental period was 75 days, which was divided into three periods according to feed changes and nutritional levels proposed by Rostagno et al. (2017). The treatments used were: Positive control (PC) with a 5% of reduction in the amino acid requirement (T1); Negative control (NC) with a 7.5% of reduction in amino acid requirement (T2); T2 + 100 g/ton of an acid protease - EnzyPAC PRO (T3); T2 + 150 g/ton de EnzyPAC PRO (T4) e T2 + 200 g/ of other acid protease (T5). The diet was provided *ad libitum* and leftovers were weighed daily. The animals were weighed on days 63, 89, 112 and 138 of life and 0.5% titanium oxide was provided 3 days before switching from phase 1 to 2 for fecal collection for digestibility analysis. For economic analysis, production costs and sales price stipulated in the swine exchange of the Associação Paulista dos Criadores de Suínos (APCS), were evaluated for the month of June 2021 (R$7,20).

All variables were submitted to analysis of variance. When there was a statistical difference, the Tukey test was used to compare the means, adopting p<0,05. The SAS software (2009) was used through the MIXED procedure.

Results

There was a statistical difference for body weight (BW) average daily weight gain (ADWG) and feed conversion (FC). Crude protein digestibility coefficient (CPDC) was higher for animals from treatments T2, T3, T4 and T5 when compared to T1. The cost per kg produced was lower for animals from T4 and T5, followed by T3, T2 and T1. As for profit per animal produced, T4 provided the highest value, followed by T5, T3, T2 and T1 (Table 1).

Table 1. Performance, crude protein digestibility coefficient (CPCD) and economic analysis of growing and finishing swine fed diets with proteases

Variable	Treatments					*P*
	T1	T2	T3	T4	T5	
BW 138d (kg)	89b	97ab	100a	103a	101a	0,00
ADWG 63-138d (kg)	0,8b	0,9ab	1,0a	1,0a	1,0a	0,00
FC 63-138d	2,6a	2,6ab	2,5bc	2,4c	2,5bc	0,04
CPDC%	52b	66a	64a	62a	60a	0,00
Cost Kg R$	8,19	8,09	7,93	7,76	7,76	-
Profit R$	84	89	102	121	118	-

Weight 138 = weight at 138 days of life; ADG 163-138= Daily weight gain from 63 to 138 days of life; FC 63-138= Feed conversion from 63 to 138 days of life CPDC= Crude protein digestibility coefficient; Cost Kg = Cost per kg produced and Profit = Profit per animal produced. P = P value in Tukey's test, considered significant when P<0.05.

Discussion and Conclusion

The treatments with the lowest inclusion of CP and amino acids in the diet reflected in the highest CPDC, which suggests that the availability of CP alters the digestive capacity of pigs in the growing and finishing phases. The inclusion of 150g protease (T4) in the diet did not differ the FC from T3 and T5, however, it improved when compared to PC and NC, which may have been the key point to be the treatment with the best economic result (profit R$).

The inclusion of protease in the NC reduced costs, which, added to the better productive performance, improved the profitability of production. Therefore, the use of proteases can be recommended in order to make pork production more viable.

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Real time auditory sensor-based monitoring supports objective decision on possible interventions in finishing facilities based on accurate diagnostic detection of respiratory pathogens

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Introduction

Coughing in itself (although easily recognized) does not provide any objective information regarding causality. Therefore, accurate diagnostics are needed to guide optimal interventions. The objective of this study was to improve the quality of diagnostic information to optimize intervention efficacy. This objective was achieved by using 24-hour real time sound monitoring provided by SoundTalks® (ST) to determine a change in respiratory health status (ReHS) and trigger an Oral Fluid (OF) sampling at the time of the coughing event (ReHS+).

Materials and Methods

From March 2020 – July 2021 a finisher site with 1200 pen places in 4 separate rooms were monitored by ST. Growth performance was also monitored by 2 real-time 3D camera sensor GrowPro (Skov). Blood (20 samples) and 4 OF per room were taken from the first 4batches at placement to determine health status. In thecase of a warning (yellow light) or increased cough (red light) signal from the monitors indicating respiratory health problems (ReHS+), 4 OF were obtained from thepens below the SoundTalks™ monitor to determine the pathogens involved. The OF samples were submitted cooled to a German lab (AniCon) and tested for PRRSV antibodies and App, M-hyopneumonia, M-hyorhinis, Glaesserella parasuis (GSP), Influenza A Virus (IAV) and PCV2 by PCR.

Results

Results from this study demonstrated that all batches were serological positive to App 12 and Mhyo and negative to PRRS. No ReHS+ events were detected by ST between March and July of 2020. A short event in July in room 1 and 3 was followed by heavy cough in August in both rooms. Until April 2021 several ReHS+ events were detected, mostly in 2 – 3 rooms at the same time (Figure 1). The OF sampling revealed that IAV and GSP were involved in every event. Mhyo was only detected once in room 4. Heavy clinical signs (high body temperature and no eating) were observed in the 2 first batches with IAV break in room 1 and 3. Later on, the symptoms were milder. Clinically affected pigs from room 1 were treated by 3 days of penicillin injection with antibiotics. For the next batches the owner checked the ReSH status and the OF dx results before intervention and to decide on the need for medication. No later batches turned out to be treated.
Despite of clinical signs related to the coughing events no significant growth retardation was identified during the outbreak periods (Figure 2).

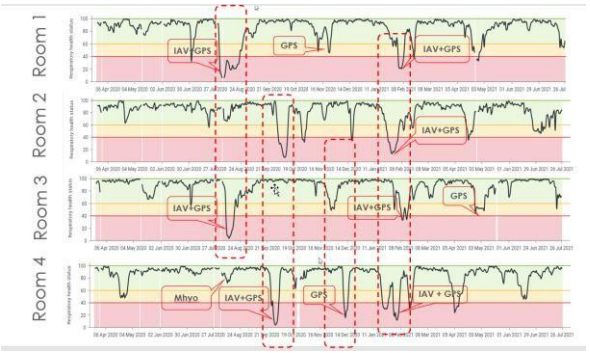

Figure 1. ST Respiratory Health Status (ReHS) related to monitor color for each of the rooms monitored. Main pathogens involved in the coughing event are shown in the comment box.

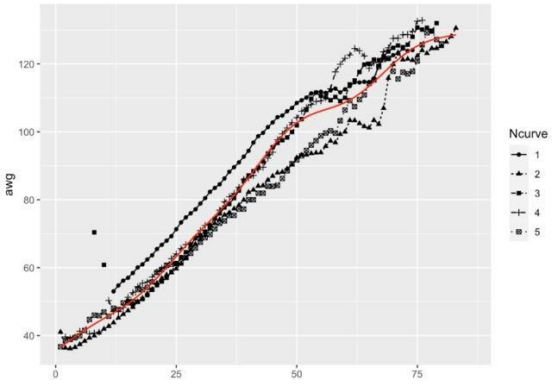

Figure 2. Average image technology growth curves from the 5 batches monitored. The red reference growth line is established from batches without any coughing events.

Discussion and Conclusion

With ST it was possible to target the diagnostics to the actual time of cough, determine the pathogens involved in ReHS+ and decide on intervention. ST information, in combination to the provided diagnostic result, is helping the producer to intervene, or in this case even more important, not to intervene, based on objective information and thereby saving unnecessary antibiotics.

Reproductive consequences of an abnormal feed intake during the lactation period

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Introduction

Insufficient nutrient intake of sows during lactation has serious reproductive and productive consequences (1). The early detection of sows with poor feed intake during lactation would allow us to anticipate possible problems and maximize their production. In this sense, the use of electronic feeders permits to have real-time data available for a more accurate evaluation. The aim of this work was to evaluate production of sows based on their feed intake pattern during lactation.

Materials and Methods

Daily lactation feed intake records were collected in a commercial farm in Segovia, Spain. A total of 1058 daily feed intake records were collected from 585 Topigs sows (from parity 2 to parity 6). Data were collected using a computerized feeding system (Gestal Solo, JYGA Technologies, Quebec, Canada). In order to unify the data, lactation was set to 28 days and sows with lactation less than 21 days were removed from the database.

The detection of sows with low start and low global feed intake during the lactation period was done by clustering using k-medoids which are from the family of unsupervised classification machine learning algorithms. Clustering was applied in 6 standarized variables determined according the averages of feed consumption in the different sub-periods of the lactation phase. Consequently, two clusters were defined, with very different feed intake pattern: 1/ Control group (CG): 1017 sows (96.1%); Normal feed intake pattern, and 2/ Low consumption group (LG): 41 sows (3.9%); Low feed intake during the whole period.

Reproductive parameters of the current cycle were analyzed including prolificacy (total number of piglets and born alive, percentage of stillborn), preweaning mortality (PWM) and weaning-to-first service interval (WFSI). Farrowing rate of sows was also analyzed. Data analyses were conducted by using the proc GLIMMIX (ANOVA parametric test) and proc NPAR1WAY1 (Wilcoxon non-parametric test) of the SAS software (version 9.4; SAS Inst. Inc., Cary, NC).

Results

Feed intake during the lactation period of both groups is presented in the Figure 1. The CG sows showed a normal feed intake pattern during lactation, while the feed consumption in the LG group was very low especially during the first third of lactation. After then, the feed intake increased but it kept also low throughout the rest of lactation. In total, sows in CG consumed 35.8% more kg of feed during the whole lactation period than the LG sows (180.4 kg vs 115.8 kg; $p > 0.0001$).

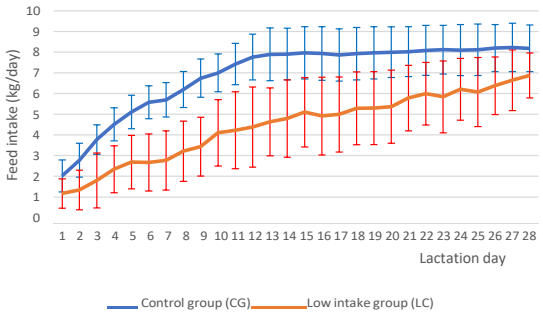

Figure 1. Mean (± standard deviation) feed intake of sows during the lactation period.

Reproductive performance results are presented in table 1. There were no differences in the total number of piglets born between groups. However, LG showed a lower number of born alive piglets and a higher percentage of stillborn. In addition, this group also showed higher PWM percentage and WFSI and lower farrowing rate than the CG sows.

Table 1. Reproductive performance of Control (CG) and Low feed intake (LG) lactating sows

	CG	*LG*	SEM
Total born (nº)	16.6	16.8	*1.141*
Born alive (nº)	14.7[a]	13.2[b]	*0.716*
Stillborn (%)	11.1[b]	20.3[a]	*3.369*
PWM (%)	15.6	16.9	*2.688*
WFSI (days)	6.33[b]	13.11[a]	*1.310*
Farrowing rate (%)	90.8[a]	75.0[b]	-

PWM: pre-weaning mortality; WFSI: wean-to-firt service interval; SEM: standard error of mean; The values with different superscript letters in a row are significantly different ($p < 0.05$)

Discussion and conclusions

The absence of pain and the wellbeing of the sow after farrowing is a direct determinant of her intake during lactation, especially during the first days. Therefore, low feed intake start of LG sows was probably associated with some difficulties during farrowing, as it is reflected by their higher percentage of stillborn. The deficit of nutrients during lactation had severe consequences in weaning-to-first service interval and farrowing rate of the following cycle. Therefore, identifying these feed consumption problems on time will allow us the adequate decision making to maximize the production in swine industry.

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Respiratory health status measured by a sound-based monitoring technology impacts growth of finishing pigs in the Netherlands

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Introduction

Respiratory disease outbreaks continue to be one of the major problems in modern pig production worldwide, but of particular importance is the commercial pig production impacting the overall health as well as productivity and animal welfare.[1] SoundTalks® is a cloud-based sensor technology that monitors 24/7 the sound emitted from pigs. Based on artificial intelligence, this technology processes the sound data collected at the farm and transforms it into a metric (ranging between 0 and 100) that represents the animals' respiratory health status (ReHS). When the ReHS value falls below a certain threshold, the system emits early warnings (yellow/red LED alerts) allowing producers to intervene prior to clinical signs of respiratory disease are evident for care givers[2]. Despite the early warning evidence, further research is needed to fully understand the impact of ReHS on production performance (i.e. average daily gain, ADG). Therefore, this study aimed to describe ReHS in a commercial farm monitored by SoundTalks® and investigate the potential association of such data with pig growth.

Materials and Methods

The study was performed in a finishing farm in the Netherlands (11 rooms, 105-300 pigs/room) that SoundTalks® continuously monitored (1 monitor/room) for approx. 1-year period. To determine ADG, weight scale systems (MS Pigscale) were used to monitor individual daily weights in rooms 3 and 7. Data from these 2 rooms were analyzed with R statistical software. Linear regression models were used to study the association between ADG and respiratory health indicators by SoundTalks® (i.e. daily ReHS value, SoundTalks® green, yellow and red monitor color and combinations). Other environmental and production parameters (i.e. pigs age represented as months after placement, room temperature, mortality, and number of treatments) and their possible interactions were also considered as predictors in the model.

Results

Results from this study showed that, in all rooms, on 28% (max=74,5%; min=4,8%) and 14,5% (max=38,2%; min=2,1%) of days the farms had respiratory alarms, as indicated by yellow or red warning signals, respectively. There was an evident room effect as the percentage of days with green, yellow or red ReHS significantly differed between rooms. The ADG of a total of 6 finishing batches were closely monitored during the study period. Fig 1. describes the distribution of ADG by months after placement for two of the monitored rooms. The graph shows that pigs' ADG under red or yellow alarms (outbreak period) was lower compared to pigs' ADG under green monitor color (period of free respiratory disease). In addition, the estimated linear model for room 7 showed that ADG was lower during outbreak periods compared to non-outbreak periods being that difference statistically significant at 10%. Results regarding pig's age demonstrated that, during an outbreak period, the average ADG was statistically different depending on whether the pigs were at the beginning or end of the finishing period (room 7). Although more information is undoubtedly needed to confirm these assumptions, it seemed that the outbreak impacted the ADG more at the beginning (month 1) rather than at the end (month 2-3).

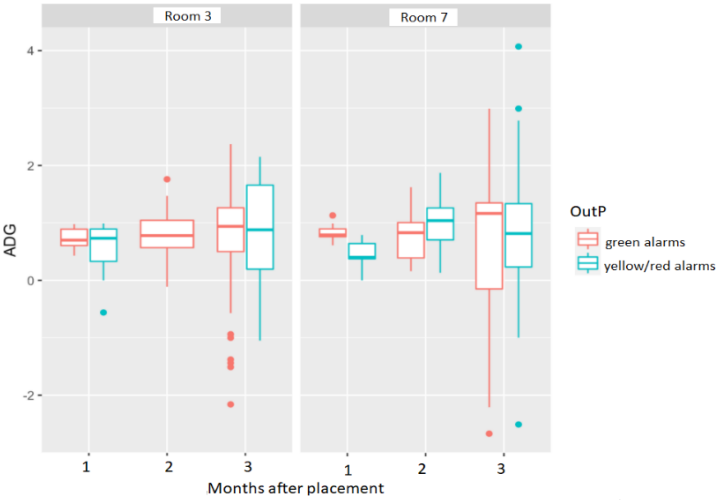

Fig 1. Average daily gain (ADG) distributions in piglets, by month after placement, during respiratory outbreak period (yellow/red alarms) and non-outbreak period (green alarms) measured by SoundTalks® in 2 of the monitored rooms.

Conclusions and Discussion

This study showed the impact of respiratory health status in ADG in swine growing population. Results from these 3 monitored batches/rooms demonstrated that age at the time of the outbreak is a key variable since observed ADG difference was higher in younger animals compared to older ones. Despite the importance of these results, further studies are needed to understand room effect during these respiratory outbreaks (13% of yellow or redSoundTalks® alarms in room 3 compared to 62% in room7), individual pig behavior during disease episodes, and frequency of weighting events on real sick animals compared to healthier animals, and its implication in ADG calculations.

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Standardized economic assessment of a sound-based precision livestock farming tool (SoundTalks) comparingtiming of intervention after a dual *Mycoplasma hyopneumoniae* and PRRS virus seeder challenge in pigs

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Introduction

Respiratory disease outbreaks continue to be a major pig production problem, impacting antibiotic use, welfare, productivity and profitability.[1] SoundTalks is an audio-based technology that continuously identifies and quantifies respiratory problems in pigs, generating alerts (yellow warnings, red alarms) when respiratory outbreak onset is detected. These alerts have enabled triggering earlier caregiver awareness than caregiver observations alone[2]. However, further research is needed to quantify the economic impact of this technology. The objective of this study was to evaluate the performance and economic differences resulting from earlier detection and intervention following the onset of a clinically detectable respiratory disease episode measured by SoundTalks.

Materials and Methods

Eleven-week-old pigs (n=1655) were placed in 72 pens across two rooms. Each room contained three SoundTalks monitors, one monitor per 12 pens. Study groups were randomly allocated within each zone. In every pen, three randomly selected seeder pigs were challenged with *Mycoplasma hyopneumoniae* followed by PRRS virus seven days later. The three groups were defined as: SoundTalks (ST) alert day zero (G0), day 5 (G5) and day 10 (G10). Alert day 0 was defined as the daythat the first ST yellow/red alerts were reported post- challenge. All pigs received the same treatment protocol
– differentiated only by the date the treatment protocol was started. Continuous sensor data was registered at zone level (e.g., audio, temperature) and pen level (e.g., water use, temperature). Performance was measured at both the individual pig and pen levels. Linear regression mixed models were used to study the association between the production parameters and treatment groups after controlling for other independent variables. Economic differences among treatment groups were calculated using a "Standardized Economic Index" (SEI) based on a partial budget model. The SEI is a function of finished pig performance measures, historical feed ingredient costs and market pig prices and the cost-to-operate (CTO) of the technology being evaluated. Pig performance measures utilized in the SEI were average daily gain (ADG), feed conversion rate (FCR), average daily feed (ADF), mortality, and individual pig treatments. Historical monthly market prices and feed ingredientcosts were obtained for the most recent 10-year time period (January 2011 through December 2020). In addition to hardware installation costs, a weighted SoundTalks hardware lifespan of 48 months was used to calculate the hardware cost for inclusion in the CTO. Torepresent the impact of a more natural (contact) exposureand infection dynamic, data for contact challenged pigs that did not experience exceptional handling (e.g., did notexperience snaring, bleeding, tracheal catheterization) were used to model SEI.

Results

After seeder pig challenge, two respiratory outbreaks caused by swine A influenza virus (IAV) were registered throughout the study. Pigs were treated accordingly to study design. Contact challenged pigs from G0 had 12,7 and 20,4 grams higher ADG compared to those from G5 and G10 respectively. Similarly, contact challenged pigs from G0 had a 23.4% and 10.1% decrease in individual treatments when compared to G5 and G10 respectively. Contact challenged pigs from G0 had a 0.26% higher and 1.22% lower percent mortality compared to those from G5 and G10 respectively. All production variables were introduced into the SEI model and the resulting Benefit:Cost (B:C) ratio 10 year time series is shown in Fig 1.

Figure 1. Ten year monthly Benefit:Cost ratio (green and blue dotted lines) as well as its 48 month rolling average (green and blue lines) for SoundTalks investment based on performance group differences.

(Group 0 vs 5 represented as blue lines; and Group 0 vs 10 as green lines) using North American monthly market price and feed costs
Throughout the 120 month period (January 2011 through December 2020), and using a CTO of USD $0.254/pig marketed (inclusive of installation, hardware and software subscription for a single barn 4800 head grow-finish site) the mean monthly B:C ratio was 4.27, ranging from 2.52 to 7.90 and exceeding 2:1 for 120 of 120 (100%) months (G0:G5). For the same 120 month period, the 48 month rolling B:C Ratio ranged from 3.37 to 5.06, exceeded 2:1 for 120 of 120 (100%) intervals (G0:G5).

Conclusions and Discussion

A 48 month rolling average was used to allow evaluation of the B:C ratio across the estimated average lifespan of SoundTalks hardware. This study suggests that there can be a favorable and consistent economic impact based on aggregate performance differences using a technology that enables earlier detection and treatment intervention.

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Sustainable use of agro-industrial by-products as feed in finishing pigs' diets

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Introduction

Recording to the International Feed Industry the world population will be more than 9,5 billion people until 2050 (1). As a result, larger quantities of food and especially of protein will be needed. FAO makes a special reference on the importance of finding alternative animal feeds (2). Every day a wide variety of agro-industry by-products are disposed to the environment and are accused of their high pollutant load. Some of them are rich in bioactive compounds and can be useful as feed ingredients for farm animals.

In this dietary experiment a special silage of olive mill wastewater, grape pomace and deproteinized cheese whey was used on finishing pigs' diets. The purpose of the trial was to investigate the potential beneficial or adverse effects on the growth performance and health of pigs.

Materials and Methods

All experimental procedures were in accordance withthe National guidelines for animal trials (3).

The silage was based on corn supplemented withgrape pomace, deproteinized cheese whey and olive mill wastewater at a relative ratio of 20/20/60. The silage was packed under vacuum and fermented for 30 days, after inoculation with *Lactobacillus buchneri.*

For this experiment 18 crossbred (1/4 Large White × 1/4 Landrace × 1/2 Duroc) finishing pigs were individually ear-tagged randomly allocated to 3 dietary treatments (control-0%, 5% and 10% silage). Theexperiment lasted 60 days, from start of the fattening phase until the slaughter of the pigs. The diets wereformulated according to the recommendations of NRC (4). Access to feed and drinking water was *ad libitum*. Atthe beginning and end of the trial, the weight of pigs wasmeasured. Also, before the slaughter blood samples were taken to investigate hematological (WBC, Lym., Mon., Gra., RBC, Hct, Hb and THR) and biochemical (AST, ALB, TRIG, GLU, ALKP, ALT, CK and CHOL)
parameters.

The collected data were subjected to one-way ANOVA. Significance level was set at 5% ($P<0.05$). Data homogeneity was tested using Levene's test.

Results

Table 1 presents the differences that the inclusion of the silage caused on pig performance parameters. Final body weight and body weight gain were not affected by the inclusion of the silage ($P>0.05$). The other performance parameters, feed intake per pig and feed conversion ratio, were within the normal ranges. In addition, the hematological values did not differ statistically significantly ($P>0.05$). Regarding the biochemical parameters, values were statistical similar between groups ($P>0.05$), except of the alanine aminotransferase (ALT), which was decreased in group C (10% inclusion of silage) compared to the control group ($P<0.05$).

Table 1. Effect of the dietary supplementation ofsilage on performance parameters of weaned piglets.

	Silage inclusion				
	0%	5%	10%	SEM	P
Live weight, kg					
Initial weight	57.75	59.48	61.18	0.85	0.27
Final weight	122.08	123.60	127.95	1.51	0.28
Weight gain, kg	64.33	64.11	66.76	1.25	0.66
Feed intake per pig (kg)	187.72	189.15	191.15	-	-
Feed conversion ratio	2.92	2.92	2.86	-	-

[a,b] Mean values with different superscripts differ significantly ($P\leq0.05$).

Discussion and Conclusions

Nowadays there is a great interest on finding natural antioxidants to protect the animals from the harmful consequences of oxidative stress. Grape pomace and olive mill waste water are rich in polyphenolic compounds, that protect from the oxidation caused by free radicals (5). Deproteinized cheese whey has high concentration of lactose and can increase the *Lactobacillus* populations in swine gut (6).

The results of our dietary experiment showed that the silage, which was created by three different agro-industrial by-products with high pollutant load, can be used on finishing pigs' diets without negative effects on growth performance and health status, contributingat the same time to the environmental protection and circular economy.

Acknowledgments

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User perception of a PCV-2 / M. hyo Vaccine Mixing System Innovation

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Introduction

To date, the fresh mixing of PCV2 and M. hyo vaccines requires a transfer needle for liquid transfer. Boehringer Ingelheim is preparing the launch of its new PCV2 / M. hyo vaccine mixing system TwistPak®, aiming to provide an innovative, more efficient, and safer mixing procedure retaining the flexibility of using the products as a monovalent or combined vaccine. TwistPak® will be the first bottle system which enables the safe and hygienic mixing of 2 mono-vaccines provided in 2 separate bottles without use of a transfer needle.

Materials and Methods

An empirical market research study was conducted with a random selection of n=131 swine production owners, production managers and farm staff veterinarians across Germany (n=50), USA (n=31) and China (n=50). Interviews were conducted in person from September through December 2019 following a strictly standardized survey to observe the handling of the 2 bottle systems as described below in Figures 1 and 2.

Figure 1. Mixing processes with transfer needle

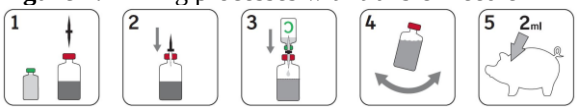

Figure 2. Mixing process with TwistPak®

50-dose bottles were used for each system and handled up to 3 times. Repeat handlings were performed to capture the impact of learning effects, changes in time needed for handling (measured in seconds), and the user perception of each system.
Respondents rated a pre-specified list of mixing system attributes in terms of importance. After the final handling, they were asked to allocate a total of 100 preference points to each system alongside the list of attributes. With an allocation of more than 50 points to a specific mixing system that system is categorized as preferred whereas the allocation of 50 points to each system equals no preference. SPSS V. 25 was used for data analysis.

Results

Overall the impression of TwistPak® after the final handling experience was rated positive by 95% of the respondents.

Table 1. Respondents' preference for mixing systems

	% of respondents in ...	TOTAL		DE		US		CN	
		TP	TN	TP	TN	TP	TN	TP	TN
Importance ▲	Confidence, substances are mixed properly	47	6	56	2	29	16	50	4
	Clean preparation and hygienic mixing	82	2	86	0	84	0	76	4
	Safety for user	83	2	94	0	87	0	70	6
	User compliance	48	5	44	4	61	3	44	6
	Time to mix substances	82	3	92	0	87	3	68	6
	Very low risk of product loss	82	3	92	2	77	0	74	6
	Easy to handle	66	11	72	8	77	16	52	12
	Not too many handling steps	56	9	60	8	65	13	48	8
	Not too much waste	43	18	24	34	74	6	42	10
	Not too many parts	53	11	58	16	58	3	44	12
	Appealing / innovative design	56	2	20	2	81	3	76	2

Data shows % of respondents; TP=TwistPak; TN=Transfer Needle

The results of the preference point allocation (cf. Table 1) provide empirical evidence that TwistPak® is perceived superior to transfer needle systems across all assessment factors at global level. The greatest advantages are perceived for user safety, time needed to mix the substances and low risk of product loss. Further evidence to the latter is provided by the significantly lower average handling time for TwistPak (TP) vs the transfer needle system.

Table 2. Handling time requirement

	TOTAL		DE		US		CN	
	TP	TN	TP	TN	TP	TN	TP	TN
AVG (s)*	20	72	14	67	21	77	26	75
Min (s)	5	21	5	21	8	33	11	30
Max (s)	133	197	27	182	104	169	133	197

* paired t-test between TP (=TwistPak) and TN (=Transfer Needle) resulted in p≤0.001 (2-tailed) for the total and all countries

Conclusions and Discussion

The empirical data shows how innovations in medical product presentation / bottling can improve the overall user experience with established medical products. Compared to existing PCV2/M. hyo mixing solutions TwistPak offers the advantage of flexible (mono or combined) use with significantly less compromise on handling speed, safety and other factors compared to traditional mixing procedures.
PCV2/ M. hyo vaccine mixing represents just one example where the benefits of the new mixing technology can be utilized. We recommend exploring more areas of use in livestock production, pet, and human healthcare.

Using a sound-based monitoring of respiratory health status to improve grow-finish production performance

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Introduction

The swine industry continues to experience substantial economic losses due to respiratory disease outbreaks in modern pig production worldwide impacting productivity, antibiotic usage, and animal welfare.[1] SoundTalks®, an available Precision Livestock Farming (PLF) technology for swine producers, is a cloud-based sensor technology that monitors the sound emitted from pigs on a 24/7 basis. Based on artificial intelligence, this technology processes the sound data collected at the farm and transforms it into a metric (0 - 100) that represents the animals' respiratory health status (ReHS). When the ReHS value falls below a certain threshold, the system emits early warnings (yellow/red LED alerts) allowing producers to intervene prior to the time when clinical signs of respiratory disease are evident to care givers[2]. Despite the early warning evidence, further research is needed to fully understand the impact of ReHS on production performance in the swine growing populations (i.e. mortality, average daily gain (ADG), and feed conversion (FC)). Therefore, this study aimed to describe the respiratory health status (ReHS) in the nurseries and finishers of a commercial farm monitored by SoundTalks® and to investigate the potential association with production performance.

Materials and Methods

The study was performed in the growing population of a large farrow to finish sow farm in Spain (8 nursery rooms, 16 finishing barns) continuously monitored by SoundTalks® for a period of 11 months. Information regarding production performance at a batch level (i.e. weeks after placement, mortality %, finisher ADG, and finisher FC) was consolidated to be analyzed. Linear regression models were used to study the association between production variables and respiratory health markers (i.e. average ReHS value, SoundTalks green, yellow and red monitor color and combinations). Other environmental (i.e. daily room temperatures as well as calendar-month) were also considered for the descriptive models.

Results

A total of 25 nursery and 25 finishers batches were analyzed from Nov 2020 to Sept 2021 for the study. Overall, results from this study demonstrated that the respiratory health (i.e. Average ReHS value of the batch) evaluated during the nursery period was significantly associated with batch nursery mortality % (R^2=0,3532, p value=xx) (Fig1A). With regards to the finishing groups, significant positive association was found between pig growth (i.e. ADG) and the respiratory health measured as a percentage of days without yellow/red alarms (i.e. % green) (R2=0,2585, p value=0,0065) (Fig1B). On the other hand, there was no significant association between the respiratory health and FC or % mortality in the finisher groups. Furthermore, there was an evident room effect as the percentage of days with green, yellow or red ReHS significantly differed among nursery and finisher airspaces. Other important variables were calendarmonths, since the % of yellow and red alarms significantly increased during the summer months (July-Sept); as well as months after placement, since there was a significantly lower ReHS during the first and third month after placement compared to the second month of the finisher period (p-value<0,001 Tukey method comparison).

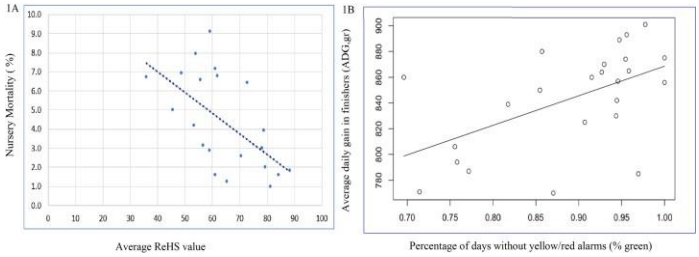

Fig 1A. Nursery negative correlations between mortality and average Respiratory Health Status per batch. (p valor=0,0075); R^2=0,3515.

Fig 1B. Finishing batch correlation between average daily gain (gr) and days without respiratory alarms (%) measured by SoundTalks® (p valor=0,0065); R^2=0,2585.

Conclusions and Discussion

This is the first study, to the author's knowledge, that shows the direct impact that the respiratory health status (ReHS) measured by SoundTalks® has on key production performance parameters during the growth and finishing phase of the pig. Results from this study demonstrated that continuous sound monitoring of pigs used to implement an early intervention against respiratory challenges will directly improve the production parameters of the growing and finishing populations. Further studies are needed to understand the implications and comparison of different interventions following SoundTalks® alarms when different pathogens are involved.

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REPRODUCTION

Achieving breeding targets in weaned sows by not breeding or oestrus checking on day 3 post-weaning. Saving time in a busy world

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Introduction

The purpose of breeding a batch of sows is to achieve the target quality and quality of weaned pigs at the allotted weaning age.

Reviewing the physiology of oestrus and ovulation allows for time savings in a busy breeding unit. Ovulation occurs at 70% of the way through standing oestrus and the length of standing oestrus is longer shortly after weaning period than later. Peak conception rates occur 4-6 days postweaning, with mating before day 4 postweaning having a conception rate below 80%[1]. Whilst sperm will survive in the oviducts for 24-48 hours post-insemination the ovulated egg must be impregnated within 6 hours.

Null hypothesis

There is a herd performance advantage in checking for oestrus 1,2, and 3 days post-weaning and breeding any sows in oestrus from day 3 post-weaning.

Materials and Methods

A trial was conducted on a commercial farm with a weekly batch target of 1420; 7.5kg weaners at 27 days of age. This equated to 11 weaned per batch weaning sow from 120 weaned sows per batch. The sows were pure bred Large White and Landrace and the farm was SPF but PRRS positive. The trials were conducted consecutively.

Group 1

Sows were weaned and oestrus checked each day twice post-weaning. Oestrus sows were mated twice (am/am) from day 3 of weaning. There were 156 batches in Group 1 records (3 years)

Group 2

Boar exposure and oestrus-checking only commenced from day 4 post-weaning. Oestrus checking was only conducted once daily in the morning the sows were mated twice (am/am) if seen in oestrus.

There were 104 batches in Group 2 records (2 years). Note that sows bred after day 7 were considered late sows, as they had missed the batch requirements; breeding targets were achieved withweaned sows, gilts, late sows and returns; and sows were mated bycervical artificial insemination.

The time required for oestrus checking and mating for each trial groups was compared.

Results

Breeding day distribution for weaned sows

Day post-wean	3	4	5	6	7
Group 1 %	10.5	65.6	16.5	4.7	2.7
Group 2 %		64.5	28.4	5.5	1.6

Batch Farrowing Rate analysis

	Group 1	Group 2
μ	87.2	85.4
σ	3.8	3.8
Max	98	98
Min	70	78
10 percentile	83 %	81 %

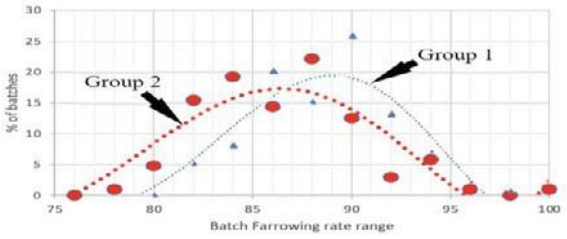

Figure 1. The distribution of batch Farrowing Rate results. Triangles Group 1 mating included day 3 post-weaning. Circles Group 2 mating not including day 3 post-weaning Difference p<0.01.

Weaning number analysis

	Group 1	Group 2
μ	10.4	11.0
σ	8	9.1
Max	13.5	13.7
Min	8	9.1

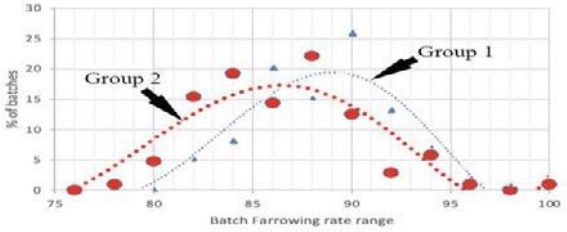

Figure 2. The distribution of batch weaning results. Triangles Group 1 mating included day 3 post-weaning. Circles Group 2 mating not including day 3 post-weaning.Difference p<0.02

Time management

Minimum batch breeding target determined by the 10% percentile (1st decile), to ensure batch is full 90% of the time.

	Minimum breeding females	Breeding time h	Oestrus detection h	Total hours
Group 1	145	17.4	10	27.4
Group 2	149	11.9	3	14.9

Discussion and Conclusion

Checking oestrus in sows from day 1 post-weaning and mating from day 3 resulted in a higher farrowing rate %. However, not oestrus checking of sows and mating only from day 4 resulted in a higher number of piglets weaned per sow. As the farm was batching, both systems achieved the required goal of filling all the farrowing places and weaning sufficient piglets.

Leaving the weaned sows until day 4 for oestrus detection and simultaneous mating saves the farm considerable time, while still allowing for batch targetsto be achieved, with minimal impact on performance indicators. Single serving would decrease the man-hourtime requirements further to only 10 hours.

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Altrenogest supplementation in sows from day 6 to 12 of pregnancy improves piglet performance at birth

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Introduction

Although it has long been recognized that increasing litter size would increase production's efficiency, significant improvements have not been achieved due to a disproportional increase in prenatal mortality (1). The causes have been linked to embryonic heterogeneity, competition for space and/or nutrients and compromised placental development. All these aspects may influence piglet performance (1,2). Thus, improve early embryo development is essential to achieve a better reproductive performance. Progesterone (P4) plays a crucial role on initial conceptus development once it modulates a specific intrauterine environment (2). However, the effects of P4 or its analogues supplementation on litter performance at birth are unknown. Therefore, the aim of this study was to evaluate the effects of altrenogest supplementation from day 6 to 12 of pregnancy on the number of piglets born, stillbirth rate, piglet birth weight and percentage of piglets born under 800g.

Materials and Methods

A total of 301 females were randomly allocated in two groups: non-supplemented females (NS; n = 163) or females supplemented orally with 20 mg of altrenogest (Regumate® - MSD Saúde Animal) from day 6-12 of pregnancy (ALT; n = 138). The ovulation was considered as occurred 48 hours after the estrus detection to determine the first day of pregnancy. The results are presented as mean ± SEM and were considered significant at $p < 0.05$

Results

The results are shown in Table 1. The treatment increased ($p < 0.05$) the number of total piglets born. The stillbirth rate was lower ($p < 0.05$) in ALT-sows. The sows from both groups had piglets with similar ($p > 0.05$) average birth weight. Additionally, ALT-sows had lower percentage of piglets born under 800 g compared to sows from CON ($p < 0.05$).

Discussion and conclusion

In the present study, the altrenogest supplementation from day 6-12 of pregnancy increased the number of total piglets born. In contrast with our findings, Soede et al. (2012) treated sows with altrenogest prior to day 6 of pregnancy and found reduced number of foetuses at day 42 of pregnancy and litter size at birth. The differences between the studies may be related to the period of altrenogest supplementation. Mathew et al., (2011) demonstrated that progesterone supplementation performed prior to day 6 of pregnancy impairs embryo survival.

Table 1. Effects of altrenogest (Regumate®, MSD Saúde Animal) supplementation from day 6 to 12 of pregnancy on litter performance at birth

VARIABLE	CON[1]	ALT[2]	P-VALUE
Total piglets born (n)	16.6 ± 0.36	17.3 ± 0.37	0.03
Stillbirth rate (%)	7.6 ± 0.58	5.9 ± 0.56	0.02
Birth weight (kg)	1.288 ± 0.02	1.293 ± 0.02	0.80
Piglets born < 800 g (%)	8.0 ± 0.60	6.6 ± 0.56	0.02

[1]CON: non-treated sows; [2]ALT: sows treated with altrenogest (Regumate®, MSD Saúde Animal) from day 6 to 12 of pregnancy

In our study, the altrenogest supplementation from days 6-12 of pregnancy reduced the stillbirth rate and the percentage of piglets born under 800g. Similarly, Muro et al. (2020) demonstrated that sows treated with altrenogest from day 6-12 of pregnancy had greater embryo size and weight at day 28 of pregnancy with no impacts on embryo survival (5). Indeed, other studies demonstrated the positive impacts on uterine environment of P4 or its analogues supplementation during early pregnancy (6,7). An enriched uterine environment may be related to an improvement on conceptus development and, consequently, number of piglets born under 800g and stillbirth rate. In conclusion, altrenogest (Regumate® - MSD Saúde Animal) supplementation from day 6-12 of pregnancy may be used to increase the number of total piglets born as well to decrease the stillbirth rate and the percentage of piglets born under 800g in order to improve productivity and welfare at maternity.

Acknowledgments

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Are uterine lymph nodes appropriate specimens for the diagnosis of genital diseases? – A preliminary report

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Introduction

Post mortem analyses (PMA) of genital tracts are appropriate in order to help determining reasons for reproductive problems. PMA usually includes gross-morphology and histology of different genital organs (i.e. ovaries, uteri etc.), as well as bacteriology (i.e. of uteri) and mykotoxicology [i.e. for zearalenone (ZEA) and DON], but virology is commonly not. Lymphatic organs are appropriate for the detection of bacterial and viral pathogens for e.g. intestinal or respiratory diseases, but have never been included into PMA. Regional lymph nodes (Lnn) supplying the uterus are often enlarged in females suffering from reproductive problems. It appeared thus reasonable to include these Lnn into PMA, which is reported in this preliminary study.

Materials and Methods

Fifteen genital tracts (including the urinary bladder) of reproductively failed gilts and different parity sows of six farms (1–7 tracts/farm) were submitted for PMA. Fertility problems included e.g. high rates of returns, low/fluctuating pregnancy rates or vaginal discharge. PMA included gross-morphology and histology of different parts of the genital tract (i.e. uterus, cervix, vagina etc.). Uterine specimens (n=8) were submitted for bacteriology and bile samples (n=8) for the analysis of ZEA and DON by HPLC/MS. The regional Lnn located within the broad ligament were collected (bilaterally if available) and stored at -80°C until analysis for PCV2, PRRSV, PPV and also for different chlamydial species by real-time PCR. While detailed information on PCV2 and PRRSV vaccination is not available, all animals had been routinely vaccinated against PPV.

Results

All genital tracts had inflammations in one or more organs tested (Tab. 1), which were mostly sub-acute and moderate to high in severity. All uteri were bacteriologically positive with up to ten different bacterial species, and 6/8 bile specimens mostlyseverely positive for DON (while ZEA was found only in a few samples in neglectable concentrations; not shown in Tab. 1). Some of the uterine Lnn collected were markedly enlarged. None of them were positive for PCV2, PRRSV and Chlamydia. In contrast, Lnn of 4 animals from 3 farms were tested positive for PPV.

Discussion and Conclusion

Results of this report confirm the validity of PMA in cases of reproductive disorders. While PMA usually includes gross-morphology, histology, bacteriology as well as occasionally also mycotoxicology, a virological examination of genital specimens is rather uncommon. This preliminary report demonstrats that PPV, i.e. a virus that can be linked to reproductive disordes, is detectable in uterine Lnn. This was inspite the fact that animals had been routinely vaccinated with an attenuated PPV vaccine suggesting that infection and replication were still possible. Another interesting observation was that 3/4 PPV positive anaimls were also highly contaminated with DON suggesting that DON may have facilitated a PPV infection. As to whether PPV contributed to the reproductive problems remains, however, unanswered. The fact that other pathogens were not detected in uterine Lnn does not exclude their principal ability to colonize them. Further studies are requested.

Table 1. Results of histology, bacteriology (B; uterus only; number species) and analysis for DON (bile; µg/l), as well as of RT-PCR for PPV (uterine Lnn; ct-value) (n = 15)

n/Farm	Inflamed organs[1]	B	DON[3]	PPV[4]
1/1	V, C, U, S	2	>200	34.0
2/1	V, C, U, S	3	>200	26.4
1/2	V, C, U	5	<10	neg
1/3	V, C, U	2	63.0	neg
1/4	V, C, U, S	---[2]	---[2]	neg
2/4	V, C, U, S	---[2]	---[2]	neg
3/4	V, C, U, S	---[2]	---[2]	neg
4/4	C, U, S	---[2]	---[2]	neg
5/4	V, U, S	---[2]	---[2]	neg
6/4	V, C, U	---[2]	---[2]	34.6
7/4	V, C, U	---[2]	---[2]	neg
1/5	U	1	24.9	neg
2/5	U	3	12.4	neg
1/6	V, C, U, S	10	>200	neg
1/6	V, C, U, S	7	>200	34.0

[1]V = vagina; C = cervix; U = uterus; S = salpinx; [2]--- = not tested; [3]DON plus Deepoxydeoxynivalenol/ 3-Acetyl-Deoxynivalenolund/ 15-Acetyl-Deoxynivalenol; [4] neg = negative

Acknowledgments

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Comparison of reproductive performance of sows in herds with partial and complete reproductive vaccination schedules

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Introduction

Porcine parvovirus (PPV) is a primary cause of reproductive failure in pigs (1,2). In a susceptible herd, sows between 1 to 60 days of gestation are susceptible to PPV infection (2). Under field conditions, a cross-sectional study reveals that 99.0% of the replacement gilts in Thai swine commercial herds are infected with PPV before entering the breeding herd (3). Therefore, awareness on the PPV infection and associated clinical symptoms should be raised. The present study aims to evaluate reproductive performances, especially the evidence of mummified fetuses in sows in relation to PPV vaccination schedule (partial vaccination and complete vaccinations) in large scale swine breeding herds in Thailand.

Materials and Methods

A retrospective study was conducted in two commercial swine breeding herds in Thailand and included data of 78,492 litters from 28,996 Landrace x Yorkshire crossbred sow. The numbers of sow-on-production in each herd were 4,800 and 8,000 sows, respectively. Reproductive performance data including total born, born alive, stillbirth (%) and mummified fetuses (%) were collected and analyzed. The analyses were based on individual records of 44,262 litters from 13,301 sows from PPV partial vaccination schedule herd (i.e., the PPV vaccination is performed only in replacement gilts but not in sows) and 34,230 litters from 15,695 sows from PPV complete vaccination schedule herds (i.e., the PPV vaccination is performed in both replacement gilts and all parities of sows). Multiple analyses of variance (ANOVA) and least-squares means procedure were used to analyze the data.

Results

Reproductive performance of sows from herds with complete and partial vaccination schedules for PPV are presented in Table 1.

Table 1. Reproductive performance of sows in completed and partial PPV vaccination schedules

Variables	PPV vaccination schedule	
	Complete	Partial
Observations	34,227	44,262
Sows	15,695	13,301
Parity	3.7 ± 2.1	3.6 ± 2.0
Total born	13.2 ± 3.4[a]	13.1 ± 3.6[b]
Live born	12.0 ± 3.2[a]	11.8 ± 3.8[b]
Stillbirths (%)	6.5[a]	5.6[b]
Mummified (%)	2.6[a]	4.6[b]
Litters with mummified piglets >30%	0.9[a]	3.3[b]

[a,b] Different superscripts differ at $P<0.05$

Conclusions and Discussion

The incidence of mummified fetuses and the percentage of litters with >30% of mummies in the herd that uses a partial PPV vaccination schedule was significantly higher than the herd that uses a complete PPV vaccination schedule. Therefore, to reduce the percentage of mummified fetuses per litter in the partial PPV vaccination herds, a completed PPV vaccination schedule in all parities of sows every 4 – 6 months is strongly recommended (2).

Acknowledgements

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Comparison of two commercial altrenogest based products on estrus synchronisation in gilts under a field conditions.

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Introduction

The proper age at first service in gilts is directly connected to the future performance results and longevity of sows. Effective gilt management programs allow to meet replacement targets and to maintain optimal size of gilt pool. This gives the opportunity to plan precisely mating of the gilts which are to be introduced into the batch of weaned sows (1).

Altrenogest is the synthetic steroid with the progestagenic activity, which acts the similar way as progesterone from the corpus luteum (2). The use of altrenogest for estrus synchronization in gilts is widely used in swine farms and proven effective management tool (1). There are several commercial altrenogest based products registered and available in Thailand. Formulation of pharmaceutical products and their pharmaceutical properties are important parameters with significant influence on the drug absorption, concentrations in the target tissues and consequently the therapeutic effect (3). Therefore, the objective of the presented study was to compare the synchronization efficacy and effect on wean estrus interval (W-E) of two selected products under the field conditions.

Materials and Methods

The study was conducted on integrated swine farms in Thailand. Cycling gilts after confirmed first estrus were divided into 2 groups and treated according to SPC of products (20 mg altrenogest / animal), 18 days with Altresyn® (group 1) and competitor altrenogest product (group 2). After the withdrawal of altrenogest treatment, standard estrus detection was performed every day same way in both groups. Number of detected gilts on heat and W-E interval were recorded. The estrus rate (%) and average W-E interval were calculated for both groups.

Results

The results are presented in table 1. There are no significantly differences observed between Altresyn and second altrenogest product that farm used. Numerically difference and positive trend has been observed in the estrus rate of group 1 (1.9% higher thangroup 2) and average W-E interval in group 1 was 7.68h shorter than group 2.

Table 1. Comparison of the results of two treatment groups.

Parameter	Group1	Group 2	p-value
No. of gilts	424	321	
No. of detected gilts on heat	399	296	
Estrus rate (%)	94.1%	92.2%	0.705
Average wean- estrus interval (days)	5.43	5.57	0.820

Conclusions and Discussion

In our study we have proven the product of Group 1 as effective tool for synchronization of heat of gilts under the field condition in Thailand. Numerically higher % of synchronization rate and shorter W-E interval was recorded in group 1.

Optimal and predictable estrus rate enable moreeffective introduction of optimal number of replacement gilts.

Short W-E interval is reducing the cost of the production and is consider as predictor of high breeding efficiency.

Nevertheless, the use of effective gilt synchronisation must be done in parallel with the good management in gilt development unit to achieve the highest efficacy and performance.

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Effects of an energy supplement on farrowing duration and blood glucose concentration of parturient sows

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Introduction

Delivering piglets is one of the most energy demanding activities hyperprolific sows undergo in their lifetime (1). Extended farrowing leads the sows to exhaustion and plasma glucose concentrations below those required, which can impair uterine contractions and, consequently, farrowing outcomes (2). Therefore, the objective of the present study was to provide an energy supplement based on carbohydrates and glycerol, administered orally to sows at the beginning of farrowing, to decrease farrowing duration.

Materials and Methods

The sows were blocked according to parity and allocated to one of the following groups: SUP (sows supplemented with energy supplement; n = 85) and CON (sows not supplemented; n = 95). The energy supplement was provided to the females of the SUP group at the beginning of farrowing (birth of the first piglet). The farrowing duration (FD) was defined as the time elapsed between the birth of the first and last piglets in the litter. The number of total born (TB) was recorded. Glucose concentrations was measured in the ear vein with a digital glucometer (Accu-Chek Guide®, Roche) at five moments during farrowing: T0 (immediately after expulsion of first piglet and prior supplementation to the SUP-sows), T20 (20 minutes after T0), T40 (40 minutes after T0), T80 (80 minutes after T0), (20 minutes after T0) T180 (180 minutes after T0). Statistical analyzes were performed using the software R (version 4.1.0). All data were tested for normality and when necessary, they were transformed. Statistical significance was considered when $p < 0.05$.

Results

The TB was similar between both groups ($p > 0.05$) (table 1). FD was shorter ($p < 0.05$) for SUP-sows compared to CON-sows (table 1). Blood glucose concentration at T0 was similar for both groups ($p > 0.05$) (4.31mmol/L vs 4.38 mmol/L for CON and SUP respectively). Sows which received the energy supplement had higher blood glucose ($p < 0.05$) at T20 (4.33 mmol/L vs 4.69 mmol/L) and T40 (4.39 mmol/L vs 4.65 mmol/L). At T80 (CON = 4.35 mmol/L; SUP = 4.39 mmol/L) and T180 (CON = 4.46 mmol/L; SUP = 4.66 mmol/L) blood glucose concentration did not differ ($p > 0.05$) between CON-sows and SUP-sows as shown in figure 1.

Discussion and Conclusion

The increased blood glucose concentration observed in SUP sows until at least 40 minutes after the energy supplement was associated with a decrease of 20 minutes in FD. It is noteworth that the farrowing duration was short in the present study even for sows from CON. In agreement with these results, Oliveira et al. (3) observed a reduction of 44 minutes in farrowing duration in sows fed an energy supplement based on lactation diet (250g) and sugar (250g) at day of farrowing. All these findings support the notion that the gravid uterus is reliant on energy from glucose oxidation to support its intense contractions (2). Collectively, these results demonstrate the feasibility of using nutritional interventions to reduce FD and enhance farrowing outcomes.

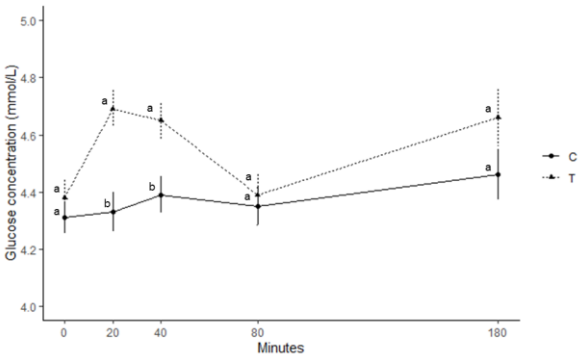

Figure 1 Blood glucose concentration during farrowing
Superscripts indicate statistically significant differences ($p \leq 0.05$).

Table 1. Effects of energy supplement on farrowing traits.

Variables	Groups	
	CON	SUP
Farrowing Duration (min)	228 ±8.6[a]	208 ±9.6[b]
Total born	17.5 ± 0.3	17.4 ± 0.4

Data are presented as mean ±SEM.
Superscripts indicate statistically significant differences within the columns ($p \leq 0.05$).

Acknowledgments

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Effects of gilt growth rates from birth to breed on subsequent performance

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Introduction

The modern gilts have showed a high growth rate and sexual precocity. Age at puberty has been suggested as an indicator of lifetime reproductive performance (1). It has been shown that gilts mated at a younger ageare culled later in life than gilts mated at an older age (2). The objective was to evaluate the effects of gilt growth rate from birth to breed on subsequent reproductive performance and retention rate until parity 3.

Materials and Methods

Data on 1,962 gilts (Camborough®) collected at a sow farm in South of Brazil. Weight (with a scale) and ageat first breeding were obtained. Gilt growth rate from birth to breed was calculated as follow: Growth rate, kg/d = (Gilt weight at breeding – 1.35)/(Gilt age at breeding). Females were followed until their third farrow. Reproductive performance in each cycle was recorded. Growth Rate Categories Gilts were categorized based on their growth rate from birth to breed as follow: Bellow 650g (average AGD g/d 622– N 382), 650 to 750(average AGD g/d 696 – N 1161)and Above 750 (average AGD g/d 622 – N 364). All gilts were breed with >135kg, second estrus, 20 days after second shot reproductive vaccine and 14 days oncage to adaptation. Data were analyzed using linear orlogistic regression models in R, gilt was the experimental unit. Explanatory variable: growth rate categories. Total born and weaned pigs were analyzedfollowing a normal distribution. Retention rate was analyzed following a binomial distribution. Tukey multiplicity adjustment was used to avoid type I error. Results were considered significant at $P \leq 0.05$.

Results

Gilt growth rate from birth to breeding was positively correlated with weight at first breeding and negatively correlated with age at first breeding. Total pigs born inthe first parity was higher for gilts with birth to breedgrowth rate above 750 g/d, followed by gilts with 650to 750 g/d ADG then gilts <650 g/d ADG (Figure 1). Total pigs born up to parity 3 was higher for gilts witha birth to breed growth rate above 750 g/d compared to gilts with a growth rate between 650 to 750 g/d or below 650 g/d (Figure 2). There was no evidence for differences in retention rate and weaned pigs up to parity 3 according to the different birth to breed growth rate categories (Figure 3 and 4).

Conclusions and Discussion

Gilts with high growth rate are more productive in terms of total born on the first parity and up to parity 3. No differences were evidenced in terms of retention rate.

These results show an important economic opportunity, as high grow rate gilts produce more, they can be breed earlier (respect weight >135kg, second estrus and sanity acclimatation).

Acknowledgments

Lar, UFPR, UFMG and Agroceres PIC.

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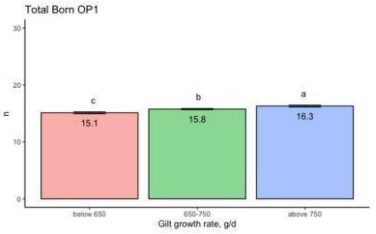

Fig. 1. Results from total born Parity 1.

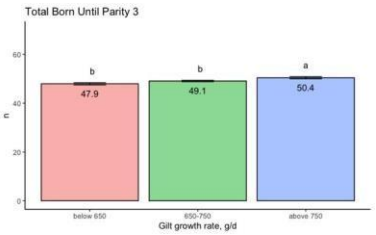

Fig. 2. Results from total born until Parity 3.

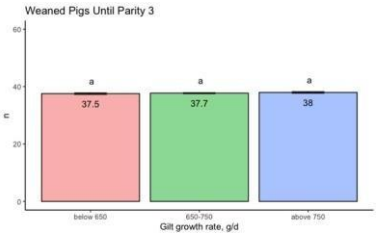

Fig. 3. Results from weaned pigs until Parity 3.

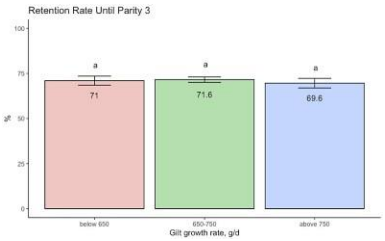

Fig. 4. Results retention rate until Parity 3.

Encapsulation of boar semen in alginate beads with natural extracts and silver nanoparticles as an antioxidant and antimicrobial agents

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Introduction

The boar semen encapsulation in alginate beads is one of the main innovations in Artificial Insemination (A.I.) techniques. In this methodology usually used of the seminal doses is with commercial- swine extender (1). However, a low sperm viability it has been seen once that the spermatozoa were released of the alginate beads, because of that we propose the use of natural extracts like swine extender (2,3). On the other hand, due the bacterial load in the seminal doses and the resistance of them for the excessive use of the antibiotics commonly uses, for those reasons we propose the incorporation of Silver Nanoparticles (AgNPs) like antimicrobial agent against of two principal strains: *Pseudomonas spp.* and *Staphilococcus aureus*, which are related with reproductive problems in the pigs (4).

Materials and Methods

The seminal doses were collected from a Yorkshire boar. The natural extracts were synthetized with two different dehydrate leaves of plants: *Cymbopongo citratus* (Cc) e *Hipericum perforatum* (Hp). The synthesis of AgNPs were made with green chemistry using like reducing agents the natural extracts and 1 mM of Silver nitrate ($AgNO_3$) in 25 ml of distilled water in two different solutions. According to a previously reported method of Torre, M.L., Barium chloride (0.07M), Hydroxypropyl-metylcellulose (HPMC) (0.03gr) and the natural extracts with the seminal doses were dropped into a Sodium alginate (0.5%) solution with AgNPs to obtain alginate beads with liquid matrix. The evaluation of sperm motility was determined by Optical microscopy and by a Seminal Quality System (SQS). The AgNPs solution was evaluated by UV-Vis spectroscopy, Scanning and Transmission Electron Microscopy (SEM and TEM) for the exact size of the NPs and their antimicrobial effect in two different strains: *Pseudomonas spp*. and *Staphilococcus* in TBS culture media. The size and the morphology of the beads were analyzed by SEM.

Results

The viability of the seminal doses was analyzed previous of the encapsulation while obtained 80% of viability. The samples of AgNPs were analyzed by UV-Vis spectroscopy gave an absorbance peak between 440 nm for *Cc* and 460 nm for *Hp* according to the literature for silver colloid solutions, the presence of NPs in the surface of the alginate beads by SEM with the technique of backscattered electrons (fig.1b) and their size lower than 50 nm by TEM (fig.1c). The antimicrobial effect of the AgNPs was evaluated by well diffusion method obtaining inhibition halos between 0.34 and 0.64 cm after their incubation. The interaction and the seminal viability with both natural extracts were evaluated for two consecutive days, we observed their viability at 5% after 48 hours. Finally, we obtained a good percentage of motility for the spermatozoa after being released from the alginate beads with liquid matrix (fig.1a).

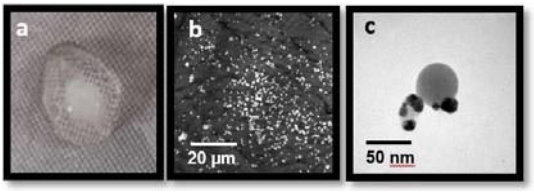

Figure 1. a) alginate bead with liquid matrix with boar semen, b) AgNPs in the Surface of the alginate bead with backscattered electrons by SEM and c) Size of AgNPs by TEM less than 50 nm.

Conclusions and Discussion

The encapsulation was achieved in alginate beads with a liquid matrix of Silver Nanoparticles, that have antimicrobial properties which are released into the sow reproductive tract acting against specific strains reducing reproductive problems, and semen in natural extracts maintaining an appropriate sperm viability for their use due to the antioxidant properties of the plants chosen to be released at specific times for 3 consecutive days due to temperature destabilization of the alginate membrane.

Acknowledgments

To CONACYT for my scolarship and the MIPE for the help that they bring to me.

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Endometrial cytology applied to the diagnosis of subclinical endometritis in sow

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Introduction

Endometritis is characterized by inflammation of the endometrium. A negative relationship between endometritis and production level in dairy cows [1] and low fertility in mares has been demonstrated[2]. The early stage of the disease without clinical manifestation is considered subclinical endometritis. The correlation of endometrial inflammation and reproductive performance is normally assessed by endometrial cytology *in vivo*. Cervical cytology is unexpensive, quick and sensitive diagnostic tool not only used diagnosis of clinical endometritis but also during early or subclinical endometritis. Due to current husbandry practice, (i.e farrowing assistance, large litter size, confined farrows) sows are prone to develop acute/chronic endometritis. The role of endometritis in the reproductive performance of the sows has not been evaluated, making it imperative to develop an *in vivo* diagnostic method that allows develop and early detection method to determine the role of this pathology on the sow reproductive performance. **The objective of this study was to evaluate the clinical use of endometrial cytology *in vivo* as a diagnostic method for endometritis in post-weaning sows.**

Material and methods

A total of 46 females of different parities allocated in individual farrowing/gestation crates in a commercial farm were assigned into three groups: 16 females at the day of weaning (D1), 18 females sampled three days post-weaning (D3) and 12 females were sampled twice at weaning date and third day post-weaning (D1-3). Cytology samples were collected with an endocervical brush (Medibrush Plus®, Medical Engineering Corporation S.A.) The brushes were immediately spread on a glass slide, stained with Romanovsky (Stain 15®, Biopur SRL) and mounted for cytological preservation. Cytological evaluation was performed under a light microscope (100X) in immersion oil. A total of 200 cells were counted including endometrial cells and neutrophil. The cut-off value for presence/absence of subclinical endometritis in sows has not been determined yet, therefore the presence of 7% of neutrophils was used based on average cut-off values previously reported in cows [2,3]. The presence/absence of purulent material in the endocervical brushes, presence/absence of vulvar discharge, was recorded during sample collection. In addition, dates of last estrus and culled sows was recorded.

Results

The sample day resulted in a variation in the proportion of sows detected with endometritis. Thus, endometritis was diagnosed in a 33.3 in D1, 50 % D3. The sows from the group D1-3 showed that 50% (6/12) had a reduction in the number of neutrophils with no changes in cellular composition of the samples, while in 41% (5/12) there was an increase in number of neutrophils. There was not a significant difference (p=0.63) in the proportion of sow with cytological diagnosis of endometritis and vulvar discharge (Table 1)

Table 1. Relationship between sows with vulvar discharge and those with a positive diagnosis for endometritis

		Vulvar discharge		Total
		Yes	No	
Endometritis	Yes	2	8	10
	No	5	31	36
Total		7	39	46

Is important to highlight that 80% of the sows diagnosed with endometritis 80% (8/10) did not show vulvar discharge. Therefore, all of them were A.I, but 30% were culled due to reproductive reasons (3/10). When the weaning-to-estrus interval (WEI) was analyzed, the greatest WEI was found in females with vulvar discharge (5.42 days), while the group of females with the presence of pus in the brush had the shorter WEI (4.28 days).

Conclusions

Multiples sample collection seems not to affect the proportion of inflammatory cells due to mechanical irritation of the cervical mucosa. Differences in the proportion detected at weaning and post-weaning can be due to physiological changes and not purely to the technique's sensitivity. In addition, 80% of the females diagnosed with endometritis did not present vulvar discharge, and 30% were culled due to reproductive reasons. Thus it can be concluded that further studies are necessary to define a cut-off value for the diagnosis of subclinical endometritis in sows and its importance as a predictive parameter to evaluate the future reproductive performance of the sows

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Enhancement of reproductive parameters on two Vietnamese farms using ERYSENG® PARVO/LEPTO

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Introduction

Sow vaccination against Swine Erysipelas (SE), Porcine Parvovirus (PPV) and *Leptospira* spp. to prevent reproductive disorders is commonly used in the vaccination plan of breeding farms (1, 2). Apart from stimulating protective immunity, vaccines must also have no or minimal adverse effects that can potentially impair the sows' performance (3).

The aim of this study was to compare the safety and the efficacy of two different reproductive vaccines on two commercial farms in Vietnam.

Material & Methods

The study was carried out on two farms of 1,200 sows each one (Farm A and Farm B) located in the North of Vietnam. On each farm, during 3 consecutive weeks, a total of 90 sows were divided into 2 groups. Group EPL, 15 sows/weeks (N=45, G. EPL) were vaccinated with ERYSENG® PARVO/LEPTO (EPL), a 2 ml (dosage) trivalent vaccine including antigens of SE, PPV, 6 serovars of *Leptospira* spp and adjuvanted with HIPRAMUNE® G[d]. Group B (G. B), vaccinated with a trivalent vaccine but including 5 serovars of *Leptospira* spp., 5 ml (dosage) and an aluminium hydroxide adjuvant. Both groups were vaccinated at day 10 after farrowing. Animals were randomly assigned to each group, based on the parity and previous reproductive data. There were no other differences in management between groups, except the vaccine.

For the safety evaluation, rectal temperature (RT) was measured the day before vaccination (D-1), at vaccination moment (D0), and +6, +24 and +48 hours later. Feed intake (FI) was also measured at the same points in time as RT, except +6 hours. In terms of efficacy, reproductive parameters in the subsequent cycle were recorded. For the statistical analysis an ANOVA of a logistic regression with the farm as random farm effect and, a Poisson regression for reproductive parameters.

Results

Regarding the safety parameters (FI and RT), no statistical differences were observed between any of the groups at any of the different time points (Table 1). In terms of efficacy, the percentages of mummified piglets were statistically significantly (*P-value≤0.05*) lower on Farm A (0.76% vs 2.08%) and the stillborn on Farm B (0.72% vs 2.19%) in the group vaccinated with EPL (Table 2).

Table 1. Rectal temperature and feed intake at different time points.

Parameter	Farm A		Farm B	
	G. EPL	G. B	G. EPL	G. B
RT (ºC)				
D-1	38.53	38.48	38.51	38.51
D0	38.63	38.62	38.65	38.66
+6h	38.54	38.53	38.7	38.69
+24h	38.58	38.6	38.65	38.6
+48h	38.52	3.54	38.57	38.5
FI (kg)				
D-1	6.45	7.02	6.97	7.09
D0	6.3	6.91	7.08	7.14
+24h	6.12	6.7	7.19	7.19
+48h	6.38	6.8	7.18	7.19

No significant statistical differences were observed (*P-value>0.05*)

Table 2. Reproductive parameters in the subsequent gestation.

Parameter	Farm A		Farm B	
	G. EPL	G. B	G. EPL	G. B
Total born	16.95	16.86	18.21	18.05
Mummified	**0.76%[a]**	**2.08%[b]**	1.73%	1.80%
Stillborn	1.06%	0.48%	**0.72%[a]**	**2.19%[b]**

Different superscripts (a, b) indicate statistically significant differences within the main parameters (*P-value ≤ 0.05*)

Discussion & Conclusion

According to these results, ERYSENG® PARVO/LEPTO is as safe as the vaccine used in G. B and offers a greater efficacy in the control of reproductive diseases, based on the reduction of the mummified and stillborn piglets observed. Furthermore, this trial demonstrates the importance of having a good and efficacious vaccination program against SE, PPV and *Leptospira* spp. infections in swine farms.

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Heatwaves on gilts insemination days impair pregnancy

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Introduction

The occurrence of events such as heatwaves (HW) will become more frequent, more intense, and longer-lasting due to climate change. This outlook represents a constraint on pig production since heat stress can impair sow fertility (1). However, there are few studies on the effects of heat stress over the reproductive indices of gilts reared in the tropics, especially in the Brazilian *Cerrado* biome region. Therefore, this study aimed to verify the effects of heatwaves on the insemination days of gilts reared in a tropical environment.

Materials and Methods

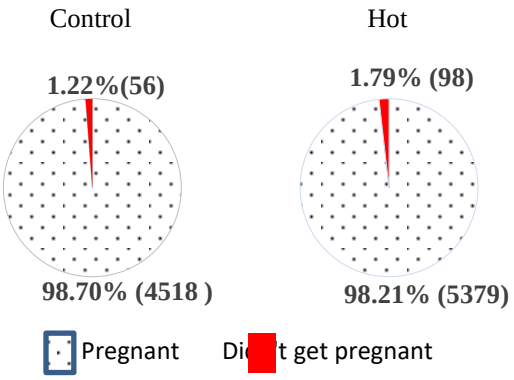
The data were collected on a commercial piglet-producing farm located in the Brazilian *Cerrado* biome (18º 91' S, 48º 25' W, and 875 m altitude) over five years. The daily air temperature and relative humidity data at 9 am, 3 pm, and 9 pm were obtained from the National Meteorological Institute and the temperature-humidity index (THI) was calculated. Three or more consecutive days of temperatures equal to or higher than 25 °C at least one of the aforementioned times together with a THI > 74 were considered a heatwave. The pregnancy and abortion numbers were calculated based on 10,051 inseminations. The gilts were divided into two groups: control (without HW) (4,574) and hot (with HW) (5,477) on the day of insemination. The data were analyzed using the chi-squared test ($P < 0.05$).

Results

A predominance of higher temperatures and THI was found at 3 pm. In the study period, 1,163 days with temperatures ≥ 25 °C and THI > 74 and 160 HW were verified. May, June, July, and August presented the lowest mean air temperatures (22.1 ºC to 24.4 ºC), THI (65 to 68), and consequently the lowest number of HW (1 to 12). The number of pregnancies of gilts inseminated during HW was lower in relation to those inseminated in thermal comfort ($P = 0.0267$) (Figure 1). The number of abortions of gilts inseminated during HW (3.20%) did not differ from those inseminated in thermal comfort (2.42%) ($P = 0.1065$) (Figure 2).

Conclusions and Discussion

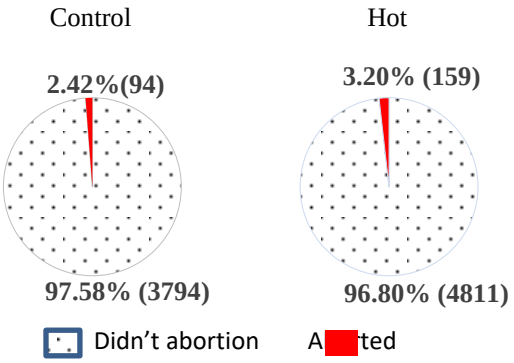
In this study it was verified that HW on gilt insemination days negatively influenced pregnancy numbers. One possible explanation for this result would be the sensitivity of the hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal axes to heat stress, resulting in alterations in glucocorticoid levels, impairing the production of the gonadotropin-releasing hormone (2). Moreover, gilts present greater sensitivity to high temperatures, due to them still being in the growth phase, which increases their metabolic rate (1).



Chi-Square Test: P = 0.0267

Figure 1. Number of pregnant or non-pregnant gilts in the control (inseminated without HW) and hot (inseminated during HW) groups.

The number of abortions was not influenced by HW on gilts insemination days (Figure 2).



Chi-Square Test: P = 0.1065

Figure 2. Number of gilts that aborted or did not abortion in the control (inseminated without HW) and hot (inseminated during HW) groups.

In a tropical environment, particularly in the Brazilian *Cerrado* biome, HW on insemination days impair the number of gilts pregnancies. Thus, the use of air-conditioning systems and adequate installations are needed to reduce the detrimental effects of heat stress in gilts.

Acknowledgments

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Increasing detection of *porcine parvovirus* as cause of reproductive failure

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Introduction

The porcine parvovirus (PPV), more correctly named Ungulate protoparvovirus 1 species, is a single-stranded DNA virus potentially causing great losses due to reproductive failure characterised by: stillbirths, mummification, embryonic death, and infertility (SMEDI). PPV viruses are prone to constant mutations and are classified into several strains of variable pathogenicity, some of them with very high virulence. As needs are to achieve clinical protection against all relevant field strains, this poses a challenge on the protective capacity of PPV vaccines. In several countries, an increase of PPV occurrence in the field has been reported within recent years. Also in France, a tremendous increase of PPV detection frequency has been noticed in 2021 compared to 2009-2017 period (1). Here we report several recent clinical cases of PPV-induced SMEDI in properly vaccinated animals.

Materials and Methods

As a part of our routine investigational service to farms experiencing reproductive failure, four SMEDI cases have been collected in which suspicion of PPV vaccine misused could be rejected following thorough investigation. These cases occurred in France in 2021 and in early 2022 in farms free of ADV/PRV, CSFV, and ASFV. All the four farrow-to-finish farms counting from 250 to 800 sows reported a severe increase in mummified foetuses. For example, in one farm, all the 1st parity sows (n=16) belonging to two consecutive batches gave birth to only mummified piglets and two 2nd parity sows gave birth to 5 mummified piglets each. For repro-prophylaxis in these farms, the gilts were vaccinated twice pre-mating and boosted every three to six months either by a commercial PPV-NADL2 plus Erysipelas rhusiopathiae (Ery) combo-vaccine or by a commercial NADL2-like PPV plus Ery plus hexa-valent Leptospira spp. combo-vaccine.
In all farms, mummies from several selected gilts and/or sows, were submitted for laboratory investigations.

Picture 1: mummified foetuses from one of the farms (14.5 to 17.5 cm long) – photo credit: Labocea

Results

All farms demonstrated strongly and only PPV-PCR positive findings in the submitted foetal material. No PCV2 was detected by PCR when investigated in the same foetal material.

Discussion and Conclusion

Vaccination against PPV and Ery is one of the most basic protocols for breeding stock worldwide. As mentioned previously, PPV strains can have different levels of pathogenicity, genetic clusters, and variable antigenicity. When vaccinating against PPV there are indications that the PPV-Kresse-like strain K22 as an antigen confers a wider and more efficient clinical PPV protection compared to other PPV vaccine strains, particularly against more virulent strains. This has been demonstrated in controlled challenge studies (2), as well as in field investigations of farms with a reliable vaccination procedure in accordance with recommendations in other countries (3,4). In conclusion, all these data confirm that a vaccine based on the PPV-K22 is a more reliable alternative in preventing PPV clinical signs and losses against all relevant field strains including the most recent ones.

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Interaction between the administration of mefepronic acid to gilts at farrow, the back fat thickness and the wean-to-estrus period

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Introduction

The body composition of gilts and sows has a deep influence on performances during lactation. The back fat thickness is a common measure to define the body condition. One of the major factors influencing on unproductive days in farms is the weaning-to-estrus period. The shortening of this period could result up to in 2.65 € per sow per day. There are a lot of factors that could improve this measure, and one of them is the body condition of the sow, and how the body resources have been used during lactation. We have investigated the influence of an administration of mefepronic acid (MA) 24 hours after farrow. Thus, fibrate can influence on the lipidic and proteic metabolism of the sows and gilts.

Materials and Methods

Two hundred gilts were involved in this trial, being 100 treated into 24 hours after farrow with a 15 ml IM injection of Liverfine® (Fatro Ibérica, Spain), but only data from 192 were recovered. This dose corresponds to 1,500 mg of total MA. The gilts were randomly allotted, and were used all the gilts farrowing into two consecutive weeks to avoid environmental factors. Two different genetic lines were evaluated; being 68 gilts from genetic A and 124 from genetic B. The fat back thickness (FBT) and the deep of loin (LD) was measured at farrow and weekly up to weaning, using a wireless ultrasonography device (Tecnoscan, USA). The wean-to-estrus (WTE) period was recorded for every animal, and a gilt was considered as in anoestrus 7 days after weaning without showing heat signs.

The comparison of data was performed using Student 's t test, considering significant a p-value<0.05. The frequencies were analyzed by Squared Chi test with adjusted residues analysis.

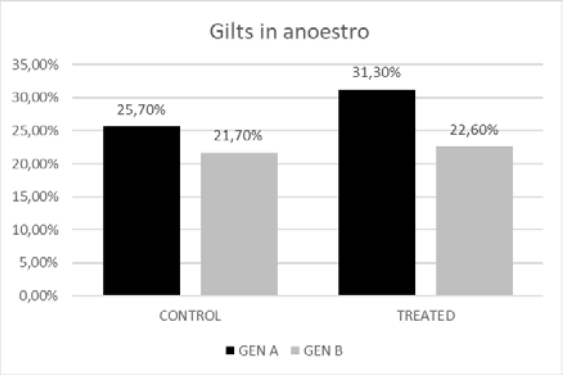
Results

The results for WTE period appear un table 1.

Table 1. Performance for WTE

GEN	GROUP	Mean	SEM
A	CONTROL	5,5769	0,33362
	TREATED	4,9524	0,25332
	p-value	NS	
B	CONTROL	5,0213	0,33935
	TREATED	4,8958	0,16642
	p-value	NS	

There was not significant difference even when theWTE was smaller in the treated group for both genotypes.



There was no difference between expected and observed frequency for anoestrum in none of the groups, even when in genotype B there was an 8.7% more of gilts not going in heat into the first week after weaning.

The correlations obtained per genotype and group between WTE and fat and muscle consumption parameters appear in the following table:

Table 2. Correlations among WTE and BFT and LD parameters

Genotype	Group	Parameter	WTE
A	CONTROL	BFT2-3	**-,443***
		DAYS	**,402***
	TREATED	BFT3-4	**,457***
		LD2-3	**-,467***
B	CONTROL	BFT3-4	**-,290***
		LW1	**-,310***

Interestingly, the 2nd and 3rd week after farrow seems to be key for the WTE, since in the control and treated group of genotype A there was a significant correlation, but whilst the loss of fat in the second week is negatively correlated to WTE, in treated group the loss of fat in the 3rd week is positively correlated to WTE. In genotype B only in control group has been assessed a positive correlation between loss of fat in the 3rd week and WTE, and interestingly negatively between the weight of litter at 24 hours of life.

Discussion and Conclusion

Apparently, the way to use the fat during lactation is related to the WTE length, and thus the use of MA, which influence the fat and muscle mobilization (data not shown in this communication) could help to reduce the WTE. Even when the differences are not significant (due, probably to variability), the reduction of half day in WTE in a 9,600 sows farm (as in this study) means an important economical save over the year, but this term need for a big scale experiment to be corroborated.

Intrauterine growth restriction (IUGR) alters intestinal microbiota from birth to the growing-finishing phase in swine

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Introduction

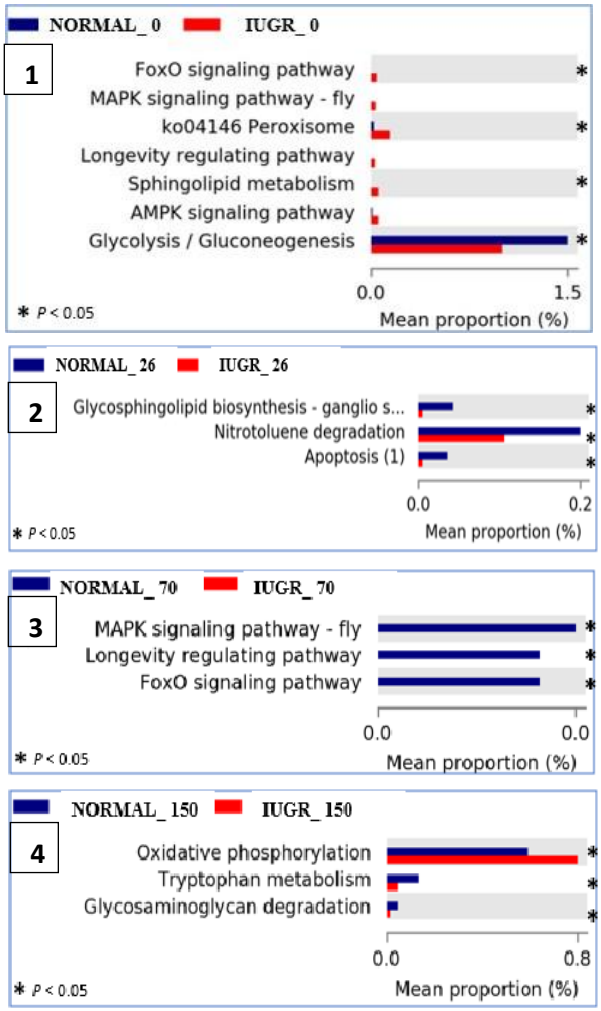
In pigs, IUGR is responsible for high mortality rates in the first weeks of life and reduced body weight gain throughout development, which leads to large economic losses to the industry (1). Homeostasis of the gut microbiota critically influences host health and development. However, recent studies have revealed that IUGR negatively compromises the composition of the microbiota in newborns, but it is still controversial whether these signs remain in the growth and fattening phases, periods in which growth development should be maximum (2,3). In this sense, in the present study, we hypothesized that IUGR alters intestinal microbiota from birth to the growing-finishing phase in swine.

Materials and Methods

One hundred sixty-two littermate male piglets were selected at birth and allocated into two treatment groups: normal weight (NW; birthweight range 1.6 - 1.9 kg) and intrauterine growth restricted (IUGR; birthweight range 0.7 – 1.0 kg). A subgroup of 10 littermate pairs were randomly selected and euthanized at birth, and on days 26 (48 hours after weaning), 70 (grower period) and 150 (finisher period), for duodenum collections. For the characterization of the intestinal microbiota, new generation sequencing was performed using the Ion Torrent 16S Metagenomics kit that amplifies the hypervariable region V4 of the bacterial 16S rRNA gene, according to the manufacturer's instructions. Data were submitted to analysis of variance (ANOVA) and LS means, compared by the Student T test, using the software SAS (Statistical Analysis System Institute Inc., Cary, NC, 2003).

Results

Figures 1, 2, 3 and 4 show the amount of bacterial populations responsible for various metabolic functions in NW and IUGR pigs' small intestine. Interestingly, newborn IUGR pigs show a reduction in the group responsible for gluconeogenesis but a marked increase in many cellular physiological events such as apoptosis, cell-cycle control, glucose metabolism, oxidative stress resistance, and longevity. In contrast, in IUGR pigs up to 70 days, it is observed that groups of bacteria important in signaling pathways that regulate a wide variety of cellular processes such as proliferation, differentiation, apoptosis and stress responses are practically non-existent. At 150 days there is an increase in oxidative phosphorylation pathways, which indicates energy source for metabolic activities.



Discussion and Conclusion

This metabolomic study of the intestinal microbiota in IUGR pigs can support the hypothesis that the effects of IUGR on the microbiota are persistent in pigs at all stages of development. Metabolomic studies in gut microbiota-related research could lead to the development of mechanistic hypotheses potentially applicable to the development of nutritional and personalized therapies.

Acknowledgments

We would like to thank our sponsors, the agencies CAPES, CNPq and FAPEMIG.

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Liver Morphofunctional Alterations Throughout Postnatal Development in Intrauterine Growth Restricted Pigs

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Introduction

Intrauterine Growth Restriction (IUGR) is a condition in which the fetus does not express its growth potential relative to gestational age. These individuals present low body weight at birth, higher risk of mortality, predisposition to short and long term impairments and chronic diseases, such as type II diabetes, gastrointestinal inefficiency and metabolic syndrome (1). In most cases this condition is called asymmetric, as the body prioritizes the development of the brain at the expense of the other organs. Thus, the individual presents normal skull circumference but a less developed body. Among the organs which may suffer from IUGR effects, the liver stands out as the most affected one. Although IUGR effects on hepaticfunction have been investigated, information on liver morphology is limited (2). Thus, the objective of this study was to evaluate hepatic morphofunctional alterations throughout postnatal development in IUGR pigs.

Materials and Methods

One hundred sixty-two littermate male piglets were selected at birth and allocated into two treatment groups: normal birth weight (NBW; birthweight range 1.6 - 1.9 kg) and intrauterine growth restricted (IUGR; birthweight range 0.7 – 1.0 kg). A subgroup of 10 littermate pairs were randomly selected and euthanized at birth, and on days 26 (48 hours after weaning), 65 (grower period) and 150 (finisher period), for blood and tissues collections. Blood samples were harvested for biochemical analysis of cholesterol (total LDL and HDL), glucose, and hepatic enzymes aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) levels via photocolorimetric method. Livers were weighted and fragments were processed for histomorphometrical analysis (hepatocyte and nucleus area in newborns, cord width and nuclei diameter at other ages) and density (nuclei number/mm²)..

Data were submitted to analysis of variance (ANOVA) and LS means, compared by the Student T test, using the software SAS (Statistical Analysis System Institute Inc., Cary, NC, 2003).

Results

IUGR pigs presented lower body weights at all ages($P<0.05$). Livers were also lighter at birth, 26 and 65 days old ($P<0.05$), but were similar at 150 days of age. Growth restriction in uterus did not affect the histomorphometrical parameters assessed regardless of age. The enzyme AST levels were higher in NBW newborns ($P<0.05$), but similar at other ages, and ALT levels were similar between the two experimental groups at all four ages, as shown in Table 1. Biochemical analysis also showed that total cholesterol and LDL levels were higher in IUGR pigs at 65 days ($P<0.05$), but HDL and glucose levels remained similar between IUGR and NBW pigs at all ages. Additionally,body weight was negatively correlated with total cholesterol at 65 days ($r = -0.56$, $P<0.05$) and glucose at 150 days ($r = -0.59$, $P<0.05$).

Table 1. ALT and AST levels (U/L)

Age/ mate	Birth		26 days		65 days		150 days	
	N	I	N	I	N	I	N	I
ALT	44.5 ^{a}	35.1 ^{a}	50^{a}	48^{a}	40.6 ^{a}	39.7 ^{a}	39.5 ^{a}	40.7 ^{a}
AST	139^{a}	95.4 ^{b}	69.8 ^{a}	66.3 ^{a}	44.2 ^{a}	49.3 ^{a}	43^{a}.	42.5 ^{a}

[ab]At the same age on the same line, means statically different ($P<0.05$). N: NBW/I: IUGR

Discussion and Conclusion

Even though IUGR affected liver weight it did not alter histomorphometrical parameters, suggesting a commitment in cell proliferation, since IUGR liver presents the same cellular density but less weight. Biochemical data indicate that growth restiction in uterus severely compromises metabolic function, which may predispose to long-term metabolicdisorders.

Aknowledgments

We would like to thank our sponsors, the agencies CAPES, CNPq and FAPEMIG.

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Monitoring status of bacterial contamination on boar stud: a case report

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Introduction

Bacterial contamination is one of the most important issues within a swine semen processing center, which must be constantly monitored. Mainly because the quality of the insemination dose is related to individual factors of the males and inherent to the semen technology, such as collection, handling, and storage of the dose, it becomes increasingly necessary for production process improvement (1).

Materials and Methods

The case report happened at a multi-genetic boar stud in Chapecó, south-west region of Santa Catarina State. However, we will present a case of only one specific genetic (39 animals). There was a routine of once a month sending of samples of fresh, extended, and stored semen, besides water (inlet water, osmosis, animal drinking water, stored and extender), to the laboratory to evaluate the contamination status of each sample. The goal for bacterial status for fresh semen is <2000 cfu/mL, for diluted and stored semen < 500 cfu/mL and for water is 0 cfu/mL. Because of this type of monitoring, inlet and stored water samples were detected last June with values higher than the established, but this did notreflect on microbiological quality of the dose that month. After that some actions were performed to suppress the contamination and procedures were carried out to identify problems. Identifying perforated tank bags, osmosis with low water production capacity (and immediately request for new equipment). Cleaning of the water tanks was carried out, and alignment of processes with the team. Even so, in July it was detected that 55% of stored semen sample was contaminated.

However, the samples of water were good. In August, the identification of males with higher contamination and application of antibiotics in the foreskin to reduce local contamination were included in the procedure, as well as cleaning of the boar housing and cleaning animals. The doses were normally produced with long term extender (Vitasem, Magapor®), and to reduce the fresh semen contamination an extender during the collection phase was used (Dicol, Magapor®). Monitoring first packaging dose, mainly because of the filling hose quality. After that, in September, plating was carried out at several points in the laboratory, and all collection points showed controlled contamination. October was the end of use Dicol on fresh semen because the contamination was controlled. And in this period we started to send more samples that we used for bacteriological control. In August we sent 54, in September 39, in October 34 and November 30 stored semen samples.

Results

Table 1 shows the percentage of samples in compliance based on the established target of 95%. In July it was 45%, in August 45,6% and in September 43,6% of stored samples in compliance. Of the 39 animals, 15 showed contamination. After that, with the effectiveness of the actions, the months of September and October have already returned to normality with 100% compliance of the stored samples.

Discussion and Conclusion

In our case, after cleaning the boar housing and boars, the contaminantion on fresh semen was controled. It is effective to monitor the status contamination once a month, but to discover the source of the contamination it was necessary to investigate all phases involved in the semen collection system. It is possible to adopt action plans, providing support to design better strategies, adjusting the procedures (2).

So some questions that remain here are, how importantis the bacterial monitoring status of dose? How many times is it necessary to proceed monitoring (once a month, every week)? How many samples should be sent to the lab, and how to decide?

This case showed us that monitoring every week couldbe a good strategy. Monitoring the main critical points is extremely important to ensure the quality of the dose.And doing this kind of periodic monitoring of samples could save time if you consider that the diagnostic of theproblem can be faster and more effective for decision making, saving time and money.

Table 1. Number of samples: compliance/total and percent (%) fresh, extended and stored semen

Months	Samples		
	Fresh	Extended	Stored
July	4/5 (80)	5/5 (100)	9/20 (45)
August	4/5 (80)	5/5 (100)	4/54 (44,4)
September	15/20 (75)	6/20 (30)	17/39 (43,6)
October	26/26 (100)	10/10 (100)	34/34 (100)
November	9/11 (82)	5/5 (100)	30/30 (100)

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Ovarian morphometrical evaluation in silent estrus and anestrus gilts

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Introduction

The domestic pig is a polyestrous species, which was developed along its domestication process. However, females may experience pathological periods of anestrus or silent estrus throughout their productive lives. Both pathologies increase the number of non-productive days, with the need of hormonal interventions or culling from the breeding herd, which leads to economic losses (1,2). Despite being relatively common in swine farming, there are few studies that have evaluated ovarian histomorphometry in those pathologies. Therefore, the objective of this study was to investigate through morphometrical analysis the ovarian alterations in silent estrus and anestrus gilts.

Materials and Methods

Thirty gilts (Landrace x Large White) were selected and allocated to the following experimental groups: control (CT; n = 10), anestrus (AN; n = 10), and silent heat (SH; n = 10). At 23 weeks of age, females were exposed to boar stimulation. Animals were weighed, backfat thickness was measured, and were sent to slaughter, where the ovaries and uterus were collected and weighed. Macroscopically visible follicles, corpus luteum and albicans were measured and follicles were classified into three classes (F1: < 3mm, F2: 3-5mm, F3: > 5mm). Subsequently, the ovaries were processed for histomorphometrical evaluation. Follicles were divided into different classes, according to the morphological characteristics established by Ross & Pawlina (3). Areas of follicles, oocyte, antrum and granulosa layer of secondary and mature follicles were measured using the ImageJ® software.

Results

Although AN animals had similar body weights to the other groups, they showed the smallest backfat thickness, as well as the smallest and lightest ovaries (Table 1) and uterus (P≤0.05). The classification of macroscopically visible follicles showed that females in anestrus have a greater number of follicles in the F1 and F2 (P.≤0.05), and absence of corpora lutea and albicans. The analysis of the follicular population revealed that the animals in anestrus had not only the highest number of secondary and tertiary follicles, but also the highest number of follicles in both early and late atresia (P≤0.05- Table 2). On the other hand, SH females showed similar behavior as the CT, except for the number of corpora lutea, which was higher (P≤0.05- Table 2). No differences in the areas of follicular components were observed among experimental groups.

Table 1 Ovarian biometrical data in Control (CT), Silent Heat (SH) and Anestrus (AN) gilts

Parameter	CT	SH	AN	SEM	p-value
Length, cm	35^{b}	34.0^{ab}	29.2^{a}	1.5	0.002
Width, cm	27^{b}	24.4^{ab}	21.6^{a}	1.1	0.008
Thickness, cm	19.3^{a}	19.0^{a}	13.4^{b}	1.2	0.003
Weight, cm	8.9^{ab}	9.6^{b}	5.3^{a}	1.2	0.04

^{a,b} Within a row, different superscripts differ (P≤0.05)

Table 2 Number of ovarian follicles at different stages of development in Control (CT), Silent Heat (SH) and Anestrus (AN) gilts

Parameter	CT	SH	AN	SEM	p-value
Preantral	26.0	25.5	10.5	11.0	NS
Antral	5^{ab}	3^{ab}	9^{a}	2	0.05
Tertiary	5^{b}	5^{b}	17^{a}	2	< 0.01
Early atretic	4^{b}	3^{b}	16^{a}	4	< 0.05
Late atretic	7.0^{b}	5.0^{b}	20.0^{a}	2.5	< 0.01
Corpura lutea	2.0^{b}	5.0^{a}	0.0^{c}	0.4	< 0.01
Corpura albicantia	2.5^{a}	2.3^{a}	0.0^{b}	0.6	< 0.05

^{a,b} Within a row, different superscripts differ (P≤0.05)

Discussion and Conclusions

It is clear that animals in anestrus present reproductive failure due to lack of ovulation, which is evidenced by the high number of atretic follicles and the absence of corpora lutea and albicantia. However, silent estrus females have normal ovaries, which indicates that the lack of estrus behavior may have other origins or is a consequence of estrus detection failure.

Acknowledgments

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Ovarian stimulation with FSH improves ovarian follicular response, oocyte quality and meiotic maturation in 140 and 160 days old prepubertal gilts

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Introduction

Oocyte competence is refer as the ability of the oocyte to resume meiosis, cleave following fertilization and develop into a viable embryo (1). *In vitro* study showed that oocytes obtained from porcine prepubertal females has a lower embryo development capacity compared to adult females (2). Strategies have been adopted in an attempt to increase developmental competence of prepubertal oocytes, such as FSH treatment in the oocyte donor prior follicular aspiration (3). However, it is unknown if FSH treatment and increasing of age could improve oocyte numbers and quality in prepubertal gilts. Thus, the purpose of this study was to examine the effects of age (140 vs 160 days) and FSH treatment on: i) density of preantral follicles; ii) biochemical composition of follicular fluid (FF), and iii) oocyte quality and nuclear maturation rate in cumulus-oocyte complexes (COCs) collected from prepubertal gilts.

Materials and Methods

Thirty-five prepubertal gilts were separated according to the age (140 ± 4 days and 160 ± 4 days) and within each age, gilts were allotted to received six injections given every 8 h of FSH [treated; 100 mg of FSH; G140+FSH (n = 10) and G160+FSH (n = 7)] or saline solution [control; 0.9% sterile saline solution; G140+control (n = 10) and G160+control (n = 8)]. After 24 h of the last FSH injection, ovaries were recovered and the number of small (1-3 mm), medium (3-6.49 mm) and large (≥6.5 mm) follicles were counted and COCs were aspirated from medium follicles. Then, the COCs recovered were morphologically classified (grade I-IV) and COCs grades I-II were used to brilliant cresyl blue (BCB) staining and *in vitro* oocyte maturation (IVM). After IVM, the oocyte meiotic maturation was evaluated by orcein staining. In addition, FF was used for analysis of biochemical parameters in an automatic biochemistry analyzer. Data were analyzed by the GLM procedure of SAS 9.4® ($p < 0.05$).

Results

Results of COC morphological classification and oocyte nuclear maturation are shown in Table 1. The results showed a significant effect ($p < 0.05$) of FSH and donor age in the ovarian follicle population. The percentage of medium follicles increased ($p < 0.0001$) as the same proportion that the percentage of small follicles reduced ($p < 0.0001$) in FSH-treated and younger gilts. In addition, the concentration of glucose in FF increased ($p < 0.05$) in FSH-treated and older gilts; in contrast, the concentration of triglycerides decreased ($p < 0.05$) in these same groups of animals.

Table 1. Morphological classification, BCB test and meiotic maturation rate of COCs from gilts at 140 and 160 days of age submitted or not (control) to a FSH stimulation (Mean ± SEM).

Parameter	Treatment			
	160 days of age		140 days of age	
	Control	FSH	Control	FSH
Total COCs (n)	36.5 ± 9.3^{Bb}	64.9 ± 16.3^{Ba}	56.2 ± 5.2^{Ab}	88.5 ± 10.1^{Aa}
GI oocytes (n)	4.5 ± 1.7^{b}	17.3 ± 4.8^{a}	7.9 ± 1.3^{b}	19.6 ± 3.4^{a}
GII oocytes (n)	11.7 ± 3.1	17.0 ± 5.2	18.6 ± 3.0	23.9 ± 4.3
GIII oocytes (n)	11.6 ± 3.0	17.8 ± 6.9	16.9 ± 2.5	25.1 ± 3.9
GIV oocytes (n)	8.6 ± 2.5^{B}	12.7 ± 3.7^{B}	12.8 ± 2.4^{A}	19.9 ± 2.4^{A}
BCB + % (n)*	39.1 (43)^{Ab}	87.9 (174)^{Aa}	20.8 (42)^{Bb}	52.2 (165)^{Ba}
Meiotic maturation % (n)*	59.2 (61)^{Ab}	73.4 (138)^{Aa}	42.9 (79)^{Bb}	62.8 (179)^{Bb}

Within a row, mean of values followed by lower-case (FSH vs control) and uppercase (140 days vs 160 days) letters differed ($p < 0.05$) among them by Tukey-Kramer or Chi-square* test.

Discussion and Conclusion

FSH treatment led to an increase in the follicular population available for oocyte aspiration in 140 and 160 days old prepubertal gilts, showing that prepubertal gilts are able to respond to exogenous FSH injections. Also, FSH seems to have an positive impact on oocyte quality and competence since FSH-treated gilts presented greater number of COCs with better quality and meiotic maturation rate. Increased FF concentrations of glucose in FSH-treated gilts indicate that glucose is used as a source of energy metabolism, assisting the oocyte maturation. In conclusion, FSH treatment is effective to improve the oocyte quantity, quality and nuclear maturation in 140 and 160 days old prepubertal gilts. Moreover, oocytes obatined from 140 days prepubertal gilts apperared less meiotically competent than 160 days old prepubertal gilts-derived oocytes.

Acknowledgments

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Plasma testosterone levels and *17α-hydroxylase* expression kinetics in different birth weight boars

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Introduction

Low birthweight (LW) piglets are a reality in commercial farms and have been associated with functional disorders of several organs systems. There is strong evidence that LW pigs present compromised postnatal growth and performance and poor meat quality (1). However, reports of the effects of birthweight on the reproductive system are scarce, especially in boars (2, 3, 4). Impairment of testis development compromises the efficiency of spermatogenesis and steroidogenesis. Testosterone is required for normal development of the male reproductive tract and sexual maturation. Synthesis of testosterone occurs in the Leydig cells and is dependent upon the expression of several enzymes, including the 17α-hydroxylase (17α-OH), which is highly regulated within the testis (5). In this context, the effects of birthweight on testicular development and its implications on sperm production in boars deserves further investigation. Therefore, the aim of the present study was to evaluate the evolution of plasma testosterone levels and 17α-OH expression from 8days old to 10 months old in different birthweight boars.

Materials and Methods

Sixty male littermate pigs were selected immediately after birth and divided into two birthweight categories: high (n=30 HW; 1.85 to 2.15 kg), and low (n=30 LW; 0.85 to 1.15 kg) birthweight. A subgroup of 20 littermate boars was orchiectomized at 8 days, and 8 and 10 months of age and a testicular sample was collected for the analysis of the steroidogenic enzyme 17α-OH. The relative expression of 17α-OH was measured by quantitative polymerase chain reaction (qPCR) Additionally, blood samples were withdrawn from the jugular vein for analysis of plasma testosterone concentrations, which was quantified using a commercially available electrochemiluminescence immunoassay (ECLIA) kit (Roche Diagnostics USA). Both parameters were statistically evaluated within the same age and among the three ages within the same birthweight category (kinetics). using the general linear model (GLM) procedure of SAS.

Results

Birthweight did not affect plasma testosterone concentrations nor 17α-OH relative expression at all ages evaluated (Table 1). On the other hand, 17α-OH kinetics showed higher levels at birth, which dramatically decreased overtime, regardless of birthweight (Table 2; P<0.05). Although a decrease in 17α-OH was observed, testosterone levels remained constant overtime (P>0.05).

Discussion and Conclusion

The expression of 17α-OH and plasma testosterone concentrations in LW and HW boars at 8 days, 8 and 10 months of age suggest that sexual maturation and spermatogenesis may not be compromised by altered fetal growth. Furthermore, this enzyme, which catalyses the production of precursors for glucocorticoid, oestrogen, and androgen synthesis, is involved in sexual development during fetal life and at puberty (5). The absence of birthweight effects on circulating testosterone levels have also been reported in 12-month-old boars (3). The importance and effect of the decrease in the 17α-OH activity is not clear in boars. Therefore, future studies on the evolution of plasma testosterone levels and 17α-OH expression are necessary to explain their effects on sperm production.

Table 1. Testosterone plasma concentration in high and low birthweight boars

Age	Testosterone plasma concentration	
	HW (n=10 for each age)	LW (n=10 for each age)
8 Days	2,4 ± 0,9 [a]	2,4 ± 0,9 [a]
8 Months	3,9 ± 0,9 [a]	3,4 ± 0,9 [a]
10 Months	4,2 ± 0,8 [a]	4,5 ± 0,9 [a]

Table 2. Relative expression of 17α-OH in high and low birthweight boars

Age	Relative expression of 17α-OH	
	HW (n=10 for each age)	LW (n=10 for each age)
8 days	31,0 ± 2 [a]	25,5 ± 4,2 [a]
8 months	6,2 ± 2 [b]	6,3 ± 2 [b]
10 months	4,0 ± 2 [b]	1,9 ± 2 [b]

[a,b] Within a column, LSmeans with different superscripts differ P*p* <0.05)

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Agroceres PIC, Fapemig, CNPq and Capes

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Reproductive performance of gilts with reduced age at first breeding and high growth rates

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Introduction

Gilts have an important role on the farm, as they represent the largest category (19-20%) of female pigsin a breeding herd (1). The genetic selection constantly promotes evolution that may change certain traits of these animals (2). However, there is lack of information on management strategies suitable formodern sows. This study, therefore, aimed to evaluatethe effect of age and growth rate of gilts at first mating on productive performance and retention rate until third farrowing.

Materials and Methods

The study was performed with 1,962 gilts (Camborough®) at a farm with 10k sow stock *(MHY* and *APP* positive, stabilized in terms of health*)* in the south of Brazil. The groups were retrospectively created according to age at first mating: T1- (190 to 200d – n= 290); T2 (200 to 210d – n= 370); T3 (210 to 220d – n= 591) and T4 – (>220d – n= 620). In all treatments, the following criteria were established for breeding: >135kg body weight, second estrus, 20 days after reproductive vaccines and 15 days of cage adaptation. All females were weighted at birth, weaning, nursery, selection, flushing, breeding, and inbreeding/weaning (Parity 2 and Parity 3) to obtain a growth curve. Additionally, measures of backfat thickness and body condition (caliper) were performed at selection, flushing, breeding, and onweaning/breeding (Parity 2 and Parity 3). Blood samples were collected to evaluate estrogen on day 16and 18 of the second cycle, and progesterone, tree days after the end of estrus (10 animals per treatment). Retention rate was calculated as follow: Retention rate (%): (Stock after farrowing Parity 3)/ (Stock at the beginning) *100. Data were analyzed using linear or logistic regression models in R, gilt was the experimental unit. Results were considered significantat P≤0.05.

Results

The growth results from each treatment group are summarized in Table 1. The total number of piglets born, born alive, weaned, stillborn and mummies along three parties were not affected by age at first mating (Table 2). Retention rate until parity three in T1 gilts was slightly higher than T4 females, but it didnot reach the level of significance (Figure 1); however,no statistical difference (P > 0.05) was detected. No statistic differences were detected on estrogen and progesterone (Table 3).

Table 1. Growth performance of females from birth to breeding of the four experimental groups

Variables	Treatment			
	T1	T2	T3	T4
Birth weight (kg)	1.55±0.32 ^{A}	1.49±0.32 ^{B}	1.40±0.32 ^{C}	1.35±0.32 ^{D}
Weaning weight (kg)	6.6±1.09 ^{A}	6.48±1.08 ^{A}	6.24±1.11 ^{B}	6.23±1.18 ^{B}
Age at weaning (days)	22±2 ^{AB}	22±3 ^{B}	22±3 ^{B}	23±4 ^{A}
ADG at weaning (kg/day)	0.23±0.04 ^{A}	0.23±0.04 ^{B}	0.22±0.05 ^{C}	0.21±0.05 ^{D}
Nursery weight (kg)	23.05±4.51 ^{A}	22.49±4.84 ^{A}	21.32±4.94 ^{B}	20.7±5.13 ^{C}
Nursery age (days)	60±4 ^{A}	61±4 ^{A}	61±4 ^{A}	62±5 ^{A}
ADG nursery (kg/day)	0.6±0.09 ^{A}	0.57±0.1 ^{B}	0.55±0.1 ^{C}	0.53±0.11 ^{D}
Selection weight (kg)	108.38±8.25 ^{A}	101.44±10.97 ^{B}	98.54±9.92 ^{B}	95.38±10.49 ^{C}
Selection age (day)	146±3 ^{A}	145±4 ^{A}	146±4 ^{A}	147±5 ^{A}
ADG selection (kg/day)	0.74±0.05 ^{A}	0.7±0.07 ^{B}	0.67±0.06 ^{C}	0.65±0.07 ^{D}
Age at 1st heat (days)	161±11 ^{C}	166±14 ^{B}	167±16 ^{B}	175±21 ^{A}
Flushing weight (kg)	134.29±8.94 ^{AB}	137.4±10.25 ^{A}	134.32±11.5 ^{A}	129.75±13.28 ^{B}
Flushing age (days)	177±4 ^{D}	188±5 ^{C}	192±8 ^{B}	198±12 ^{A}
Backfat thickness at flushing (mm)	13.85±2.23 ^{A}	14.07±2.2 ^{A}	13.80±2.47 ^{A}	13.18±2.76 ^{B}
Caliper flushing (points)	11.8±1.65 ^{AB}	11.9±1.85 ^{A}	11.46±1.91 ^{B}	10.69±2.15 ^{C}
1st Breeding age (days)	195±3 ^{D}	207±3 ^{C}	215±3 ^{B}	235±15 ^{A}
1st Breeding weight (kg)	147.52±8.42 ^{C}	150.72±9.79 ^{B}	150.97±10.46 ^{B}	155.13±12.53 ^{A}

*Different letters within a row indicate statistical difference among treatments. Were conducted by Tukey's test based on a linear model (p<0.05).

Table 2. Mean and standard deviation of performance of females along the moments before mating for the four treatments.

	Variable	Treatment			
		T1	T2	T3	T4
Parity 1	Weaned (n)	13±3 ^{a}	13±3 ^{a}	12±3 ^{a}	12±3 ^{a}
	Total born (n)	15.68±3.29 ^{a}	15.56±3.06 ^{a}	15.74±2.83 ^{a}	15.4±3.15 ^{a}
	Born alive (n)	14.66±3.17 ^{a}	14.59±2.94 ^{a}	14.64±2.89 ^{a}	14.26±3.32 ^{a}
	Stillbirths (%)	3.91±5.7 ^{a}	3.52±5.23 ^{a}	3.93±7.01 ^{a}	4.61±8.82 ^{a}
	Mummified (%)	2.54±4.83 ^{a}	2.53±4.54 ^{a}	2.96±5.39 ^{a}	2.83±5.61 ^{a}
Parity 2	Weaned (n)	12±3 ^{a}	12±3 ^{a}	12±3 ^{a}	12±3 ^{a}
	Total born (n)	15.63±4.1 ^{a}	15.55±3.85 ^{a}	15.89±3.83 ^{a}	15.46±3.75 ^{a}
	Born alive (n)	14.01±4.31 ^{a}	14.07±3.91 ^{a}	14.21±4.17 ^{a}	14.03±3.83 ^{a}
	Stillbirths (%)	5.91±8.34 ^{a}	5.24±7.76 ^{a}	5.66±9.55 ^{a}	5.6±9.2 ^{a}
	Mummified (%)	4.41±10.34 ^{a}	4.08±7.85 ^{a}	4.57±9.01 ^{a}	3.76±8.48 ^{a}
Parity 3	Weaned (n)	12±4 ^{a}	12±3 ^{a}	13±3 ^{a}	12±3 ^{a}
	Total born (n)	16.7±4.48 ^{a}	16.8±4.36 ^{a}	16.88±3.92 ^{a}	16.68±4.04 ^{a}
	Born alive (n)	15.23±4.01 ^{a}	15.47±3.91 ^{a}	15.54±3.6 ^{a}	15.38±3.75 ^{a}
	Stillbirths (%)	6.27±7.45 ^{a}	5.66±7.72 ^{a}	5.52±8.17 ^{a}	5.25±6.95 ^{a}
	Mummified (%)	2.43±4.44 ^{a}	2.06±3.69 ^{a}	2.39±4.08 ^{a}	2.39±4.26 ^{a}

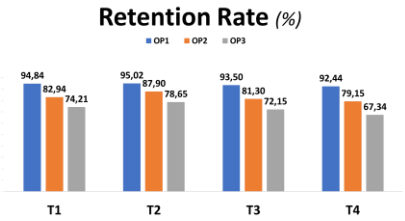
*Different letters indicate difference between treatments. Were conducted by Tukey's test based on a linear model (p<0.05).

Table 3. Mean and standard deviation of female progesterone for the four treatments throughout the collections.

Variable		Treatment			
		T1	T2	T3	T4
Progesterone (ng/mL)**	1	6.86±2.43 ^{c}	5.74±1.82 ^{c}	7.06±2.61 ^{c}	8.02±2.54 ^{c}
	2	14.04±4.45 ^{b}	12.25±3.96 ^{b}	14.59±5.86 ^{b}	16.16±4.98 ^{b}
	3	19.01±2.74 ^{a}	20.83±2.21 ^{a}	19.91±2.79 ^{a}	18.72±3.57 ^{a}
Estrogen (pg/mL)*	1	29.15±8.14 ^{b}	35.99±6.63 ^{b}	26.08±8.01 ^{b}	27.61±8.44 ^{b}
	2	38.06±3.63 ^{a}	34.82±4.78 ^{a}	40.27±1.06 ^{a}	37.23±7.02 ^{a}

*Different letters indicate difference between treatments. Were conducted by Tukey's test based on a linear model (p<0.05). ** Estrogen levels were assessed at days 16and 18 of the second cycle. *** Progesterone levels were assessed for three consecutive days after the end of estrus behavior.

Figure 1. Retention rate (%).



*Different letters indicate difference between treatments. Were conducted by Tukey's test based on a linear model (p<0.05).

Discussion and Conclusionsn

Gilts having at least one estrus before mating, minimum 135 kg bodyweight are eligible for insemination with a minimum of 195 days of age without commitments in litter size, farrowing andretention rates until the third parity.

Acknowledgments

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Risk factors associated with pelvic organ prolapse incidence in a Brazilian sow farm

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Introduction

The pelvic organ prolapses (POP) in sows has shown a considerable increase in the last years, occurring in the vagina, rectum, uterus and bladder (1). In a recent study carried out in the United States, with 104 farms, the incidence on the herd was 2.7%, being the POP responsible for 21% of the total mortality (2). Among the possible factors observed for the occurrence of prolapses are nutritional management, genetic, environment and health (1). Our objective was to evaluate and identify the relationship of different factors with the occurrence of pelvic organ prolapse in sows.

Materials and Methods

Data from 1,028 sows (PIC Landrace and PIC Camborough) was collected at the final third of gestation, pre-farrowing, at farrowing, and post-farrowing, from July to September of 2021 in two production units located in southern Brazil. Whole-herd and individual sow information were collected, including prolapse incidence, body condition score (BC) measured by caliper, perineal score (PS) classified into PS1, PS2 and PS3, tail length, fecal score (FS), oxytocin use, and performance records. For statistical analysis, a logistic regression model using PROC LOGISTIC on SAS® (SAS Institute, Inc., Cary, NC) (4) was used to assess risk factors associated with the incidence rate of POP, with sow as the experimental unit.

Results

Sows with PS3 had higher POP incidence compared with sows with PS2 and PS1 (38.46 vs 9,41 and 0,96%, respectively). Sows with dry feces had higher POP incidence compared to sows with normal feces (9.09 and 1.64%, respectively; $p<0.01$). Sows with tail length < 13cm had higher POP incidence compared to sows with tail length >13 cm (5.18 and 2.25%, respectively; $p<0.01$). There was no association of BCS and POP incidence, although sows with BSC "thin" had higher POP incidence ($p<0.01$) compared with "fat + ideal" sows (Table 2). There was an association among sows with BCS "thin", fecal score "dry" and POP incidence (Table 3). There was also no evidence of an association between use of oxytocin, total born or litter weight and POP incidence.

Discussion and Conclusion

The prolapse incidence was 4 times higher in sows with PS3 than in PS2 sows. Potential causes of the PS occurrence are still not clear. However, injuries of the perineal ligaments, eg tail docking, would correlate with the neuromas and neuroanatomical alterations on the peripheral nervous (1) and POP ocurrence. The prolapse incidence was 5 times higher in sows with dry feces and there was an association among dry feces, BSC thin and POP incidence that would be directly related to low water intake, leading to tenesmus, constipation and an increase on intra-abdominal pressure.

Table 1. Parameters analyzed and prolapse incidence in sows

Item	POP incidence (%) [1]			*p value* [2]
Perineal score (1; 2 and 3)	0.96 [a]	9.41 [b]	38.46 [b]	<0.01
Faecal Score (Normal; Dry)	1.64 [a]	9.09 [b]	-	<0.01
Tail length (> 13cm; < 13cm)	2.25 [a]	5.18 [b]	-	<0.01
Prepartum body score (Fat; Ideal and Thin)	1.79	1.74	3.98	0.13
Use of oxytocin (No; Yes)	3.25	4.84	-	0.38
Total Born (>16; <16)	3.26	4.28	-	0.38
Litter weight (>19; <19 kg)	3.26	1.82	-	0.53

Table 2. Effect of body score on POP incidence

	Ideal + Fat	Thin	*P value*[2]
POP incidence (%) [1]	1.76 [a]	3.98 [b]	< 0.01
Sow (n)	511	503	-

Table 3. Effect of prepartum body score and faecal score on POP incidence[1]

BCS	Faecal score		n	*p value*[2]
	Normal	Dry		
Fat	1.56	3.23	223	0.99
Ideal	1.44	2.53	288	0.99
Thin	1.81 [a]	8.09 [b]	503	0.04

[1]Lsmeans for the incidence or probability of the prolapse occurrence. [2]General model with binomial distribution to measure the probability of the prolapse occurrence ($p< 0.05$).

In summary, PS, FS and tail lenght appeared to be contributing factors associated with POP incidence in this system.

Acknowledgments

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The administration of 2-Methyl-2-phenoxypropanoic acid after farrow to gilts improve theuse of fat and muscle during lactation

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Introduction

The usage of fat during lactation is a main factor to keep the body condition of gilts and sows and achieve an adequate growth rate of litter. Especially in nulliparous whom are still growing themselves. The hepatic metabolism is critical to have a correct lipidic metabolism, meeting then the requirements of breeder and litter. We have investigated the mobilization of fat and muscle in gilts after administration of 2-Methyl-2-phenoxypropanoic acid, also known as mefepronic acid (MA).

Materials and Methods

Two hundred gilts were involved in this trial, being 100 treated into 24 hours after farrow with an IM injection of Liverfine® (Fatro Iberica, Spain), but only data from 192 were recovered. The gilts were randomly allotted, and were used all the gilts farrowing into two consecutive weeks to avoid environmental factors. The fat back thickness (FBT) and the deep of loin (DL)was measured at farrow and weekly up to weaning, using a wireless ultrasonography device (Tecnoscan, USA). Moreover, the piglets were weighted at birth+24h, after fostering and at weaning. Two different genetic lines were evaluated; being 68 gilts from genetic A and 124 from genetic B. The difference between FBT and DL were calculated, among each week of lactation. The comparison of data was performed using Student 'st test, and squared Chi for frequencies.

Results

The values for BFT and LD appears in tables 1 and 2 performance appears in table 1 and 2

Table 1. Average for BFT at each measure

GEN	GROUP	BFT1	BFT2	BFT3	BFT4
A	CONTROL	11.9771	10.5286	9.3443	8.9257
	TREATED	13.5742	11.3955	10.2818	9.4636
	p-value	NS	NS	NS	NS
B	CONTROL	14.218	13.4157	12.0287	11.4787
	TREATED	14.873	13.6651	12.4198	12.0881
	p-value	NS	NS	NS	NS

Table 2. Average for LD at each measure

GEN	GROUP	LD1	LD2	LD3	LD4
A	CONTROL	46.7357	44.4629	41.7129	40.9271
	TREATED	46.9409	46.4061	41.7803	41.4561
	p-value	NS	NS	NS	NS
B	CONTROL	46.2992	44.9492	41.8811	38.1861
	TREATED	47.0675	44.823	40.2548	40.5825
	p-value	NS	NS	NS	0.035

There was no significant difference for BFT at aby measurement, but there was a significant difference for LD at weaning in the genotype B, with higher DL for treated group. The consumption of fat (as BFT decrease) and muscle (as LD decrease) every week of lactation and the whole period appears in the tables 3 and 4.

Table 1. Fat consumption

GEN.	group	ΔFBT 1st week	ΔFBT 2nd week	ΔFBT 3rd week	ΔFBT whole period
A	CONTROL	1.4486	1.1843	0.4186	3.0514
	TREATED	2.1788	1.1136	0.8182	4.1106
	p-value	NS	NS	NS	NS
B	CONTROL	0.8024	1.387	0.55	2.7393
	TREATED	1.2079	1.2452	0.3317	2.7849
	p-value	NS	NS	NS	NS

Table 2. Muscle consumption

GEN.	group	ΔDL 1st week	ΔDL 2nd week	ΔDL 3rd week	ΔDL whole period
A	CONTROL	2.2729	2.75	0.7857	5.8086
	TREATED	0.5348	4.6258	0.3242	5.4848
	p-value	NS	NS	NS	NS
B	CONTROL	1.35	3.068	3.6951	8.1131
	TREATED	2.2444	4.5683	-0.3278	6.4849
	p-value	NS	NS	NS	0.002

there was no difference in the fat consumption evenwhen in genotype A the BFT loss in treated gilts ishigher than in control group. But interestingly, the muscle consumption is higher for treated group ingenotype B during the two first weeks but in the thirdthe average animals increased LD, with a significantdifference at weaning compared to control group. Asregards the gilts that increased muscle thickness, there was lower frequency that expected in the 2nd week (19%, AR=-2.3, p=0.017) for treated group, but much higherin the 3rd week (55.6%, R=2.0, p=0.035) in genotype B.There was no influence of weaned piglets but it was significant the influence of LD at farrow.

Discussion and Conclusion

The usage of fat and muscle during lactation in gilts is critical, since the breeders are still growing and it's preferable to consume fat and not muscle. In this trial we have observed how the treated gilts use more fatand less muscle, in a different way in two different genetic lines. There is a shot literature of mefepronic acid usage, especially in cows, and commonly focused in lactational ketosis prevention, but there is a lack of information of the effects on sows. Certainly, in former trials we had not found ketosis during lactation since normally in sows and gilts, this condition use to appear in the last third of gestation. But, in this study, the MA seems to produce a differential way to use the fat and the muscle, saving muscle tissue during the last week of the lactation. The effect could not be constant in all genotypes and these terms need for further research.

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The effect of ultraviolet light chamber, used for disinfecting semen blisters, on farrowing rate and number of piglets born alive

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Introduction

Keeping a pig farm with a high heath status is essential to achieve satisfactory reproduction performance and profit. In recent years, several diseases have emerged and re-emerged, such as African swine fever and enteric delta coronavirus (1,2,3). To face this challenge, the attention given to biosecurity has increased in pig farms worldwide. Many diseases enter the farms through infected animals, people and/or fomites. Thus, several strategies have been used to prevent the spread of diseases. Among them, we can mention the quarantines, closed herd systems and disinfection tools, such as ultraviolet (UV) chamber (1,3). However, it is still unclear whether the reproduction efficiency of the treated semen dose can decrease due to the disinfection procedure using a UV chamber. Therefore, the aim of this study was to compare the reproduction performance (FR: farrowing rate and NBA: number of piglets born alive) of two treatment groups: 1) sows inseminated with semen doses disinfected using a UV chamber compared to 2) sows inseminated with semen doses without this disinfection procedure (control group).

Material and methods

The semen blisters that were disinfected were placed in a stainless-steel chamber with an ultraviolet light lamp (256 nm ultraviolet wavelength in the C range) and ozone gas spray at the day they arrived at the farm. The blisters were uniformly exposed to UV for 1 minute ($20x10^3 J/cm^2$). Before and after the disinfection, the blisters were stored at a temperature between 15°C and 18°C, in the same way as those that were not disinfected using the UV chamber. The inseminations of both treatment groups were performed by an experienced professional after the detection of estrus, according to the protocol routinely used at the farm. Sows from the same genetic line and parity number were used for both treatment groups. The inseminations were performed in three batches of females, with intervals of 28 days between them. In total, 28 sows (14 for each group) were inseminated for this study. The reproduction performance between the two groups were compared using the *lsmeans* R function (6) with a confidence level of 95%. Each phenotype was evaluated fitting, as a fixed effect in the linear model, the batch of insemination (N=3), treatment group (UV disinfected or control) and service sire (N=12), as the semen of each sire was used to inseminate at least two sows. Significant difference between the treatment groups was declared when a P value ≤0.01 was observed.

Results

All 14 sows inseminated in each of the two treatment groups farrowed successfully and therefore the FR was 100% for both. Regarding the NBA, a significant difference (P=0.009) was observed between groups (Table 1). A decrease of 2.56±0.87 NBA was observed in the group of sows inseminated with the semen doses that were disinfected using the UV chamber compared to the control group.

Table 1: Least-square means (Lsmean) and standard error (SE) for number of piglets born alive of the two treatments group.

Group[1]	Lsmean	SE	Confidence limit	
			lower	upper
UV	12.6	0.62	11.3	13.9
Control	15.2	0.62	13.9	16.5

[1]UV: group of sows inseminated with semen doses disinfected with ultraviolet chamber; Control: group of sows in the control group.

The UV light has the potential of mitigate heath outbreaks caused by viruses and bacteria present on the semen blister's surface due to its disinfection properties. Although this seems to be an important tool for biosecurity, the results of this study show that it may negatively impact NBA and therefore it needs to be considered carefully. On the other hand, it doesn't seem to have any impact on FR in the evaluated dataset. However, this study used a small sample size and the boars used in the treated and control group were different. Therefore, further studies on this subject needs to be performed for more conclusive results.

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The use and sharing of a boar semen lysate

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Introduction

PRRS virus can be transmitted vertically through boar semen, and the boar semen must remain PRRS negative, otherwise, it will lead to PRRS infection and disease in sow farms. Daily monitoring of PRRS infection status in boar studs is an important means of preventing PRRS. Semen is extracted by different methods, and the extraction method with the highest sensitivity is selected.

Materials and Methods

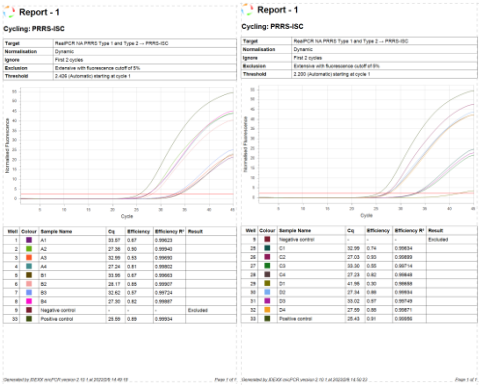
1. Sample classification: Using 4 bottles of different semen, nucleic acid extraction was performed by four different methods. Method A: Extract with manual nucleic acid extraction kit; Method B: First use RealPCR TL-60 for extraction and then with manual nucleic acid extraction kit; Method C: Extract with automatic nucleic acid extraction kit; Method D: First use RealPCR After TL-60 treatment, use an automatic nucleic acid extraction kit for extraction.

	Boar semen1	Boar semen2	Boar semen3	Boar semen4
A	A1	A2	A3	A4
B	B1	B2	B3	B4
C	C1	C2	C3	C4
D	D1	D2	D3	D4

2. Sample processing: 1ml semen samples were taken from the four groups of ABCD and centrifuged at 12000rpm for 4min. In groups B and D, the supernatant was discarded; 400ul RealPCR TL-60 buffer was added, mixed with a pipette tip, and incubated at 70°C for 10min; the lysed samples were centrifuged at 15,000rpm for 1min, and 400ul of the clarified lysate was added to a fresh centrifuge tube.
3. Nucleic acid extraction: In group A, 200uL of supernatant was added to a new 1.5mL centrifuge tube, and then 500uL of lysate from tomorrow's DNA/RNA virus extraction kit was added, shaken, and mixed for the 30S, and allowed to stand at room temperature for 5min. Transfer all the solutions of A and B to the purification column, centrifuged at 12000rpm for 1min, and discard the liquid in the collection tube; add 500uL of rinse solution 1 to the purification column, centrifuged at 12000rpm for 1min, and discard the liquid in the collection tube; add 500uL to the purification column Rinse solution 2, centrifuge at 12000rpm for 1min, discard the liquid in the collection tube; put the purification column back into the collection tube, centrifuge at 12000rpm for 2min; transfer the purification column to a new 1.5ml centrifuge tube; add 50ul of eluent to the purification column, After incubation at room temperature for 2 min, centrifuge at 12,000 rpm for 1 min, and collect the DNA/RNA eluate into a 1.5 mL centrifuge tube. Groups C and D were extracted using an automatic nucleic acid extraction kit. First, the deep-well plate was taken out, and the magnetic beads were resuspended by inverting and mixing several times. Then, the liquid on the wall was tapped on the table to slow down, and then the aluminum sealing film of the kit was carefully removed. ; Take out the proteinase K solution, and after brief centrifugation, use a pipette to add 20uL to the first 4 wells of the first column; take the samples of group C, and use a pipette to add 300uL to the corresponding 4 wells of the first column; take the samples of group D, use a pipette to add the last 4 sample wells in the first column without proteinase K; put the deep-well plate into the automatic extractor for nucleic acid extraction; after the extraction, the eluate in the fifth column is transferred to the EP tube, for the extracted nucleic acid.
4. Configuration of the reaction system: use a 25uL system (20ul reaction solution with 5ul nucleic acid) for configuration.

Results

The semen PRRS in the FAM channel was negative; the Ct value of the endogenous gene in the HEX channel was significantly lower using RealPCR TL-60 buffer.



Conclusions and Discussion

After the semen is first treated with RealPCR TL-60 buffer, the lysis will be more thorough and the sensitivity of pathogen detection will be increased. At the same time, monitoring of PRRS in boar semen will help to better prevent PRRS.

Acknowledgments

PIG PEACE (Hangzhou) Technical Service Co., Ltd.

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VIRAL DISEASES

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A double intervention to stabilize Porcine Reproductive and Respiratory Syndrome - PRRS in aone site farm with continuous flow

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Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) is the most relevant infectious disease in the swine industry. PRRS virus (PRRSv) live inoculation has been used to stabilize and even eliminate PRRS in positively confirmed farms. Batch farrowing is a production system used to maximize productivity and could be used it to interrupt disease transmission in susceptible populations. This report captures the stabilization of a one site farm with a continuous production flow by virus inoculation and implementation of batch farrowing management.

Methods

In 2018, a commercial farm (200 sows per year) experienced reproductive and respiratory problems in both sows and growing finisher pigs (PRRS positive by ELISA). Serum samples were randomly collected from 35 days old piglets with acute clinical signs to confirm PRRS infection by ELISA. PRRS positive serum was used to amplify the PRRSv in 21 days old piglets, these pigs were euthanized and bled to harvest serum to be used as PRRSv inoculum. 1 ml of serum titrated to 10^3 PRRSv /ml was inoculated by muscular injection the whole herd. Simultaneously, a batch farrowing system was implemented in groups of three. The pig production flow was moved into another barn within the same farm emptying the nursery area. All-in all-out, washing and disinfection processes were implemented along with McREBEL[1] procedures in all farrowing rooms to avoid transmission. Herd closure for 40 weeks was stablished. PRRSv surveillance was implemented throughout and after the herd closure following the AASV recommendations. Differences in production parameters between periods were assessed using paired samples t-test, repeated measures ANOVA and post-hoc Tukey test, significance level was established at $p<0.01$.

Results and Conclusions

PRRSv circulation was present before intervention. After PRRSv inoculation compatible clinical signs with infection were observed, including 12.2% abortion. Three batch farrowing groups were successfully implemented with 15 sows / group. Summary of the production results is shown in table 1 and summary statistics is presented in table 2 and 3. Farrowing rate decreased from 85.63% to 74.43% ($p<0.01$) during the PRRS outbreak, after the intervention the farrowing rate increased to 87.6% ($p<0.01$). After intervention, the abortion rate decreased from 25.57% to 3.08% ($p<0.01$), this recovery reached similar levels than before the outbreak. In addition, total number of piglets born alive increased from 8.89% to 10.7% ($p<0.01$), this increment was higher than before the outbreak; wean to finish mortality rate decreased from 55% to 5.11% ($p<0.01$); ADG increased from 726 g to 897 g ($p<0.01$).

Finally, PRRSv PCR tests were consistently negative in four consecutive sampling processes after PRRSv inoculum.

Table 3. Summary statistics of production parameters in growing finisher pigs before outbreak and after intervention

Site	Parameter	Before Outbreak	PRRS Outbreak	After Intervention
S1	Born weight (kg)	1.51a	1.17ab	1.41b
	Weaning weight (kg)	6.13a	4.80b	6.19a
	Weaning age (days)	21.25a	21.36a	21.52a
S2	Mortality rate (%)	4.89a	40.00b	4.87a
	Final age (days)	70.15a	70.47a	70.44a
	Final weigth (kg)	28.58a	24.82b	29.85a
	Average daily gain (kg)	0.46a	0.41b	0.48a
S3	Mortality rate (%)	0.20a	15.04b	0.24a
	Final age (days)	154.48a	154.18a	154.25a
	Final weigth (kg)	102.16a	85.62b	105.05a
	Average daily gain (kg)	0.87a	0.73b	0.89a

A significant production improvement was obtained after the implementation of live PRRSv inoculation and the implementation of the batch farrowing system. Moreover, after 40 weeks of herd closure, the PRRSv circulation was eliminated. Immunity development by inoculation and interruption of PRRSv transmission by modification of epidemiological subpopulation might bethe main reasons to obtain these results. Altogether, this report shows that virulent PRRSv infection can be controlled in a one site farm with a continuous production flow.

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Site	Parameter	Before Outbreak	PRRS Outbreak	After Intervention
S1	Sows (n)	174	110	125
	Mortality rate (%)	5.1	31.03	5
	Farrowing rate (%)	85.63	74.43	87.56
	Abortion rate (%)	3.02	25.57	3.08
	Total born (n)	12	11.3	12.91
	Total born alive (n)	9.98	8.89	10.66
	Mummies per sow (n)	0.59	1.11	0.49
	Still born (n)	1.43	1.38	1.76
	Pre weaning mortality rate(%)	7.5	9.05	4.05
	Born weight (kg)	1.5	1.2	1.4
	Weaning weight (kg)	6.3	4.8	6.5
	Weaning age (days)	21	21	21
S2	Mortality rate (%)	5	40	4.87
	Final age (days)	70	70	70
	Final weigth (kg)	28.59	24.82	29.85
	Average daily gain (kg)	0.455	0.409	0.477
S3	Mortality rate (%)	0.2	15	0.2
	Final age (days)	154	154	154
	Final weigth (kg)	102	85.6	105.2
	Average daily gain (kg)	0.874	0.724	0.897

A Practical Lymph Node Sampler to faciliate Diagnosis of African Swine Fever Virus

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Introduction

African swine fever (ASF) is a highly contagious hemorrhagic and transboundary animal disease. It has rapidly spread to many regions of the world and is responsible for serious economic losses (1). Recently, early detection and eradication are feasible to control the spread of this disease (2). However, diagnosis based on clinical signs is impractical to differentiate the typical symptoms of ASF from classic swine fever, swine erysipelas or highly pathogenic porcine reproductive and respiratory syndrome. How to make a confirmatory diagnosis is a top priority.

Materials and Methods

The lymph node sampler consists of a needle, a syringe, a handle and a connection rod inside the needle. The needle contains a barb, which can take outthe lymphoid tissue. The connection rod can be inserted into the needle to extrude the tissue.

The pig was restrained to access its inguinal lymph nodes. Then, the skin was punctured vertically with the sampler to ensure that the barb entirely entered the tissue. The sampler was pulled out, and the handle was pressed to push the tissue out of the needle (Figure 1). The tissue was placed into an Eppendorf (EP) tube for further detection.

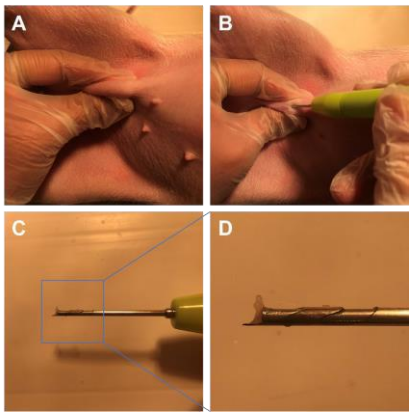

Figure 1. Three steps to obtain inguinal lymph node samples using the lymph node sampler. A. Find and pinch the inguinal lymph node. B. Puncture the skin and tissue with sampler. C. Pull out the sampler and press the handle to squeeze the tissue out. D. Enlarged view of squeezed lymph node tissue.

For serum samples, collect the blood from the porcine anterior vena cava into an anticoagulation tube. Hold for one hour and take the serum. For oral fluid samples, tie a cotton rope in front of the pig. Squeeze out the oral liquid after the sufficient chew by the pig. For throat swabs, insert a long spermaduct with a sponge on the top into the throat quickly. The cotton nasal swabs and rectal swabs were also eluted with the nucleic acid protective fluid. The nasal-rectal swab and nasal-throat-rectal swab samples were pooled from individual samples.

Results

Lymph node samples were collected by the lymph node sampler, which makes the sampling safer, more efficient and minimally invasive. The ASF virus (ASFV) DNA from lymph node sample as well as serum, oral fluid, nasal and rectal swab samples from pig production sites were detected by real-time PCR. Results demonstrated that higher level of ASFV DNA was detected in the lymph node samples than other samples (Figure 2).

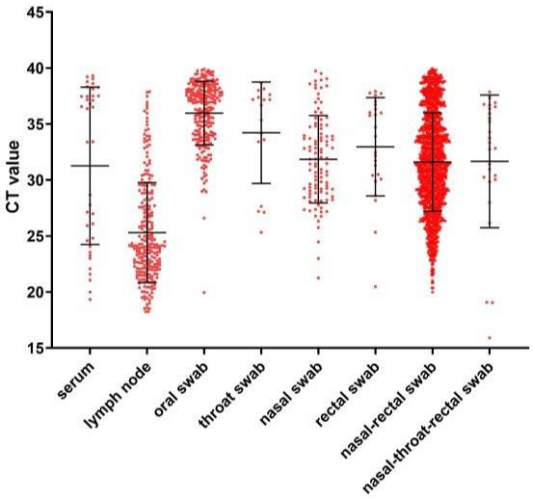

Figure 2. The ASFV DNA in lymph nodes and other kinds of collected samples. Eight kinds of porcine samples were collected, and their ASFV content was detected by qPCR shown as Ct values.

Discussion and Conclusion

Our findings suggested that the lymph node sample isan ideal tissue for confirmatory ASFV infection. The lymph node sampler is a convenient tool for lymph node sampling for practitioners.

Acknowledgments

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Adoption of an active surveillance and biosecurity program to prevent the entry of *Porcine Epidemic Diarrhea* (PEDV) through transport to the state of Yucatán-México.

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Keywords:
Porcine Epidemic Diarrhea, truck sampling, Yucatán state México.

Introduction

Porcine epidemic diarrhea virus (PEDV), which first reported in the United States in 2013 (1), was later reported in early July of the same year in México. The disease has been identified in different regions of the country, without being diagnosed in the states of the south-east, including Yucatán (2).
Indirect contact transmission of PEDV is frequent with a low biosecurity via contaminated fomites as personnel and transport trailers (3). Therefore, since 2014 an enhanced biosecurity program was implemented, between private industry (Grupo Porcícola Mexicano - Kekén & Asociación Ganadera de Porcicultores de Yucatán) and public institution (Comité Estatal para el Fomento y Protección Pecuaria del Estado de Yucatán) through a transport verification program for returned trucks from outside deliveries of marketed pigs (external processing plants). Herein we describe the adoption of an active surveillance and biosecurity program established to prevent the entry of PEDV through transport to the state of Yucatán-México.

Material & Methods

The program was stablished by a biosecurity point (BP) over the border between the state of Yucatán and Campeche, which the vehicles are verified after the cleaning and disinfection process (C&D) carried out in a Keken's truck washing station, verified with a criterion of 0% visible organic matter on BP, if this requirement is not met, the trucks receive a second C&D in another established washing facility, previous entry to state. On early 2020 the trade route was extended to states where the PEDV had a high prevalence, so it was established a new criterion on the program, based on negative tested results of surface samplings as an active surveillance program running up to date. The biosecurity protocol established in the BP is: 1. All trucks are C&D before arriving at the BP in a designated truck washing facility. 2. Once each truck arrives to the BP, samples are obtained from trailers floor and bars surfaces with sterile wipes and from chassis with swabs, both on sterile saline–moistened solution. 3. A sampling area of 10cm x 10 cm is established in every point, using new latex gloves were worn for each sample collection to minimize the risk for cross-contamination. 4. Five points are taken, two front points of the trailer per side, two midpoints per side and one point at the rear of one floor of the trailer, the same procedureis repeated for chassis. 5. Regardless of the test result, the trucks are C&D verified based on 0% organic matter criteria on surfaces before entering the state. 6. The refrigerated samples are submitted to Keken´s diagnostic laboratory, running a real-time PCR and the result is obtained in less than five hours. 7. If surfaces sample of each truck results PEDV+, it receives a second C&D process until it receives a negative result before entering to the state.

Results and Conclusions

Since early 2020, a total 233 out of 2,157 (10.8%) surface samples tested positive for PEDV after the C&D procedures. Studies have reported similar prevalence's (9.2% to 14.1%), after contact with processing plant facilities that serve different suppliers before C&D procedures (3,4), and even after C&D, up to 7.5% detection has been found (5). Higher results have described when there exists lack of good biosecurity practices (70.5 %) during the unloading of pigs, such as the use of exclusive boots, personnel segregation, useof overalls, driver getting off without sanitary measures, lack of cleanliness in unloading chutes, bad procedures of C&D on vehicles, etc. (4,5).
The results of this work suggest that by implementing biosafety control measures in transport such C&D with complete removal of feces, prior to its return to the state of Yucatán, it's not enough to significantly reduce the risk, so it is necessary to add other practices or filters such as sampling and diagnosis to reduce the spread capacity of PEDV and prevent an early outbreak to still maintaining the state as a disease-free. Collaborated work between public and private industry are encouraged to succeed on regional diseases control and prevention programs.

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African swine fever virus detection in boar semen

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Introduction

African swine fever (ASF) is a highly contagiousviral disease of swine which causes high mortality, approaching 100%, in domestic pigs. For many diseases, it can be transmitted vertically through semen; according to the latest research progress of African swine fever, the African swine fever virus can pass through the blood-brain barrier but not the blood-testis barrier, which means that African swine fever virus cannot Vertical transmission through semen. But why even do semen testing for African swine fever virus? The reasons are as follows: Whether there is contamination in the process of semen collection, semen packaging and semen transportation, and assessing contact cross-contamination; Semen is not only pure semen components but also prostatic fluid.

Materials and Methods

1. Sample processing: Take 1ml of the semen sample and centrifuge at 12000rpm for 4min, discard the supernatant; add 400ul RealPCR TL-60, mix wellwith a pipette tip, incubate at 70°C for 10min; centrifuge the lysed sample at 15000rpm for 1min, take 400ul for clarification Add lysate to a new centrifuge tube.
2. Adjust the binding conditions: add 200ul ethanol to each centrifuge tube; vortex and mix for 10s; incubate at 18-26°C for 5min; centrifuge briefly.
3. Nucleic acid extraction: transfer the above 600ul mixture to the purification column, centrifuge at 8000 rpm for 1 min, discard the liquid in the collection tube, and put the adsorption column back into the collection tube; Repeat centrifugation at the rotating speed); add 500ul of deproteinized washing solution to the purification column, centrifuge at 12,000 for 1 min, and discard the liquid in the collection tube; add 500ul of washing solution to the purification column, centrifuge at 12,000 for 1 min, and discard the liquid in the collection tube; add tothe purification column again 500ul washing solution,centrifuge at 12000 for 1min, discard the liquid in the collection tube; centrifuge again at 12000rpm for 2min, discard the collection tube, transfer the purification column to a new 1.5ml centrifuge tube; add 50ul eluent to the purification column, incubate at room temperature for 2min, Centrifuge at 8000rpm for 2min, discard the purification column, and the liquid in the 1.5ml centrifuge tube is the nucleic acid of the sample to be tested.
4. Preparation of reaction system: 5 microliters of extracted nucleic acid + 20 microliters of the reaction solution.

Results

The semen test for ASFV was negative.

Conclusions and Discussion

ASFV testing in semen can be used as an assessment tool for exposure to cross-contamination.

Acknowledgments

PIG PEACE (Hangzhou) Technical Service Co., Ltd.
Prairie Diagnostic Services (PDS) in Canada

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African Swine Fever virus inactivation by feed additives *in vitro*

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Introduction

African swine fever virus (ASFV) is currently considered the greatest threat to the swine industry. Contamination of feed and ingredients has been identified as one potential risk for transboundary transmission (1). Feed additive chemicals based in medium chain fatty acids (MCFA) and/or formaldehyde have been shown to help mitigate virus viability (2). We hypothesized that MCFA blends with organic acids would provide similar ASFV inactivation. The present objective was to study the inactivation (kinetics and doses) of feed additives against ASFV measured in porcine alveolar macrophages (PAMs) *in vitro*. Experiments were performed to test a water-soluble feed Additive 1 (including formic acid, acetic acid, propionic acid, MCFA and ammonium formate) and Additive 2 (including formic acid, propionic acid, and MCFA).

Materials and Methods

The study was conducted in a high-containment unit at Wageningen Bioveterinary Research, the Netherlands. Additives 1 and 2 were tested at different concentrations (1:10, 1:100, 1:200, 1:400, 1:800 and 1:1000) to evaluate inactivation of ASFV Georgia 2007/1 strain (genotype 2). The assays were done with duplicate samples with 0.9 ml or 1 ml solutions in culture media (RPMI 1640, 5% FBS, 1% Penicillin/Streptomycin and 1% Amphotericin). This with or without 0.1 ml ASFV porcine plasma inoculum (1:10 ASFV stock to plasma). The pH was measured. The titrations (in triplicate) were conducted by using PAMs 10-fold serial dilutions in the same cell culture media and titrated in three-fold on PAMs in 96-wells plates. Plates were incubated for 4 days at 37 °C in a humidified atmosphere with 5% CO_2. Dilutions were made at room temperature, and titrations done immediately. Presence of ASFV was detected by an immunoperoxidase monolayer assay using an anti-ASFV hyper-immune serum followed by a Horseradish Peroxidase conjugated anti-swine IgG monoclonal antibody solution. After staining, plates were read microscopically for the presence of ASFV, scoring coloured cells as positive. Titres were calculated using the method of Reed and Muench and expressed as $\log_{10}$ $TCID_{50}$/ml. In addition, qPCR was conducted to assess virus DNA presence.

Results

The validity of the assay was confirmed by the positive control (0.1 ml of ASFV in 0.9 ml medium) showing a 5.7 $\log_{10}$ $TCID_{50}$/ml titer with the expected detection limit (DL) of1.5 $\log_{10}$ $TCID_{50}$/ml. The qPCR titer confirmed this result showing an equivalent titer of 5.7 $\log_{10}$ $TCID_{50}$-eq/ml. High additive concentrations fixated the PAMs resulting in increased detection limits (DL) (Fig. 1). Additive 1, diluted 400 times, resulted in a titer of 5.0 $\log_{10}$ $TCID_{50}$/ml (DL ≤1.5), and no virus could be detected at the 1:10 (DL ≤3.5), 1:100 (DL ≤2.5) and 1:200 (DL ≤2.5) dilutions. At dilutions >1:800 no inactivating effect on ASFV and PAMs was observed. Additive 2, diluted 200 times, resulted in a titer of 4.8 $\log_{10}$ $TCID_{50}$/ml (DL ≤2.5), with no detectable virus at the 1:10 (DL ≤3.5) and 1:100 (DL ≤2.5) dilutions. At dilutions >1:400 no inactivating effect on ASFV and PAMs was observed for Additive 2. The 1:10 dilutionof both additives showed an inhibiting effect on theASFV DNA. However, the qPCR was not affected bylower concentrations. The pH showed a decreasingacidity with increasing concentration (see Fig. 1).

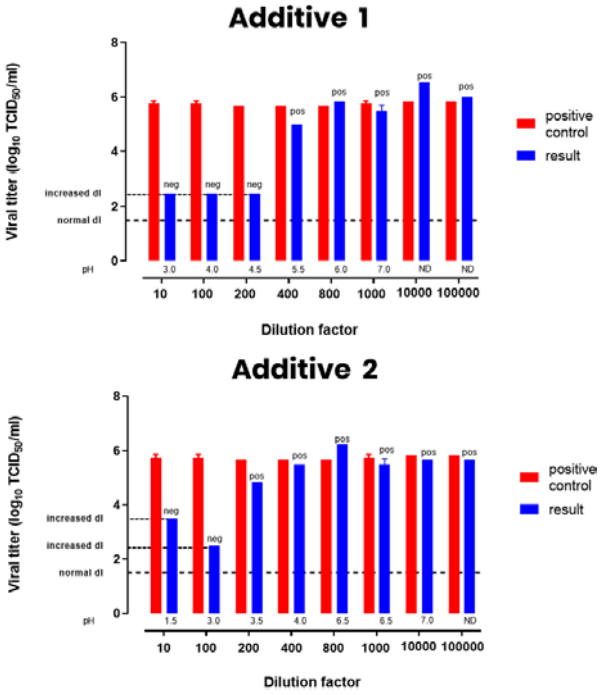

Figure 1. ASFV titration results after incubation with different dilution factors for Additive 1 and Additive 2.dl = detection limits are identified. ND = not determined.

Discussion and Conclusion

To reduce ASFV by 99.93%, Additive 1 and Additive 2 should not be diluted more than 200 and 100 times, respectively. For these doses, pH was 4.5 for Additive 1 and 3.0 for Additive 2. ASFV is inactivated at pH<3.9 in serum-free medium (3). Hence, the acidifying effect in both additives and MCFA in Additive 2 (2) could explain the results. At 1:10 dilution, the additives reduced ASFV DNA, similarly to MCFA and formaldhyde in a previous study (2). In conclusion, both additives can help to mitigate the risk of ASFV.

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An incursion of Japanese Encephalitis virus into southeastern Australian pig herds

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Introduction

An outbreak of Japanese Encephalitis virus disease has been seen in eastern Australia from mid 2021 on a few farms, resulting in prolonged gestation length, mummified and stillborn piglets. Often with severe foetal abnormalities and congenital tremors.

There has been no obvious clinical problems in piglets, weaners, grow/finishing or adult pigs.

Japanese Encephalitis virus

Japanese Encephalitis virus (JEV) is an Flavivirus, an enveloped positive strand RNA virus endemic in eastern and Southeastern Asia. This is a large group of viruses, often arthropod borne. JEV has previously been detected in trapped insects and water birds in the northern tropical regions of Australia.

JEV is a zoonotic mosquito borne pathogen, causing clinical signs in an estimated 70,000 people annually. Majority of infections are inapparent.

Humans and horses may become affected. Waterbirds are the reservoirs and pigs may act as amplification hosts. While vaccination of people is commonly practiced in Asia, the virus is notifiable in Australia and no vaccination policy is practiced.

Clinical signs

Clinical signs were first seen in April 2021 in Northern Queensland but in 2022 more cases were seen in the Southern states on a number of properties.

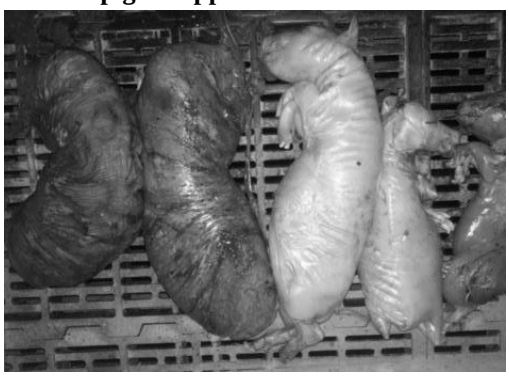
The case presentations which should raise concern were:

Prolonged gestation length

Sows failed to farrow by day 117 and were induced.At parturition they passed large number of mummified and stillborn piglets. The clinical signs are seen in allparities of sows.

Mummified and stillborn piglets in 10% of sows

Mummified piglets appeared bloated with ascites



Congenital abnormalities

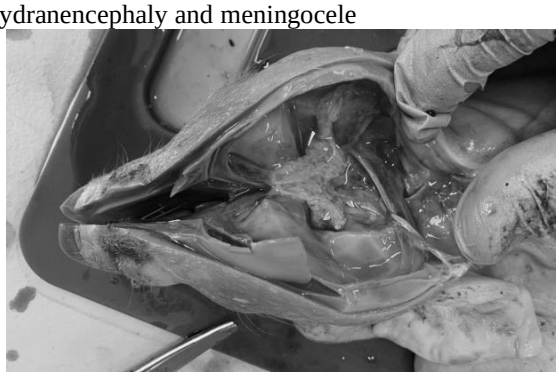
Stillborn piglets presented with an array of congenital abnormalities.

Severe arthrogryposis and changes in limb proportions and had brachygnathia.

Some of these unfortunate piglets were born alive.

Neurological abnormalities

A striking findings can be cerebral and cerebella aplasia, hydranencephaly and meningocele



Congenital tremor piglets

Some piglets were born alive exhibiting trembles.

Laboratory examination

The samples were all negative for Atypical congenital tremor; Aujeszky's disease; CSF; ASF; Menangle; IVA: MVE: Kunjin Virus and PRRSV.

JEV was found in brain, pleural fluid and spleen of submitted stillborn piglets.

Discussion and Conclusion

While these clinical signs are recognised in Asia in outbreaks, this is the first major outbreak in Australia. The fact that affected litters are seen in multiparitty sows indicates this is a recent incursion into these farms. The striking clinical signs naturally caused distress and concern in the staff of affected farms.

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Analysis of swine hemorrhagic syndrome records in the Brazilian veterinary surveillance and emergency system (e-Sisbravet) in the state of Santa Catarina (Brazil), in 2020.

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Introduction

Classical Swine Fever (CSF) is a disease caused by a pestivirus of the Flaviviridae family, has high morbidity and mortality, affects swine and wild boar ofall ages, and, when present, causes high economic damage.
Since 2020, the official system for registering disease notifications in Brazil became the e-Sisbravet, which is a specific electronic tool for managing the data obtained in the surveillance of calls to animal health notifications.

Materials and Methods

This report presents a descriptive analysis of investigations of Swine Hemorrhagic Syndrome (SHS) registered in e-Sisbravet in the State of Santa Catarina in the period 2020 and extracted through a report based on the date of notification. The analysis was carried out based on ten indicators, of which we highlight: the number of occurrences and temporal distribution; the spatial distribution; the framing of the notifier and the framing in the initial service.

Results

During the year of 2020, the State of Santa Catarina registered 242 occurrences of SHS in the e-Sisbravet, with the vast majority of investigations taking place in the western mesoregion (78.63%).
From Figure 1, it is possible to observe that the occurrences were attended during all months of the year, with a similar distribution in most months, to demonstrate that the State Veterinarian Service (SVS) has been promptly investigating the notifications submitted.

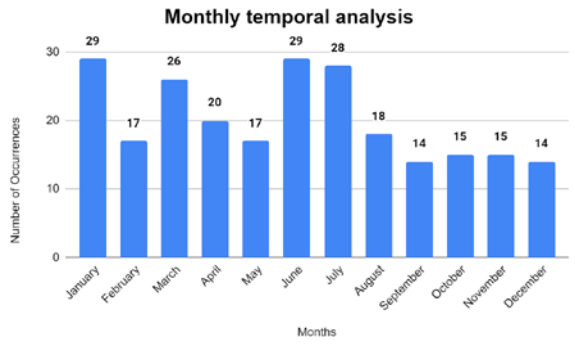

Figure 1. Monthly temporal analysis of swine hemorrhagic syndrome occurrences in 2020 – SC.

Among the types of notifiers of SHS occurrences, the veterinarian qualified by the SVS stands out with 64.88% of the classifications. This type of notifier predominates since it is responsible for monitoring thezootechnical indices of swine farms, following up on existing sanitary occurrences, and making notifications when appropriate.
Of the total number of recorded occurrences, 241 (99.6%) were classified as a discharged suspecting the initial care and only one (0.4%) as a probable case of SHS. Discarding of the initial care occurs due to the lack of clinical signs compatible with SHS since high mortality is already a trigger for notification.

Discussion and Conclusion

We observed that the investigations are concentrated in the municipalities of the west of Santa Catarina, which is consistent with the distribution of the swine population in the State, considering that this region concentrates more than 70% of the Santa Catarina herd. However, it is necessary to intensify surveillance in other parts of the State that also have a significant swine population, such as the southern region, composed mostly of swine farmers who are not dedicated to the export market and who generally do not have assistance from a continuous veterinary doctor on the farm.
With the implementation of e-Sisbravet, there was an improvement in the quality and management of data for the animal health surveillance system, as it is a specific tool that allows managing the data obtained in the attendance to notifications of suspected diseases that affect pigs.
In 2019, the State of Santa Catarina had several training courses for the use of this new tool, the e-Sisbravet, and started the year 2020 with the full implementation of the calls being recorded electronically. At the end of the first year of use, wehad 100% of SHS calls closed in the system, with 93.38% of investigations launched and closed on the same day, demonstrating a clear understanding by the users.

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Analysis of the genetic diversity of PCV2 in Mexico from 2016 to 2021

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Introduction

Porcine circovirus associated disease (PCVAD) caused by Porcine circovirus type 2 (PCV2), is one of the most important diseases, due to the economic impact that it causes. PCV2 is a DNA virus with a 17 nm of diameter belonging to the Family *Circoviridae* (1). Its genome contains 1766-1768 nucleotides with at least 4 ORFs, being the ORF2 gene recognized as phylogenetic and epidemiologic marker (2). PCV2 continuously evolves through the mutation point, as well as the genetic recombination, which can lead to the emergence of new variants (3), there are at least 8 recognized genotypes (PCV-2a / PCV-2h) (4). Despite the increasing diversity of PCV2, the recorded genotypes with more prevalence worldwide are PCV2a, PCV2b, and PCV2d, being also those with more clinic importance (5, 6). In Mexico, there are few reports about the evolving dynamic of PCV2. The objective of this study is to analyze the genetic variants of PCV2 in Mexico from 2016 to 2021.

Materials and methods

From 2016 to 2021, 5,077 PCR tests were performed, in order to detect the (ORF1) of PCV2 in different states of Mexico, such as: Sonora, Jalisco, Guanajuato, Veracruz, Puebla, Queretaro, Nayarit, Aguascalientes, Michoacan, Nuevo Leon, Chiapas, and Hidalgo. The DNA extraction was carried out with the QIAamp DNA Mini Kit (250) cat. 51306, QIAamp cador Pathogen Mini Kit (250) cat. 54106, and MagMAX™ CORE Nucleic Acid Purification Kit Catalogue. A32702. The PCR test was performed using the genesig Std RT-PCR Detection kit for PCV2 CAT. Path-PCV2-standard. 49 samples were selected with a ≤ 25 CT value. The sequencing of the complete genome of PCV2 was carried out through the end-point PCR test, amplifying the ORF1 / ORF2. After that, the Nextera XT de Illumina cat. FC-131-1096 kit was used. The analysis of the sequences was performed with the CLC Genomics Workbench 21.0.3 software of Qiagen. A phylogenetic tree was generated through the application of the Neighbor Joining model with a Bootstrap analysis of 1,000 repetitions. Classification of PCV2 variants was done according to the sample identities, as well as the phylogenetic tree, comparing it with the reference sequences. Also, the construction of a map with the variants distribution in Mexico was done.

Results

During the period mentioned above, 5,077 RT-PCR PCV2 tests were performed. 946 were positive (18.6%), 49 of them were selected (5.2%) from the total of positive samples. Once PCV2 sequencing was carried out, the results were classified (Table 1). Data show that 88% of PCV2 genome are in the PCV2-d genotype, while PCV2-a represents 10%, and finally PCV2-b genotype is 2%.

Table 1. Classification of PCV2 variants in Mexico (2016-2021).

	GENOTYPE			
ESTATE	PCV2-a	PCV2-b	PCV2-d	TOTAL
Chiapas			1	1
Guanajuato			1	1
Hidalgo			1	1
Jalisco	1	1	14	16
Michoacán			1	1
Nayarit			1	1
Nuevo León	1		2	3
Puebla			2	2
Sonora	2		18	20
Veracruz	1		2	3
TOTAL	5	1	43	49

The place where the variants were located was also analyzed (Figure 1), and it was possible to observe that PCV2-d genotype is in most of the states where there is the higher swine population. PCV2-a genotype was identified in states such as Sonora, Jalisco, and Veracruz. Finally, PCV2-b genotype was identified only in Jalisco.

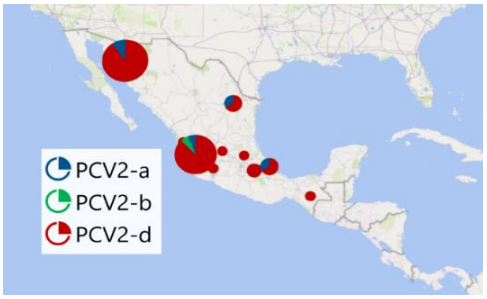

Figure 1. Geographical distribution of PCV2 sequences in Mexico.

A phylogenetic analysis was carried out, in order to observe the genetic changes between the different genotypes. The nucleotide sequences indicate that each genotype maintains up to 98% of identity in each one of the described variants (PCV2-a, PCV2-b, PCV2-d) in all the world.

Discussions and conclusions

Previous studies in Mexico (7) identified the presence of two genotypes (PCV2-a and PCV2-b), which correspond to the findings in this study. However, PCV2-d genotype was not reported in that publication, having the last one higher prevalence in this study, which corresponds to the information reported in the United States (8). This study is the second report produced in Mexico regarding PCV2 genotypes. It is necessary to track the evolutionary trend of PCV2. Despite the genetic diversity of PCV2, previous publications have shown that current vaccines continue offering cross protection against all the variants described above (6).

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Analysis of the genetic diversity of *PRRSV* and presence of more frequent variants in Jalisco, México during 2015-2021

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Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) disease, caused by PRRS virus (PRRSV), is the most economically important disease affecting pig production worldwide (1). PRRSV is an RNA virus that is 45-70 nm in diameter. The PRRSV genome has ten open reading frames (ORFs) called ORF1a, ORF1b, ORF2a, ORF2b, ORF3, ORF4, ORF5, ORF5a, ORF6, and ORF7(2). ORF5 codes for the structural glycoprotein GP5, which plays an important role in virus infection as it is the key immunogenic protein, which can induce humoral and cellular immune responses (3). The PRRSV, similarly to other RNA viruses, presents a genetic variability that is usually studied to establish associations between the virus and the epidemiological characteristics of its presentation in different geographical locations. The use of RFLP (Restriction Fragment Length Polymorphism) using enzymes Mlu I, Hinc II and Sac II can help to differentiate the cuts patterns of PRRSV variants, but if we include the sequences of ORF5, you can observe heterology between the PRRSV variants (you can detect the nucleotides changes, deletions, or insertions) (4). The objective of this study is to analyze the genetic diversity of PRRSV, and which have been the most present variants in recent years (2015-2021) in Jalisco, México.

Materials and Methods

13,792 samples from Jalisco were tested via RT-PCR for PRRSV (using the VetMAX PRRSV NA &EU Reagents kit, Catalog number 4468465) during 2015-2021, result in 6190 samples PRRSV positives and 7602 PRRSV negatives. The ORF5 of 745 positive samples (with CT below 30) from Jalisco were sequenced (Jalisco 1-Jalisco 745) using the Nextera-XT DNA Sample Preparation Kit and the Miseq System equipment. The sequences were analyzed in Qiagen's CLC Genomics Workbench 21.0.3 software. An alignment of the sequences of the 745 samples was carried out in addition to an identity comparison between the reference variants. The phylogenetic tree was analyzed through the Maximun Likelihood Philogeny model, with a Neighbor Joining construction model and a Bootstrap analysis of 1000 repetitions.

Results

The state of Jalisco currently has the largest number of PRRSV variants (76 different RFLP patterns) compared to some other states of the Mexican Republic. Table 1 shows the top 10 most present RFLP variants in the state of Jalisco during 2015-2021.

Table 1. Presence of PRRSV ORF5 RFLP in Jalisco

TOP 10	SEQUENCE RFLP	NO. OF CASES
1	2-5-2	102
2	1-3-4	88
3	1-6-4	83
4	1-8-1	63
5	1-6-3	56
6	1-7-4	37
7	1-3-3	25
8	1-2-3	18
9	1-2-4	16
10	1-8-2	16

The RFLP variants, such as 1-8-1 and 1-2-3, have been present in all years (2015-2021) as well as the 2-5-2 (vaccine virus), while the 1-6-4,1-6-3,1-7-4 variants, only since 2016. The 1-3-4 variant was introduced in 2018. Currently, in Jalisco there are at least 8 clusters of well-defined RFLP patterns identified during the last 6 years.

Discussion and Conclusion

The PRRSV variants found in Jalisco were identified and the RFLP´s was analyzed. These variants are among the most common variants in México (5). One possible reason is because Jalisco has a high number of variants compared to the rest of the country is the scattering of farms compared to other areas of Mexico. In 2020, Jalisco´s Union of Pig Farmers reported at least 1956 backyard farms, 796 semi-technical farms and 377 technical farms. The displacement of field variants and the high mutation rate of PRRSV in Jalisco depends on many factors, in order to mitigate these problems is necessary to address two important points: biosecurity and vaccination. The persistence of these strategies will support the control of PRRSV.

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Application of trimeric spike protein of porcine epidemic diarrhea virus on investigation of virus-host interaction

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Introduction

Porcine epidemic diarrhea virus (PEDV) belongs to the genus Alphacoronavirus and the family Coronavirus, and results in a highly contagious swine disease porcine epidemic diarrhea (PED) characterized by acute watery diarrhea, vomiting, and dehydration in suckling piglets. Among the structural proteins encoded in PEDV, the spike (S) glycoprotein plays a critical role in host affinity, virus-cell recognition, membrane fusion, and viral entry (1). The antigen of PEDV could not only be detected in the gastrointestinal tract, especially the villus epithelium of small intestine, but in intestinal crypt cells, goblet cells, and macrophages located in the mesenteric lymph nodes, spleen, and Peyer's patches (2). In the last two decades, porcine APN (pAPN) and sialic acid have been suggested as binding receptors for PEDV. However, several recent studies indicated that pAPN is not a functional receptor for PEDV but rather a co-receptor facilitating the PEDV infection by its protease activity (3,4). Therefore, the main host receptor of PEDV still remains unknown. In this study, we aim to use co-immunoprecipitation (co-IP) to determine potential host cell receptor of PEDV on pig enterocytes and Vero-E6 cells.

Materials and Methods

We first constructed and expressed trimeric S ectodomain glycoprotein of PEDV Pintung-52 strain using human embryonic kidney 293 cell line to obtain recombinant protein harboring structure and post-translational modifications mimicking natural virions. In co-IP, the purified S protein was applied as a probe to interact with membrane proteins derived from pig small intestine and Vero-E6 cells, which was assumed to comprise potential host cell receptor. Following, the harvested protein complexes were separated by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The suspected target protein was sent for protein identification by matrix-assisted laserdesorption ionization-time of flight (MALDI-TOF) analysis in Institute of Molecular Biology, Academia Sinica (Taipei, Taiwan). The result was blasted with the database of *sus scrofa* and primates in National Center for Biotechnology Information (NCBI).

Results

To identify (co-) receptor of PEDV, the interaction between a trimeric PEDV S protein with membrane proteins derived from neonatal piglets' enterocytes and Vero-E6 cells was investigated by co-IP and mass spectrometry. The results showed that a protein in size of approximately 75 kilodaltons was identified in both cell-derived membrane fractions. After MALDI-TOF and data analysis, glucose-regulated protein 78 (GRP78) was identified.

Discussion and Conclusion

Glucose-regulated protein 78 is traditionally considered as a kind of chaperones facilitating protein folding and assembly, and could be detected in ER lumen, cytoplasm, mitochondria, and nucleus. Additionally, it has been reported that GRP78 is expressed on the cell surface of Vero-E6 cells. Notably, recent studies revealed that GRP78 could translocate from ER lumen to the cell surface under ER stress and may plays a key role in virus attachment and entry (5), such as Dengue virus, Japanese encephalitis virus, tembusu virus, Middle East respiratory syndrome coronavirus (MERS CoV) and SARS-CoV-2. However, the role of GRP78 in PEDV still needs further investigation. In the future, overexpression of GRP78 in PEDV permissive and non-permissive cells will be conducted and viral replication will be evaluated in these cells.

Acknowledgments

This work was supported by National Taiwan University (111L891002) and the Ministry of Science and Technology, Taiwan, R.O.C. (MOST 109-2313-B-002-016-MY3).

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Assessing Senecavirus A shedding and transmission in growing pig populations

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Introduction

Senecavirus A (SVA) has been linked to vesicular disease outbreaks in pigs worldwide (1), and the number of cases appears to be increasing yearly. Several SVA outbreaks have been detected following stressful events for pigs, such as transportation (2) and farrowing (3,4). Moreover, intermittent viremia and shedding for up to 60 days post-infection after stress simulation have been reported (5). Virus transmission within farrowing rooms may persist for several weeks since processing fluids (PF) in breeding herds undergoing SVA outbreaks test SVA RNA positive for an average of ~12 weeks (6). However, there is scarce information on SVA shedding and transmission after weaning in pigs born during the outbreak (e.g. growing pig populations). This study aims to use molecular diagnostic tools to assess shedding and infection in different stages of the nursery-grow and finishing phases.

Materials and Methods

A breeding herd located in the Midwestern United States undergoing an SVA outbreak was conveniently selected for this longitudinal study. Five different cohorts of weaned piglets were being longitudinally tested for SVA at the time this abstract was being written. PF samples from cohorts 2, 3, 4, and 5 were collected 2-3 days after farrowing. After weaning, four oral fluid (OF) samples are being consecutively collected at weeks 1, 2, 3, 4, 9, 11, and 16 post-placement into a wean-to-finish barn, with one last OF sampling time-point one week before sending the pigs to market. Additionally, 60 blood samples were collected at week 2. All OF and blood samples are being tested for SVA through rRT-PCR to monitor virus shedding and viremia throughout the growing phase at the University of Minnesota Veterinary Diagnostic Laboratory.

Results

The cohorts of piglets being longitudinally tested were weaned at weeks 4, 7, 8, 9, and 10 after outbreak detection in the breeding farm (cohorts 1, 2, 3, 4, and 5, respectively). Viral RNA was found in PF samples from cohorts 2, 3, 4, and 5. Unfortunately, it was not possible to collect PF samples from cohort 1. Interestingly, preliminary results show that only cohort 1 (weaned four weeks after SVA outbreak detection in the breeding herd) was OF-positive by SVA rRT-PCR until three weeks post-placement, with suspect results at weeks 4 and 8 post-placement. Until this moment, all tested OF samples from cohorts 2, 3, 4, and 5 have tested negative even though, at farrowing, their respective PF samples tested positive. All 60 sera samples collected from each cohort at week two post-placement in a wean-to-finish barn tested negative for SVA rRT-PCR. All preliminary results are shown in Table 1.

Table 1. Preliminary results from oral fluid (OF) and sera testing by SVA rRT-PCR.

	Sample type / Week in the growing phase							
	PF	OF	Sera	OF	OF	OF	OF	OF
Cohort	--	1	2	2	3	4	8	11
1	--	P	N	P	P	S	S	N
2	P	N	N	N	N	N	N	--
3	P	N	N	--	--	--	--	--
4	P	N	N	--	--	--	--	--
5	P	--	N	--	--	--	--	--

*P = Positive - at least 1 out of 4 samples tested positive with a Ct. value of at least 36. S = Suspect - at least 1 out of 4 samples tested yielded a Ct. value ≥36 and <40. N = Negative - all four tested samples tested negative. Dashed lines are shown where no results are yet available.

Discussion and Conclusion

Preliminary results agree with earlier findings in that SVA RNA was found in PF samples from cohorts 2 to 5, which could mean that piglets weaned up until ten weeks after outbreak detection were exposed to SVA infection. However, SVA shedding in the growing phase has only been detected in cohort 1 (weaned four weeks after outbreak detection), suggesting that virus transmission may occur post-weaning. It is unknown whether our negative findings in sera are due to maternal immunity or because most pigs were already infected during the suckling period and viremia had ended at the time of sample collection, or even if transmission was not occurring at high levels in the second week of the growing phase and our sample size was not large enough to detect viremic pigs.
Preliminary results of this study shed light on the epidemiology of SVA in growing pigs as pigs clearly carry the virus into growing pig sites.

Acknowledgments

The authors would like to acknowledge participating veterinarians and pig farms staff for agreeing to participate in this study and help collect samples. This study was funded by the American Association of Swine Veterinarians Foundation.

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Assessment of a 5-step program for PRRS control in Colombia

Introduction

The porcine reproductive and respiratory syndrome virus (PRRSV) is one of the main causes of economic loses in the global swine industry and its control requires an efficient program able of risk assessment and implementation of virus stabilization strategies: management and/or vaccination (1). In the countries where PRRS vaccination is not authorized, the control strategies include the controlled exposure of the reproductive stock (use immune response to eliminate viral transmission to the progeny and wean negative piglets). The objective of the present study was to assess the implementation of a 5-step program approach in a PRRS positive farm.

Materials and Methods

A 750-sow farm with site I (sow farm) and site II (nursery) placed in Antioquia, Colombia uses a 28-day band system with weaning at 21 days and broke with PRRS despite that before the outbreak the replacements were obtained from a PRRS negative source (entered the system every two months) and that the semen used was also certified PRRS free. A 5-step PRRS control program was implemented and assessed: **Step 1.** Identify desired goals (stabilize PRRS infection in site 1). **Step 2**. Determine Current PRRS Status with diagnostics **Step 3**. Understand Current Constraints: Risk assessment (using the COMBAT APP Comprehensive Online Management and Biosecurity Assessment Tool from Boehringer Ingelheim Animal Health). **Step 4**. Develop solution options (one year replacement and acclimatation, internal/external biosecurity upgrade and the implementation of the 10 gold rules for PRRS control (2). **Step 5**. Implement and Monitor Preferred Solutions

Results

Once it was decided that the initial goal was to stabilize the site 1, a full diagnostic run was performed with PRRS ELISA test as shown, determining an ongoing PRRS contamination and classifying the farm as a positive and nonstable system. The risk evaluation tool (COMBAT) identified several weak spots associated to employee movements among production zones, piglet movements and nursing, out of site hired transportation, lack of quarantine unit and high epidemiological pressure for PRRS in the area. The implemented solutions included changes of the biosecurity and management and the natural exposure of negative gilts to PCR positive piglets showing high levels of PRRSV excretion. Twenty-one days post exposure 100% of the gilts tested positive. After 100 days of cooling (no viral excretion) the sows enter the system producing negative progeny that allows for an improvement in the productive and health parameters in the farm starting on Band 5 C in site I (Fig I). Benefits in site II are observed from May 2021 (Fig 2).

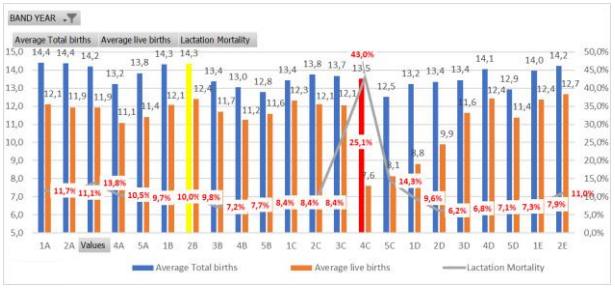

Fig 1. Productive parameters site I (2020-2021).

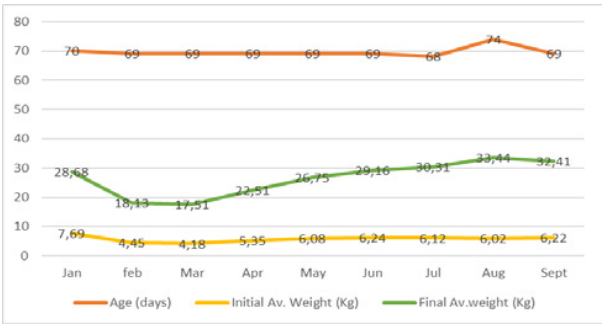

Fig 2. Productive parameters site II, January to September 2021.

Conclusion

Achieve a fast and efficacious PRRSV control requires not only knowledge about the disease and the farms affected but the use of risk assessment tools and a systemic approach. The implementation of this 5-step program allowed the stabilization of the farm PRRS status at weaning. After the control measures a significant decrease in mortality at weaning, an increase in the number of piglets at farrowing, and a higher body weight gain was observed. Currently the farm is weaning PRRSV negative piglets, keeps improving their biosecurity under the systemic 5-step approach to decrease the risk of reinfection. Colombia currently does not allow PRRSV vaccination, PCR and sequencing studies are ongoing to determine with an epidemiology assessment based on virus homology the suitability of a control program using both biosecurity and vaccination.

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Bronchointerstitial pneumonia and bronchiolitis associated with Porcine HemagglutinatingEncephalomyelitis Virus

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Introduction

Porcine hemagglutinating encephalomyelitis virus (PHEV) is the only described beta coronavirus affecting pigs. Commonly PHEV causes encephalitis and ganglioneuritis of the myenteric plexus resulting in tremors, muscle fasciculations, paddling, dullness, and vomiting. PHEV was confirmed by PCR detection in respiratory flu-like cases on show pigs in Michigan, the US, in 2015. This first description reported moderate interstitial pneumonia and damage of the airway epithelium Other epitheliotropic viruses, including swine influenza virus (IAV) and porcine parainfluenza virus 1 (PPIV-1), were ruled out in this report. Besides PHEV detection by PCR, *in situ* detection of PHEV in pulmonary lesions has not been reported. The objectives of this study are 1) confirming the presence of PHEV *in situ* in cases of bronchointerstitial pneumonia of unidentified etiology 2) determining the genetic relation of PHEV genome detected in respiratory and neurological cases.

Materials & Methods

Three cases received during 2020 diagnosed as unspecified bronchointerstitial pneumonia and bronchiolitis (no etiological diagnosis) were evaluted by next-generation sequencing (NSG). Viral homology of the newly generated virus by contig assembling was performed on the Basic Local Alignment Search Tool. A phylogenetic analysis was performed to compare the PHEV sequences detected in this case and PHEV spike genes references reported on GenBank. A retrospective evaluation of 15 cases received at ISU-VDL from 2019 through 2021 was perfomed. The inclusion criteria was based on a primary histological assessment. Animals categorized as unspecified bronchositnestitial pneumonia were tested for PHEV. PCR was performed on fixed tissues by sectioning a 40 µm scroll of paraffin embed blocks, followed by RNA extraction by standard procedures. PHEV was confirmed by qPCR against the PHEV spike gene. The presence of PHEV within the bronchiolar epithelium was confirmed by *in-situ* hybridization (ISH) using Advanced Cellular Diagnostic's RNAScope specifically targeted against the S gene mRNA.

Results

Next-generation sequence analyses demonstrated the presence of PHEV in a case with bronchointerstitial pneumonia. Further, the presence of PHEV was confirmed by specific qPCR results on 72.2% (13/18) of cases with unspecified bronchointerstitial pneumonia. The average PCR Ct value was 34.15 (2.57± SD). Phylogenetic analysis demonstrated that this virus forms a unique cluster with other PHEV previously detected on respiratory cases but slightly diverged from isolates reported on neurological cases (Figure 1).

Figure1: phylogenetic tree of respiratory cases (highlight in green-Michigan; blue-ISU) compared with neurological cases (no highlight). Maximum parsimony (MP) tree based on the nucleotide sequences of the Spike protein. The value along the branches represent substitutions per site

ISH-RNA against PHEV showed strong staining in the airway epithelium of sections that present epithelial attenuation and regeneration. Scattered PHEV positive cellswere observed in the bronchiolar lamina propria and alveolarinterstitium (Figure 2).

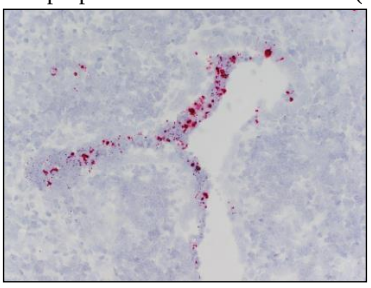

Figure 2: Bronchointerstitial pneumonia with intense positive staining against PHEV mRNA (red staining) on respiratory epithelium and sloughed necrotic epithelia.

Discussion & Conclusion

PHEV has been historically associated with neurological disease in pigs. A single report has detected PHEV by PCR on cases of IAV-like bronchointerstitial pneumonia in showpigs. Although serologic studies demonstrated the PEHV prevalence in the US is 53.35%, there are scarce reports of PHEV VWD. Thus, this retrospective study may support thatcurrent PHEV prevalence could result from undetected or undiagnosed respiratory PHEV. Epitheliotropic viruses, including IAV and PPIV, are endemic in commercial pigs inthe US. Other beta coronaviruses, including BCoV and SARS-CoV-2, have a clear epithelial tropism. Phylogenetic analysis demonstrated that PHEV associated with respiratory lesions clustered independently from neurological strains. Thus, different PHEV genotypes could lead to different phenotypes with different clinical outcomes.Further studies are necessary to evaluate the pathogenesis ofPHEV associated with respiratory disease.

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Case report: Getting back on track as good as naïve PRRS farm performance when homogenizedimmunity by PRRS MLV (VR2332)

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Introduction

This case describes a 2-site farrow-weaned system 1,700 sow farm, commercial pelleted feed and all- in all-out weekly production. The farm started withnaïve PRRS herd status and was able to maintain the naïve status for 4 years. By the end of 2020, the stillborn increased followed by high preweaning mortality rate and mummified rate respectively.

The diagnosis of PRRSV was confirmed in November 2020 by PCR and serology check combination with clinical observations. Over timethe 5-steps of PRRS control interventions were implemented and evaluated[1]. In this abstract the results of before the PRRS outbreak versus after the 5-steps control program implemented are discussed.

Materials and Methods

In November 2020, the farm encountered mainly inthe farrowing house by increased stillborn, pre- weaned death, mummified fetus and a slightly dropof farrowing rate in the month of January. PRRSV was confirmed to be the main cause of the problemthrough serology and PCR. Since the problems occurred, controlling from hot (outbreak) to stable by whole herd vaccination by Ingelvac PRRS MLV (Strain VR2332, Type II) was adopted. Due to the success of the problem solving, communication needs to be clarified. Monthly meetings andcommunication have been executed consistently to monitor the progression, constraints and the successful of each implemented step. The farm instituted initial program by a double mass vaccination of the sow herd, 30 days apart and everythree months. Along with the sow vaccination, the piglets were vaccinated at 2 weeks of age, gilts wereintroduced and vaccinated twice, with 30 days apartand kept in the gilt development unit for at least 90 days within the farm. Before entering these gilts were confirmed for the PRRSV shedding by using the oral fluid PCR every month. Stable sow herd status was measured by producing negative piglets at 2 weeks together with processing fluid at 3 days of age monthly. The comparative results were evaluated by the sow performance with the moving range of individual control chart through the Minitab Program LLC USA.

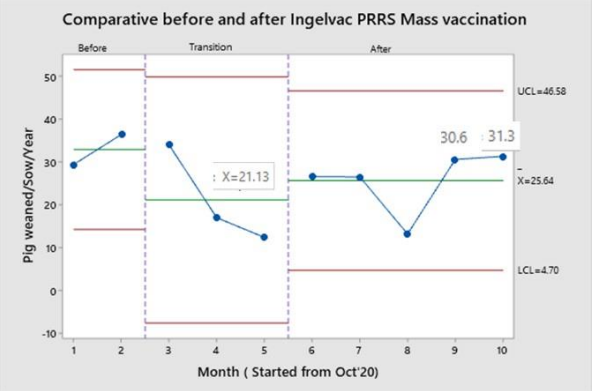
Results

The farm monitored the herd diagnostically with monthly PCRs on weaned pigs. Immediately the condition of the piglets was improved along with actual performance improvement as shown in **Table1.** The pigs weaned per sow per year resumedas good as before the outbreak period within 6 months after implementation of PRRS MLV vaccine, as shown in the **Figure1.**

Table 1. Sow performance during period before and after problems solving

	Oct-20	Nov-20	Dec-20	Jan-21	Feb-21	Mar-21	Apr-21	May-21	Jun-21	Jul-21
% SB	7.3	6.4	12.9	19.7	12.1	7.2	7.9	6.3	6.9	6.3
% MM	4.2	3.4	4.8	22	12.7	17.5	18.3	10.2	4.5	6.5
%FR	93.7	93.1	92.6	87.3	91.6	89.4	90.4	90.1	91.7	92.7
% Pre-weaning MT	5.9	8.3	10.5	36.3	25.1	11.9	9.1	5.7	6.8	7.1
PW/L	13.6	13.5	13.2	8.3	6.5	10.4	10.9	11.4	13.1	13.7
PWSY	29.4	36.4	34	17	12.4	26.6	26.5	13.2	30.6	31.3

Figure 1. The moving range of pig per sow per year (PSY)



Discussion

In this case, getting back on track to produce 31 PSY like naïve period by vaccination with Type II PRRS MLV is the most challenging task for the PRRS solutions team. The 5-Steps Process together holistic approach, combining vaccination, pig flow management by having 2 sites and good animal husbandry can help break the PRRS circulation in a production system within 6 months

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Case report: The Implementing of 5 Steps PRRS control improve farm performance evolutions in 2isolated of PRRS strain farm

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Introduction

The co-existence of PRRSV Type I and Type II ina pig herd has been reported in Thailand[1].Vaccination by modified live vaccine (MLV) is one of the most popular tools to control PRRS impacted, reproductive failure, poor farrowing performance as well as nursery and growers suffered from growth retarded and are highly susceptible to secondary bacterial and otherviral infections[2].

The objective of these observations is to evaluatethe sow performance evolutions after implementing Type I PRRS MLV vaccine (VR2332) in a farm with both Type I and Type IIPRRSV infection

Materials and Methods

The 850 sow, two-site farm farrow-to-nursery and grower-finisher sites, in the central area of Thailand,has been vaccinated with Type I PRRS MLV for over 8 years. During August-September 2019, the grower-finisher site farm encountered by PRDC. Two isolated Type I and Type II of PRRSV were confirmed through PCR from stillborn, 2-3, 5-6, 7- 8 and 10-11weeks old pigs as indicated **Table1**. The abortions had been increased since Sep'20 consequence by peak of mummified, stillborn and preweaning mortality during the Oct-Dec 2020. After confirming PRRSV infection together with clinical observation, the farm had a double mass vaccination of the sow herd, 30 days apart. Piglets' vaccination also implemented along with sow vaccination day from 7-24 days of age, then followed as the routine program at 14 days of age since then. The introduced 18 weeks old gilts were acclimatized and vaccinated 2 shots of Type II PRRS MLV 30 days apart in a Gilt Development Unit located within the farm. The virus shedding monitoring was established every quarter for the gilts and every month for the suckling piglets. The limitations of the farm were discussed with thefarm's team lead and communication room had been set up for each month. The sow performances were evaluated by computerized and analysis of evolution by Individual Moving Rage, Minitab LLC, USA.

Table1.The Serum PCR results

Age	Stillborn	2-3 wk	5-6 wks	7-8 wks	10-11 wks
PCR results	Type II	Type II	Type I &II	Type II	Type II

Results and Discussion

The farm monitored the herd diagnostically with monthly PCR testing of weaned pigs. Immediately the condition of the piglets was improved along withactual performance improvement shown as the figure 1 & 2. Moreover, the farm also improved theNon-reproductive sow day from 67 days duringbefore vaccination period by Type I PRRS MLV to45 days after herd was stabilized, with a totally reduce wasting NPD 22 days. **Figure1.** The comparative sow performance period before and after implement of Type II PRRS MLV vaccination.

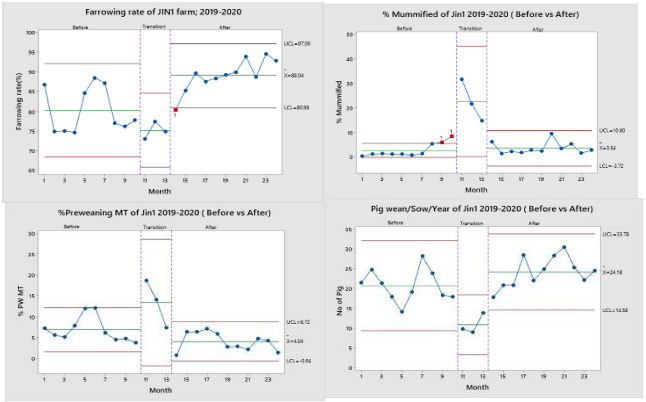

Figure 2 Pictures of piglets before and after PRRSControl

Discussion

Having 2 Types of PRRSV infections in the farm in combination with clinical and production parameter helps the farmer make the right decision to choose the right vaccine to control the PRRS based on holistic approach, the systematic 5-Steps PRRS control.

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Characterization and quantification of Rotavirus C RNA by RT-qPCR in diarrheic feces from piglets at South, Midwest, and Southeast regions of Brazil

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Introduction

Rotavirus (RV) belongs to the *Reoviridae* family, the *Rotavirus* genus that is classified into nine species (A-D and F-J). The RV species identified in pigs are RVA, RVB, RVC, and RVH. The RV genome is composed of 11 segments of double-stranded RNA (dsRNA), which encode six structural and six non-structural proteins (1). Among structural proteins, VP7 protein is on the outer capsid and induces neutralizing antibodies. In addition, the VP7 gene is used to classify RV strains into G genotypes. RVC is one of the major causes of severe neonatal diarrhea in piglets, causing significant economic losses (2). This study aims to evaluate the detection and quantification of RVC in piglets with neonatal diarrhea and to characterize the G genotype of these circulating strains in Brazilian pig herds.

Materials and Methods

Diarrheic fecal samples were previously screened by the silver-stained polyacrylamide gel (ss-PAGE) technique. Herds with fecal samples showing dsRNA electrophoretic profile of RVC (*n*=9) were selected. In these herds, between 2017 and 2019, 41 diarrheic fecal samples were obtained from suckling piglets. The pig herds were located in the South (*n*=4 farms and 20 samples), Midwest (*n*=4 farm and 14 samples), and Southeast (*n*=1 farm and 7 samples) Brazilian regions. The fecal samples from positive pig herds were confirmed RVC-positives by conventional RT-PCR technique (3) and quantified by RT-qPCR (4). For classification into G genotype (VP7 gene), six positive samples (South: *n*=1; Midwest: *n*=3; Southeast: *n*=2) were submitted to partial amplification of the VP7 gene (5) and subsequently submitted to analysis nucleotide sequencing on an ABI3500 Genetic Analyzer sequencer. The nucleotide sequences of the amplicons were determined to classify VP7 into G genotypes using the neighbor join method with the Kimura two-parameter model using MEGA7 software.

Results

A total of 31/41 (75.6%) diarrheic fecal samples were positive for RVC by conventional RT-PCR. At least one fecal sample was positive per pig herd evaluated. Comparative analyzes of the VP7 gene sequences of this study was performed with representative strains of 31 known RVC G genotypes (6). G6 genotype was identified in all six samples evaluated. The viral load ranged from 6.3 x 10^4 to 4.0 x 10^8 genomic copies/g of diarrheic feces.

Table 1. Distribution of total fecal samples from piglets with diarrhea RT-PCR-positives for RVC according to the region and respective viral load (RT-qPCR).

Region	Pig herds	Age (days)	RVC positive/total samples (%)	Range of the viral load*
South	4	1-25	14/20 (70)	$6.3x10^4$ to $1.5x10^8$
Midwest	4	1-7	10/14 (71.4)	$2,3x10^6$ to $4.0x10^8$
Southeast	1	1-7	7/7 (100)	$6.5x10^6$ to $3.0x10^8$
Total	9	-	31/41 (75.6)	-

*Genomic copies/g of diarrheic feces

Discussion and Conclusion

In this study, positive RVC pig herds were used as herd selection criteria. Despite this, high detection rates of RVC and high viral loads were identified in suckling piglets with neonatal diarrhea. Neonatal diarrhea is frequent in pig herds. Among RV species high RVC detection rates in suckling piglets have been reported to be higher than even other species, such as RVA, indicating the important role of this RV species in diarrhea outbreaks (7,8). The widespread commercial inactivated vaccines against RVA possibly favor the increase of infections caused by other RV species, such as RVC. In this study, the G6 genotype was the only one to be detected, which is consistent with a previous Brazilian study that obtained the same genotype suggesting that the G6 genotype is predominant among the RVC circulating strains in Brazilian pig herds (9). In conclusion, these data reinforce the role of RVC in the etiology of neonatal diarrhea in piglets and highlight the importance of using effective prevention and control measures to minimize production losses resulting from RV infection.

Acknowledgments

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Characterization of the clinical impact, pathogenicity, transmissibility, and antibody response of PRRSV 1-4-4 L1C variant strain in comparison with other PRRSV strains in experimentally inoculated pigs

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Introduction

PRRSV-2 is genetically classified using RFLP patterns or phylogenetic lineages/sublineages based on ORF5 sequence (1,2,3). The periodical emergence of PRRSV-2 variants is not a new thing as RNA virus has higher mutation rate and rapid evolution. Some variants were detected transiently whereas some variants established stable infection in swine populations. The emergence of PRRSV 1-7-4 L1A variant in the USA in 2014-2015 was associated with high production losses (4). Since October 2020, PRRSV 1-4-4 L1C variant emerged in the USA and appeared to be associated with extremely high production losses in mid-west swine farms based on field observations (5). However, no unequivocal experimental data is available to confirm the perception that the 1-4-4 L1C variant is more virulent than other PRRSV strains.

Materials and Methods

In this experimental inoculation pig study involving 6 groups (8 inoculated pigs and 4 contact pigs for each group), the clinical impact, pathogenicity, and transmissibility of the recently emerged PRRSV 1-4-4 L1C variant strain were compared with three other PRRSV 1-4-4 strains (L1C non-variant, L1A and L1H sublineages) and one previously described virulent PRRSV 1-7-4 L1A strain as well as a mock-inoculum in 4-week-old pigs for a duration of 28 days post inoculation (DPI). Five viruses grown in ZMAC cells were used for inoculation (6). The contact pigs were introduced at 2 DPI. Daily temperature and clinicalsigns were recorded. Serum and oral fluid samples were collected at 0, 2, 4, 7, 10, 14, 21 & 28 DPI. Pigs were necropsied at 10 DPI (4 inoculated pigs/group) and 28 DPI (all remaining pigs). Fresh and formalin-fixed tissues (lungs, tonsils, TBLN, thymus, heart, spleen, kidney, and brain) were collected at necropsy.

Temperatures, virus loads in serum and oral fluid samples, and antibody titers were analyzed using a linear mixed model. The average daily weight gain (ADG), lung gross lesion scores and PRRSV RNAlevels in tissues (e.g., lung, tonsil, and brain) wereanalyzed using a one-way ANOVA with Tukey-KramerHSD. For lung microscopic lesion scores, and IHCscores a linear mixed model was used. For all analyses SAS was used and a p-value ≤ 0.05 was considered significant.

Results

The 1-4-4 L1C variant virus-inoculated pigs became more lethargic, were off feed faster, had higher mortality, and had higher percentage of pigs with fever (>40°C) compared to other virus-inoculated groups. The 1-4-4 L1C variant virus-inoculated group had significantly higher viremia levels compared to all other virus-inoculation groups at 2 DPI. The same trend of viremia level was found in contact pigs at 2 days post contact. 4/4, 2/4, 2/4, 0/4, and 2/4 contact pigs in the L1C variant, L1C non-variant, L1A, L1H, and 1-7-4 L1A groups became viremic at 2 DPC. There were more severe gross lung lesions in the 1-4-4 L1C variant virus-inoculation group compared to other virus-inoculation groups except the 1-7-4 L1A group. Higher antibody titers were observed in 1-4-4 L1C non-variant in both the inoculated and contact group after 14 and 21 DPI respectively. The differences of ADG, microscopic lung lesion score, IHC score and RNA level in different tissues were not statistically significant between virus inoculated groups.

Discussion and Conclusion

This is the first study providing experimental data in weaned pigs regarding the clinical impact, pathogenicity, and transmissibility of the newly emergent 1-4-4 L1C variant strain along with comparisons with other PRRSV strains. The findings from this experimental study align with what field veterinarians observed for the L1C variant outbreak (7). L1C variant-inoculated pigs had more severe outcomes in certain aspects, such as fever (>40°C) during 0-10 DPI, and significantly higher viremia levels at some time points. The higher number of contact pigs becoming viremic at 2 days post contact implies that the L1C variant strain may have higher transmissibility than other PRRSV strains although it needs to be confirmed with a study involving more pigs. Future studies are needed to understand the protective efficacy of PRRS MLV vaccines against the newly emergent PRRSV 1-4-4 L1C variant strain.

Acknowledgments

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Characterizing United States boar stud biosecurity practices to assess risk of African swine fever virus entry and transmission pathways

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Introduction

There are an estimated 200 boar stud facilities in the United States which provide semen for artificial insemination of 6 million sows (1). Although standard levels of biosecurity practices are at their highest in these boar studs, there are still known incidents of venereal disease, such as Porcine Respiratory and Reproductive Syndrome (2?). There is concern thatwere African Swine Fever, another disease quite likely to be transmitted via artificial insemination, introducedinto a boar stud but remain undetected, disease could unknowingly be transmitted to sow farms via semen movements. The Secure Food Systems (SFS) team at the University of Minnesota (UMN) (3) is conducting a proactive risk assessment (RA) that systemically evaluates the potential risk of liquid, cooled semen movements from a boar stud in a control area during an African swine fever (ASF) outbreak, since information regarding this transmission risk is scarce.

Material and Methods

A working group (WG) of boar stud subject matter experts (SMEs), state and federal regulatory officials, and academicians was convened to guide the RA. Ten different potential routes of African swine fever virus (ASFV) entry into a US boar stud were evaluated (Figure 1), scientific literature regarding those pathways was reviewed, collated, and presented by the UMN SFS team to the WG, and the WG SMEs provided information back to the UMN SFS team regarding stud characteristics, existing biosecurity practices, and industry standards. The information was collected in regularly scheduled meetings and via surveys given to the WG, SMEs and a subset of boar stud managers, veterinarians, and consultants.

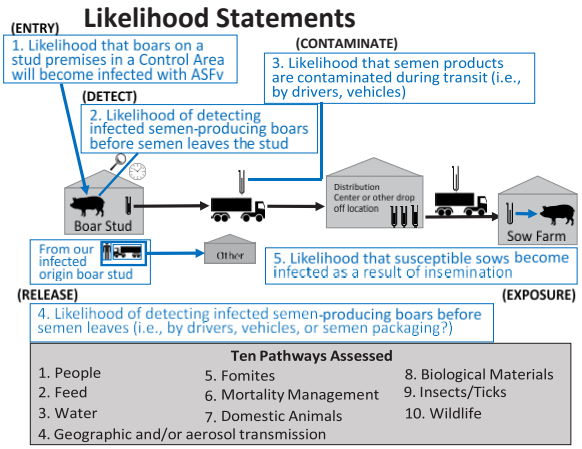

Figure 1 Likelihood statements evaluated as part of the proactive risk assessment, along with the ten pathways assessed.

For each pathway assessed, the current boar stud biosecurity practices were then evaluated for their ASFV introduction potential. To further decrease the likelihood of ASFV introduction, the WG then developed more targeted biosecurity practices that would be used in the event of an ASF outbreak. Finally, the likelihood ratings of ASFV introduction via the assessed pathways were then estimated based on these targeted mitigations by the WG, along with the enhanced biosecurity requirements (EBRs) for permitted movement of semen during an ASF outbreak, per the Secure Pork Supply Plan (SPS) (4).

Results and Discussion

The WG discussions and survey responses allowed the UMN SFS team to characterize US boar studs and their biosecurity practices. The characterizations of the biosecurity practices were considered in context with the science available, and this allowed assessment of the potential gaps through which ASFV introduction may occur. Assuming that targeted and enhanced biosecurity recommendations from the WG and SPS are in place during an ASF outbreak, the tentative likelihood ratings for ASFV entry, which varied from negligible, low, and low to moderate, were estimated for each pathway and qualitatively assessed. It is important to note that the likelihood ratings are only for the likelihood that a boar stud premises in a Control Area will become infected with ASFV and do not consider the pathways and risk of subsequent ASFV spread from the boar stud, which is currently being assessed by the UMN SFS team. In conclusion, the US boar stud population can reduce its risk of ASFV introduction by implementing more enhanced and targeted biosecurity practices. This entry analysis can be referred to when evaluating the risk of moving liquid, cooled semen movements from a not-known-to-be-infected boar stud in a control area during an ASF outbreak.

Acknowledgments

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Comparative efficacy study of freshly mixed and ready to use PCV2 and *Mycoplasma hyopneumoniae* vaccines in a commercial swine farm in Japan

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Introduction

Porcine Circovirus Type 2 (PCV2) and *Mycoplasma hyopneumoniae* (*M. hyo*) are the causative pathogens of porcine respiratory disease complex (PRDC), which causes severe economic losses in the swine industry worldwide (1,2). The objective of this study was to compare the efficacy and economic impact of commercially available, freshly mixed, and ready to use PCV2 / *M. hyo* vaccines in a commercial swine farm in Japan.

Materials and Methods

This study was conducted in a farrow to finish swine farm with 250 sows located in West Japan during Apr. 2020 to Jan. 2021. The farm was seropositive to PRRSv at around 80 days of age, but post-weaning mortality was controlled without PRRS vaccination in piglets. On the day of weaning, suckling piglets born from 11 sows were evenly divided into two groups by individual weight, sexes, and parities. Group A (n = 67) received a single 2 ml shot of Ingelvac® FLEXcombo Mix (Boehringer Ingelheim Animal Health Japan Co., Ltd.), and Group B (n = 67) received a single 2 ml shot of ready to use PCV2 / *M. hyo* vaccine at 21 days of age, respectively. Both vaccines were administered in accordance with the manufactures' instructions. Pigs were individually weighted at 21 (weaning), 87 (migrated to the finisher barn), and 147 days of age and days to market. Blood samples were collected at 21, 87, and 147 days of age (5 pigs per group) and tested for PRRS ELISA / qPCR, PCV2 ELISA / qPCR, and *M. hyo* ELISA. Post-weaning mortality and average daily weight gain (ADWG) were statistically analyzed using the Chi-square test and Student's *t*-test, respectively.

Results

There was no statistically significant difference in post-weaning mortality and ADWG between the two groups. However, Group A showed lower mortality (Group A: 2.99% and Group B: 7.46%) and higher ADWG (Group A: 624 g/day and Group B: 617 g/day). Additionally, Group A also showed higher weight uniformity (coefficient value) at 147 days of age (Group A: 15.7% and Group B: 17.4%) (Table 1). In addition to that, PCV2 ELISA titer at 87 days of age was more varied in Group B than Group A (data not shown). As a result, an estimated 10.29 USD difference per pig at the end of the study between the two groups was calculated based on production parameters.

Discussion and Conclusion

In this study, although there was no statistically significant difference in production parameters between the two groups, freshly mixed PCV2 / M. hyo vaccination showed lower post-weaning mortality,higher ADWG and higher weight uniformity than a ready to use vaccine. Interestingly, PCV2 ELISA titer at87 days of age was more varied in Group B than Group A, suggesting that PRRS field virus infection differently affected the immune response between the two groups.

Table 1. Production parameters

	Group A (Freshly mixed)	Group B (Ready to use)	*p*- value
Pigs No.	67	67	-
Average body weight (kg, 21 days of age)	5.85 ±1.38	5.97 ±1.45	0.62
Average body weight (kg, days to market)	109.57 ±4.20	109.33 ±4.16	0.74
Weight variation (CV%, 147 days of age)	15.7	17.4	-
Average study period (days, wean to market)	166.3	167.5	0.95
Post-weaning mortality (%)	2.99	7.46	0.24
ADWG (g/day)	624	617	0.38

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Comparative efficacy study of freshly mixed and ready to use PCV2 and *Mycoplasma hyopneumoniae* vaccines in a farrow to finish farm in Japan

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Introduction

Porcine Circovirus Type 2 (PCV2) and *Mycoplasma hyopneumoniae* (*M. hyo*) are the two main causative pathogens of porcine respiratory disease complex (PRDC), which causes severe economic losses in the swine industry worldwide (1,2). PCV2 / *M. hyo* combination vaccines are widely used to protects pigs against those two separate infections. The objective of this study was to compare and evaluate the efficacy and economic impact of commercially available, freshly mixed, and ready to use PCV2 / *M. hyo* vaccines in a farrow to finish farm in Japan.

Materials and Methods

This study was conducted in a farrow to finish swine farm with 250 sows located in West Japan during Apr. 2021 to Dec. 2021. The farm was seropositive to PRRSv at around 80 days of age, but post-weaning mortality was controlled without PRRS vaccination in piglets. Onthe day of weaning, suckling piglets born from 22 selected sows were evenly divided into two groups by individual weight, sexes, and parities. Group A (n=103) was intramuscularly administered a single 2 ml shot of Ingelvac® FLEXcombo Mix (Boehringer Ingelheim Animal Health Japan Co., Ltd.), and Group B (n =100) was intramuscularly administered a single 2 ml shot of ready to use PCV2 / *M. hyo* vaccine at 21 days of age, respectively. Both vaccines were administered in accordance with the manufactures' instructions. Pigs were individually weighted at 21 (weaning), 75 (migrated to the finisher barn), and days to market. Blood samples were collected at 21, 75, and 146 days of age (5 pigs per group) and tested for PRRS ELISA / qPCR, PCV2 ELISA / qPCR, and *M. hyo* ELISA. Post-weaning mortality and percentage of light-weight pigs marketed were statistically analyzed using the Chi-square test.

Results

There was statistically significant difference in post-weaning mortality between the two groups. (Group A: 0.97% and Group B: 6.00%) (Table 1 and Figure 1). However, there was no statistically significant difference in the percentage of light-weight pigs marketed between the two groups (Group A: 11.7% and Group B: 13.0%) (Table 1). Consequently, an estimated 8.16 USD difference per pig at the end of the study between the two groups was calculated based on those two production parameters.

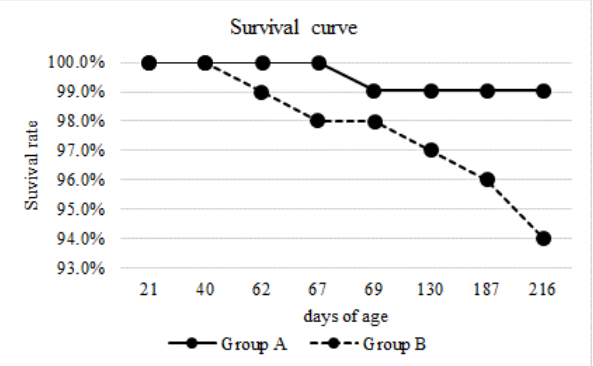
Discussion and Conclusion

In this study, freshly mixed PCV2 / M. hyo vaccination program showed statistically significant lower post-weaning mortality than a ready to use vaccine. Additionally, a lower percentage of light-weight pigsmarketed was observed in Group A than Group B even though there was no statistically significant difference between the two groups.

Table 1. Production parameters

	Group A (Freshly mixed)	Group B (Ready to use)	*p*- value
Pigs No.	103	100	-
Post-weaning mortality (%)	0.97	6.00	0.049
Percentage of light-weight pigs marketed (%)	11.7	13.0	0.76

Figure 1. Survival curve



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Comparative efficacy study of freshly mixed and ready to use PCV2 and *Mycoplasma hyopneumoniae* vaccines in a farrow to finish PRRS negative farm in Japan

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Introduction

Porcine Circovirus Type 2 (PCV2) and *Mycoplasma hyopneumoniae* (*M. hyo*) are the two main causative pathogens of porcine respiratory disease complex (PRDC), which causes severe economic losses in the swine industry worldwide (1). PCV2 / *M. hyo* combined vaccines are widely used to protects pigs against those two separate infections. Although these vaccines are effective as disease control measures, the stress of pigs, the transmission of pathogens by needles, and the workload of producers, which are factors in the onset and aggravation of post-weaning multisystemic wasting syndrome (PMWS), must be taken into consideration. In addition, influence of the transitional antibody, adjuvants, the timing and/or the number of administrations and the ability to induce humoral immunity or cell-mediated immunity vary depending on the type of vaccine. Therefore, the objective of thisstudy is to compare the productivity in a farrow-to-finish farm using a freshly mixed combination of PCV2 / M hyo vaccine, which is currently widely used in Japan.

Materials and Methods

This study was conducted in a farrow-to-finish swine PRRS negative farm with 100 sows located in East Japan during Jun.2021 to Jan.2022. On the day of weaning, suckling piglets were evenly divided into two groups by individual weight, sex, and parity**.** At 21 days of age, Group A (n=80) was intramuscularly administered a single 2 ml shot of Ingelvac® FLEXcombo Mix (Boehringer Ingelheim Animal Health Japan Co., Ltd.), and Group B (n=80) was intramuscularly administered a single 2 ml shot of a commercial ready to use PCV2 / M. hyo vaccine. The piglets were randomly allocated to 2 groups and fed under the same conditions from 3 weeks of age (test day 0) to shipment. Pigs were individually weighted at 3 weeks of age (weaning), 12 weeks of age (moved to the finisher barn) and market at an average age of 168 days. Average daily weight gain (ADG) was calculated from 3 to 20 week of age and statistically analyzed using the Student's *t*-test.

Results

The production parameters are shown in Table 1. ADG was 45 grams higher in group A (Group A: 0.745 kg/day; Group B: 0.700 kg/day), indicating statistical difference between groups. The post-weaning mortality, excluding crushing death, was reduced by 5 percent in Group A (Group A: 2.5%; Group B: 7.5%), although no significant difference was observed. The average body weight at 20 weeks of age was 2.5 kg superior in Group A (Group A: 96.6 kg, Group B: 94.1 kg) resulting in an overall higher average body weight distribution within the group (Figure 1).

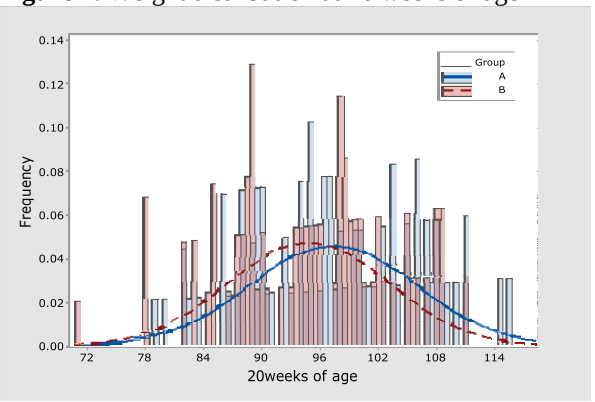
Discussion and Conclusion

Average daily weight gain is routinely used as a key performance indicator in swine farms worldwide. In this study the ADG from 3 to 20 weeks of age was significantly higher in group A compared to group B. In addition, the lower mortality rate observed in Group A also contributed positively for the overall performance. Based on the evaluated production parameters and on the current pork price in Japan, an estimated difference of 22.75 USD per pig was calculated in favor of Group A. From the above, it was shown that the improved productivity obtained with the combination vaccine Group A was satisfactory.

Table 1. Production parameters

	GroupA (Freshly mixed)	GroupB (Ready to use)	*P* -value
Pigs No.	80	80	
Post-weaning mortality (%)	2.5	7.5	0.1382
Body weight 20 weeks of age(kg)	96.6±8.7 [79.0～115.4]	94.1±8.5 [71.2～111.0]	0.0771
ADG	0.745±0.110 [0.133～0.919]	0.700±0.152 [0.044～0.867]	0.0332

Figure 1. Weight distribution at 20 weeks of age



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Comparative field trial on the effectiveness of three different PCV2 monovalent vaccines in a Portuguese swine farm

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Introduction

The commercial vaccines against Porcine Circovirus type 2 (PCV2) help to reduce the clinical manifestations and the losses associated with the subclinical infection (1,2). The purpose of this study was to assess the effectiveness of Circovac® (Vaccine A) in a commercial farm in comparison with two competitor PCV2 vaccines (Vaccines B and C).

Materials and methods

Three groups of 220 piglets each were randomly assigned into one of the groups (Vaccines A, B or C), ear tagged and vaccinated prior to weaning. The parity of the sows, piglet age and initial weight were balanced. The piglets were mixed during the nursery phase and separated by group when moved to the fattening rooms: 1 group per room. In each group, 30 pigs were chosen as sentinels and blood samples were collected at beginning, middle and end of fattening and tested for PCV2 (qPCR) and PRRSV (RT-PCR). Each group was weighed at beginning and end of fattening and the feed consumption was recorded. One of the fattening rooms was different from the other two in size, animal density, feeders, and ventilation system. To overcome these differences, the trial was replicated 3 times and each group rotated along the rooms.

Results

Although PCV2 and PRRSV infections were confirmed, no PCVD compatible signs were observed, and no significant differences were found on mortality (2% Vaccine A; 0,9% Vaccine B; 2,1% Vaccine C). No consistent differences were found on ADWG (Vaccine A: 772g/794g/ 825g; Vaccine B: 784g/841g/796g; Vaccine C: 769g/807g/794g) or FCR (Vaccine A: 2,55/2,53/2,35; Vaccine B: 2,57/2,42/2,47; Vaccine C: 2,55/2,47/2,50).

Discussion and Conclusion

All 3 vaccines protected the animals against PCV2 infection, preventing disease and mortality. The tested vaccines seem to have avoid productive losses due to PCV2 subclinical infection: no consistent differences were found among the 3 groups when we consider the results of the 3 replicates.

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Comparative phylogenetic analysis of L1C-144 PRRSV with circulating strains in Mexico

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Introduction

PRRS has represented one of the greatest challenges within the national and global pig farming during the last decades, mainly due to the virus high variability. This characteristic has led to the presence of new viral variants in swine production areas across the US, with periodic occurrence causing significant clinical impact as the appearance of an atypical PRRS virus in Iowa during 1996, to RFLP 1-7-4 virus identified in 2014 in NC and most recently L1C-144 in US midwest. Because of this, PRRS control becomes a challenge in most pig farms. The aim of this study is to compare ORF5 PRRSV sequences circulating in Mexico with US PRRSV L1C-144 (Genbank MW 525343).

Materials and Methods

117 PRRSV ORF 5 sequences were obtained from pigs blood serum, oral fluids, processing fluids and lungs all of them positive to PRRSV by RT PCR from 2016 to 2021, and corresponding to Mexican states: Puebla (4), Guanajuato (11), Jalisco (16), Michoacán (3), Querétaro (15), Veracruz (2), Sinaloa (2) and Sonora (63) (Figure 1). The average sample size was 30 per farm, obtained on different stages of production: weaning, development, growing, fattening, sows, gilts and boars. PCR products were sequenced using Ion Torrent PGM platform. Obtained sequences were submitted in GenBank, phylogenetic analysis comparing the reference sequence for PRRSV L1C-144 (GenBank MW 525343) was performed with MEGA 7.

Fig 1. Distribution of PRRSV ORF5 sequences in Mexico

State	Sample	Year	Lineage	RFLP
Puebla	4	2019	5,8	ND
Guanajuato	11	2019-2020	1,8	ND
Jalisco	16	2019-2021	1,5,8	163, 164, 252, 134, 181
Michoacán	4	2019-2020	8	123
Querétaro	15	2016-2021	1,5,7,8	173, 163, 124, 252, 131
Veracruz	2	2021	1	ND
Sinaloa	2	2020-2021	2	1104, 154
Sonora	63	2016-2021	1,5,7,9	174, 1262, 144, 252, 143, 112, 1982, 1262, 1134

ND: undeterminad

The radial phylogenetic tree was constructed using TreeView (Figure 2) and genetic distances (%) between PRRS L1C-144 sequence and all sequences obtained from Mexico, were visualized using a temporal graph with MINITAB (Figure 3).

Results

53.8% of the analyzed sequences were from Sonora, 7 of them RFLP 144 and grouped within lineage 1. However, none of them showed similarity with PRRSV L1C-144. The remaining states had strains corresponding to lineages 1, 2, 5, 7, 8 and 9 (figure 2).

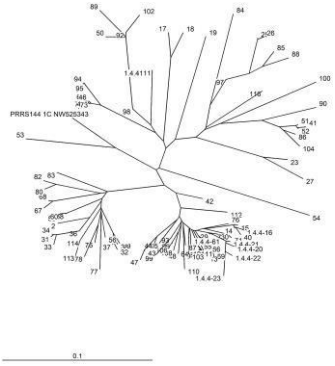
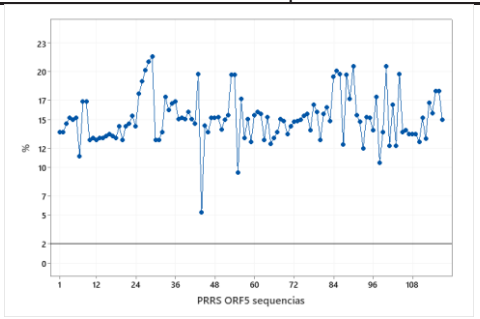


Fig. 2 Phylogenetic tree of 117 PRRSV ORF5 sequences and the reference sequence PRRSV L1C-144

Genetic distance analysis of PRRSV L1C-144compared to 117 sequences analyzed and collected between 2016 to 2021 in Mexico showed a minimum distance of 5.3% and a maximum of 21.6%.

Fig. 3 Comparison of the reference sequence PRRSV L1C-144 PRRSV with 117 sequences from Mexico



Discussion and Conclusion

Considering a cut-off point of ≤2% in genetic difference to determine the same variant of the virus in ORF5 PRRSV sequences, it can be suggested that the PRRSV virus L1C-144 was not identified in the samples obtained from Mexico and analyzed in this study. This analysis suggests evidence of no presence of PRRSV L1C-144 variant among the group of samples, but it is important to continue with active epidemiological surveillance. It is important to emphasize that good biosecurity measures are considered to be the best way to prevent the introduction of new PRRSV strains in pig farms

Acknowledgments

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Comparative Survival of Different Strains of Porcine Reproductive and Respiratory Syndrome Virus at Different temperatures

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSv) is the causal agent of one of the most important endemic diseases in the North American swine industry (1). There are two major prototypes of PRRSv, the European isolate (type 1) and the North American isolate (type 2) (2). It has been described that this virus can survive outside the host, which is related to temperature and relative humidity, increasing the risk for indirect transmission (3). Simultaneous outbreaks due to type 2 strain 1-4-4 lineage 1C has raised the concern about possible survivability features that may contribute to the dissemination of PRRSv (4). In this study, we assessed the survivability of ten different strains of PRRSv at 4°C, room temperature (~25°C) and 37°C.

Materials and Methods

The following strains were evaluated in this study: a Lelystad isolate (European type) and nine North American isolates classified by restriction fragment length polymorphism (RFLP) as 1-7-4, VR2332, 1-4-2, 1-26-2, 2-5-2, Ingelvac vaccine, 1-4-4 MN, 1-4-4 SD, and 1-8-4. The viruses were propagated and titrated in MARC 145 cell line.

For each strain, three 24-well plates were labeled appropriately. Then, the virus was applied to the bottom of all wells (100µl of virus/well) and the plates were air-dried for 4 hours. Subsequently, one plate each was placed in the fridge at 4°C, on the bench at room temperature (~25°C), and in the incubator at 37°C. Thereafter, the surviving virus was eluted (from 3 wells each) at the following time points: after 4 hours, day 1, day 3, day 7, day 14, day 21, day 28 and day 35.

For each time point, three wells were eluted using 200µl of elution buffer (3% beef extract-0.05 M glycine) per well. Serial 10-fold dilutions of all eluates were prepared in MEM and inoculated in monolayers of MARC 145 cells prepared in 96-well microtiter plates. The plates were incubated at 37°C under 5% CO_2 and were examined daily under an inverted microscope for the appearance of cytopathic effects (CPE). The 50% end points were calculated after 7 days of incubation. Virus titers were calculated using the Karber method and expressed as $\log_{10}$ $TCID_{50}$ per 100µl (5).

Results

Figures 1-3 show the survival of seven PRRSv strains at three different temperatures. All results are expressed as $\log_{10}$ $TCID_{50}$/100µl.

Discussion and Conclusion

All seven strains of PRRSv survived for at least 35 days at 4°C. Although all of them survived for 35 days at 4°C, their titers decreased by 2 $\log_{10}$ (1-7-4; VR2322; 1-4-2; and Lelystad) to 5 $\log_{10}$ (1-26-2; Ingelvac; 2-5-2). This indirectly indicates that 1-7-4; VR2322; 1-4-2; and Lelystad strains are more resistant in the environment than the other three strains. However, all seven strains survived only between 1 and 3 days at 25°C and 37°C. The results for strains 1-4-4 MN, 1-4-4 SD and 1-8-4 will be shared at the conference.

Figure 1. Comparative survival of seven PRRSv strains at 4°C.

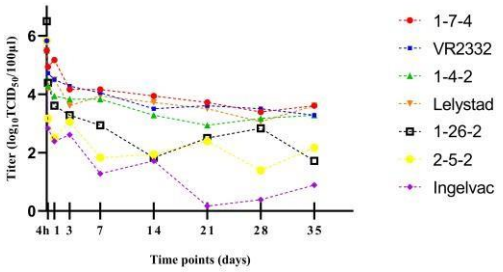


Figure 2. Comparative survival of seven PRRSv strains at room temperature (~25°C).

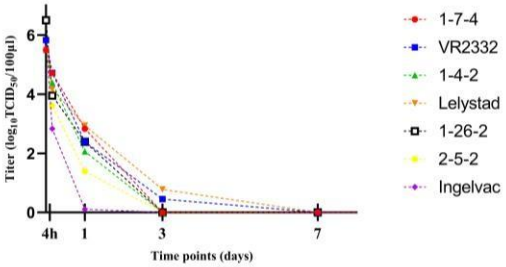
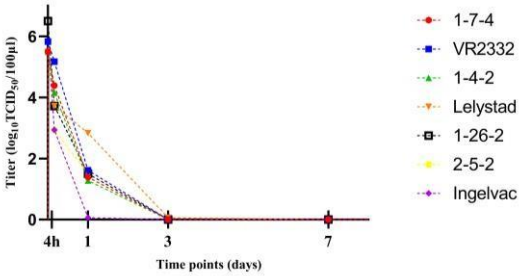


Figure 3. Comparative survival of seven PRRSv strains at 37°C.



Acknowledgments

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Comparing the Efficacy of Porcine Circovirus 2 Vaccines in Reducing Viremia Titer

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Introduction

Porcine circovirus 2 (PCV2) infection is commonly associated with a group of complex multi-factorial diseases classified under the umbrella term of Porcine circovirus associated diseases (PCVAD) (1). It has been shown that PCV2 viremia load correlates with disease severity and as a result, negatively impact zootechnical parameters such as average daily gain (ADG) in a load dependent manner (2,3,4,5). An efficacious PCV2 vaccine should be able to reduce viremia titer over the production lifetime of pigs.

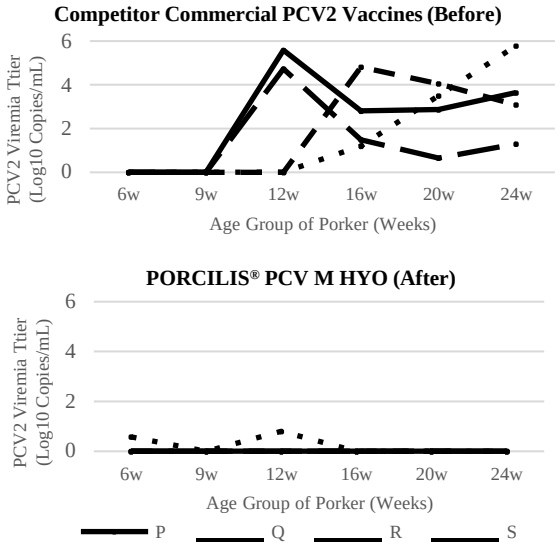
Material and Methods

Field trial was conducted in four selected commercial pig farms: Farm P, Q, R, and S. All four farms were practicing farrow-to-finish operation with 200 – 2,000 sows and husbandry practices typical of pig production in Malaysia. PCV2 vaccination was routinely done using existing competitor commercial PCV2 vaccines. PORCILIS® PCV M HYO vaccination regime was introduced to the farms via intramuscular injection in the neck region of 3 weeks old piglets. Serum samples were collected from the porker herds before and after 12 months of practicing PORCILIS® PCV M HYO vaccination regime in the farms. Ten serum samples were collected from each age group: six, nine, 12, 16, 20 to 24 weeks of age. Serum viremia titer was determined by qPCR (Intervet International B.V., Netherlands), whereas viremia titers over production lifetime was expressed as area under curve (AUC) values. To test for statistical association between PCV2 viremia titers before and after using PORCILIS® PCV M HYO, paired sample t test were performed with significance level set at $p < 0.05$.

Results

When farm routine PCV2 vaccination was done using existing competitor commercial PCV2 vaccines, viremia was detected across all four farms. Viremia was detected as early as nine weeks old, up until finisher stage of 24 weeks old with titers ranging from 0.6 – 6.0 $\log_{10}$ PCV2 virus copies / ml serum (Figure 1). After 12 months of practicing PORCILIS® PCV M HYO vaccination, Farm P, Q and R successfully achieved viremia free status across all tested age groups. Although viremia was still present in Farm S, the detected titer decreased markedly to 0.8 $\log_{10}$ PCV2 virus copies / ml (Figure 1). PCV2 viremia titers before and after using PORCILIS® PCV MHYO were statistically significant (t: 0.00066; $p < 0.05$).

Figure 1. Viremia titer detected in farms before and after 12 months of practicing PORCILIS® PCV M HYO vaccination regime



When viremia titer over the production lifetime of pigs was expressed as AUC values, the PORCILIS® PCV M HYO group clearly demonstrated reduction of viremia severity (Table 1).

Table 1. Area under curve (AUC) to quantify PCV2 viremia titer over pig production lifetime.

PCV2 Vaccine \ Farm	Area Under Curve (AUC)			
	P	Q	R	S
Competitor	49.5	27.7	41.6	30.3
PORCILIS® PCV M HYO	0	0	0	4.5

Discussion & Conclusion

PCV2 viral load in serum has been shown to be correlated with the severity of PCV2 disease manifestation (2,3,5). Further, PCV2 viremia has been shown to negatively impact the average daily gain (ADG) of porkers in a load dependent manner. The higher the serum PCV2 viremia titer, the lower the ADG of pigs (4). Hence, it is important to include herd PCV2 viremia titer as one of the aspects in evaluating field efficacy of PCV2 vaccine. In this field trial, PORCILIS® PCV M HYO proved to be an efficacious vaccine that is able to reduce PCV2 viremia titer over the production lifetime of pigs.

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Comparison of probability of detecting African Swine Fever virus among different sampletypes in the farrowing rooms

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Introduction

A rapid and consecutive 21 month increase of the breeding sows inventory to levels same to that prior ASFV outbreak in China had proved the success of the test-and-removal method in ASF control and elimination in infected herds (1). This success is partly attributed to the profound understanding of characteristics of the Georgia strain of ASFV, including early detection of ASFV DNA prior or almost same time of the onset of clinical signs (2,3), which allows early identification of infected animals and subsequent precision removal.

However the emergence of an attenuated ASFV strain causing subclinical infection was reported in late 2020(3). This poses new challenges to the early detection of the attenuated strain by the well developed within herd test removal method. In order to improve chances of early detection of the low virulent strains, we compared the probability of detection of ASFV DNA by PCR of different samples at weaning and ran simulation of different probabilities of detection at different within herd prevalence.

Materials and Methods

An attenuated ASFV-infected herd was identified. Thirty-two litters within a 120 crates farrowing room were conveniently selected to collect each of the sample types. From each litter, sow precaval vein serum (SS) samples, sow oropharyngeal (OP) swabs, family oral fluid (FOF), individual piglet precaval vein serum (PS), aggregated environmental (AE) samples refering to pooled samples of udder swabs and swabs of feeders and drippers at the time of 3 days before weaning were collected. Each sample was collected in a biosecure manner, i.e to change gloves and collectors for each sample. DNA extraction and qPCR was performed using a DNA extraction machine Gene Pure Pro 96 from Bioer company (Hangzhou, China) and the MRD company (Beijing, China) according to the manufacturer’s instructions. Data was collected and analyzed using SAS.

Results

1. **Detection of ASFV DNA by qPCR in different sample types.** Of all 32 litters, there were 30 (93.75%) PS, 22 (68.75%) AE, and 21 (65.63%) FOF samples testing PCR-positive. For individual samples, 21 (65.63%) SS, 14 (43.75%) OP swabs, and 7 (21.87%) TB samples were positive (table 1).

2. **Estimating probabilities of detecting ASFV in different within-litter prevalence scenarios**. Probabilities of detecting attenuated ASFV under different within-litter prevalence were predicted. As shown in figure 1, pooled PS samples had the highest probabilities of nearly 75% at low within litter prevalence, followed by SS samples (42%), FOF(36%) and AE(36%), OP (15%) and TB (12%). When prevalence reached almost 100%, predicted probabilities of detection for PS serum was 93.75%, followed by SS samples (83%), FOF(78%) and AE(78%), OP (39%) and TB (28%).

Discussion and Conclusion

Our result was consistent with previous reports that viral DNA of attenuated Genotype II can be detected in blood for 76 days, while that can be detected in OP for 52 days with an intermittent mode(3). This gives blood samples comparative advantage over other sample types of detecting ASFV.

As predicted, pooled PPSB samples had the highest probabilities of detection by nearly 75% at low within litter prevalence. This can be due to the long-term presence of viral DNA in blood and larger volume of samples. Because of difficulty in sampling PS samples, individual samples of SS, or group samples such as FOF and AE or a combination of all these might be an alternative in field practice. This requires further research.

In summary, our study provided useful information in how to early detect low virulent Avirus in the herd.

Table 1. Detection of ASFV DNA by qPCR in differentsample types.

	Tail Blood	OP swab	SS	PS	FOF	AE samples
No. of Pos	7	14	21	30	21	22
Positive rate	21.87%	43.75%	65.63%	93.75%	65.63%	68.75%

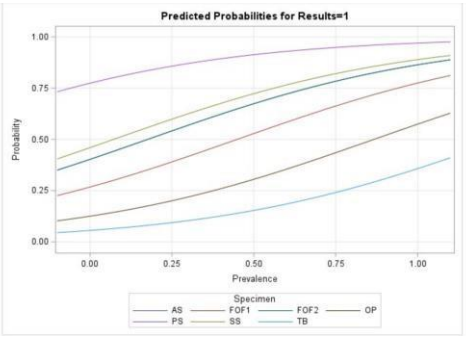

Figure 1: Probabilities of detecting A-virus by qPCR if present in samples by within-litter prevalence.

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Comparison of PRRS MLV vaccination efficacyin a low post-weaning mortality farm in Japan

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Introduction

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is one of the major pathogens in pigs and has a significant economic impact on the swine industry worldwide (1). PRRS MLV vaccination has been demonstrated as an effective tool for not only reducing clinical symptoms but also improving production losses. The objective of this study was to investigate the efficacy of PRRS vaccination in a low post-weaning mortality farm in Japan.

Materials and Methods

This study was conducted in a farrow to finish swine farm with 1,000 sows. Although this farm was seropositive for PRRSV at around 90 days of age, post-weaning mortality was managed at a low level without PRRS vaccination for piglets. To investigate whether PRRS MLV vaccination affects production performance or not in this situation, piglets were intramuscularly administered a shot of Ingelvac® PRRS MLV (Boehringer Ingelheim Animal Health Japan Co., Ltd.) 2ml at 21 days of age. This study was conducted from Jul 2018 to Jun 2020, and piglets were divided into two groups, non-vaccinated from Jul 2018 to Jun 2019 (control group) and vaccinated from Jul 2019 to Jun 2020 (vaccinated group). Post-weaning mortality, average daily weight gain (ADWG), the number of treatments by injection (times/month) and estimated injection cost were compared between these two groups. Statistical analysis was implemented by Chi-square test and Student's *t-test*, respectively.

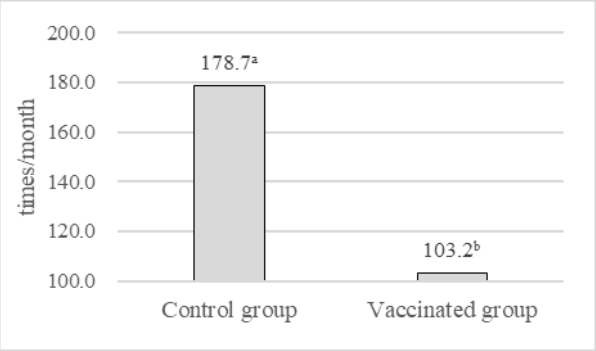
Results

The vaccinated group showed lower mortality than the control group, 2.9% vs 3.1%. ADWG was almost equal between the two groups (Control group: 730, Vaccinated group: 731). On the other hand, statistical significance in the number of treatments by injection was shown between the control group and the vaccinated group, 178.7 and 103.2, respectively (Figure 1). Furthermore, the vaccinated group showed less monthly variability than the control group (Figure 2). As a result, the estimated injection cost for the vaccinated group was reduced to 43.6% of the control group based on production parameters.

Discussion and Conclusion

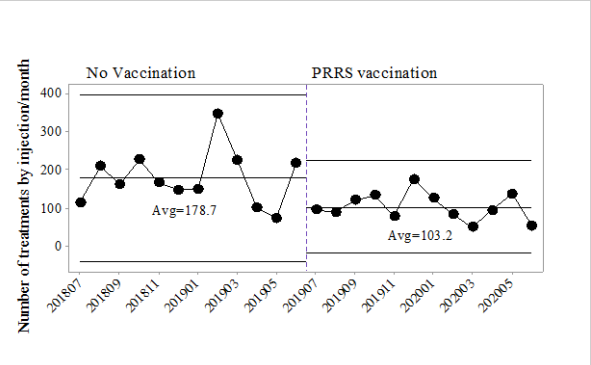
PRRSV is one of the main pathogens for porcine respiratory disease complex (PRDC) and would cause secondary infection by other pathogens on the respiratory tract. This study suggested that PRRS MLV vaccination for piglets leads to the reduction of clinical symptoms which care workers can detect on farm. Additionally, reduction of the estimated injection treatment cost may reduce not only medication cost, but also care workers' treatment-related labor, allowing them to have more time to observe their pigs carefully. Furthermore, there was no statistical significance in post-weaning mortality between the two groups, but if fattening pigs were shipped based on 0.2point improvement, estimated profit would be generated in such amount of sows. In conclusion, this comparison of before and after vaccination suggests that PRRS vaccination for piglets may lead to a profit in swine farm management, even on farms with low post-weaning mortality.

Figure 1. Number of treatments by injection (times/month)



a, b: different character shows statistical significance ($p<0.05$)

Figure 2. Monthly records of the number of injections (I-MR chart)



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Comparison of two ELISA tests for detection of serum antibodies against PRRSV and theiruse in routine monitoring testing programs

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) continues to be one of the most economicallysignificant diseases affecting the swine industryworldwide. In the US alone, its impact has been estimated at $4.67 for every pig marketed (1). Routine monitoring of the antibody response against PRRSV is essential to understand disease dynamics and optimize control measures in infected farms.

This study investigates the sensitivity of two PRRSV ELISA tests using serum samples from pigs of known status and compares their use for monitoring purposes.

Materials and Methods

A total 182 serum samples were used to evaluate the sensitivity of two commercially available PRRSV antibody ELISA tests. Samples originated fromPRRSV vaccinated pigs or from PRRSV fieldinfection cases. Serum samples were tested in thesame laboratory with (i) ELISA-1 [PRRS X3 Ab Test,IDEXX Laboratories Inc.] and with (ii) another commercial PRRSV indirect ELISA (ELISA-2), according to the manufacturer's recommendations.Results were calculated as sample to positive ratio (S/P). A linear regression analysis was used to study the correlation of S/P values between both ELISA tests.

Results

One hundred and eighty-two samples tested with ELISA-1 produced valid results that could be converted to S/P values. However, 16.5% of the samples (30 out of 182) tested with ELISA-2 produced invalid results (ELISA detector was oversaturated during reading) and S/P values could not be calculated.These 30 samples were excluded from further analysis. ELISA-1 correctly detected all the characterized samples as positive (sensitivity 100%). ELISA-2 failed to detect 8.5% (13 out of 153) of positive samples (sensitivity 91.50%). Average S/P value, standard deviation, and coefficient of variation (CV%)for both ELISA tests are shown in Table 1. Non- perfect correlation was found between both tests (R^2= 0.79, $p<0.05$).

Analysis of the CV% according to ELISA-1 S/P value groups (S/P 0 to <1, S/P 1 to <2, S/P 2 to <3, S/P ≥3) showed a higher variation of results for ELISA-2 in every group (Table 2).

Table 1. Average S/P value, standard deviation (SD), coefficient of variation (CV%) and minimum and maximum S/P values for PRRSV positive serum samples tested with two ELISA tests.

	ELISA-1	ELISA-2
Average S/P	2.29	2.93
SD	0.82	1.48
CV%	36%	51%
Minimum S/P value	0.45	0.19
Maximum S/P value	3.96	5.16

Table 2. Coefficient of variation (CV%) by S/P value groups for PRRSV positive serum samples tested with two ELISA tests.

S/P group	ELISA-1	ELISA-2
0 to <1	23%	50%
1 to <2	18%	47%
2 to <3	11%	29%
≥ 3	8%	25%

Discussion and conclusion

Monitoring programs for PRRSV require ELISA antibody tests with a good dynamic range but without excessive variation in the S/P values, for reproducible results that allow a better interpretation of the situationin the farm. Overall, ELISA-1 showed an excellent dynamic range with lower variability than ELISA-2. The reduced sensitivity of ELISA-2, 91.5% in thisstudy, has been described before (2) and could pose arisk for accurate detection of positive samples,particularly in outbreak situations.

The high rate of invalid results showed in this study with ELISA-2 (16.5%) was unexpected. This finding adds uncertainty to routine monitoring programs interpretation based on ELISA-2 testing and increases costs due to retesting and/or additional samplings to satisfy program requirements for sample size.

Under the conditions of this comparative study, ELISA-1 showed better sensitivity and demonstrated to be suitable for routine PRRSV antibody monitoring programs.

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Control of PRRS Using PORCILIS® PRRS in 3 Koreans Commercial Farms

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Introduction

Mixed PRRSV infection with both NA and EU strains of PRRSV is common in Asia. In such a situation, producers face difficulty selecting between NA or EU vaccines. The use of multiple PRRSV live PRRSV vaccines on farm may increase changes of reversion to virulence or recombination mutants arising1. PRRSV viremia is correlated with decreased zootechnical performance and increased incidence of respiratory lesions2. Monitoring the PRRSV viremia status of the farm after vaccination with PRRSV vaccines is therefore a good way to evaluate the efficacy. We performed this study to assess if Porcilis PRRS can be used to stabilize farms with PRRS NA/EU mix-infection by sow vaccination.

Materials and Methods

The study was carried out in South Korea and involved 3 commercial farrow to finish pig farms with active virus transmission from sows to piglets and viremia in the nursery. Only the farms implementing an immunization program using Porcilis PRRS were included in the present study. Vaccine used in this study was Porcilis PRRS vaccine (manufacturer: MSD Animal Health, Boxmeer). Blood samples were obtained from 20, 40, 70, 100 & 130 day-age old pigs, as well as breeding animals (sows & gilts). Serological (ELISA) and virological (PCR) tests were performed on blood samples from different age groups to determine the status of infection. PRRSV ELISA tests from pig sera were carried out applying the IDEXX PRRSV ELISA Kit (IDEXX, USA) according to the recommendations of the manufacturer. RNA from serum samples was extracted and PCR was performed by real- time PCR machine. Samples positive by PCR were subjected to sequencing. The viral ORF5 was sequenced preferably.

Sequencing was performed with the Sanger method on ABI 3500 sequencer (Applied Biosystems). Chromatograms were analyzed and edited manually using the BioEdit software version 7.2. In this study, PRRSV ORF5 sequences (606 nt) were analyzed.

Sequence analysis was performed using the “similarity network” diagram to identify the closest, most similar sequences to reference strains. Subsequently, the percentile similarity of these sequences to ORF5 was evaluated relative to that of the PRRSV reference strain in GenBank.

Results and Conclusion

In Farm 1 and Farm 2, the herd remained unstable but the overall antigen detection rate decreased after Porcilis PRRS vaccination. It was confirmed that stabilization was achieved in Farm 3 as antigens were not detected at 20 and 40 days age-old after vaccination. As a result of the productivity in the test farms, there was little significance between the PRRSV fluctuation and the herd productivity. These means that actual productivity could be affected by complex factors, such as breeding management, seasonal factors, and other diseases including PRRSV infection. If combined with herd management strategy improvements such as biosecurity, we found that Porcilis PRRS can be applied to farms with mixed PRRSV infection and can be effective in stabilizing mixed PRRS infected farms.

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Current trends and patterns of PEDV in the United States

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Introduction

Since the initial Porcine epidemic diarrhea (PED) epidemic in 2013, PED virus (PEDV) has persisted in the U.S. breeding herd with low incidence during the post-epidemic period. There is minimal research and understanding of the endemic phase of PEDV in the country. During the epidemic, different publications cited several risk factors associated with the rapid spread of PEDV (animal movements,truck contamination, slaughter plant contamination, contaminated feed and feed-mills among others)[1]–[3]. However, after containment of the epidemic (around mid-2014) minimal research has been published on the endemic state of PED in the U.S. Our objective was to characterize PED in the U.S. breeding herd in the post epidemic period (spatial-temporal distribution of cases and associated factors).

Materials and methods

We used data from 1100 breeding farms in 27 states, whose PED statuses were routinely reported to the Morrison Swine Health Monitoring Project (MSHMP) between July-2014 and June-2021. We stratified the data into six regions over which mixed-effects logistic regression analyses and spatial-temporal analyses were done.

Results

625 PEDV outbreaks were recorded on 373 farms. The total number of farms breaking annually reduced from 95 farms in 32 counties between July-2014 and June-2015 to 53 farms in 28 counties July-2020 and June-2021. The mean incidence risk declined from 8% ± 7% (July-2014 & June-2015) to5% ± 3% (July-2020 & June-2021). Outbreaks wereseasonal, with most outbreaks occurring during winter (January - March – p = 0.001, relative risk =2.2). Ten spatial-temporal clusters of PED cases (p < 0.05), spanning 2.5 km2 to 833.7 km2 and 1-5 months, were recorded in four regions. The occurrence of PED cases on farms was associated with county-farm-density, with farms located in medium-density counties (0.013-0.031 farms/km2) twice more likely to experience outbreaks than farms in low-density counties (< 0.013 farms/km2) (p < 0.001) (Figure 1).

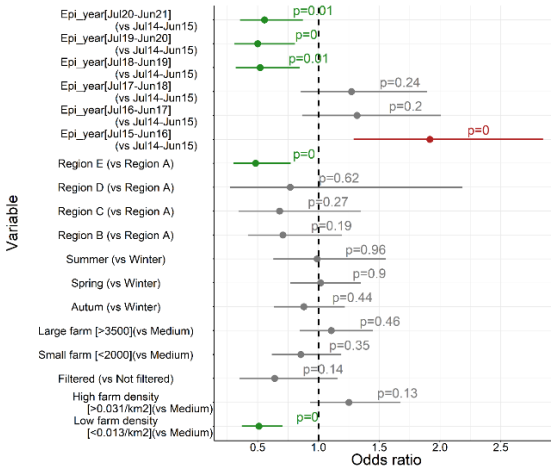

Figure 1: Summary of mixed effects logistic regression analysis controlling for different risk factors for PEDV occurrence in the U.S. *(color code: green – significant negatively associated factors, red – significant positively associated factors, grey – factors not significantly associated with PEDV occurrence)*

Discussion and conclusion

The overall decline in PED cases over the years andthe decrease in spatial extent likely reflects ongoingefforts employed by production systems to control PEDV during the post-epidemic period. This presents an opportunity for concerted and coordinated efforts to understand PEDV dynamics in nursery and growing herds and initiate strategic steps towards regional and subsequently national elimination of PED in the U.S.

Acknowledgements

We thank the MSHMP team and participating pig production systems for data sharing and curation. This study was partially funded by the Swine Health Information Center (SHIC).

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Daily monitoring and analysis of African swine fever virus in a large-scale pig farm in China

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Introduction

In the post-ASF era, more and more large-scale pig farms in China have established their ASF testing laboratories to assess the loopholes in the existing biosecurity system.

Materials and Methods

1. Sampling method: Sampling of personnel, materials, environment, and vehicles is carried out by using sterile gauze dipped in physiological saline and then wiping the surface.
2. Nucleic acid extraction: (1) Take a centrifuge tube (numbered according to the corresponding sample), take 400 μl of the liquid sample, centrifuge for 10-30 seconds, and directly take 200 μl of the supernatant as the sample to be tested. (2) Take a clean 1.5ml centrifuge tube, add 200μl of the sample to be tested, add 20μl proteinase K and 500μl buffer J1 in turn, mix up and down (6-8 times), and incubate at room temperature for 5 minutes, that is, the sample lysis mixture. (3) Take the adsorption column and the collection tube (the adsorption column is placed in the collection tube), suck all the sample mixture into the adsorption column, cover the tube, centrifuge for 10-15 seconds, discard the liquid in the collection tube, put the adsorption column Put it back into the collection tube. (4) Open the cap of the adsorption column, add 700 μl of buffer J2, close the cap, centrifuge for 10-15 seconds, discard the liquid in the collection tube, and put the adsorption column back into the collection tube. (5) Carefully open the cap of the adsorption column, add 700 μl of buffer J3, close the cap, centrifuge for 10-15 seconds, discard the liquid in the collection tube, and put the adsorption column back into the collection tube. (6) Centrifuge again for 10-15 seconds, discard the collection tube, and put the adsorption column into a new 1.5ml centrifuge tube. (6) Carefully open the cover of the adsorption column, drop 50 μl of buffer J4 into the middle of the adsorption membrane, cover the cover, stand at room temperature for 1 minute, centrifuge for 10-15 seconds, discard the adsorption column, and cover the centrifuge tube cap, the liquid in the tube is the nucleic acid of the sample to be tested.
3. PCR: use the African swine fever virus detection kit to detect the African swine fever virus.

Results

Positive rate	June	July	August	September	October	November	December	Annual
Personnel	0.00%	12.73%	1.01%	3.48%	3.17%	2.79%	0.87%	2.78%
Materials	0.00%	0.00%	0.00%	0.00%	3.80%	1.23%	1.89%	1.23%
Surroundings	0.00%	0.00%	0.00%	0.00%	1.75%	0.00%	0.00%	0.24%
Pig	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Vehicle	0.00%	0.00%	0.00%	0.00%	4.60%	0.00%	1.04%	1.37%
Total	0.00%	2.65%	0.27%	1.53%	3.13%	1.07%	0.74%	1.37%

From June to December, a total of 2,928 samples were tested. The positive rate of the test data was ranked in order: personnel, vehicles, materials, environment, and pigs. Among them, because it is a new pig farm, there are few foreign pigs pulling vehicles, and the frequency of testing is low, but the positive rate of foreign pig pulling vehicles is still thehighest; the feed truck has been tested for half a year,and the number of positive detections is zero; Amongthe several types of vehicles involved in the site, the ranking of positive rates is: foreign pig-pulling vehicles, purchased vehicles, internal transfer vehicles, and feed vehicles. In terms of the overall testing and testing frequency: the ranking of risk factors is: foreign pig-pulling vehicles, personnel (thepositive rate detected by foreign equipment maintenance personnel is much higher than that of furloughed employees), vehicles, materials (vegetables purchased from the vegetable market) The positive rate detected is far greater than that of express), pigs.

Conclusions and Discussion

Each pig farm can share the detection status of its pigfarm and set a warning line for the positive rate. For example, the positive rate in October was greater than 3%, indicating that there were many loopholes in biosafety; it was mainly due to a large number of procurement of materials and the cross-contamination of procurement personnel, office personnel, and isolation personnel in public areas. In November, we began to standardize procurement, stagger the contact of the three types of personnel,and at the same time provide special isolation rooms and did a good job in disinfection and biosafety training in public areas, and the positive rate dropped directly.
Through daily monitoring, we can find loopholes in biosecurity, ensure that all incoming material personnel is negative, and reduce the risk of infection.The daily detection of ASF is very important in the post-ASF era.

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Description of a new recombinant clade within the subtype 1 of Betaarterivirus suid [1] (PRRSV1) causing severe outbreaks in Spain

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Introduction

This report describes 28 complete genomes of a new recombinant clade within *Betaarterivirus suid 1* (porcine reproductive and respiratory syndrome virus 1 (PRRSV1) (1). PRRSV1 is one of the most important pathogens of pigs and cause serious economic losses (2). Within PRRSV1 at least 3 subtypes are recognised. Subtype 1 is the most widely distributed (3), while subtypes 2 and 3 were described in Western Siberia and Eastern Europe, respectively (4). Within each subtype the genetic diversity is considerable. Recombination is an important evolutionary mechanism in RNA viruses. For PRRSV1, recombinant strains have been repeatedly reported along European countries, occasionally involving different Modified Live Virus (MLV) vaccines.

Most often, sequencing of viral ORF5 is used to monitor PRRSV infection is pig farms. However, recombination may involve any viral segment and thus, requires another sequencing approach, namely full genome sequencing.

Materials and Methods

Sera samples were collected between February and November 2021from sows and piglets in 11 breeding farms located in Catalonia (NE Spain) and owned by different companies. All farms were suffering severe PRRS outbreaks characterized by high abortion rates, mortality in sows and increased mortality in weaners and growers (>20%). Complete genomes were obtained after isolation of PRRSV1 in porcine alveolar macrophages (PAMs), applying a tailor-made NGS protocol (5). Briefly, the total RNA, extracted with Trizol, is directly sequenced without using primers.NGS reads are trimmed and mapped against a reference genome. Sequences are available in GenBank with the Accession Numbers OM893828 to OM893855. Obtained sequences were compared with other PRRSV1 sequences available in Genbank as well as in the database of the Veterinary Laboratory for the Diagnosis of Infectious Diseases of the *Universitat Autònoma de Barcelona*. Phylogenetic analyses were performed using MEGA X. Detection of recombination was performed using the RDP 5 software and GARD (www.datamonkey.org/GARD).

Results, Discussion and Conclusion

The analysis of the sequences, revealed that all farms affected by the severe PRRS outbreaks were infected by different variants of the same strain. Recombination analyses based on RDP and GARD revealed a common pattern among all isolates. A similarity analysis of the recombinant segments with BLAST identified an Italian highly pathogenic strain (6) as the major parental sequence (MF346695-PR40/2014, 80% of the genome in 5 segments, with a 86-89% nucleotide identity), and three minor parental strains: KC862570-Olot-91 (11% of the genome in 2 segments with a 86% nucleotide identity), KY434184-D40 (7% of the genome in a singlesegment with a 86% nucleotide identity), and an unknown strain (2% of the genome in a single segment with a nucleotide identity lower than 85%).

A maximum likelihood (ML) phylogenetic tree placed all the genomes in a monophyletic branch within PRRSV1 subtype 1, pointing to a single event in the origin of all recombinant strains. Besides, the epidemiologic insights available suggest that thisrecombinant strain, that has been circulating in Spain since January-February 2020, is expanding and becoming epidemic.

To sum up, the strains isolated in all those outbreaks, characterized by a high virulence, consistently shows evidence of recombination with a highly virulent Italian strain and other subtype 1 strains. Its apparent greater pathogenicity should be evaluated in future studies.Also, the regular detection of recombinant PRRSV strains illustrate the plasticity of this virus, together withthe need to use whole genome sequences as analytical tool.

Acknowledgments

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Detection and correlation of PCV2 by PCR and IHC in tissues in a PCV2d/PRRSV challenge model in the new paradigm of PCV2d infection

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Introduction

Porcine circovirus type 2 (PCV2) is economically significant, highly prevalent, and the cause of porcine circovirus-associated disease (PCVAD). Lymphoid depletion and histiocytic inflammation are lesions of PCVAD, which are often exacerbated by co-infection (1). Therefore, PCVAD diagnosis should consider the clinical context and presence of lesions and besupported by laboratory testing. However, the dynamics of virus replication, tissue localizations during infection,stage of infection, particular animal(s) sampled, sampletype(s), diagnostic techniques applied, and vaccination status can compromise accurate diagnosis. Increased PCV2d prevalence and vaccinated herds reporting morePCVAD cases and/or greater viral detection questions vaccine efficacy (2). The objective was to evaluate the relationship of sample types, duration of infection, and results of different diagnostic techniques in vaccinated and unvaccinated pigs in a PCV2d/PRRSV co-infection model under field conditions.

Materials and Methods

On study day 0 (D0), 100 pigs from a PRRSV/Mhp negative, PCV2 low prevalence, commercial sow farm were weaned and vaccinated with Ingelvac PRRSV MLV. Eighty of the 100 pigs also received commercial PCV2 vaccine (Vacc), leaving 20 pigs as unvaccinated controls (NoVacc). On D28 (49 days-of-age), all pigs were inoculated with 1 mL IM and 1 mL IN of PCV2d (5 $\log_{10}$/2ml dose) as well as 2 mL IM of 1-7-4 PRRSV (4.0 TCID50/mL). Blood samples were collectedweekly for PCR and ELISA for both PCV2 and PRRSV. At termination (D56), tissues were collected (tonsil, lung, lymph nodes) for histopathology, IHC and PCR.

Results

The challenge resulted in mortality of 60% NoVacc and 9.09% Vacc prior to D56. Deceased NoVacc before D56 had histopathology average lesion scores of 3 (severe inflammation and lymphoid depletion) and IHC scores of 3 (>50% positive cells) in tonsil and lymph node, compatible with severe PCVAD. Vacc IHC and lesion scores were 1-2 (10-50% positive cells and mild-moderate inflammation/depletion). Vacc necropsied at D56 had average lesion scores of 0-1 (none-minimal inflammation/depletion) and IHC scores 0-1 (<10% positive cells). NoVacc had average scores of 3 (severe) for histological lesions and IHC. The average PCR Ct values on lung, lymph node, and tonsil in NoVacc were 8.1, 8.4, and 9.9, respectively whereas Vacc had significantly higher Ct values of 25.4, 22.6, and 23.5, respectively. Animals challenged with PCV2 had positive correlation of the IHC score and PCR Ct values in lung, lymph node, and tonsil. However, the individual animal evaluation by vaccination status demonstrated that the lack of vaccine drives the correlation between IHC score and PCR Ct due to a strong positive correlation in NoVacc and a linear correlation in Vacc (Figure 1). The Ct values in feces varied from 18.7 to 24.1 between 46 and 56 DPI in NoVacc and 25.6 and 27.6 in Vacc. Viral load in serum was consistently 2-3 logs higher in NoVacc compared to Vacc.

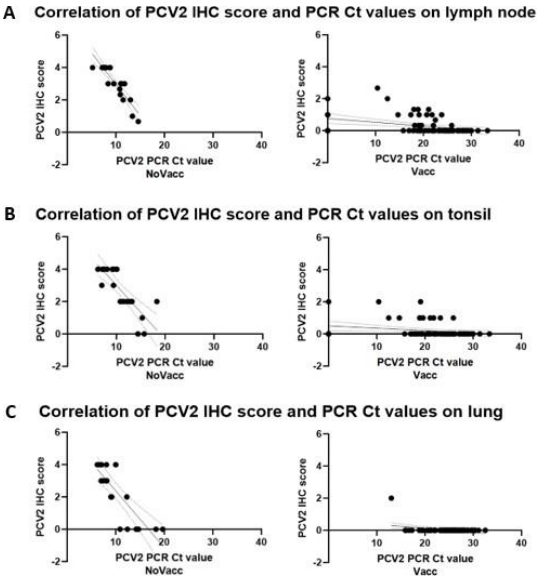

Figure 1. Comparison of diagnostic correlation of antigen detection by IHC and nucleic acid detection by PCR in tonsil (A), lymph node (B), and lung (C) in NoVacc (left) and Vacc (right).

Discussion and Conclusion

Experimental co-infection by inoculation with contemporary PCV2d/PRRSV isolates resulted in high mortality and severe PCVAD. Co-infection can affect PCVAD diagnosis, but NoVacc and Vacc present a different viral detection threshold in tissues and serum. In NoVacc, the correlation of viral detection by PCR and *in situ* detection by IHC seems to be less affected by tissue type than by vaccination status. NoVacc had strong IHC positivity within an average Ct of 10, whereas Vacc had less positive staining and an average Ct of 23.3. Viral shedding in feces was higher in NoVacc for several weeks post-infection but is not reliable for diagnosis of PCVAD. Death was highly correlated to viral load confirmed by both IHC and PCR. Continuing production impact of PCV2 on survivors is likely less in Vacc than in NoVacc, with variation influenced by particular and present ongoing co-infections.

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Detection and differentiation of PCV2 and PCV3 using a multiplex real-time PCR test

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Introduction

Porcine circovirus type 2 (PCV2) is an economically important pathogen that has been associated with a broad range of clinical diseases including PCV2 systemic disease (PCV2-SD), respiratory and enteric disease, reproductive failure, and porcine dermatitis and nephropathy syndrome. PCV2-SD and subclinical infections are highly prevalent worldwide. Infection of pigs with porcine circovirus type 3 (PCV3) is thought to be linked to porcine circovirus diseases-like scenarios (1). PCV3 has been identified in most pig-producing countries and retrospective studies have shown that it has been circulating in swine populations for decades.

This study reports on the detection and differentiation of PCV2 and PCV3 DNA using a multiplex real-time PCR and compares it with existing detection methods at established veterinary diagnostics laboratories.

Materials and Methods

The analytical sensitivity (limit of detection), of the RealPCR PCV2/PCV3 Multiplex DNA Test was determined. Ten-fold dilutions of synthetic nucleic acid ($1x10^7$ to 1 copy/ reaction) representing the target sequences of PCV2 and PCV3 were used and tested in duplicate or triplicate.

For diagnostic testing, the RealPCR PCV2/PCV3 Multiplex DNA Test was compared with existing PCR methods at multiple veterinary diagnostic laboratories using field samples (serum, oral fluid, processing fluid, tissue, lung lavage and fetal tissue) sourced from the United States. A total of 161 and 190 samples were used for PCV2 and PCV3 testing, respectively.

Table 1. Analytical sensitivity of the RealPCR PCV2/PCV3 Multiplex DNA Test

	DNA copies/ reaction	Mean Ct value	Replicates detected
PCV2	$1x10^7$	14.0	3/3
	$1x10^6$	17.8	3/3
	$1x10^5$	20.7	3/3
	$1x10^4$	24.2	3/3
	$1x10^3$	27.1	3/3
	$1x10^2$	30.3	3/3
	$1x10^1$	32.9	3/3
	1	36.9	3/3
PCV3	$1x10^7$	15.2	2/2
	$1x10^6$	18.1	2/2
	$1x10^5$	21.5	2/2
	$1x10^4$	25.0	2/2
	$1x10^3$	28.0	2/2
	$1x10^2$	31.3	2/2
	$1x10^1$	35.8	2/2
	1	38.7	2/2

Ct = Cycle threshold

Results

Investigation of the limit of detection showed that the RealPCR PCV2/PCV3 Multiplex DNA Test consistently detected PCV2 and PCV3 targets at concentrations between 10 copies and one copy per reaction (Table 1).

Comparison of the RealPCR PCV2/PCV3 Multiplex DNA Test with existing PCR tests at veterinary diagnostic laboratories showed that the percent agreement for testing diagnostic samples was 97.5 and 97.9% for PCV2 and PCV3, respectively.

Discussion and conclusion

This study reports on the detection and differentiationof PCV2 and PCV3 DNA in a multiplex reaction usinga novel real-time PCR test. This is a cost-effective approach for testing relevant porcine circovirusinfections, as PCV2 and PCV3 can be identified simultaneously in a single well, with laboratory workflow advantages. In addition, the use of real-time PCR allows for measuring the viral load present in thesample by using quantification standards to produce astandard curve. This has practical relevance as the viral load has been correlated with porcine circovirus associated diseases and its use proposed for monitoring PCV2 vaccination efficacy (2, 3).

As analytical sensitivity testing demonstrated, the RealPCR PCV2/PCV3 Multiplex DNA Test consistently detected ≤ 10 copies per reaction of PCV2 and PCV3 DNA. Similarly, a high agreement with other PCR tests used at several locations for testing field samples was shown. The few samples showing discrepant PCR results between tests had late Ct values in one or the other test. Discrepant samples were not available for retesting or further investigations.

In conclusion, this study shows high analytical sensitivity of a new real-time PCR for detection and differentiation of PCV2 and PCV3 DNA. In addition,comparable results to existing PCR tests in use in veterinary diagnostic laboratories were found, with the added benefit of detection and differentiation of PCV2and PCV3 DNA in a single well.

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Detection of influenza A virus in pig farmworkers

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Introduction

Influenza A virus (IAV) has many hosts, including pigs and people, which is a public health concern [1]. Bidirectional transmission of IAV may cause disease and may have potential to generate novel IAV strains. United States (US) swine herds experience frequent outbreaks of acute influenza that can impact both swine and human health. Despite the recognition that bidirectional transmission of IAV occurs between people and pigs, little is known about how frequently that transmission or exposure event takes place. Therefore, the objectives of this study were to a) implement a surveillance system at the farmworker-swine interface, b) quantify how frequently farmworkers tested positive for IAV and c) characterize the IAV positive samples to assess risk of interspecies transmission.

Materials and Methods

Seven sow farms located in the Midwestern-US were selected for the study. The farms were representative of US commercial farms with an average of 4,000 sows. The farms had a history of IAV infections and 5 of them tested IAV positive during the course of the study. The study took place during the 2019/2020 peak of human influenza season (January-March). After collecting baseline information, each participant was asked to self-collect a nasal swab before entering the farm and at the end of the working day, specifically after completing the daily chores, twice a week for 8 weeks. At each sampling point, participants also recorded their body temperature using disposable thermometers, answered a short survey regarding the chores performed during the day, and reported whether they had influenza-like illness (ILI).

Pigs were also sampled at three time points during the study. Each time 30 nasal swabs were collected from 20 day-old pigs prior to weaning. Farmworker samples were tested individually with an IAV specific rRT-PCR test that detects both human and swine IAVs [2]. Pig samples were tested in pools of 3 using a rRT-PCR targeting the conserved IAV matrix gene [3]. A subset of IAV positive samples from pigs and farmworkers were selected for whole genome sequencing.

Results

There were 58 farmworkers from the seven participating farms who completed the sampling protocol, and a total of 1,785 nasal swabs were obtained from the farmworkers. Out of the samples collected, 58 samples (3.2%) tested IAV rRT-PCR positive, 20 (34.4%) of them from samples taken before entering the farm and 38 (65.6%) from samples taken at the end of the working day**.** From the 64 enrolled workers, 33 of them tested IAV positive at least once during the study. Pigs from five of the seven participating farms were IAV positive at the three sampling points during the course of the study. Whole genome sequencing from samples obtained from farmworker nasal passages indicated evidence of infection of a worker with human seasonal H1N1 IAV (H1 pandemic 2009-like clade 1A 3.3.2) when reporting to work, and several workers had evidence of exposure to a swine H1N2 IAV (H1-alpha of clade 1A 1.1) circulating in the pigs on the farm where they were employed. There were no statistically significant association between farmworker survey responses and their IAV test results.

Discussion and Conclusion

Our study provides evidence that farmworkers can report to work infected with human-seasonal IAV and that farmworkers are exposed to swine IAV that can be detected in their nasal passages. Both of these represent a risk for bidirectional transmission between humans and pigs. We also found that self-reporting ILI symptoms to aid in the detection of IAV-infected workers had low sensitivity. Overall, our results emphasize the need to implement surveillance and adopt transmission mitigation measures at the pig/human interface.

Acknowledgments

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Detection of multiple lineages of PRRSV in breeding and growing swine farms

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Introduction

The detection and co-circulation of multiple variants of porcine reproductive and respiratory syndrome virus (PRRSV) have been observed and reported in swine. However, longitudinal studies analyzing large numbers of samples to access the long-term prevalence of PRRSV variants and its potential impact on pig performance are lacking in the literature. The primary objective of this study was to describe the genetic variation of PRRSV in processing fluid (PF), oral fluid (OF), and tonsil scraping (TS) specimens from five swine farms with different types of production stages and PRRS status over a period of time (approximately one year). Furthermore, the association between PRRSV prevalence and production parameters was investigated.

Materials and Methods

Three farrow-to-wean, one wean-to-finish, and one finisher farm were recruited (Table 1) and sampled monthly over a time period of approximately one year (February 2019 – March 2020), i.e., with a goal of 12 sampling events per farm. At each monthly sampling event, PF (n = 8) and TS (n = 8) samples were collected from Farms 1 – 3 while OF (n = 8) and TS samples (n = 8) were collected from Farms 4 and 5. Viral RNA extraction and reverse-transcription quantitative PCR (RT-qPCR) were conducted for all samples, and samples with Cq < 31 were submitted for ORF5 gene sequencing. The resulting sequences were aligned to 690 ORF5 reference sequences of typical PRRSV type 1 and 2 strains species that served as anchors for PRRSV ORF5 lineage classification previously (1,2).

Results

Results showed that PRRSV was detected by RT-PCR in 21-25% of all types of specimens. In breeding farms, PRRSV detection in PF and/or TS samples was correlated with stillborn and mummified fetuses, and pre-weaning mortality throughout the study period. Although ORF5 sequences were obtained in <16% of all sample types, simultaneous detection of PRRSV variants including field and vaccine strains within a single sampling event was identified in both breeding and growing pig farms. Phylogenic analyses based on ORF5 sequences classified detected field PRRSV into L1A and L1H, two sub-lineages of lineage 1 (L1) (Table 2).

Discussion and Conclusion

Our study demonstrated the presence of multiple PRRSV lineages, sub-lineages, and variants in swine herds and its potential association with swine reproductive performance under field conditions.

Table 1. Characteristics of five U.S. swine farms enrolle the study.

Farm	Production type	Inventory	PRRS infection status	Vaccination at sampling	Late ou
1	Farrow-wean	5,000 sows	Previously infected	No	Au
2	Farrow-wean	6,000 sows	Recently infected	Yes	Ja
3	Farrow-wean	2,500 sows	Actively vaccinated	Yes	
4	Wean-finish	3,550 pigs	Recently infected	No	Mar
5	Finisher	2,800 pigs	Actively vaccinated	Yes	

Table 2. Lineages of PRRSV ORF5 sequences isolated overtime

		Sampling event									
Farm	Sample type[1]	1	2	3	4	5	6	7	8	9	10
1	PF	L1H		L1H							
	TS										
2	PF	L1H	L1H	L1A L1H L8†	L1H		L1H				
	TS		L1H	L1H							
3	PF	L1H	L1H	L1H	L1H	L1H					
	TS										
4	OF							L5†		L1A L5†	
	TS	L1A					L5†		L5†	L1A L5†	
5	OF								L1A	L1A	L5
	TS				L1A	L1A					

[1] PF: processing fluid samples; TS: tonsil scraping samples; oral fluid samples
*Samples were not collected on grey shaded sampling events.
†Sequences > 98% identical to L8 (Fostera® PRRS; Zoetis and L5 (Ingelvac PRRS® MLV, Boehringer Ingelheim) va strains.

Acknowledgments

National Pork Board, Des Moines, Iowa 50325 USA

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Detection of PCV2 genotypes present in Colombia, sequencing of ORF2 and prediction of epitopes for T cells

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Introduction

Porcine circovirus type 2 (PCV2) belongs to the Circoviridae family, genus Circovirus. It is a primary causative agent of Porcine Circovirus 2 Associated Diseases (PCAVD) (1). A striking feature of PCV2 is its high-rate evolution. Despite being a DNA virus,PCV2 has an evolutionary rate similar to RNA virus. The virus has evolved rapidly, and a classification system based on the DNA sequence of the ORF2 gene of the virus has identified eight different genotypes(PCV2a - PCV2h) (2). The endemic nature of all PCV2 genotypes is perhaps the cause of the sporadic clinical and subclinical appearance of clinical signs associated with potential vaccine/vaccination due to insufficient protection. This study aimed to determine the genotypes of PCV2 circulating in Colombia by sequencing the ORF2 gene. Additionally, the interaction of epitopes of the Cap protein of the Colombian strains with T cells through EpiVax was analyzed in-silico.

Materials and Methods

Eighty-six farms from different swine production regions were selected with different production systems and vaccination protocols for PCV2 control. One hundred sixty-six cases with signs suggesting PCV2 were collected from these farms. Samples included blood, lymph nodes, spleen, and thymus. DNA was extracted from all samples using a commercial kit. Then, conventional PCR was performed using primers that amplify a region of 550 bp of PCV2; all PCV2 genotypes are detected with thisamplicon. Circulating PCV2 strains were then characterized through ORF2 sequencing. Alignment was carried out with the ClustalW method andcompared with other 45 ORF2-PCV2 nucleotide sequences retrieved from the GeneBank. Then, phylogenetic analyzes were performed by using MEGA7. Finally, the obtained PCV2 sequences were used to predict T cell epitopes (PigMatrix) andcompare the epitope content of T cell (EpicC) to determine epitope coverage.

Results

PCV2-DNA was detected by conventional PCR in 54.8% (91/166) of cases. From those, 57 ORF2-PCV2 sequences were obtained. The phylogenetic analysis revealed that 96.5% (55/57) belong to the PCV2d genotype and 3.5% (2/57) to the PCV2a genotype. (Figure 1)

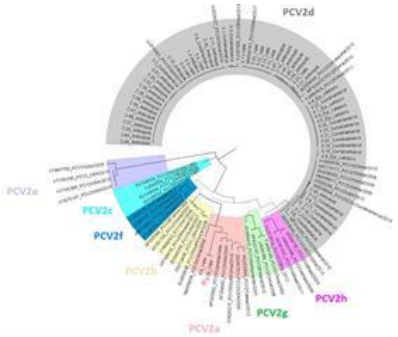

Figure 1. Phylogenetic analysis of ORF2 PCV2 strains circulating in Colombia during 2021.

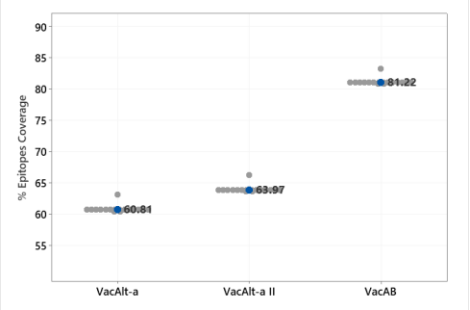

Figure 2. Epitope Coverage from EpiCC analysis for PCV2 vaccines

According to the results from the epitopes analysis, it was found that a bivalent vaccine containing PCV2a/b may have a better immune coverage against the circulating strains of PCV2 in our country.

Discussion

PCV2 was detected with high prevalence in most of our country's important pig rearing areas. Despite the continued use of the PCV2 vaccines, the PCV2d genotype appears to be the most common in the pig population studied and may be associated with clinical or subclinical cases in immunized pigs. Previous studies have shown that the predominant genotype was PCV2b. the above indicates that the virus has shifted over time in all regions studied. In addition, the bivalent vaccine (VacAB) containing PCV2a and PCV2b revealed greater epitope coverage against PCV2d, suggesting better immune protection.

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Detection of porcine circovirus type 2 and type 3 by oral fluid samples in swine herds from Brazil

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Introduction

Porcine circovirus 2 (PCV2) is the etiological agent of the porcine circovirus associated diseases (PCVAD), which includes postweaning multisystemic wasting syndrome (PMWS), proliferative and necrotizing pneumonia, granulomatous enteritis, and occasionally porcine dermatitis and nephropathy syndrome (PDNS) and reproductive failure in pigs. Worldwide, the main pathogenic subtypes are: PCV2a, PCV2b, PCV2d, associated with major economic losses in the swine industry. *Porcine circovirus 3* (PCV3) is one of the most recently described species, and has been detected in pigs with different clinical and pathological manifestations , mainly reproductive and respiratory disorders, and occasionally multisystemic inflammation and myocarditis. The diagnosis of both viruses' infection is made through the analysis of serum, organs and secretions samples. Currently, oral fluid has been widely used as it is a less stressful, easy and quick wayto collect samples for diagnosis. Initially, the most prevalent PCV2 subtype in Brazil was PCV2a and soon PCV2b became a matter of concern, however, in recent years, PCV2d has been detected in severalBrazilian herds (2). Moreover, PCV3 has been shownto be present, alone and in co-infections with PCV2b and PCV2d, in several symptomatic animals in different farms. The present work aims to detect and genotype PCV2 and PCV3 using the PCR technique in routine samples, in order to trace the current profileof the infection in Brazilian swine commercial herds.

Materials and Methods

Samples of oral fluid and organ fragments were collected from commercial pig farms in the statesof Espírito Santo, Goiás, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Paraná, Rio Grande do Sul andSanta Catarina and sent for diagnosis and genototyping at the Research Laboratory in Animal Virology, UFMG. The samples were collected from May 2019 to December 2021, obtained from farms that presented clinical disease compatible with PCV2and PCV3 infection. Eighty-three oral fluid samples were collected using the cotton string collection method (3). The samples were immediately refrigerated after collection and frozen until delivery to the laboratory. These samples were the focus of theresearch for diagnosis, due to the practicality involvedin the sampling process. One hundred twenty-two samples of organ fragments (lung, lymph node, spleen) and two hundred ten blood serum samples from animals showing signs of PCVAD were sent, along with oral fluids. PCR assays were performed using specific primers for each genotype (PCV2a, PCV2b, PCV2d and PCV3) (4,5).

Results

Table 1. Results of PCV2 (a, b, d) and PCV3 genotyping, obtained from samples of blood serum, oral fluid and organ fragments**.**

RESULTS - PCR GENOTYPING						
SOURCE	PCV2A	PCV2B	PCV2D	PCV3	CO-INFECTION	NEGATIVE
ORAL FLUID	0/83 (0%)	8/83 (9.64%)	27/83 (32.53%)	34/83 (40.96%)	15/83 (18.07%)	30/83 (36.14%)
ORGAN FRAGMENTS	0/122 (0%)	17/122 (13.93%)	24/122 (19.67%)	31/122 (25.40%)	14/122 (11.47%)	89/122 (72.95%)
BLOOD SERUM	0/210 (0%)	21/210 (10.00%)	33/210 (15.71%)	51/210 (24.28%)	31/210 (14.76%)	136/210 (64.76%)
TOTAL	0/415 (0%)	46/415 (11.08%)	84/415 (20.24%)	116/415 (27.95%)	60/415 (14.45%)	255/415 (61.44%)

In all sampled farms, viral DNA was detected through PCR, confirming the circovirus circulation. In all farms there were animals that presented clinical disease, where at least four farms were vaccinated against PCV2. The species and genotypes most present in the farms were PCV2d and PCV3. The PCV2b genotype was also present in forty-sixsamples. The PCV2a genotype was not detected in the samples, suggesting there was no circulation of this virus in the sampled period (Table 1).

Discussion and conclusions

The detection and genotyping of PCV2 and PCV3 in oral fluid samples demonstrated the efficiency of oralfluid as a collection material for molecular diagnosis of PCV2 and PCV3 in Brazil, even under inappropriate shipping conditions, with periods of material transport greater than 2-3 days. This efficiency in the PCR test was ensured through the detection of the viruses nucleic acid in organ fragments, considered as good sample models. PCV2b, PCV2d and PCV3 are circulating in Brazilianpig farms, causing clinical disease, even in vaccinatedfarms. Oral fluid samples are effective in the detectionand genotyping of PCV2 and PCV3 in Brazil.

Acknowledgments

CNPq and FAPEMIG

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Effect of AuraPig® on reduction of porcine reproductive and respiratory syndrome virus (PRRSV) in nursery pigs under field condition

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) causes reproductive failure, respiratory diseases due to secondary infection of bacteria, and reduced growth performance in pigs worldwide (1,2). The outbreak of PRRS occurs in nursery period as horizontal transmission from pigs-to-pigs throughoutthe herd. Nowadays, vaccination is the common strategyto control PRRSV. However, the vaccine efficacy is not sufficient to reduce the shedding of the virus. To date, there are several natural products have been shown to inhibit PRRSV replication in pigs (3, 4). AuraPig®, a mixture of natural antimicrobial compounds, consist of polyphenol, and organic acids that had antimicrobial activity against *Escherichia coli* in ruminant (5). Therefore, the objective of this pilot study was to study the efficacy of AuraPig®, on PRRSV infection in nursery pigs.

Materials and Methods

A total of 2400 piglets were selected based on the history outbreak of PRRSV circulating in nursery population. The infected piglets were randomly divided into 4 groups according to AuraPig®, dosing for treatment. Infected pigs were treated with AuraPig at concentration of 1:250, 1:500, and 1:1,000 in drinking water for 7 hours a day from 5 to 10 weeks of age. Serum samples were randomly collected once a week until 10 weeks of age and tested for real-time reverse transcription PCR (Real time RT-PCR) to determine thequantity of total PRRSV RNA using virotype® PRRSV RT-PCR Kit (QIAGEN, Germany). Moreover, serum was also used to evaluate for PRRSV antibody using PRRS Ab ELISA 4.0 (BioNote, Hwasung, South Korea). The presence of PRRSV antibody was detected by the sample to positive (S/P) ratio.

The difference in means between control and AuraPig® -treated groups were analyzed using One-wayANOVA with post hoc Tukery's test.

Results

The mean value of viremia in pig serum was statistically significant decreased in pigs after treated with AuraPig® at concentration of 1:250 and 1:500 compared to the control group (p=0.047 and p=0.001, respectively) (**Figure 1**). This finding suggested that AuraPig® (at concentration of 1:250 and 1:500) was sufficient to reduce viremia in blood sera of the infected pigs.

The average S/P ratio of 1:250 and 1:500 AuraPig® -treated groups were significantly increased when compared to the control (p<0.0001) (**Figure 2**). This result suggested that pigs treated with either 1:250 or 1:500 AuraPig® had a potential to produce antibody against PRRSV infection.

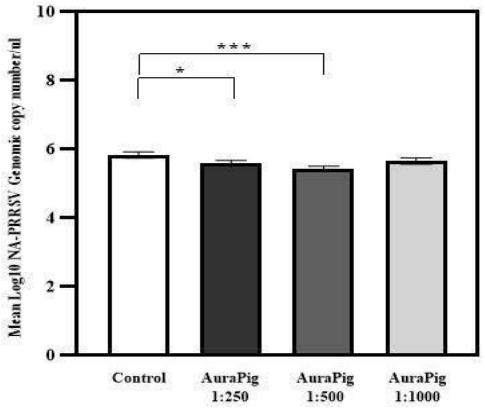

Figure 1.The average PRRSV RNA levels in pig serum. The values are means ± SEM. An asterisk indicates statistically significant difference (* p<0.05; *** p<0.001).

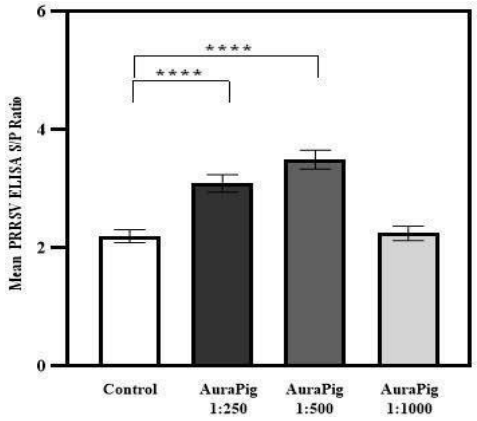

Figure 2. The mean values of anti-PRRSV antibody response from serum samples. Asterisks represents the levels of statistical significance at p<0.0001.

Discussion and Conclusion

The data suggested that AuraPig® reduced the level of viral loads in serum and also stimulated high levels of antibody response to PRRSV. Proposed mechanisms of AuraPig® against PRRSV might be enhancing of the protective immune responses and suppress ongoing inflammation to prevent secondary PRRSV infection as the same mechanisms to other plant extracts (6).

These findings demonstrated that AuraPig® has the potential to be used for controlling PRRSV infection. Thus, the use of AuraPig® provided good protection against PRRSV in piglets and effectively suppressed the PRRSV infection.

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Effect of Pooling Family Oral Fluids on the Probability of PRRSV RNA Detection in Weaning Age Pig Populations

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Introduction

Family oral fluids (FOF) are oral fluids obtained from a sow and her litter. Almeida *et al.* [1] demonstrated FOFs are a convenient and cost-efficient alternative to serum for PRRSV surveillance in weaning age pigs. Routine PRRSV surveillance in large herds can be expensive even for FOF, The objective of this study therefore were: 1) investigate the impact of pooling this sample type on the probality of PRRSV detection by Rt-qPCR, and 2) estimate the number of FOF pools required to have a ≥ 95% confidence of at least one positive RT-qPCR test.

Materials and Methods

FOF samples were obtained from PRRSV-endemic and PRRSV-naïve herds and tested for PRRSV RNA by reverse-transcriptase quantitative polymerase chain reaction (Rt-qPCR). Thirty PRRSV-positive FOF samples were each pooled with varying amounts of PRRSV negative FOF samples (in the ratios 1:3, 1:5, 1:10, and 1:20) and tested for PRRSV RNA in replicates of 6. A probit regression model was used to estimate the probability of PRRSV RNA detection in the tested pools using the brglm package on R. The mean cycle threshold (Ct) values for each undiluted sample was used to assign them into one of three categories, accounting for viral concentration within PRRSV-positive FOF samples pre-pooling (Category A for Ct values < 34, Category B for Ct values ≥ 34 ≤ 36, and Category C for Ct values > 36). Predictions from the aforementioned fitted statistical model, the principles of counting, and fundamentals of probability were applied to estimate the number of FOF pools required to have a ≥ 95% confidence of at least one positive RT-qPCR test (assuming a 56-crate room, a given prevalence, and a perfect test).

Results

The mean probabilities of detection for 1:3, 1:5, 1:10, and 1:20 dilution levels were respectively 99%, 99%, 97%, 87% for Ct Category A; 97%, 93%, 91%, 68% for Ct Category B; and 79%, 65%, 54%, 26% for Ct Category C. Table 1 represents the number of pools needed to be sampled to have a ≥ 95% probability of having at least one positive RT-qPCR test assuming; a 56-crate room, a given prevalence, and a perfect diagnostic test.

Table 1: minimum number of pools needed to detect PRRSV RNA by RT-PCR with at least 95% certainty in a 56-crate room.

PRRSV-positive litters	Inter-litter prevalence[1]	Level of pooling[2]			
		3	5	10	20
1	1.79%	18	11	6[3]	NP[4]
2	3.57%	15	10	5	NP[4]
3	5.36%	12	8	4	3[3]
4	7.14%	10	7	4	3[3]
5	8.93%	9	6	3	2
6	10.71%	7	5	3	2
7	12.50%	7	4	3	2
8	14.29%	6	4	2	2
9	16.07%	6	4	2	2
10	17.86%	5	3	2	2

[1](Number of positive litters/Total number of litters).
[2]Number of FOF per pool.
[3]One pool will have fewer FOFs.
[4]NP = Not possible.

Discussion and conclusion

Overall, the probability of PRRSV detection decreased with increasing Ct of positive samples within pools, and with increasing proportion of negative FOFs within pools

As shown in Table 1, fewer samples (larger pool sizes) can be submitted for PRRSV RT-qPCR and still have an over 95% probability of having at least one positive test. When there is cost constraint for monitoring, or in fixed budget scenarios, pooling FOFs is here demonstrated to be a valid option for PRRSV surveillance.

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Effect of Porcine circovirus 2 (PCV-2) vaccine on PCV-2 viremia in vaccinated piglets under field conditions

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Introduction

Porcine circovirus type 2 (PCV2) is the smallest, non-enveloped, single-stranded DNA virus belonging to the Circovirus Genus of the Family of Circoviridae. PCV-2 is one of the most economically relevant viruses for the swine industry (1). PCV-2 vaccines have shown a drastic effect on the control of PCV2 associated clinical disease as well as the improvement of production parameters (2). Consequently, analysis of the effect of vaccinations against PCV2 is important for the swine industry. The objective of this study was to evaluate the effect of PCV2 vaccines on PCV2-viremic in piglets born from the same sow under the same management conditions in the farm.

Materials and Methods

This study was conducted in a farrow to finish swine farm with 250 sows located in West Japan during Apr. 2021 to Oct. 2021. On the day of weaning, suckling piglets born from 4 selected sows were evenly divided into two groups by individual weight, sexes, and parities. The PCV2 / M. hyo vaccines were freshly mixed and administered as a single 2 mL dose to 21-day-old piglets. Blood samples (N=5) from Vaccinated (N=22) and Non-vaccinated (N=22) groups were collected on 21, 28, 35, 41, 48, 63, 91, 145 and 173 days old. Serum samples were examined for the presence of PCV2 DNA by TaqMan-based real-time PCR (qPCR) test and inverse PCR to confirm the presence of the circularized DNA. On PCR-positive samples collected at 173 days old, virus isolation was performed with three blind passages on PK-15 cells. The last passage for each sample was confirmed for the presence of PCV2 using qPCR and IFA. Post-weaning mortality was statistically analyzed using the Chi-square test.

Results

The PCV2 genomic copy number from vaccinated was not significantly different from the non-vaccinated group (Fig 1 and 2). However, post-weaning mortality rate of non-vaccinated group (18.2%) was significantly higher than that of vaccinated group (0%, $p = 0.036$). PCV2 was isolated from the non-vaccinated group (isolation rate 50%) but not the vaccinated group (0%) (Table 1).

Discussion and Conclusion

The results from this study reveal that qPCR detected the presence of small fragments of PCV2 gene but did not correlate the PCV2 viremia level. Furthermore, the PCV2 vaccine used in this study showed effectiveness in reducing PCV2 viremia and mortality in vaccinated pigs. The present data supports that vaccination against PCV2 results in a significant reduction in piglet viremia, cannot be assessed by qPCR. This reduction of infectious PCV2 by vaccination may relate with the pig productivity improvement.

Figure 1. Positive rates for PCV2-PCR between non-vaccinated group and vaccinated group in pigs

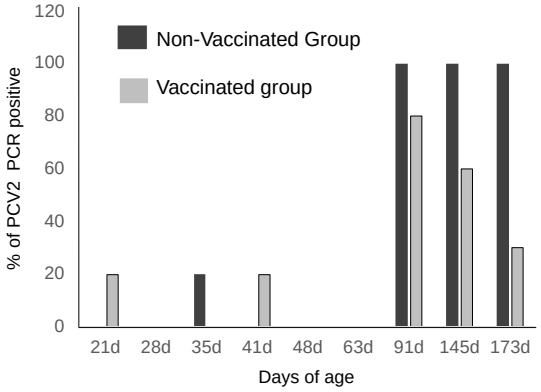

Figure 2. Kinetics of PCV2 gene in non-vaccinated group and vaccinated group in pigs

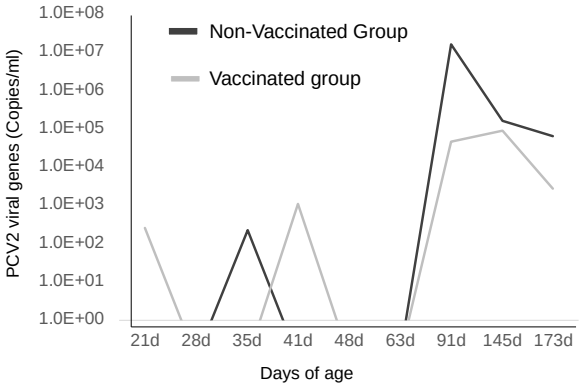

Table 1. Results of PCV2 isolation from pig serum samples.

Group	Range of PCV2 copies/ml in (+ve no.)		IFA +ve no.	Isolation no.	Mortality rate
	Serum	Supernaat of isolates			
Non-Vaccine	9.15E+02-1.17E+05 (10)	8.94E+01-3.52E+02(6)	5	5	18.2%(4/22)
Vaccine	4.55E+02-5.00E+03 (3)	8.87E+01(1)	0	0	0%(0/22)

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Efficacy of a combined PCV2 and PRRS vaccine against a virulent PRRSV heterologous challenge

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Introduction

The objective of this study was to evaluate the efficacy of a new combination vaccine FLEX CircoPRRS® containing INGELVAC PRRS® MLV and adjuvanted Porcine Circovirus (PCV2, killed baculovirus vector) in a heterologous type-2, lineage 1 PRRSV respiratory challenge model.

Materials and Methods

At ~ 3 weeks of age (D0), 126 PRRSV naïve pigs, randomized into 3 groups (2 Vaccination groups and 1 Placebo), blocked by weight, were intramuscularly (IM) vaccinated with either 2 ml of PBS as Placebo (N=42), 2 ml INGELVAC PRRS MLV (N=42) or 1 ml FLEX CircoPRRS® (N=42). Pigs were housed in rooms by group during the vaccination phase. On D27, all pigs were comingled and challenged with a virulent type 2 PRRSV (RFLP 1-7-4, lineage 1) at $10^{4.55}$ $\log_{10}$ $TCID_{50}$/dose with 2.0 ml IM and 2.0 ml intranasally (IN, 1 ml per nostril). Serum samples and weight data were collected periodically from D0 to D49. On D42 (14 days post-challenge), half of the pigs from each group were necropsied, and lungs were scored for the presence of macroscopic lesions. Remaining pigs were evaluated for viremia through termination of the study (D49). Serum samples were tested by RT-PCR for PRRSV viremia and by ELISA for PRRSV antibody. Pairwise comparisons between groups were conducted using a level of confidence of 0.05 to indicate statistical significance.

Results

Both vaccination groups demonstrated a significant reduction in gross lung lesions (mean percentage) compared to the Placebo group (Table 1). There was no difference in average daily weight gain (ADWG) between either vaccination group and Placebo group during the vaccination phase (D0 – D27). In the challenge phase (D27 – D42), both vaccination groups had a significantly higher ADWG than the Placebo group (Table 2). Both vaccination groups had a significant reduction in post-challenge PRRSV viremia (D42 -D49) compared to the placebo group (Figure 1).

Table 1 : D42 Percent Lung Lesions (mean)

Treatment	Lung Lesions (95% CI)
INGELVAC PRRS MLV (N=21)	1.20[a] (0.07, 3.66)
FLEX CIRCOPRRS (N=22)	1.98[a] (0.36, 4.86)
Placebo (N=21)	17.92[b] (12.03, 24.69)

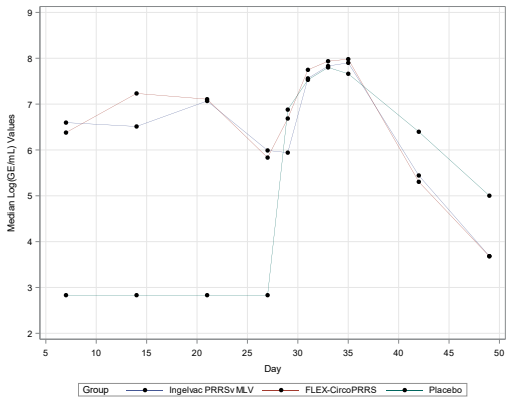
*Different letters indicate significant (P≤0.05) differences

Table 2: Pre and Post-challenge ADWG (Gram; values originally calculated in lb.)

Treatment	Pre-challenge ADWG, D0 – 27 (95% CI)	Post-challenge ADWG, D27- 42 (95% CI)
INGELVAC PRRS MLV (N=42)	467[a] (435, 494)	553[a] (494, 616)
FLEX CIRCOPRRS (N=42)	453[a] (421, 480)	521[a] (462, 585)
Placebo (N=42)	444[a] (412, 476)	430[b] (367, 489)

*Different letters indicate significant (P≤0.05) differences

Figure 1 : Post-challenge Viremia-Median Log Genomic Copies/ml by Group and Day



Discussion

This study fully demonstrated that FLEX CircoPRRS® is efficacious against a heterologous type-2, lineage 1 PRRSV respiratory challenge. Efficacy is supported by significant differences in lung lesions, ADWG and magnitude of viremia between vaccinated and Placebo pigs. There was no difference in ADWG between treatment groups during the vaccination phase demonstrating no performance impact from vaccination prior to challenge.

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Efficacy of the PRRSV-2 MLV in commercial farms infected with relatively low genetic similarity

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Introduction

Vaccines are one of an important defense measures for Porcine reproductive and respiratory syndrome (PRRS). Recent studies have shown that it is difficult to predict the efficacy of vaccines only with genetic identity (1). But in many cases, vaccines with higher genetic similarity with field virus circulating in pig farms are considered more efficacious. In this experiment, when the PRRSV-2 modified live vaccine was inoculated at a Korean domestic pig farm, the difference of viremia and growth performance according to genetic similarity was investigated. Injection route comparison between IDand IM did not influence immune response to the PRRSV vaccine.

Materials and Methods

Three pig farms with PRRS infection were selected and each was named A farm, B farm, and C farm. Genetic identity for ORF 2-7 (ORF 5) of the experimental A, B and C farms PRRSV with the vaccine strain are 85.2% (83.3%), 88.0% (88.1%), 88.4% (87.4%), respectively. Sixty pigs (3 weeks old) were divided into ID, IM vaccination group, and control group for each farm. The analysis of PRRSV circulating in the blood was performed by real-time PCR. Blood collection was performed at 0, 3, 5, 11, and 16 weeks post inoculation(wpi). Since the necropsy was conducted for each group at 11wpi, Average daily gain (ADG) was calculated by measuring up to 11wpi.

Results

In farm A, which has relatively low identity with vaccine strain, there was no significant difference in viral titer between the vaccination group and the control group throughout experimental period, and there was no difference in viral infection age between two groups (see Figure 1-A).

In farm B and C, which has relatively high identity with vaccine strain, vaccination group demonstrated low viral titer compared to control group and it was confirmed that the vaccination group showed delayed infection with the field virus than the control group (see Figure 1-B, 1-C). Especially, the viral titer of vaccination group was significantly low in 3wpi (p <0.05).

In the case of ADG, all vaccination groups except the IM group of farm A were higher than the control group (see Table 1). Especially, the ID group of farm A, the ID group and IM group of farm B showed significant differences compared to the control group(p<0.05). Additionally, there was no significant difference in efficacy of vaccine between ID group and IM group.

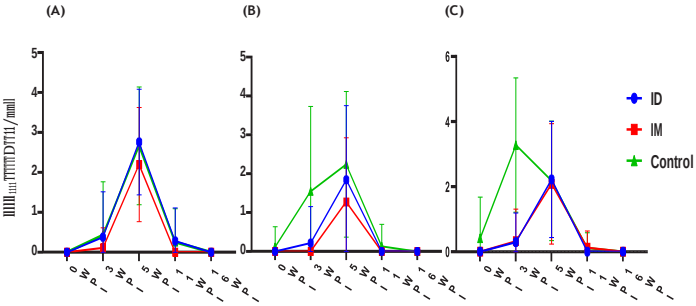

Figure 1. Mean PRRSV titers ($TCID_{50}$/ml) in serum of the each group.

Table 1. ADG(g) of pigs in A, B, and C farm

	A farm	B farm	C farm
ID	540.0	426.8	470.3
IM	476.9*	431.1	467.2
Control	510.4	385.5	464.5

*IM group in farm A showed low average weight before vaccination compared to other groups.

Discussion and Conclusion

The efficacy of the PRRSV vaccine was evaluated in commercial farms with low viral genetic similarities to that of vaccine strain.

Although ADG improved most dramatically in farm B, ADG seems to be difficult to predict only with genetic similarity, because there are so many factors affecting the ADG in the field trial.

Overall, it is difficult to assure that the higher nucleotide identity between vaccine strain and field strain leads to higher efficacy of vaccine in commercial pig farms. However, there is a tendancy to have a better production performance and low in viremia when selecting a PRRSV vaccine, the identity can be one of the considerable options according to this study.

Acknowledgments

This work was supported by iPET(320005042SB080) and MSD Korea, Inc., Republic of Korea.

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Efficacy of "Tooth Extraction" for ASFV elimination and relevance of point of care testing for ASFV to the field

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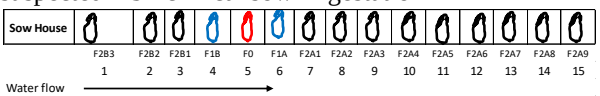
Introduction

In Vietnam, African Swine Fever was officially reported by the Ministry of Agriculture and Rural Development (MARD) February 19, 2019 (1). Subsequently an estimated 6 million pigs were lost, at least 20% of Vietnamese swine industry (2). As ASF outbreaks continue, the "Tooth Extraction" protocol used to control ASFV in China has been widely adopted in Vietnam as an alternative to sow herd depopulation. The protocol is: remove the sow exhibiting ASF clinical signs (index case) plus the sow stalled on each side of her (sampling whole blood for ASFV PCR testing when possible) (3). The objective of this study was to test the efficacy of this "Tooth Extraction" protocol, then use the collected blood samples to compare commercial point of care (POC) assays (quick tests (QT) and PCR) against the laboratory based OIE approved ASF PCR.

Materials and Methods

764 samples from 52 suspected ASF events were collected. A "Suspected ASF event" was defined as a sow exhibiting OIE-established clinical signs as determined by a farm caregiver (4). For each suspected ASFV event, whole blood was collected from the index sow plus 14 animals housed around the index sow (Figure 1). Samples were tested for ASFV DNA by an OIE-approved real-time ASFV PCR (STAND) within 24-hours of arrival to the laboratory. The proportion of positive animals was analyzed as a function of the gestation stall distance from the index sow. 723 of the samples (46 events) plus 50 known ASFV negative samples were further tested in the laboratory by 2 commercial quick tests (POC QT A&B) and 3 POC PCR (POC PCR A, B and C) according to product instructions, and compared with STAND.

Figure 1. Example of blood sampling protocol around a suspected ASF clinical sow in gestation†‡



†F0=index sow, F1= direct/closest contact neighbors, F2= indirect contact neighbors; A= "down" row, B= "up" row
‡The rationale for the sampling distribution was due to an assumption of a common water trough

Results

In 17 of the 52 events (33%), the index sow and 14 neighbor sows were ASFV PCR negative. Of the 35 events where ASF PCR was positive, in 19 (54 %), removal of the index sow and her direct contact neighbors still left one or more ASFV-positive sow (Figure 2). Table 1 details POC test results for 637 ASF negative and 86 ASF DNA positive sows.

Figure 2. ASF positive sows by location from index sow

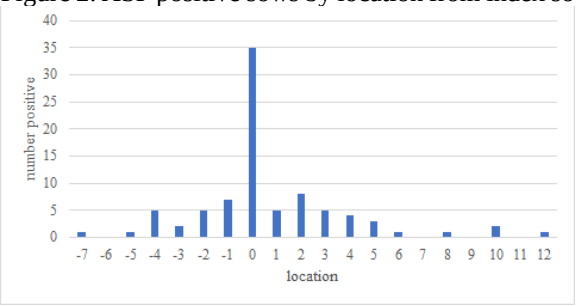


Table 1. POC Test performance compared to OIE (p72) real time PCR

STAND PCR Comparison Field Samples (637 neg, 86 pos)	Overall (n=723)		Known Neg (n=50)
	Se	Sp	Sp
POC QT A	60%	88%	100%
POC QT B	53%	74%	100%
POC PCR A	85%	98%	100%
POC PCR B	84%	95%	100%
POC PCR C	84%	98%	98%

Conclusions

"Tooth Extraction" is not sufficient to eliminate ASFV from a sow farm. ASFV DNA was detected in blood from sows showing no clinical signs. Point of care tests are not sufficient for ASFV elimination. POC PCR was 85% sensitive in detecting ASF positive sows (both clinical and non-clinical). POC PCR may be considered if access to laboratory-based PCR testing is unavailable. QT are unreliable for on farm use in ASFV detection.

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Silver Lake Research Corp. Irwindale CA USA
Eurofins Technologies Ingenasa, Madrid Spain
Collaborating Vietnamese producers

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Eight years of African swine fever (ASF) in Poland – domestic pigs outbreaks

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Introduction

African swine fever (ASF) is lethal disease of *Suidae* caused by dsDNA virus (ASFV). The disease is harmless to humans but it has devastating effect on pig production in affected countries and international economy. ASF was introduced to Poland in February 2014 (1, 2, 3). Since than the disease spread from the east of country to the west, in 2021 finally affecting all sixteen Polish provinces (4).

The aim of this work was to analyze the dynamics of the ASF spread in the domestic pigs population across the territory of Poland, from 2014 to 2021.

Materials and Methods

The data were collected from official announcements of Chief Veterinary Officer in Poland in the years 2014-2021 (4). The data were clustered yearly in pairs: number of outbreaks and number of pigs in these outbreaks. In addition, the number of small (up to 20 pigs), medium (from 21 to 100 pigs) and large farms (above 100 pigs) affected by ASF was compared in the analyzed period of time (2014-2021).

Results

In the years 2014-2021 a total of 488 ASF outbreaks in domestic pigs in Poland (Table 1) were confirmed. The elimination of the disease resulted in euthanasians and utilization of 166,763 pigs in Poland.

In the first five years of ASF the disease was mainly limited to small farms (up to 20 pigs). In contrast, in the last three years ASF affected mainly medium and large farms (Table 2). Since 2017 outbreaks in large commercial farms holding above 1000 pigs were noted. It was 2 farms in 2017, 9 in 2018, 9 in 2019, 8 in 2020 and 7 in 2021, respectively. The alarming fact was that there were 3 confirmed outbreaks in farms with total number of pigs above 10,000, in which the highest number of pigs in individual ASF outbreak reached 27,908 (Lubuskie province).

Discussion and Conclusion

In the first three years of epidemic the disease was limited to the eastern part of country and affected small number of farms. In next years, ASF spread to the west affecting in 2019 Lubuskie, a province neighboring to Germany (2, 3).

The increased number of ASF outbreaks in domestic pigs directly affected the structure of pig herds in Poland (1). Despite the fact that the total number of pigs in Poland before the ASF epidemic (10 992,9 pigs in 2013) and today (11 033,3 pigs in 2021) remained at almost the same level (5), the total number of pig herds dramatically decreased. In 2013 it was 278,4 pig herds in Poland (5), while the current number is 80,0, which is almost 200 thousand less than 9 years before (6).

The fight with ASF affects the economy and pig production in Poland, changing pig herds structure and limiting the number of small farms holding up to 20 animals (5, 6). Despite the current efforts of farmers and the General Veterinary Inspectorate connected with the implementation of the biosecurity measures in farms, the ASF outbreaks affected also large commercial farms. The problem with the disease will still be the present, till the effective and safe vaccine will be commercially available. Untill that time the main effort should be focused on the maintaining strict rules of biosecurity in the farms as well as controlling ASF vectors.

Table 1. ASF outbreaks in Poland in the years 2014-2021.

Year	Number of ASF outbreaks	Number of pigs in ASF outbreaks
2014	2	9
2015	1	7
2016	20	1 333
2017	81	5 311
2018	109	25 395
2019	48	35 360
2020	103	57 095
2021	124	42 253
Total:	488	166 763

Table 2. ASF outbreaks in Poland in the years 2014-2021in farms differing in size

Year	Small farms[a]	Medium farms[b]	Large farms[c]
2014	2 (100%)	0	0
2015	1 (100%)	0	0
2016	11 (55%)	6 (30%)	3 (15%)
2017	44 (54%)	32 (40%)	5 (6%)
2018	54 (50%)	32 (29%)	23 (21%)
2019	13 (27%)	14 (29%)	21 (44%)
2020	50 (49%)	32 (31%)	21 (20%)
2021	29 (23%)	58 (47%)	37 (30%)
Total:	204 (42%)	174 (35%)	110 (23%)

[a]small farm:- up to 20 pigs; [b]medium farm: 21 - 100 pigs; [c]large farm: above 100 pigs

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Elaboration and evaluation of an autogenous inoculum as a PRRS control tool: An experience in Peru

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Introduction

The aim of this study was to summarize the methodology for the elaboration of a homologous inoculum from a strain of Porcine Reproductive and Respiratory Syndrome Virus (PRRSv) as part of a large-scale PRRS acclimatization program in commercial pig farms, and to describe its impact in productive and reproductive parameters in 5 200 animals, after single-dose inoculation of 10^4 viral copies/ml of inoculum (1, 2).

Materials and Methods

Blood samples were collected from PRRSv qPCR positive piglets from a recent outbreak of PRRSv North American (Type 2). Samples were also pre-screened by PCR for other major swine pathogens circulating in Peru, including Porcine Circovirus type 2 (PCV-2), Porcine Parvovirus (PPV), Influenza A Virus (IAV) and Classical Swine Fever Virus (CSFV). The number of viral copies/ml were estimated as follow: cDNA was synthesized from positive RNA samples and controls, the ORF7 gene fragment of PRRSv was amplified by end-point PCR (3), then purified from an agarose gel using the Wizard SV Gel and PCR Clean-Up System Kit (Promega, USA). The amplicon concentration was quantified in a Quantus Fluorometer (Promega, USA). A standard curve was generated by qPCR based on serial dilutions of the quantified amplicon, which allowed the calculation of the number of viral copies/ml for each positive sample. Samples were diluted with PBS 1X to a concentration of 10^4 viral copies/ml, Gentamycin was added to the inoculum and then it was stored at -80°C until use. After progressive thawing, 2 ml of inoculum was applied intramuscularly to 5 200 animals including replacement gilts, boars, and all sows of the farm, independently of the reproductive status (pregnant or lactating). According to previous reference(4), clinical signs were observed, and reproductive and productive parameters were measured at 3-, 7- and 10- weeks post-inoculation (pi). Seroconversion was measured by ELISA (IDEXX) at 21 days pi.

Results

Between day 1 and 2 pi, inappetence was observed in 0.5% of the animals. Neither fever nor other clinical signs were observed. Appetite was restored after 24 hours. PRRSv antibodies were detected 21 days pi by ELISA. Pre-weaning mortality (PWM) decreased 29.9%, 58% and 69% at 3-, 7- and 10-weeks pi, respectively (figure 1). Wean-to-finish mortality increased in rearing (21-70 days), which was the group most affected by the outbreak, unlike fattening (71-150 days) whose average remains, so that the use of inoculum seems not having influenced these age groups. Moreover, while no variation in total born piglets was observed, the stillbirth piglets decreased 34.7% and 56.8% at 7- and 10-weeks pi, respectively (figure 2).

Discussion and Conclusion

A single-dose intramuscular injection of 2 ml of PRRSv autogenous inoculum containing 10^4 viral copies/ml induceed seroconversion without any considerable adverse effect. A short-term evaluation suggested an improvement in productive and reproductive parameters on the farm.

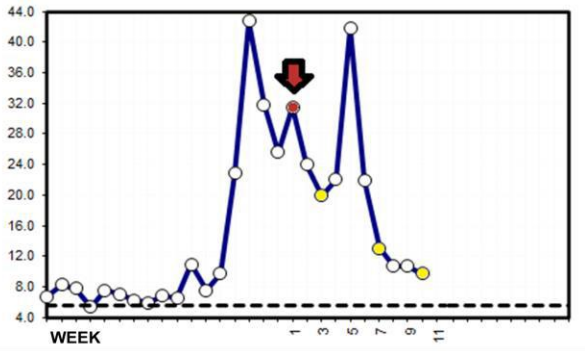

Figure 1. Percentage of Pre-weaning mortality (PWM). Inoculation date is pointed by the red arrow and Week 3, 7 and 10 pi are pointed by yellow circules.

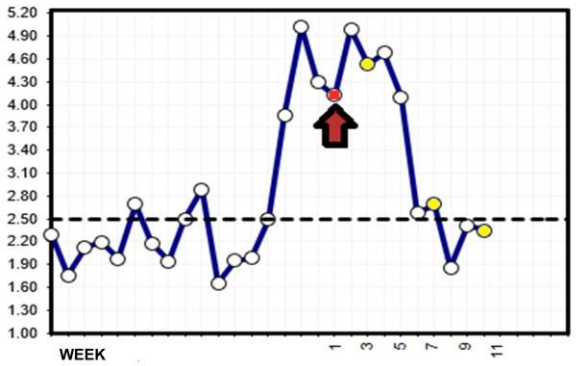

Figure 2. Percentage of Stillbirth piglets. Inoculation date is pointed by the red arrow and Week 3, 7 and 10 pi are pointed by yellow circules.

Note: At 3 weeks pi there was a PEDV outbreak.

Acknowledgments

Dr. Luis Gimenez-Lirola for his great support and kind recommendations.

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Eliminating African Swine Fever Virues in Four Large Sow Herds by New Generation Test - Removal Technology in China from 2018 to 2019

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Introduction

Since the first report of ASFV in China in 2018, conventional stamping out method to control ASF has proved unwieldly because of high production intensity and complex trade network (1). It was reported that ASFV spreads relatively slowly within a herd following introduction, with estimated within-pen basic reproduction ratio (R0) of approximately 2.8 and between-pen R0 of approximately 1.4 (2). Moreover, ASFv DNA detection in oral, nasal and rectal swab samples occurred between zero to two days before onset of clinical signs (3,4), suggesting that early detection of ASFv by qPCR following introduction within herdsmay be possible. To provide an alternative to conventional stamping out method, we reported the feasibility of implementating an extensive sampling method and qPCR tests to determine the status of ASFV in herds, with a rapid removal measures to successfullyeliminate the virus from four large swine herds in China from October 2018 to June 2019.

Materials and Methods

When first detection of ASFv occured in the farm,whole herd sampling and qPCR tests were carried out to evaluate the disease status in the herd. Then infected pigs were accurately removed and environment decontaminated by the precision removal process. One or more rounds of this process were applied until the whole herd remained negative for 7-14days.

Specifically for the sampling protocol, detailed electronic maps showing the precise layout of each barn were produced to facilitate the sampling protocol. For early detection, all clinically abnormal pigs with signs including off-feed, fever, lethargy, hemorrhagic diarrhea, redness of skin, lameness, and abortion were sampled and tested. Swabs from nasal, oral, rectal (NOR), trough lips, and defecation area surface were pooled in a 2ml microtube as one sample. For confirmation of first ASFV DNA detection, especially when samples showed Ct values of higher than 35, lymph node samples were collected using an innovative lymph node sample collector.

Pigs were removed in a bio-secure manner by using sealed U-shape tunnel made from waterproof polyester cloth and exclusive carts.

Data including qPCR result and TTNH (time to negative herd, in which both pigs and environment were negative) were collected from each farm. TTNH was determined by calculating the days from the first ASFV positive qPCR result until the last positive result.

Results

We successfully controlled ASF and eliminated the virus from four large swine herds. The time to negative herd was 19, 28, 14, and 1 days in farms 1 to farm 4 respectively. Retention rates of pigs of farm 1 to farm 4 was 69.7% , 65%, 99.4% and 99.72% respectively.

Discussion and Conclusion

We firstly developed a new generation test - removal technology to eradicate ASFv in four farms with most herds retained for normal production. The successful eradication of ASFV in herds would greatly facilitate the control and eradication of ASFV in China and worldwide.

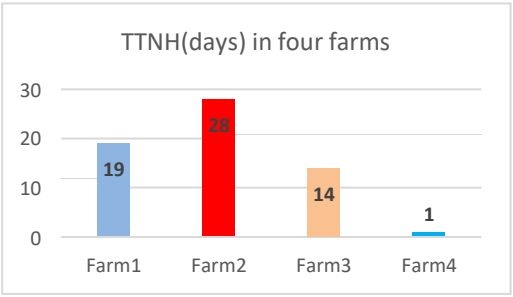

Figure1. TTNH (time to negative herd, in which both pigs and environment were negative) in four herds.

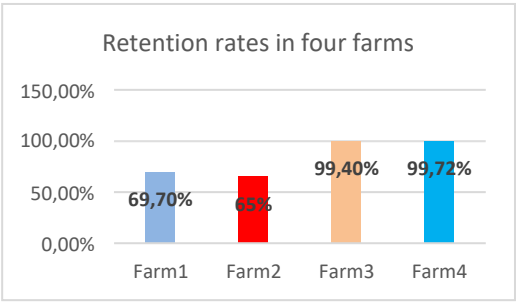

Figure2. Retention rate (=number of retained sows/ number of sows prior to detection) in four herds.

Acknowledgments

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Elimination of African Swine Fever type 2 MFG360 and CD2v variant from a boar stud and breeding farm

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Introduction

Following the spread of African Swine Fever type 2 into Georgia in 2007 the pathogen has become pandemic. In China a type 2 gene deleted variant MGF360 and CD2v and a ASF type 1 virus has been recognised. This report illustrates the recognition of the ASF type 2 variant on a pig farm and successful method to eliminate the virus from the farm.

Materials and Methods

Twenty four boars in a 180 boar stud on a breeding farm suddenly presented depressed with blood vomiting and incoordination. From the 24 clinically abnormal boars, 6 were PCR positive for ASF in saliva and blood samples (Ct 30+) and 15 had detectable antibodies to ASF type 2. From the 156 non-clinical boars, 24 were also saliva PCR positive. The virus was sequenced revealing the variant MGF360 and CD2v.

Results

Once the cause of the problem was recognised, sales from the farm was suspended the farm was depopulated.

Successful method of cleaning and repopulation.

Standard down time: 3 months followed by one month of sentinels

Cleaning protocols:

There will be three cleaning cycles with a limewashing and fumigation after each cleaning cycle.

1. Soap and water around the farm to start killing and deactivating the virus
2. Dispose of all medicines, needles and syringes. This should include all medicine.
3. Remove all disposables from the farm, including all feed. Empty all feed hoppers and feed bins these will need to be disposed cannot be fed to other pigs.
4. Rodent control should start and be vigorous. Place water near baits to encourage intake
5. The farm is going to be cleaned thoroughly including the slurry passageways and the loading areas and driveways.
6. Pay particular attention to the removal of all faecal material. The building should be brushed down thoroughly and then dry cleaned using a knife and scrape to remove all visible faeces. The small amounts should be removed with a dustpan and brush. This has to be very thorough and on your hands and knees
7. Remove dust by vacuuming where possible
8. Areas of particular note – pigs have long tongues Under and around gate posts and gates. Corners at theback of pens. Around fittings i.e. farrowing pens Under drinkers and troughs. Where cracks and holes exitin the concrete
9. Repair all large cracks and holes in concrete by cleaning out where possible and pouring a suitable disinfectant. Once dry repair by screeding over with concrete.
10. All wooden partitions and removable objects should be soaked in disinfectant for a period of 3 to 5 days using metal baths. Place outside in sunlight to dry
11. Drain and clean the slurry channels and pits. Remove all available faeces.
12. Lime wash all surfaces especially up to 2 metres and spray with suitable disinfectant using a knap sack sprayer into the ceiling and loft areas.
13. Ensure that the water supplies are adequately disinfected
14. Bird proof all buildings where possible
15. Ensure unit perimeter secure
16. Remove all disposables from the farm, including all feed. Empty all feed hoppers and feed bins. Ideally all feed should have been eaten
17. Dispose of all brushes, shovels and scrapes
18. Dispose of all overalls, boots and protective clothing

Surfaces

Ensure all surfaces are cleaned. This must include the fridge, chemical store, feed stores, changing rooms and staff room. All surfaces to be limewashed.

Midden area. Spread all the midden materials and lagoons and slurry store. The soil within the proximityof the midden area has faeces still remaining from the old unit. Skim off this area to a depth of 80 cm.Spray the soil with suitable disinfectant and then re- scree over the 80 cm of soil

Straw and other bedding. Old straw remaining from the old unit should be moved and disposed off as this can harbour mice/rats from the old unit

Dogs and cats. Discuss dog and cat protocols.

Tractors. Ensure all tractors and equipment, in particular muck spreading and bob cats, are thoroughly cleaned and disinfected

Lime wash all surfaces

Once whole farm fumigated.

Repeat from 1 again

Repeat from 1 again. Three thorough cleaning has proven to be very effective.

Sentinels

A month of sentinel study for each of the buildings is essential

The sentinels must be ASF and CSF Free. PCR and ELISA at the beginning

They must be tested free at the end – all the pigs. Examine all deaths carefully.

New stock

Entered the farm and there has been no reoccurance.

Further reading:

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Epidemiological and molecular retrospective analysis of Porcine Circovirus Type 2 and 3 in the United States grower-finisher herd

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Introduction

Porcine Circovirus 2 (PCV2) and 3 (PCV3) are single-stranded DNA viruses. PCV2 was described in the 1990s associated with wasting disease and has become an economic burden for the swine industry. PCV2 vaccines became commercially available in the US in 2006. PCV3 was discovered in 2016 and has been associated with reproductive failure and multisystemic inflammation (1,2). However, the prevalence of PCV2/3 coinfection has not been retrospectively assessed. Therefore, the objectives of this study were 1) asses retrospectively the PCV2/PCV3 coinfection farm prevalence and geographical distribution in the US grower-finisher herd in 2000, 2006, and 2012 2) evaluate the PCV2 and PCV3 phylogenetic difference over time at the whole genome and ORF2 level.

Materials and Methods

7,230 serum samples representing 865 premises collected during 2000, 2006, and 2012 were obtained from the National Health Monitoring System (NAHMS) biobank. A variable number of sites per year were selected based on unknown herd or within-herd PCV2/PCV3 coinfection prevalence of 50% with a minimum of 15% precision and 95% confidence interval. Per farm, 15 serum samples were divided into 3 pools of 5 serums for PCV2 and PCV3 qPCR detection (ISU VDL). In addition, subsets of PCV2 and PCV3 positive samples were analyzed by genomic sequencing and phylogenetic analysis (ISU).

Results

In 2000 and 2006, most farms had a PCV2/PCV3 coinfection or PCV2 single infection. By 2012, most farms were double negative while an equal proportion were single positive for one pathogen (Figure 1). The average PCV2 PCR Ct was not significantly different between all years, while the average PCV3 PCR Ct was significantly higher in 2012 compared to 2000. In 2012, PCV2 vaccinated farms with clinical PCVAD weremost commonly PCV2 single positive. In contrast, PCV2 vaccinated farms with no clinical PCVAD were most commonly double negative for both pathogens.. Thirty-nine complete PCV2 genome sequences clustered into 3 subtypes: 32/39 PCV2a, 6/32 PCV2b, and 1/39 PCV2d. All sequences obtained in 2000 belonged to the PCV2a subtype. In the 2012 sequences, a dramatic shift in subtypes was observed with 6/8 PCV2b, 1/8 PCV2a, and 1/8 PCV2d.. A similar shift in subtypes was observed for the 50 PCV2 ORF2sequences obtained. Mutations in the PCV2 ORF2 full length Cap protein differed between subtypes. These mutations were present in regions of hypothesized immunological importance including linear and conformational epitopes, and critical neutralizing residues. However, fewer mutation were located in a suggested decoy epitope. Thirteen complete PCV3 genome sequences clustered into three subtypes: 3/13 PCV3a, 2/13 PCV3c, and 7/13 unclassified. In contrast, the PCV3 ORF2 clustered into three subtypes: 21/28 PCV3a1, 5/28 PCV3a2, and 2/28 PCV3. The prevalence of PCV3a1 decreased and shifted to PCV3a2 and PCV3c by 2012. Within the PCV3a2 subtypes, a I150L mutation was consistently observed, which is located in a B cell epitope.

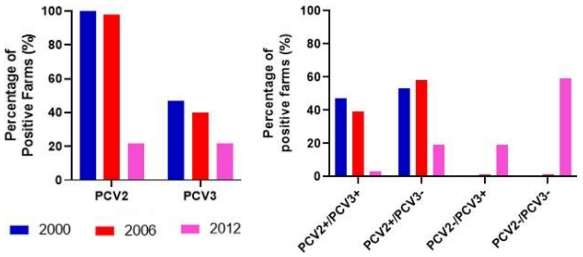

Figure 1. Farm prevalence (left) and coinfection rate (right) of PCV2 and PCV3 in 2000, 2006, and 2012.

Discussion and Conclusion

PCV3 was endemic in the US swine industry before the first description in 2016. PCV2 and PCV2/PCV3 coinfected farms were highly prevalent before 2006. After the introduction of PCV2 vaccines in 2006, the percentage of PCV2 and PCV2/PCV3 coinfected farms decreased. In 2012, PCV2 vaccinated farms with clinical PCVAD were highly associated with PCV2 infection. The prevalance of the PCV2a subtype reduced dramatically after the introduction of commercials vaccines. Then, the prevalence of PCV2b and PCV2d gradually increased by 2012. Consistent amino acid changes were located in regions of immunological importance and mutations were less frequent in a suggested decoy epitiope. These specific amino acid changes that differ between PCV2a and PCV2b may suggest vaccination with one subtype may not offer complete cross protection against other subtypes. More genetic diversity was observed in PCV3 with the subtypes of PCV3a1, PCV3a2, and PCV3c prevalent in 2012. A mutation located within a B cell epitope at residue 150 was common in the PCV3a2 subtype, suggesting potetial virus escape from the immune system.

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Evaluation of air filters in swine farms as a surveillance method to assess the spread of porcine reproductive and respiratory syndrome and influenza A viruses

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) and influenza A virus (IAV) are two major respiratory pathogens that result in significant economic losses to the pig industry in the US. Airborne transmission is considered an important route of PRRSV and IAV spread, which makes the two pathogens costly and difficult to control. Air filtration of incoming air has been widely used in swine farms in the Midwestern US, and has effectively reduced the incidence of airborne PRRSV infections in breeding herds (1). Therefore, detection of viruses in used air filters from farms offers a unique largely unexplored opportunity to monitor the regional spread of PRRSV and IAV. In this study, we aimed to evaluate the use of air filters as a surveillance method to monitor the regional spread of PRRSV and IAV, and to enhance our understanding of the epidemiology and control of airborne diseases.

Materials and Methods

We selected 7 breeding herds from high pig density areas which were either PRRSV negative or stable (i.e not weaning positive pigs) at the beginning of the study. Twenty brand new air filters were installed in each farm, and five filters were removed each time atapproximately 6, 8, 11 and 14 months post installation.Five samples were cut from each filter, with each sample consisting of six 2-by-2-inch squares. Thesesamples were ground in liquid nitrogen and mixed with minimum essential media. Viral RNA was extracted from the supernatant of each sample and quantified by real time RT-PCR for PRRSV and IAV (2). A filter wasconsidered positive if at least one sample tested positive. Samples positive for PRRSV or IAV were further analyzed with whole genome sequencing.

Results

A total of 136 air filters were analyzed. These filters were installed in July 2019 at the earliest, and removed successively until October 2020. Filters were cut into 680 samples for testing. Out of the 136 filters, ten (1.5%) samples corresponding to seven (5%) filters from three farms tested positive for PRRSV. During the study, PRRS outbreaks were reported in four farms, however, only one PRRSV positive filter originated from farms that had PRRS outbreaks. In contrast, sixty five (47.8%) filters from all seven farms tested positive for IAV, with a total of 131 samples positive (19.3%). Six IAV positive samples were sequenced and one sample was successfully subtyped as an H3N2 human-like influenza virus. In addition, multiple lineages were identified for the influenza internal genes from different samples.

Conclusion

Testing of used air filters in swine farms did not result in an enhanced surveillance method for airborne PRRSV. However, used filters for influenza surveillance should be further evaluated to represent more farms and locations. Overall, detection of PRRSV and IAV in the air filters showed some potential evidence of regional airborne transmission for these viruses, but additional investigations are needed to better understand the source of the detected viruses and the factors that contribute to airborne transmission of these viruses.

Acknowledgments

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Evaluation of ASF and PRRS virus transmission between pigs when using conventional needles and a needle-free device

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Introduction

Porcine reproductive and respiratory syndrome (PRRS), a devastating disease in pigs characterized by respiratory and reproductive disease, has been present inSoutheast Asia (SEA) since the 1990's [1]. In addition to PRRS, the spread of African swine fever (ASF) in the SEA region has increased the threat to ASF-free herds [2]. Intramuscular administration using needles has been the main route of vaccination in pigs although the risks associated with conventional needles are high. PRRS virus (PRRSV), for instance, was transmitted by conventional needles and was able to induce the disease in naïve pigs [3]. Therefore, the objectives of this study were to evaluate African swine fever virus (ASF) and porcine reproductive and respiratory syndrome virus (PRRSV) transmission between pigs when using conventional needles and a needle-free device.

Materials and Methods

In the present study, forty-two 3-week-old pigs were procured from a herd free of ASF and PRRSV. Their negative status against both pathogens was confirmed by PCR in blood samples upon arrival. Eighteen pigs were randomly allocated into 6 groups called seeders, of 3 pigs each, namely IM/ASF, ID/ASF, IM/PRRSV, ID/PRRSV and 2 control groups, NoChal/IM and NoChal/ID. Twenty-four age-matched pigs were divided into 4 groups of 6 pigs each as sentinels: IM/ASFsent, ID/ASFsent, IM/PRRSVsent and ID/PRRSVsent.

At 0 days post exposure (DPE), the IM/ASF and ID/ASF groups were exposed to ASF-infected pigs. The IM/PRRSV and ID/PRRSV groups were inoculated intranasally with 4 ml of HP-PRRSV-2 (10^6 $TCID_{50}$/ml, 2 ml/nostril). At 7 DPE (0 days post injection (DPI)), the IM/ASF and IM/PRRSV groups were given 2 ml of a bivalent porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* (Mhyo) vaccine via the intramuscular route using conventional needles. The ID/ASF and ID/PRRSV groups were given 0.2 ml of a PCV2 and Mhyo vaccine (Mhyosphere® PCV ID, HIPRA) intradermally using a needle-free device (Hipradermic®, HIPRA). Also, at 7 DPE the same conventional needles and needle-free device were used to inject the same volume of the vaccine into the animals in the sentinel groups (1 exposed pig to 2 sentinels) withthe same route of injection for each. Blood samples were collected from the seeders at 0, 7, 14, 21 and 28 DPE, and from the sentinels at 0, 7, 14, 21 and 28 DPI. ASF and PRRSV antibodies and viraemia were evaluated using ELISA and RT-qPCR respectively.

Results

The results demonstrated that the ASF and PRRSV seeder groups had the highest viraemia at 7 DPE. Following injection, sentinel pigs of the IM/ASFsent and IM/PRRSVsent groups were PCR positive at 7 DPI. In contrast, sentinel pigs of both the ID/ASFsent and ID/PRRSVsent groups were PCR negative throughout the experiment. Seroconversion results show that the IM/ASFsent and IM/PRRSsent groups had positive animals at 14 DPI, whilst the ID/ASFsent and ID/PRRSsent groups were negative throughout the experiment (Table 1).

Table 1. Seroconversion of age-matched sentinel pigs following injection.

Groups	Days post injection				
	0	**7**	**14**	**21**	**28**
IM/ASFsent	0/6*	0/6	**4/6**	**6/6**	**6/6**
ID/ASFsent	0/6	0/6	0/6	0/6	0/6
IM/PRRSsent	0/6	0/6	**2/6**	**6/6**	**6/6**
ID/PRRSsent	0/6	0/6	0/6	0/6	0/6

*Asterisk indicates number of positive pigs/total pigs.

Discussion and Conclusions

Our findings revealed the potential for ASF and PRRSV transmission through needles during vaccination.

On the other hand, the possibility of applying intradermal vaccines with a needle-free device such as Hipradermic® inhibits both ASF and PRRSV transmission and could be used as an alternative vaccination route, avoiding iatrogenic transfer of pathogens between animals with shared needles.

Acknowledgments

This study was supported by Chulalongkorn University (Fundamental fund, grant number 65413100200013, CUFRB65_food(26)190_31_09).

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Evaluation of internal farm biosecurity measures combined with sow vaccination to prevent influenza A virus infection of suckling piglets in breeding herds

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Introduction

Influenza A virus (IAV) is widespread in swine worldwide [1]. Piglets of weaning age are one of the sub-populations most likely to test IAV positive in breeding herds [2]. Commonly at weaning, pigs are transported to distant locations where they may be commingled with pigs from other herds creating an opportunity for co-circulation of distinct influenza viruses. As a result, new strains of IAV may emerge.
Vaccination of pregnant sows has been the main tool for controlling IAV infections in breeding herds [3]. This strategy has been effective at reducing IAV prevalence at weaning, but is not sufficient to consistently wean IAV negative pigs [4]. A recent field study that evaluated enhanced biosecurity measures resulted in a significant delay in IAV infections during lactation, but it was not enough to reduce IAV prevalence at weaning [5]. The objective of the present study was to evaluate the impact of combining both, internal biosecurity practices directed at minimizing IAV infection in piglets and sow vaccination, in IAV prevalence at weaning.

Materials and Methods

Six IAV positive breeding herds were selected for the study. Five herds were assigned to the treatment group, which consisted in implementing an enhanced internal biosecurity protocol combined with mass sow vaccination. The internal biosecurity protocol consisted of implementing practices of no cross fostering after the piglet's first 3 days of age, no usage of nurse sows, changing of disposable gloves between litters when handling piglets and daily disinfection of tools used in farrowing rooms for a period of 8 weeks. The mass sow vaccination included vaccinating all females (sows and gilts) with an autogenous herd-specific vaccine with a booster 3 weeks later. The autogenous vaccine included two strains of the H1 subtype (delta-1-1B.2.2.1 andpdm-1A.3.3.2) and one of the H3 subtype (Cluster IVA 3.1990.4.1) and strains had been selected by genetically analyzing IAV isolates recovered from the pig production company's farms and selecting the most prevalent. One farm served as control, in which there was no change in management or vaccination practices. Prior to the intervention, all farms were screened for IAV for 3 consecutive weeks, collecting 90 udder skin wipes from litters of weaning age [6]. Six weeks after the booster vaccination, litters at weaning were sampled for 3 more consecutive weeks to assess IAV prevalence after intervention. All collected samples were then tested individually using an IAV rRT-PCR test. Differences in IAV prevalence before and after the intervention by farm were assessed with a chi-square test using R statistical software (version 4.1.1) [7].

Results

Three of the five farms (60%) that were assigned to the treatment group tested IAV negative in all 3 sampling points post-intervention. One of the treatment farms had a significant decrease in IAV prevalence post intervention but IAV could still be detected in low levels in the last sampling of the study. In contrast, IAV prevalence in one of the treatment farms was not altered indicating that the treatment failed and had no effect on decreasing prevalence at weaning. As expected, the control farm tested IAV positive throughout the study.

Discussion and Conclusion

The study presented here provides a protocol that combines sow vaccination and enhanced internal biosecurity practices to help wean negative pigs to IAV. This protocol can serve as a guide to pork producers that have the goal of controlling and/or eliminating IAV infections in their breeding herds.

Acknowledgments

Pipestone Veterinary Services

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Evaluation of parity and management practices in influenza virus infection in suckling pigs

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Introduction

Infection of piglets prior to weaning is one of the key factors that contribute to IAV persistence in breeding herds. Understanding how piglets become infected, what factors contribute to piglet infection and what are the most relevant sources of IAV infection to piglets is key to control IAV. In this study, we evaluated whether changes in management practices during the pre-weaning period change IAV prevalence. Changes in management practices included farm workers behavior related to the movement of pigs between litters (i.e cross-fostering) and biosecurity measures implemented by the workers while handling piglets. We also evaluated the effect of sow parity in IAV prevalence during the course of lactation.

Materials and Methods

Three farms known to be IAV positive at weaning were selected for the study. These farms were selected based on their willingness to participate in the study, ability to follow the study protocols, lack of influenza vaccination in pigs prior to weaning and history of piglets testing IAV positive at weaning for 3 months prior to start the study. At enrollment, farrowing rooms were allocated to either "control" or "treatment" groups. Litters within the control rooms were processed (tail-docking and castration), handled and treated according to existing farm protocols. Litters in the treatment rooms were not cross-fostered after processing, and processing or any other handling of the pigs was done by farm personnel changing gloves, and wearing boot covers if personnel was to enter the crates. A total of 28 farrowing rooms distributed across 3 farms were enrolled into the study. Within each farrowing room litters were stratified into young parities (P0, P1 and P2) and older parities (>P3), random selection of litters within each strata was performed. The selected litters were sampled throughout the preweaning period to assess IAV status.A total of 360 litters (120 per farm) were enrolled into the study. The samples collected were udder skin wipesfrom study litters and surface samples from airborne particles deposited on the surfaces of the studyfarrowing rooms [1]. Sampling of litters and the environment took place at 1, 8, 13 and 18 days of age of the piglets, approximately. Samples were also collected from materials used in the farrowing rooms and from worker's hands after piglet handling using a cotton gauze impregnated in DMEM transport media.

Samples were tested using rRT-PCR that targets the conserved matrix gene of IAV [2]. Statistical associations of experimental groups and dam paritywere explored with a generalized linear model using statistical software [3].

Results

The udder wipes collected from study litters showed a lower IAV prevalence in the treatment group 209/720 (29%) compared to the control group 318/720 (43%) (p-value < 0.001). At day 8 of age the litters from thecontrol group had 7.5 times higher IAV prevalence than the litters from the treatment group. However, at weaning, differences in IAV prevalence had disappeared (77.2% vs. 81% for treatment vs. control, respectively, p-value = 0.41). There were no differences in IAV detection between parity groups (young parity sows 254/676 (37.5%) vs older parity sows 273/764 (35.7%) (p-value = 0.86). Samples collected from farm worker's hands and materials had 58% (65/111) and 46%(13/28) positivity to IAV respectively.

Discussion and Conclusion

Our results indicate that specific management practices directed at minimizing spread of IAV in pigs prior to weaning can slow down IAV transmission within farrowing rooms, however they are not sufficient to result in a significant decrease of IAV prevalence at weaning. We did not find an association between sow parity and IAV litter prevalence during the lactation period. The high prevalence of IAV in the samples collected from farm worker's hands and fomites is most likely facilitating the indirect transmission of IAV within farrowing rooms while piglet handling.

Acknowledgments

This study was funded by Boehringer Ingelheim Animal Health USA Inc. Duluth, GA 30096.

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Evaluation of shedding and effect on pig performance of Prevacent® PRRS vaccine

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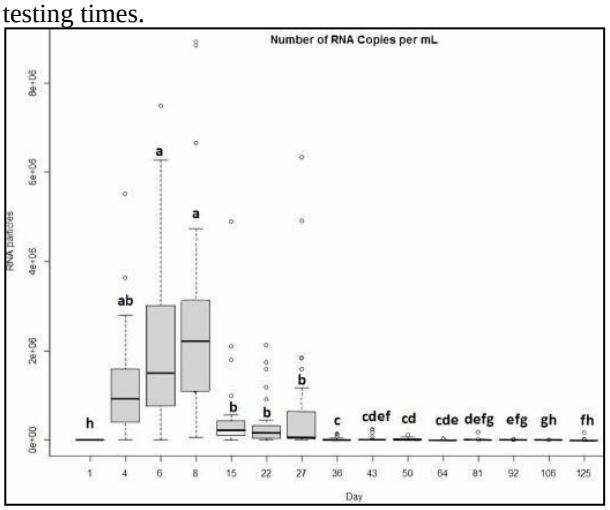
Introduction:
Many swine production systems today are vaccinating PRRS negative pigs from at risk populations with PRRS vaccine. Vaccination has been shown to improve performance in the face of field virus infections.[1,2,3,4] However, vaccination has reduced post-weaning growth performance in absence of PRRS exposure.[5] Protecting PRRS negative weaned pigs placed in high PRRS risk areashelps reduce shedding of field virus if infection occurs and helps reduce area PRRS spread. The use of PRRS vaccine may be one of these tools. Elanco licensing data for Prevacent® PRRS from a small group of pigs in a research setting showed limited transmission from vaccinates to unvaccinated sentinels.[6]

Objective:
Objectives of this study included: (1) identifying the level of viremia and shedding in pigs following vaccination with Prevacent PRRS® vaccine in a commercial setting, and (2) measuring any impact of vaccination on performance of the pigs.

Materials and methods:
- 30 sentinel pigs in each treatment were identifiedand tagged in each ear and blood tested at various times in the study.
- Oral fluids were collected from each pen at the same time blood samples were collected.
- Day 0: Prevacent® PRRS vaccine administered at labeled dose (1 ml/pig). Controls administered1 ml saline.
- Body weights collected start at (day 0) and end (day 50) and prior to marketing at the end of finishing phase.
- Cyclonic Air collector was used to collect air samples at daily intervals from Day 0- Day 28; samples were collected from inside the barn, atpit fan, and 1 mile away from the site in the downwind direction for the day.

Results:
All air samples collected were negative from all locations demonstrating that there was no detectable shedding by this method of sample collection.
Pigs serologically converted to vaccine showing an immune response to the vaccine.
The levels of virus dropped quickly following exposure which may explain that there was limited shedding detected from the population.

Figure 1. Number of RNA copies per ml at various testing times.



Vaccination did not affect growth performance wean to market.

Figure 2: Performance of vaccinated groups

	Treatm			
	None Vaccinated	Vaccinated	SEM	*P*- value
Weight (kg)				
day 0	**6.3**	**6.3**	**0.15**	**0.99**
Day 50	**34.1**	**34.4**	**0.63**	**0.27**
Day 125	**105.4**	**105.7**	**1.39**	**0.64**
ADG (grams/ Day)				
Day 0-50	**553.4**	**557.9**	**0.01**	**0.19**
Day 51-125	**952.5**	**952.5**	**0.01**	**0.96**
Day 0-125	**789.3**	**793.8**	**0.01**	**0.63**
Moraltiy %				
Day 50	**2.90**	**2.80**	**0.37**	**0.91**
Day 125	**5.20**	**4.80**	**0.29**	**0.79**

Conclusions and Discussion:
Vaccine virus did not shed into the air in this study based upon sampling completed. Viremia decreased quickly after vaccination and may be in part becausethere was limited spread. Vaccination did not affect the wean to market growth performance.

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Evolution of PRRS RT-QPCR Analysis from 2018 to 2021 in an European Laboratory

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Introduction

PRRS diagnosis provides valuable information for decision-making for the control and prevention of the disease. Consequently, new sampling methods (processing fluids and tongues) are proposed to facilitate sampling and increase PRRSV detection.The objective of this study was to analyse the evolution of RT-qPCR results and type of samples received in Diagnos (HIPRA diagnostic laboratory, Spain) from 2018 to 2021.

Materials and Methods

Between January 2018 and December 2021, 39,269 RT-qPCRs were done in the lab on samples coming from different European countries. Samples were grouped as: semen (S), blood (B), oral fluids (OF), tissues (TI) (lungs, lymph nodes, etc.), processing fluids (PF) and tongues (TO). Evolution of % positivity was studied according to the type of sample. Moreover, positivity of the results, divided into four periods from December to February (Dec-Feb), March to May (Mar-May), June to August (Jun-Aug) and September to November (Sep-Nov), was analysed to evaluate seasonal incidence.

Results

6,388, 7,527, 11,270 and 14,084 RT-qPCRs were performed yearly from 2018 to 2021. B, OF and TI accounted for 71.4%, 20.3% and 4.6% of the samples received since 2018, respectively. PF accounted for 2.4% and TO for 1.2% of the samples (Table 1).
Regarding positivity, 24%, 20.4%, 44.1%, 18.4%, 34.8% and 1.7% of the samples were positive in the case of B, OF, TI, PF, TO and S, respectively, statistical significant differences were found between type of samples (Table 2). From 2018 to 2021, the seasonal incidence was 24.9%, 22.1%, 22.7% and 27.2% in Mar-May, Jun-Aug, Sep-Nov and Dec-Feb respectively and statistical significance was found between seasons (Table 3).

Table 1. Type of sample per year.

	S	TO	PF	OF	B	TI
2018	30	0	0	1858	4082	418
2019	12	0	9	2070	5001	435
2020	3	44	119	2023	8649	432
2021	14	427	796	2014	10297	536
Total	**59**	**471**	**924**	**7965**	**28029**	**1821**
%	**0.1**	**1.2**	**2.4**	**20.3**	**71.4**	**4.6**

Table 2. Positivity by type of sample

	S	TO	PF	OF	B	TI
% Positivity	1.7	34.8	18.4	20.4	24	44.1
Post-hoc Tukey	D	A	AB CD	CD	B	AB C

P-value < 0.001 ***
Model= Logistic Regression Model

Table 3. Positivity by period

	Mar-May	Jun-Aug	Sep-Nov	Dec-Feb
% Positivity	24.9	22.1	22.7	27.2
Post-hoc Tukey	B	C	C	A

P-value < 0.001
Model= Logistic Regression Model

Conclusions and Discussion

TO represent an alternative for monitoring piglets at early ages to detect vertical transmission of the virus as it is a convenient and easy sampling method with a high detection rate, higher than B, OF and PF. Moreover, sampling at early ages with PF cannot be used on all farms because the castration rate is low in some countries. Thus, sampling with TO represents a suitable alternative with a good level of detection. Regarding seasonality of the disease, as expected and previously reported, the highest incidence was in Dec-Feb which are the coldest, foggy months of the year that facilitate the dissemination of the PRRS virus. The season with the second highest detection of PRRSwas spring, which was higher than the hottest monthsof the year. Seasonality of the disease helps us to anticipate and create strategies to minimize the effect of PRRS in the coldest months of the year.

Experimental intravenous, intratracheal, and intranasal inoculation of swine withSARS-CoV-2

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is novel coronavirus that causes disease (COVID-19) in humans, and it is hypothesized to have a zoonotic origin. Since swine and other livestock species are susceptible to other coronaviruses, research has been initiated to determine the susceptibility of livestock species to SARS-CoV-2 and if livestock can play a role in spread of the virus. Multiple groups have reported that swine are not susceptible to SARS-CoV-2 via the intranasal inoculation route; therefore, two different routes of inoculation (intravenous and intratracheal) were evaluated to determine if swine were susceptible to SARS-CoV-2.

Materials and Methods

Twenty-four 3-week-old pigs were purchased from a commercial swine herd and transported to the National Animal Disease Center in Ames, IA. Control pigs were housed in ABSL-2, while challenged pigs and their contacts were housed in a BSL-3AG facility. Three challenge routes were performed: intravenous (IV, n=4, 344-347), intratracheal (IT, n=4, 348-351) and intranasal (IN, n=4, 352-355). Sham cell culture lysate challenge was administered to control pigs (n=2/route) with 2 control pigs untreated. Finally, one contact pig was added to each challenge group and the control room on day 2 post inoculation. Virus was isolated from a Malayan tiger that developed respiratory signs after infection with SARS-CoV-2 (TGR/NY/20). IV pigs received 2 mL, while both IT and IN groups received 5 mL of 6.8 x 10^6 $TCID_{50}$/ml virus solution. Temperatures were recorded daily using a subcutaneous microchip. Nasal/oral swabs, rectal swabs, and group oral fluids were collected on 0-7, 10,12, 14, 18 and 21 days post inoculation (dpi). Serum and whole blood were obtained at 0, 3, 7, 14, and 21 dpi. Samples were tested by PCR for the presence of virus and serum was utilized in a serum virus neutralization assay to test for neutralizing antibodies. At 21 dpi animals were euthanized and tissues were collected for PCR testing and histology.

Results

Immediately after IV challenge, pigs that received either virus or sham cell culture lysate began to vomit, but quickly recovered. Two IT pigs had increased temperatures 2-3 dpi, but only one pig had a temperature recorded greater than 40.5°C on 13 dpi from the IV group. Otherwise, pigs in this study did not demonstrate any clinical signs.

The IV group had two pigs (344, 347) PCR positive on 3 dpi in the buffy coat and one pig (345) positive in a nasal/oral swab on 4 dpi. The remaining samples were all negative by PCR. All pigs in the IV group developed virus neutralizing antibody (VN) titers of 1:32 and 64 on 7 dpi but a reduction in those titers was observed on 14 dpi (1:8 to 1:32). By 21 dpi all pigs had titers of 1:8.

In the IT group, all pigs were positive in nasal/oral swabs on 1 dpi, suggesting likely detection of the inoculum virus. On 2 dpi, SARS-CoV-2 RNA was detected in only two pigs (350, 351). Beyond 2 dpi, one pig (351) was PCR positive on 7 dpi, with RNA being detected on nasal/oral swabs. In rectal swabs, pig 350 was positive on 1, 2, and 7 dpi. Pig 351 had positive rectal swabs on 1 and 3 dpi. In addition, the IT group had positive oral fluids on 6 dpi. Pig 350 developed a titer of 1:32 on 21, while pig 351 had a titer of 1:16 on 14 dpi and dropped back down to 1:8 on 21 dpi.

All animals in the IN group were positive in nasal/oral swabs on 1 dpi, which again could be detection of inoculum given the route of inoculation. Two animals (353, 355) were positive on 2 dpi and one animal (354) on 3 dpi. In rectal swabs, pig 355 had positive swabs on 1 and 2 dpi. Of note, pig 352 had positive rectal swabs on 2, 3, 4, 5, 10, and 12. In addition, this group was PCR positive in oral fluids on 4 dpi. Only pig 352 seroconverted with a titer of 1:32 on 14 dpi and 1:16 on 21 dpi.

The contact pigs did not present any PCR positive samples and seroconversion was not observed in any of the contact animals.

There was no gross or histologic pathology observed in the pigs. Some tonsil and lymph node tissues were PCR positive from at least one pig in each challenge group and in a contact pig in the IV group, but Ct values were relatively high. RNAScope was performed on tissues that were PCR positive and no staining to indicate the presence of SARS-CoV-2 RNA was observed. All PCR positive samples were subjected to virus isolation. All samples were virus isolation negative after three blind passages indicating a lack of infectious virus.

Discussion and Conclusion

Our results corroborate studies from China, Germany, Canada, and the US showing that pigs are likely not susceptible to SARS-CoV-2 infection. Detection of SARS-CoV-2 RNA in secretions at early time points post-infection suggest likely detection of residual inoculum virus. The lack of sustained antibody responses in inoculated animals are most likely a result of a response to non-replicating virus antigen. Not surprisingly, contact animals remain negative throughout the experiment, demonstrating lack of transmission between swine. Thus, it is unlikely swine are a reservoir and contributing to the epidemiology and spread of SARS-CoV-2 in the human population.

Expression levels of CD163, CD169, and CD151 in PRRSV infected peccaries

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Introduction

PRRSV is an enveloped, positive-sense, single-strand RNA virus that targets pulmonary and intravascular macrophages, and blood differentiated monocytes. Clinical sings associated with PRRSV infection range from respiratory disease and mortality and reproductive failure. Infection occurs through interaction with a complex of cell surface receptors including CD163, CD169, CD151 (2,3). PRRSV infection has been widely studied in swine and it is the only reported natural host. Peccaries (*Pecari tajacu*) have also demonstrated susceptibility to infection, developing a prolonged and sustained viremia and antibody response (1). However, no evident clinical sings were observed under experimental conditions. The objective of this study was to evaluate morphologic pulmonary changes as well as gene expression levels of CD163, CD169 and CD151 receptors in pulmonary and lymphoid tissues collected from PRRSV experimentally infected peccaries.

Materials and Methods

Histological evaluation of lung was performed to assess microscopic changes in peccaries and porcine experimentally infected with PRRSV. Detection of PRRSV antigen and CD163 cellular expression was performed by immunohistochemistry (IHC). The CD163 mRNA expression in situ was performed with RNAScope. Quantification and comparison of mRNA expression levels of CD163, CD169 and CD151 genes on lung, tonsil, lymph node, and spleen from three experimental groups comprised of PRRSV infected pigs, PRRSV infected peccaries, and PRRSV negative pigs. SYBR Green PCR was performed and the data was evaluated using the ΔCt method for relative quantification.

Results

Both, PRRSV positive peccaries and pig exhibit marked interstitial pneumonia with clusters of necrotic macrophages, and lymphoplasmacytic infiltrate.Despite interstitial pneumonia observed in PRRSV positive pigs and peccaries there was no detectablePRRSV or CD163 antigen in peccaries. Although there was a strong intracytoplasmic CD163 mRNA signal in PRRSV positive pigs no *in situ* detection of CD163 wasobserved in peccaries. mRNA expression levels of CD163 were approximately 10 fold less across tissue types between the PRRSV infected peccaries and PRRSpositive and negative porcine. Levels of CD151 mRNA were significantly lower in PRRSV positive pigs than negative PRRSV, and it was 6 to 11 fold lower in peccaries. Swine PRRS positive pigs showed lower CD169 levels in tonsil compared with no infected pigs and peccaries and significant increment was observed in lymph nodes on PRRSV negative pigs.

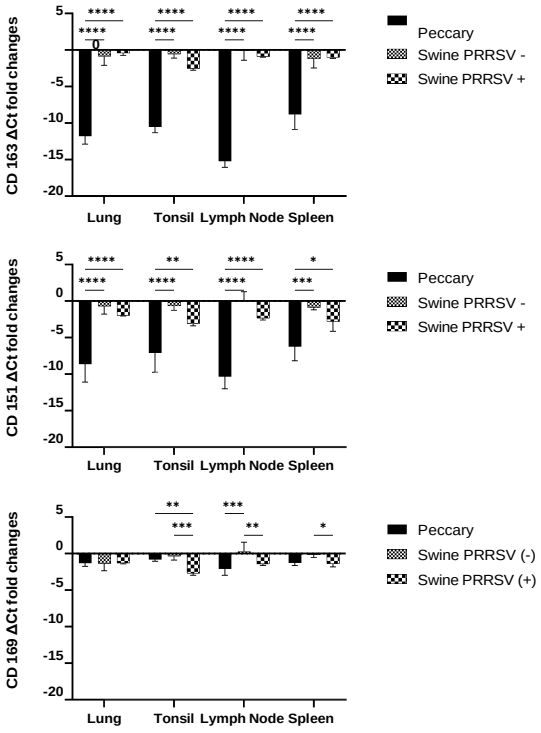

Figure 1: mRNA expression levels of cell membrane receptors associated with PRRSV entry evaluated on lung and lymphoid tissues

Discussion and Conclusion

CD163 and CD151 mRNA levels differ significantly during PRRSV infection in peccaries compared with conventional pigs, but further studies are necessary to evaluate whether this is a species-specific difference oran exacerbated regulatory response during PRRSV infection of peccaries. Although viral replication occursit may not be entirely dependent on CD163 and CD151receptors. No major difference in CD169 expression were observed across all three experimental groups which may suggest in may not play a critical role in viral penetration in pulmonary and lymphoid tissues. Furtherstudies to evaluate structural and functional differencesof PRRSV entry receptors on peccaries are necessary tounderstand the mechanism of viral replication this species.

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Farm management practices associated with influenza A virus contamination of worker's hands and clothing

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Introduction

Influenza A virus (IAV) is endemic in pigs and causes important respiratory disease in pigs causing important economic losses to swine producers [1]–[3]. In breeding herds, piglets are handled during the birthing process and throughout the pre-weaning period to ensure their care and prevent losses and injuries. Activities such as drying piglets shortly after birth, handling piglets to ensure colostrum intake and prevention of injuries, or applying treatments and vaccinations are common examples of care. Piglets may also be moved between litters. Because IAV can be transmitted through contaminated fomites, we hypothesize that there are specific management practices involving the handling of piglets that can facilitate the contamination of farmworkers' hands and clothes representing a high-risk for spreading IAV to other piglets. The objective of our study was to investigate the association of specific management practices that require intensive handling of pigs, that is processing, vaccination, and weaning with risk of IAV contamination of farmworkers' hands and clothes.

Materials and Methods

Three breeding herds with confirmed IAV detection among the suckling piglets were selected for the study. Workers were requested to wash their hands thoroughly with water and soap before initiating the activities of processing, vaccination or weaning. Workers were then provided with new PPE consisting of disposable coveralls (DuPont^{tm} Tyvek®, Wilmington, Delaware, USA) and a pair of latex gloves to be worn while performing the activities. After completion of the selected activities, samples were collected from hands and coveralls by wiping thoroughly pre-designated surfaces of the hands and coveralls using a gauze wet with transport media. An area from the coverall of approximately 30 cm x 30 cm with direct contact with the piglets, which included arms, chest, and groin area was sampled. Both palmar areas from the hands and fingers were also sampled. Samples were tested by rRT-PCR targeting the highly conserved IAV matrix gene, following previously described procedures [4]. Positive samples were further tested for virus isolation using Madin-Darby canine kidney (MDCK) cells [5]. To identify activities with increased risk of IAV detection, a multivariate generalized linear model using R statistical software (version 4.1.1) was used [6].

Results

There were 155 samples collected immediately after the activities were concluded. Seventy-six samples were collected immediately after processing with 12 (15.8%) of them testing IAV rRT-PCR positive, nine from coveralls and 3 from hands. Forty-eight samples were collected after vaccination of piglets and 45 (93.8%) of them were positive to IAV. All coveralls (19/19; 100%) were IAV positive after vaccination as were the majority (26/29; 89.7%) of the samples collected from hands surfaces. From the weaning activity, 31 samples were collected and 29 of them tested IAV positive, 14 from farmworkers' hands and 15 from their coveralls. Viable IAV was isolated from five samples with four of these from samples collected after piglet vaccination (three from hands and one from coveralls) and one from a coverall at weaning. Samples collected immediatelyafter vaccination and weaning had approximately 6 times higher risk of testing IAV positive than samples collected after processing, with prevalence ratios of 6.20 and 5.98, for vaccination and weaning, respectively (p- value < 0.0001). However, there was no significant differences in IAV detection between farmworkers' hands and coveralls (p-value = 0.42)

Discussion and Conclusion

Our results indicate that activities that involve handlingof piglets before weaning and requiring close contact between farmworkers and pigs likely represent a significant risk for IAV dissemination during the preweaning period. Our results can be used to provide recommendations to improve management protocols directed at limiting the transmission of pathogens in pigs before weaning.

Acknowledgments

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Feeding spray-dried porcine plasma to pigs contributed to delayed experimental African swine fever transmission and progression

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Introduction

African swine fever virus (ASFV) is a dsDNA virus that can cause high mortality in pigs of all ages. ASFV is primarily transmitted oro-nasally or indirectly by feeding garbage containing infected ingredients, biological vectors or fomites. Spray-dried plasma (SDP) is a highly digestible, high-protein level ingredient that is widely used in feed because it benefits growth performance, gut function, and immune parameters (1). The objective of this study was to evaluate the potential benefits of feeding SDP to naïve pigs in direct contact with pigs infected with ASFV *Georgia 2007/01* strain**.**

Material and Methods

A total of 24 pigs (24 d of age) were randomly assigned to either a control or porcine SDP (8%) feed treatment group. At housing, pigs were divided into two groups of 12 and fed their respective diets for the entire study. After 4 d of acclimation, 2 pigs in each box (trojans) were intramuscular (IM) injected with 10^3 GEC of ASFV strain Georgia 2007/01. The ratio of trojans to naïve pigs was 1:5 aiming to reproduce the slow transmission of the disease. Due to the unexpected lack of transmission to naïve pigs, by d 23, 3 additional pigs from each feed treatment group were selected as trojans, and IM injected on d 23 with the same indicated dose of Georgia 2007/01 strain. The ratio of trojans to naïve pigs after the second exposure was 3:7. Blood samples were collected at d4, 8, 15, 22, 29 and 35 of the study. Nasal (NS) and rectal (RS) swabs were collected on d4, 8, 11, 18, 25, 32 and 35 of the study. On d15 the T-cells response to ASFV exposure was analyzed. At the end of the study (d35), all pigs were euthanized and samples of submaxillary, retropharyngeal, and gastro-hepatic lymph nodes (LN), spleen, and tonsils collected. Taken samples were analyzed by RT-PCR.

Results and discussion:

During the first trojan exposure, no in-contact animals in either group became infected with ASFV and did not develop ASFV-specific antibodies (probably due to the short exposure time). However, all pigs fed with SDP showed low, albeit detectable, specific T-cells responses on d15. In clear contrast, only 2 of the 10 pigs fed the control diet showed detectable T-cell responses, indicating a lower immune priming. With these unexpected results, we decided to add additional trojans on d23. New trojans in the control group died 5 to 8 days post injection. However, 2 of 3 trojans in the SDP group survived until the end of the study (d12 post-challenge or d35 of the study). After the second trojan exposure (d23 of study), rectal temperature (RT) of contact pigs in the control group increased > 40.5ºC by day 30 of the study, however, in the SDP group of pigs this increase in RT was not observed until 4 days later (d 34 of study). Average cycle threshold (Ct) values for PCR results in blood, NS and RS at d35 (d 12 post-challenge) were 15.83±5.74, 25.47±4.72 and 33.14±5.13, respectively, for control contact pigs and 22.49±9.89, 28.19±4.07 and 31.99±5.94, respectively, for the SDP contact pigs. Average PCR Ct values were lower in the different tissues of control contact pigs compared with the SDP group (Table 1).

Table 1: Average ASFV PCR+ Ct values of different tissue samples from contact pigs at the end (d 35 or d12 after second exposure) of the study

	Treatment Groups	
ASFV PCR+ Tissue	Control	SDP
Submaxillary LN, Ct	19.15±2.35	24.10±7.48
Retropharyn. LN, Ct	20.70±2.91**	30.32±4.21
Gastrohepatic LN, Ct	22.14±5.47*	30.97±4.33
Spleen, Ct	16.86±2.69$^{\#}$	22.64±7.48
Tonsil, Ct	20.29±2.94*	26.48±5.71

Ct values of groups within a row differ; $^{\#}P<0.1$; $*P<0.05$; $**P< 0.01$.

Conclusions:

Under the conditions of this study, feeding spray-dried porcine plasma to pigs contributed to reducing ASFV transmission and progression, most likely by enhancing the ASF-specific T-cell responses, previously demonstrated as key rulers for ASFV protection (2). Feeding SDP can be a strategic nutritional intervention to improve protection against ASFV. In addition, feeding SDP during endemic ASFV situations with lessvirulent strains may help to reduce and delay transmission by direct contact.

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Field studies demonstrating the efficacy of combined PCV2 and Type I PRRS MLV vaccines

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Introduction

The combination of vaccines for single injection administration can reduce the number of injections given to pigs and improve the efficiency of the vaccination process. Four studies were conducted to investigate the efficacy of a porcine circovirus 2 (PCV2) vaccine (Ingelvac CircoFLEX®) and a Type 1 porcine reproductive and respiratory syndrome virus (PRRSV) modified live virus (MLV) vaccine (Ingelvac PRRSFLEX® EU) combined immediately prior to intramuscular injection, when compared to monovalent injections.

Materials and Methods

Randomised, controlled, blinded studies were conducted on four farms with serological and/or virological evidence of circulating Type 1 PRRSV and PCV2. Each study included between 742 and 845 pigs per treatment group, vaccinated at 13-18 days of age. Two studies (A & B) compared the combined products against the monovalent PRRS vaccine, and two studies (C & D) compared the combined products against the monovalent PCV2 vaccine. To ensure that pigs in the monovalent groups were not adversely affected by a potential viral challenge other than the field challenge of interest, they were also vaccinated with the other relevant monovalent vaccine at weaning (14 days after first vaccination).
Group least squares means of weight gain, estimated from a General Linear Model, were analysed using an ANCOVA derived t-test. The effect of treatment group on frequency of clinical signs, mortality, percentage of virus positive animals, prevalence of runts and pig treatments were analysed using Fisher's exact test and serum viral load using Wilcoxon Mann-Whitney test (1).

Results

In all four studies, there were no significant differences between combined vaccine groups and the monovalent vaccine groups in terms of weight gain from inclusion to end of study (finishing), clinical signs, mortality, pig treatments (PRRSV field challenge studies) and number of runts (PCV2 field challenge studies). Further secondary efficacy parameters included viraemia prevalence and serum viral load. Although occasional statistically significant differences between groups were seen at individual timepoints for these two parameters, differences were small and not deemed biologically relevant.

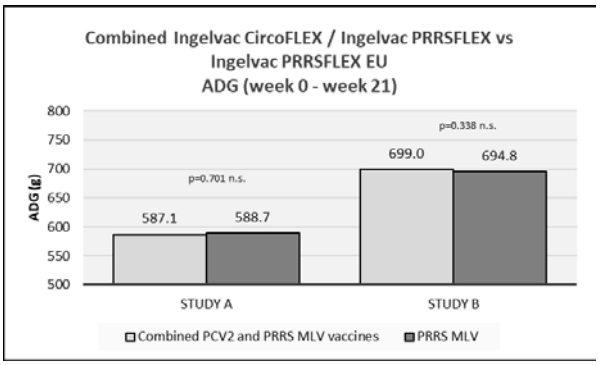

Figure 1. PRRSV field challenge studies. Average daily gain of pigs vaccinated with combined Ingelvac CircoFLEX/Ingelvac PRRSFLEX EU and pigs vaccinated with Ingelvac PRRSFLEX. (n.s. – not significant)

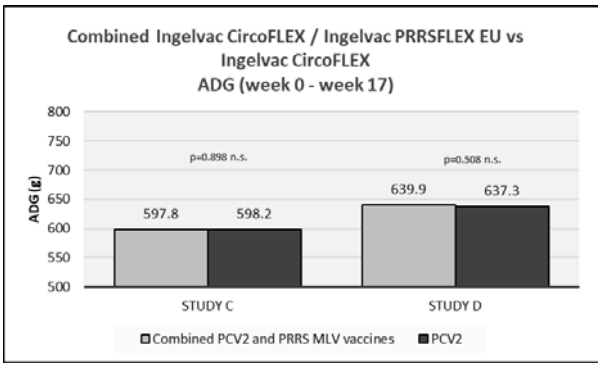

Figure 2. PCV2 field challenge studies. Average daily gain of pigs vaccinated with combined Ingelvac CircoFLEX/Ingelvac PRRSFLEX EU and pigs vaccinated with Ingelvac CircoFLEX. (n.s. – not significant)

Discussion and Conclusion

The results of these studies support the efficacy of the combined use of Ingelvac CircoFLEX and Ingelvac PRRSFLEX EU in the face of field challenges of both PCV2 and Type 1 PRRSV and its non-inferiority compared to the individual vaccines administered separately.

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First detection of an emerging strain of porcine circovirus type 3 in Peru

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Introduction

Porcine circovirus 3 (PCV3) is a recently described virus belonging to the family *Circoviridae*. It represents the third member of genus Circovirus able to infect swine, together with PCV1, considered non-pathogenic, and PCV2, one of the most economically relevant viruses for the swine worldwide industry (1). PCV3 was originally found by metagenomics analyses in 2015 in tissues of pigs suffering from porcine dermatitis, nephropathy syndrome, reproductive failure, myocarditis and multisystemic inflammation in the USA (2). The viral DNA has been found in healthy and diseased pigs; among different pathologic conditions, the strongest evidence of association comes from reproductive problems (3).

PCV3 has been considered an exotic disease for Peru, because there was no evidence of its presence. However, in November 2021 one well-managed pig farm localized in Amazonas department was presenting watery diarrhea and dehydration in animals of 120-121 days old. High morbidity and mortality were observed at that time. Several animals in the pen were sick but only two lymph nodes were obtained and sent for PCV2 diagnostics. This farm applies a commercial vaccine against PCV2 at 28 days of age. Therefore, the objective of this study was detecting the presence of PCV3 in one farm of Peru.

Materials and Methods

We worked with two samples of lymph nodes (L1 and L2) from animals of 120 and 121 days of age on one pig farm of Amazonas department in Peru. These samples were received in November 2021 and were negative for PCV2 by conventional PCR (*Manual proceedings of the virology section College of Veterinary Medicine, National University of San Marcos*).

Both samples were processed using a specific kit multiplex real time RealPCR™ PCV2/PCV3 multiplex DNA Mix (IDEXX, Westbrook- USA). For DNA extraction, we used a commercial kit extraction E.Z.N.A Universal Pathogen Kit (Omega BIO-TEK.Inc-USA) which was run following the tissue extraction protocol according to the manufacturer's indications.The simultaneous detection and differentiation of PCV2and PCV3 were run following the manufacturer's instructions (IDEXX, USA). The fluorescence signals were read for PCV2 and PCV3 with FAM and Cy5 channel, respectively. The internal positive control was read with the VIC channel.

Results

Both samples (L1 and L2) were positives to PCV3 amplification. There was no amplification to PCV2. In addition, L1 and L2 showed a threshold of 27,2 and 28,3 cycles to PCV3, respectively. Samples and controls showed an amplification curve between 25.5 and 27.9 cycles to internal positive control.

Conclusions and Discussion

PCV3 has been considered an exotic viral disease to Peru, because there was no evidence of its presence until now. Nowadays, these samples are being sequenced in order to define the origin of the virus and its genetic characterization. Preliminaries studies are showing that there is strong genetic relationship between Peruvian and North American strains as it has been reported with PEDV, PRRSV (American specie) and PDCoV when they entered to the country for the first time in 2013 (4), 2014 (5) and 2020 (6), respectively. Studies on epidemiology and diagnosis tests that include PCV3 should be implemented as part of the differential diagnosis with PCV2. This abstract represents the first documented report of the PCV3 presence in Peru by real time RT-PCR in animals with enteric problems.

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Genotyping of PCV2 field strains isolated from swine in Mexico

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Introduction

Genotype shift of PCV2 has been reported worldwide during the last years, with the emergence and spread of PCV2d (1). Though cross-protection of the current PCV2a based vaccines has been proven, emergence of immune escape mutants is feasible (2). At least four publications from USA, Korea, Germany and Brazil reported clinical cases of PCV2 disease in herds routinely vaccinating against PCV2, linked to isolation of PCV2b variant strains recognized later as PCV2d (3,4,5,6). However no PCV2 genotyping data are publicly available so far in Mexico. Thus the objective of this study was to determine the genotype of PCV2 field strains from swine clinical cases in Mexico.

Materials and Methods

Blood samples were taken from 243 pigs issued from 11 farms in 4 Mexican states (Sonora, Puebla, Queretaro, Guanajuato) between July and September 2021. The selected farms were routinely vaccinating piglets against PCV2 and sampled pigs showed respiratory signs or growth retardation. These sampled pigs were between 5 and 26 weeks old: 12% between 5 and 9 weeks, 43% between 11 and 14 weeks, 45% between 16 and 26 weeks (Table 1). The serum samples from each farm were pooled by 5 in average, according to pig age, before quantitative PCR analysis. Briefly the DNA was isolated using a commercial system column-based following the manufacturer recommendations (Qiagen®, CA, USA). The real time primers for quantification of PCV2 have been described previously (7, 8). PCR was performed using Brilliant II SYBR® Green qPCR core reagent kit of Stratagene following manufacturer instructions. The PCR reaction consisted of 25 µL of PCR mixtures (100 ng of DNA), forward and reverse primers concentrations at 150 nm, respectively. Amplification conditions were as follows: 95 °C for 10 min; and 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. In order to quantify PCV2 load, a standard curve was prepared using serial dilutions of the previously cloned PCR products, the Ten-fold dilutions were made in order to attain 10^8-10^1 genomic copies/µL sample for the PCR. Positive serum pools were directly sequenced for PCV2 genotyping when the cycle threshold (Ct) was below 30. When the Ct was higher than 30, individual serum samples from the pool were processed for sequencing. The products were gel purified using the QIA quick gel extraction kit (Qiagen®, Hilden, Germany) and sequenced in both senses with the Big Dye Terminator Cycle Sequencing Kit v2.0 and the ABI Prism 3130xl gene analyzer (Applied Biosystems, Foster City, CA) using the iPCV2-Fw and iPCV2-Rv primers. All sequences were carefully checked for quality, and only sequences encoding ORF2 without intermediate stop codons were included. The reconstruction of the phylogeny of the ORF2 sequences was performed by the neighbour-joining method (1000 iterations for bootstrapping) and the maximum likelihood method in the MEGA 5.0 package (9).

Table 1. Characteristics of sampled pigs

Region	Farms (number)	Pigs (number)	Pigs age (weeks)
Sonora	4	78	11-14
Puebla	3	69	12-26
Queretaro	1	26	7-20
Guanajuato	3	70	5-24

Results

A total of 49 serum pools were analyzed, among which 14 (29%) were positive for PCV2 from 7 (64%) of the 11 tested farms. Thirteen strains could be sequenced from either pooled or individual serum samples. These strains came from the 4 regions and from 5 farms. PCV2d genotype accounted for 92% of the sequenced strains, while only one strain belonged to PCV2a genotype. The sequenced strains came from pigs aged between 5 and 20 weeks (62% of them from pigs between 16 and 20 weeks of age, Table 2).

Table 2. Distribution of sequenced strains according to pigs age and PCV2 genotype

Pigs age (weeks)	5-9	12	16-20
PCV2a	0	1	0
PCV2d	2	2	8

Discussion and Conclusion

PCV2d was isolated in this study as the most frequent genotype, confirming the presence of this genotype in different Mexican regions of swine production. Two-third of the sequenced strains came from pigs aged between 16 and 20 weeks, reflecting field circulation of the virus during fattening period. This first study could be completed by larger scale studies covering all regions of swine production in Mexico.

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Heat treatment of oral fluid samples is not the answer for direct real-time PCR

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Introduction

Based on reports in the literature, direct PCR, i.e., PCR without nucleic acid extraction, can be achieved by heating samples and then performing the amplification step (1,2,3,4). For example, Ranoa et al. (2020) described direct PCR for SARS-CoV-2 on samples heated at 95°C for 30 minutes; lower temperatures and/or shorter incubation times did not achieve the same results.

More efficient detection of nucleic acids in swine oral fluid specimens would be highly desirable. Therefore, the purpose of this study was to evaluate the effect of heat treatment on the detection of porcine reproductive and respiratory syndrome virus (PRRSV), influenza A virus (IAV) and *Mycoplasma hyopneumoniae* (*MHP*) nucleic acids in swine oral fluids.

Materials and Methods

Oral fluid samples known to contain PRRSV (n = 8), IAV (n = 8), or *MHP* (n = 8) were thawed overnight at 4°C. Thereafter, each of the 8 samples were serially diluted (neat, 1:2, 1:4, 1:8) using swine oral fluid known to be free of PRRSV, IAV and *MHP* as diluent. Each dilution was then split into 4 aliquots and each aliquot randomly assigned to one of four procedures (Table 1). Real-time PCRs (qPCR) were performed using commercial kits (IDEXX Laboratories, Inc., Westbrook, Maine, USA) and the Magnetic Induction Cycler (MIC) qPCR Cycler (Bio Molecular Systems, Australia). Results were reported as quantification cycles (Cq) and samples with Cq values < 38 were considered positive.

Table 1. Experimental design

Procedures	Heat[a]	Cool[b]	Extraction[c]	qPCR
1	Yes	No	No	Yes
2	Yes	Yes	No	Yes
3	Yes	Yes	Yes	Yes
4 (control)	No	Yes	Yes	Yes

[a] Samples heated at 95°C for 30 min in a dry block heater
[b] Samples cooled at 25°C for 20 min in an incubator
[c] RealPCR*DNA/RNA Spin Column Kit, IDEXX Laboratories, Inc.

Results

A summary of the results is shown in Table 2. Using Procedure 4 (control) as a comparison, it can be seen that heating the sample (Procedures 1 and 2) and even heating followed by extraction (Procedure 3) negatively affected nucleic acid detection both qualitatively (pos, neg) and quantitatively (Cq values).

Table 2. Number of positives among 8 samples tested at each dilution (mean Cq response)

Pathogen by procedure	Dilutions "Neat"	1:2	1:4	1:8
PRRSV[a]				
1	0	0	0	0
2	0	0	0	1 (36.5)
3	0	0	0	0
4	8 (29.4)	8 (30.7)	8 (31.4)	8 (31.9)
IAV[b]				
1	3 (35.3)	2 (36.5)	0	0
2	0	0	0	0
3	0	0	0	0
4	8 (25.2)	8 (25.6)	8 (26.6)	8 (27.3)
MHP[c]				
1	4 (35.3)	2 (35.2)	3 (35.9)	2 (36.1)
2	3 (36.1)	2 (36.0)	2 (35.7)	1 (36.4)
3	7 (34.4)	7 (35.1)	7 (35.2)	7 (37.4)
4	8 (34.7)	8 (35.1)	8 (32.1)	7 (36.7)

[a] RealPCR*RNA Master Mix and RealPCR* NA PRRS Types 1-2 RNA Mix, IDEXX Laboratories, Inc.
[b] RealPCR*RNA Master Mix and RealPCR*Influenza A RNA Mix, IDEXX Laboratories, Inc.
[c] RealPCR*DNA Master Mix and RealPCR**M. hyo*, IDEXX Laboratories, Inc.

Discussion and Conclusion

This study showed that heat treatment was highly detrimental to the detection of PRRSV, IAV, and *MHP* in oral fluid samples by PCR. While seemingly contrary to some reports, examination of the literature showed that investigators reporting the use of direct PCR often did not include comparisons with standard methods. That is, quantitative measures of the gain or loss in performance achieved by alternative methods was often lacking. In this study, comparisons showed that the best results were obtained using standard extraction and amplification methods.

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Human-to-swine spillover and onward transmission of H1N1pdm09 in Brazil

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Introduction

Human-to-swine influenza A virus (IAV) spillover events occur with regular frequency (1). A subset of 2009 H1N1 pandemic viruses (pdm09) continue to be transmitted from humans to swine and evolve within susceptible pigs (2). Additionally, despite a swine-origin, the H1N1pdm09 lineage is now the predominant H1 human seasonal virus, and sustained transmission of the lineage in swine likely poses a public health risk if swine-adapted viruses with H1N1pdm09 genes have antigenically drifted from human seasonal H1 vaccines (3). The objective of the present study was to identify and characterize human-to-swine H1N1pdm09 spillovers in the Brazilian swine herd.

Materials and Methods

Nasal swabs and lung samples were collected from pigs between 2010 and 2020 and tested for IAV/M gene by RT-qPCR (4). Positive samples were submitted for viral isolation in SPF embryonated chicken eggs or in MDCK cells, and sequencing. Seventy H1 hemagglutinin (HA) and 55 N1 neuraminidase (NA) gene sequences were generated for this study. BLASTn was used to identify the 1000 most similar HA and NA sequences to the Brazilian swine isolates. In addition, 2008-2009 human seasonal sequences, vaccine isolates, and H1N1pdm09 sequences identified as ancestral to the pandemic (5) were included in the dataset. Nucleotide alignments were generated with MUSCLE v3.8.4 software after excluding duplicate sequences. H1pdm09 and N1pdm09 final alignments included, respectively, 5,151 and 4,805 sequences.

A maximum likelihood tree was inferred following automatic nucleotide substitution model selection for each gene using IQ-TREE v1.6.1. Statistical support for the inferred tree was evaluated using the rapid bootstrap algorithm with 1,000 pseudoreplicate datasets.

To estimate the time of the most recent common ancestor (tMRCA) of unique human-to-swine spillovers (2 sequences or more), we subsampled the larger human and swine HA data using TARDiS. This algorithm generated H1pdm09 and N1pdm09 datasets to 49 and 50 sequences, respectively, optimizing genetic diversity and temporal coverage. Each new alignment was evaluated in TempEst v1.5.3 toquantify temporal signal. Sequences from each spillover clade were combined individually with the subsampled datasets. These alignments were analyzedin BEAST v1.10.4 under a relaxed lognormal molecular clock and a non-parametric Bayesian Skyline demographic model. The MCMC chain was run for 4 x 10^8 chain steps and convergence was evaluated in TRACER v1.6 after excluding an initial 10% burn-in.

Results

Phylogenetic analyses identified multiple human-to-swine introductions of the H1N1pdm09 lineage in Brazil swine herds. However, not all these spillovers demonstrated sustained onward transmission. Our analyses suggest 8 genetic clades in which the virus maintained sustained transmission in Brazilian swine, in addition to 9 events of self-limited infections in which the virus was introduced into pigs but did not persist. The N1pdm09 results were generally concordant with the HA data and demonstrated 7 sustained spillover events and 8 events of dead-end infections.

Phylodynamic analysis indicates that the H1N1pdm09 in Brazilian pigs evolved from human seasonal viruses that were transmitted between 2007 and 2018. Most of these introductions occurred between 2007 and 2011 and only a few were detected after 2016.

Conclusions and Discussion

Human-to-swine transmission of the H1N1pdm09 virus has been reported globally (2). The H1pdm09 and N1pdm09 gene phylogenetic analysis conducted in this study show that these segments were repeatedly introduced into swine populations in Brazil since 2007. Approximately half of these introductions resulted in self-limited transmission with consequent extinction. However, several clades showed sustained onward transmission over multiple years, suggesting that the virus was continuously transmitted between pigs. The H1N1pdm09 lineage is shared with humans, consequently, the evolution of this lineage within swine poses a potential risk to public health. Thus, determining how interspecies transmission of the H1N1pdm09 affected genetic diversity across the genome, reassortment patterns, and antigenic phenotype is a critical future research direction (6).

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Immunoperoxidase monolayer assay for the detection of antibodies against *Senecavirus A*

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Introduction

Senecavirus A (SVA) is an emerging picornavirus related to vesicular disease in young and adult pigs, and neonatal multisystemic disease in piglets up to five days old. Outbreaks of SVA infection have been reported in countries such as Brazil, United States and China since 2014. During this period, the first cases of *Senecavirus A* were detected in Brazilian farms in the Southeast, South and Midwest regions. High mortality rates (15 to 30%) were observed in newborn piglets. Vesicular lesions caused by SVA are clinically indistinguishable from those caused by classic vesicular diseases, such as foot-and-mouth disease, which requires a *compulsory notification* (1). Outbreaks of SVA represent a risk for Brazilian meat production chain, since the country is the fourth largest pork producer in the world. Therefore, it is necessary to develop diagnostic tests to monitor the viral circulation in swine herds. Immunoperoxidase monolayer assay (IPMA) is an easy method to perform and can be executed on a large-scale basis (2), however, currently, there is no IPMA test available for SVA´s infection diagnosis. The aim of this study was to develop an IPMA assay and compare it with the virus neutralization (VN) test, which is the standard serological method used to diagnosis SVA infection.

Materials and Methods

To determine the performance of the IPMA test, samples of swine serum (n=100), with suspected vesicular disease, from Brazilian farms, were analyzed. The IPMA was developed based on pre-existing protocols for other agents such as *Porcine circovirus 2* (2) and *Vaccinia virus* (3). The test performance was compared and evaluated using the *Kappa coefficient*, and the area under the ROC curve was associated with the Youden's Index (*J*). Statistical analyzes were performed using Epitools software (Sergeant, ESG, 2018. Ausvet).

Results

The IPMA demonstrated ability to distinguish positive and negative results. The new method presented a kappa value of 0.22 (p<0,01), which represents reasonable agreement (60%) in relation to virus neutralization (Table 1). IPMA performance was determined by analyzing the ROC curve, with an area under the curve (AUC) of 0.601 (95%CI) being observed. According to the Youden's index, the best cut-off value for antibody titers was 1:1280, with sensitivity and specificity of 51% and 71%, respectively, compared to the virus neutralization assay.

Table 1. Comparison of the immunoperoxidase monolayer assay (IPMA) with virus neutralization (VN) testing 100 swine serum samples.

IPMA	VN Results		
	Positive	**Negative**	**Total**
Positive	30	12	42
Negative	28	30	58
Total	58	42	100

Discussion and Conclusion

The differences found between the methods may be correlated with the pattern of humoral immune response observed in SVA infection. The response is characterized by a rapid and robust increase in neutralizing antibodies strongly associated with the IgM, with subsequent elevation of the IgG isotype, detected in VN and IPMA, respectively (4).

Different levels of agreement (40-100%) between diagnostic methods were also observed in a validation study of the indirect ELISA test, compared to indirect immunofluorescence (IFI) for detection of IgG antibodies against SVA, according to the stage of infection (5).

In conclusion, despite the low performance of the IPMA compared to the reference method, the test was able to differentiate negative and positive samples. When analyzing herds with unknown epidemiological data, the use of IPMA as the only diagnostic method is not recommended. Therefore, it is still necessary to assess the IPMA performance in well-established populations.

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Impact of an agglomerate of sodium diformate and monolaurate on the reduction of African Swine Fever virus in commercial pig feed

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Introduction

The African Swine Fever virus (ASFv) causes lethal disease in pigs with mortality rates up to 100%. The virus has spread in Asia and Europe (1) and hasmeanwhile reached the Caribbean. There is mountingevidence that feed or feed materials can serve aspotential vectors for the introduction and transmission of AFSv (2). The application of various acids and theirsalts to diets for pigs has been studied extensively over decades. Numerous trials have demonstrated the mode and magnitude of action of organic acids as antimicrobials in feed for pigs and have established effective doses for piglets, fattening pigs and sows, among them the use of diformates (3). Recently, information has appeared that organic acids, e.g. formic acid (4) and medium-chain fatty acids, in particular monolaurate, may exert a certain anti-viral impact, also against the ASFv (5). However, there are some limitations (high dosages, in-vitro data). Data ona combined approach of organic acids and medium chain fatty acids are scarce. The current study therefore investigates the impact of an agglomerate of sodium diformate and monolaurate - an approved andtested feed additive for swine - on its ability to reducethe activity of the ASFv in feed.

Materials and Methods

The experiment was designed to evaluate the viabilityof ASFv (p72, genotype II) over time (0, 1, 3 and7 days post-inoculation) in commercial swine feed containing either 0% or 0.3% of an agglomerate of sodium diformate and monolaurate (Formi 3G, ADDCON, hereafter abbreviated to 3G). The feed bags were incubated at room temperature (25°C) witha viral concentration of 10^8 HAD_{50}/mL. After the appropriate post-inoculation incubation period, the surviving virus was eluted from the samples using RPMI 1640 medium with 5% fetal bovine serum. Each treatment used a set of triplicate samples that were combined and used for a single titration and inoculation into cells. Virus titers (HAD_{50}/mL) were calculated by the Karber method (6). The quantity of ASFv was determined by real-time PCR to measure C_t-value. A significance level of 0.05 was used in all tests.

Results

Mean abundance rates of ASFv in the positive controlas well as 3G-feed are shown in Table 1. The ASFv titration assay on cell cultures showed that the feed acidifier had a significant reduction activity against ASFv throughout the whole trial period, beginning only a few hours after the initiation of the trial. The 0.3% 3G inclusion into the diet was able to inhibit the virus within less than one hour significantly ($P=0.013$),from 4.72 to 3.99 Log_{10} HAD_{50}. From day 1 onwards, the reduction was highly significant ($P<0.001$). On day 7, the ASFv was inhibited completely.

Table 1. Relative abundance (Log_{10} HAD_{50}) of ASFv in positive control and 0.3% 3G-swine diets over time

Time	PC	Formi3G	Diff. (%)	*p*
Day 0	4.72^a	3.99^b	-81.4	0.013
Day 1	4.60^a	3.52^b	-91.7	0.0001
Day 3	4.07^a	2.15^b	-98.8	0.0002
Day 7	3.59^a	0^b	-100	0.0000

(a, b) Superscripts indicate statistically significant differences ($p \leq 0.05$)

Conclusions and Discussion

The addition of the agglomerate of sodium diformate and monolaurate caused a highly significant reduction of the viral load in swine feed – achieving complete inhibition of the virus after 7 days. The additive is therefore able to reduce ASFv infectivity in commercial feed at low dosages and can be consequently an economical and sustainable approach to curb the disease transmission while offering a strongly reduced infection probability for pigs that might consume virus-contaminated feed (7).

Acknowledgments

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Improvement and validation of the HIPRA biosecurity scoring tool: Focus on what can be improved to avoid PRRSv entry.

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Introduction

A PRRS control programme cannot be conceived without a biosecurity plan[1]. A good biosecurity plan involves a periodic assessment to detect which areas need improvement in order to prevent the entrance and minimize the spread of PRRSv strains within a farm. In order to detect and prioritize those areas of improvement, biosecurity surveys may help. Sometimes, risk assessment tools are used to check the farm biosecurity. Even though they are very similar, it is important to distinguish between risk and biosecurity assessments. Risk assessments try to identify potential hazards or risk factors that may predispose your farm to a PRRSv outbreak. However, sometimes there is nothing you can do to avoid them (e.g., farm location or animal density around it). On the other hand, biosecurity assessments focus on the actions that you take to minimize those risks. As a result, the biosecurity weaknesses on the farm can be highlighted and actions can be taken to correct them. In the present study the HIPRA biosecurity scoring tool was re-evaluated, improved andvalidated in order to be more precise in detecting biosecurity failures, to be able to compare biosecurity status between pig farms and to prioritize the critical points for improvement.

Materials and Methods

The tool is based on a question-and-scored-answer model. Although the questions covered the most relevant points concerning PRRSv introduction and transmission, different improvements were implemented. Firstly, all the listed questions were reviewed, removed or extended based on a PRRS expert panel opinion. Secondly, the weighting of the answers was re-evaluated in order to minimize the impact of those risk factors, such as location, that could not be improved and to give greater relevance to the critical points that needed attention and improvement. Changes were applied to an existing database (34 biosecurity surveys) to compare the effect of the modifications on the farm ranking and the biosecurity characteristics. Finally, a report was automatically generated after the survey was completed. This report includes the identification of the 5 most critical points as well as graphs benchmarking the biosecurity (Figure 1).

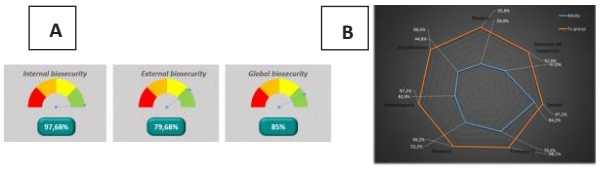

Figure 1. Example of the final report visualizations A) the internal, external and overall farm biosecurity and B) the benchmarking of the farm biosecurity compared to the company's average biosecurity using a radar chart.

Results

As a result of the question review, the total number of questions was increased from 66 to 84, extending the loading-bay, transport and personnel categories. The relative weighting of internal and external biosecurity in relation to the total biosecurity score went from 36% to 27% and from 64% to 73%, respectively. Questions relating to general risks, such as farm location or general characteristics of the farm, were still considered but their weighting in the final score was reduced from 14.7% to 3.3% and from 5% to 2.3% respectively. On the other hand, questions relating to frequent routes of PRRSv introduction, such as semen or personnel, had their relative importance increased or maintained, from 5.9% to 20% and from 14.5% to 13.2%, respectively. (Table 1).

	Category	Before	After	Change
Internal	Piglets management	9.87	11.24	↑
	Gilts management	14.68	13.37	≈
External	Location	5.06	2.31	↓
	General characteristics	14.66	3.34	↓↓
	Replacement animals	5.87	20.04	↑↑
	Semen	18.9	21.51	↑
	Trucks and vehicles	16.46	14.99	≈
	Personnel and supplies	14.49	13.2	≈

Table 1. Relative weighting (%) by subcategory in internal and external biosecurity on the total maximum score before and after the modification and degree of change between the two versions.

After the re-evaluation of the 34 biosecurity surveys, with the improved scoring tool, the biosecurity ranking of the farms changed. Farms with the worst biosecurity were at the top of the list once location and herd characteristics were minimized. For example, a farmthat was the 2nd least risky farm out of the 34 farms, moved up 9 positions once the changes were applied. This showed that most of its absence of risk was due to the farm's good location, despite the fact that its biosecurity was worse (especially in the semen category)than other riskier farms (due to location). The changes put more emphasis on semen management and biosecurity in this specific case.

Discussion and Conclusion

The tool was shown to be useful for establishing priorities. The changes implemented helped farmers to focus on what could be improved in their biosecurity plans rather than focusing mainly on their risks.

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Improving the efficiency of a candidate ASFV vaccine through feeding spray-dried plasmato pigs

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Introduction

Spray-dried plasma (SDP) is a highly digestible, high-protein level ingredient widely used in feed for weaned pigs. The benefits of SDP on growth performance, gut function, and immunity are well known (1). The objectives of this study were to evaluate the effects of feeding 8% porcine SDP in feed to pigs on the efficacy of a candidate African swine fever virus (ASFV) vaccine and protection of naïve pigs in direct contact with animals infected with ASFV Georgia 2007/01.

Material and Methods

A total of 24 weaned pigs (24 d of age) were randomly assigned to either a control (n=16) or porcine SDP (n=8) feed treatment group. Eight pigs fed the control diet were selected to serve as directly inoculated animals (trojans). At housing, test pigs were divided into two groups of 8 pigs and continued to be fed their respective diets for the entire study. On d 24 of the study, all test pigs were intranasally vaccinated with 2 mL of 10^5 PFU of IRTA-CReSA ASFV vaccine (BA71ΔCD2) (2). At d 19 post vaccination (pv) the 8 non-vaccinated trojan pigs were inoculated by intramuscular injection with 1 mL of 10^3 GEC of ASFV strain Georgia 2007/01. Fourtrojans were introduced per treatment group 2 d post inoculation (dpi) to expose the vaccinated test pigs to ASFV by direct contact. Trojans pigs were euthanized when they showed clinical symptoms of the disease. Blood samples and nasal and rectal swabs were collected weekly until the end of the study (d 41 pv). Allpigs were euthanized and submaxillary, retropharyngeal, and gastro-hepatic lymph nodes (LN), spleen, and tonsil samples collected. All samples were analyzed by RT-PCR and by a new DIVA-PCR todifferentiate between ASFV vaccine and wild strain. Inaddition, specific IgG and IgA antibodies against ASFVin all serum samples were analyzed.

Results and discussion:

In the vaccinated control diet group (V+Control), 1 pig died before vaccination and another pig died d 31 pv (d 10 post exposure, pe) due to acute meningitis. In the vaccinated SDP group (V+SDP) one pig was euthanized d 21 pv to balance the number of pigs within each group to 7 during the exposure period to maintain the ratio of trojans:in-contact pigs in both experimental groups Another pig was euthanized on d 35 pv (d 14 pe) due to intestinal prolapse. Both groups finished the experiment at day 41 pv (d 20 pe) with 6 pigs each. As expected, trojan pigs in both groups died between 5 to 8 dpi with similar onset of fever, viremia, nasal and fecal virus excretion and Ct values in tissue samples. During the exposure period 4/6 from the control diet group did not show fever and the other two showed a peak rectal temperature > 40.5ºC before d 20 pe at the end of the study. The V+SDP group did not show fever, neither PCR+ in blood nor rectal swab. The number of pigs withat least one day with PCR+ in blood, nasal or rectal swabs was higher for the control diet group during the exposure period. Tissue samples at 20 d pe from 5/6 pigsfed with the control diet were PCR+ for ASFV (P< 0.05), albeit Ct values were much higher than in trojanspigs. None of the tissue samples from SDP fed pigs were PCR+ for ASFV at any given time after challenge (Table 1). IgG or IgA titers against ASFV were not different between treatment groups. As expected, the few PCR+ samples found before exposure were the vaccine (BA71ΔCD2) ASFV strain, but all samplesPCR+ post exposure was Georgia 2007/01 strain DNA.

Table 1: Percentage of pigs with tissue samples ASFV PCR+ at the end of the study (d 41)

	Treatment Groups	
ASFV PCR+ Tissue	V+Control	V+SDP
Submaxillary LN, %	67[b]	0[a]
Retropharyngeal LN, %	50[b]	0[a]
Gastro-hepatic LN, %	50[b]	0[a]
Spleen, %	67[b]	0[a]
Tonsil, %	83[b]	0[a]

Row with uncommon superscript differ; [a,b] (P< 0.05).

In previous studies it was found that vaccinated pigs clear most of the virus at d28 pe (2). However, feeding SDP improved the efficacy of the candidate vaccine likely by improving mucosa integrity and the cell mediated immunity (1).

Conclusions:

Under the conditions of this study, 8% porcine SDP in feed improved the ASFV vaccine prototype efficacy. Thus, pigs fed with SDP showed lower virus load in blood, nasal, and rectal virus secretion after Georgia 2007/01 challenge than those fed with a control diet. Furthermore, no virus was detected in any organ of the SDP fed pigs at the time of sacrifice (d20 pe), thus offering a novel nutritional strategy using SDP to enhance the efficacy of a candidate ASFV vaccine and improve health status of pigs under ASFV conditions.

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Inactivation of two swine viruses on shoes by BioSec, a shoe-sanitizing station

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Introduction

Enhancement of biosecurity measures is critical to prevent and control pathogen transmission in swine farms (1). Indirect transmission of high economic impact diseases such as those caused by porcine reproductive and respiratory syndrome virus (PRRSv) and porcine epidemic diarrhea virus (PEDv) by shoes and other fomites, has been described (2, 3). BioSec is a shoe sanitizing station that combines Ozone + UVC (UVZone) technology and has been found effective against bacterial pathogens. In this study, we assessed the inactivation of PRRSv and PEDv on rubber soles and polyblend boot material by BioSec.

Materials and Methods

Two different approaches were used in this study. In first approach, coupons made of materials such as rubber sole and polyblend boot material were contaminated with 40 µl of PRRSv or PEDv per coupon. After air drying, four of the virus-spiked coupons were placed in an inverted position (virus side down) on the right side of the BioSec machine. An operator stepped on the left side of the machine to activate it for astandard time of eight seconds. As control, four of the virus-spiked coupons were not treated with the machine. After treatment, coupons were removed aseptically, and the surviving virus was eluted from them using an eluent solution (3% beef extract-0.05M glycine) for further processing.

In the second approach, 40 µl of PRRSv or PEDv were applied on three different areas of a polyblend boot sole. A fourth sample was applied on the boot's groove (1.5 centimeters from the bottom of the sole). After air drying, an operator wore the boots and stepped on the machine to activate the standard cycle for eight second. A polyblend boot sole contaminated with PRRSv or PEDv without turning the machine on served as control. Then, eluates from treated and non-treated sole samples were taken with a swab moistened with the eluent solution followed by further processing.

Serial 10-fold dilutions of eluates from both approaches were prepared in MEM and inoculated in monolayers of appropriate cells (MARC 145 for PRRSv and Vero 81 for PEDv) in 96-well microtiter plates. Virus titers were calculated by the Karber method and expressed as $\log_{10}$ $TCID_{50}$/sample after 7 days of incubation (4).

Results

Average virus titers for coupons and sole approaches and the percent of inactivation for each virus are shown in Tables 1, 2, and 3.

Table 1. Inactivation of PRRSv on coupons

MATERIAL	Non-treated coupons*	Treated coupons	% inactivation
Rubber sole Experiment 1	3.66	1. 58	99.16
Rubber sole Experiment 2	4.91	2.58	99.54
Polyblend boot	4.99	1.50	99.96

*Virus titers are expressed as $\log_{10}$ $TCID_{50}$/sample

Table 2. Inactivation of PEDv on coupons

MATERIAL	Non-treated coupons*	Treated coupons	% inactivation
Rubber sole	4.92	3. 08	98.55
Polyblend boot	4.83	2.50	99.53

*Virus titers are expressed as $\log_{10}$ $TCID_{50}$/sample

Table 3. Inactivation of PRRSv and PEDv on boot sole

Area contaminated	Control*	Treatment	% inactivation
Sole (PRRSv)	3.61	1.00	99.49
Groove (PRRSv)	4.50	1.17	99.95
Sole (PEDv)	4.50	1.39	99.91
Groove (PEDv)	4.50	2.50	99.00

*Virus titers are expressed as $\log_{10}$ $TCID_{50}$/sample

For the coupon assessment, an average of $\geq$ 99% of the PRSSv was inactivated on both rubber sole and polyblend boot material. In addition, an average of 98.55% and $\geq$ 99% of PEDv was inactivated on rubber sole and polyblend material, respectively. For the sole assessment, $\geq$ 99% of the PRRSv and PEDv were inactivated on the sole and the groove.

Discussion and Conclusion

These findings demonstrate the efficacy of BioSec in inactivating swine pathogens on shoe materials and its potential use in enhancing biosecurity practices in swine farms.

Acknowledgments

The authors would like to thank $PathO_3$gen Solutions for sponsoring the study.

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Increase of mummified piglets caused by Porcine Parvovirus in a Spanish swine herd

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Introduction

Porcine Parvovirus (PPV) is an endemic disease and, in vaccinated herds reproductive losses are typically low[1]. Farm monitoring data and statistical process control (SPC) can show the impact of changes in vaccination protocols related to reproductive vaccines. In this study, an increase of mummified foetuses in a Spanish swine herd using a trivalent vaccine against PPV, Swine Erysipelas and *Leptospira* spp. was reported.

Materials and Methods

From March to August 2021, a 400-sow farm located in Toledo (Spain) experienced a severe increased in the percentage of mummified foetuses, from an average basal percentage of 2.43% to 3.54% during this period. Mummified foetuses were submitted to laboratory for PPV and PRRSv PCR detection and, vaginal swabs for bacterial growth. For the prevention of this type of reproductive disorder, a trivalent vaccine containing PPV, Swine Erysipelas, *Leptospira* spp. antigens and, adjuvanted with α-tocopherol-acetate (Vaccine A) was used in the farm, according to the product label. Vaccine management and application were also reviewed.
To reverse the clinical symptoms, the vaccination with ERYSENG® PARVO (bivalent vaccine including a PPV-NADL-2 strain + SE antigen and, adjuvanted with HIPRAMUNE® G[d]) was implemented during May 2021.
A Poisson regression for percentage of mummified piglets and litters with 0, 1, 2 or ≥3 mummies, including vaccine period and parity was performed.

Results

The samples submitted were positive by PCR for PPV and negative for PRRSv. There was no bacterial growth of pathogenic bacteria such as *Leptospira* spp. or *Chlamidya* spp. The percentage of mummified piglets in farrows of sows vaccinated with ERYSENG® PARVO during the months of September and October decreased to 2.57%, being statistically different ($p<0.001$), which supposed a reduction of 37.7% (Figure 1). The percentage of litters with ≥3 mummies (Figure 2) was 6.4% from March-August and 1.2% from September-October ($p=0.05$).

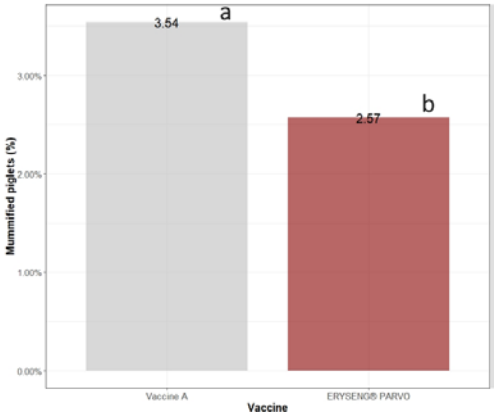

Figure 1. Percentage of mummified piglets during the periods vaccinating with the Vaccine A and ERYSENG® PARVO. Different superscripts mean significant statistically differences between groups ($p\leq0.05$).

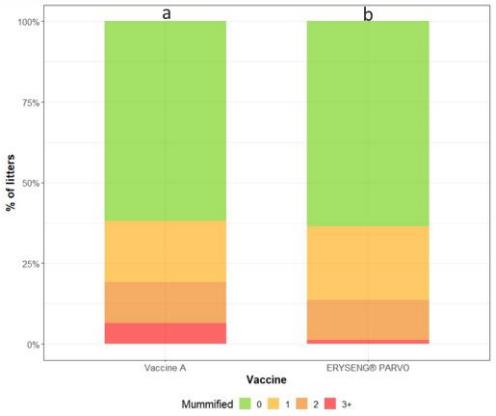

Figure 2. Percentage of litters with 0, 1, 2 or ≥3 mummies during the period vaccinating with Vaccine A and ERYSENG® PARVO. Different superscripts mean significant statistically differences between groups ($p\leq0.05$).

Discussion and Conclusion

Different PPV outbreaks have been reported in European farms using a new trivalent vaccine containing *Leptospira* spp. antigens[2, 3]. The reason of this lack of efficacy is not clear and needs to be further investigated. For the control of PPV clinical symptoms, the goal is to maintain a homogeneous herd immunity against PPV by vaccination[1].
In this case, the change to a bivalent vaccine against SE and PPV reduced the percentage of mummified piglets and the incidence of litters with a high number of mummies.

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Increased prevalence of 27a-like porcine parvovirus type 1 strains in Europe and reactivity of vaccination-antisera against different PPV-1 strains.

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Background & Objectives

Porcine parvovirus type 1 (PPV-1) is one of the causative agents of stillbirth, mummification, embryonic death, and infertility (SMEDI) in swine. The PPV-1 virus was first isolated in 1965 in Germany. Currently, it is circulating all over the world as various genetic lineages. Despitesignificant efforts of vaccination over the years, newstrains have emerged. In Europe, PPV-1 strains are shifting from cluster A to cluster B strains, with the 27a-like European lineage strains being predominant in Europe to date (1). Therefore, aPPV-1 VP1-based molecular epidemiology analysis and a reactivity analysis of sera from vaccinated animals against different PPV-1 field strains were performed in this study.

Material and Methods

All PPV-1 positive samples were collected between 2006 and 2021 at several farms in The Netherlands (n=18), France (n=4), Belgium (n=2) and Denmark (n=64). While sows were routinely vaccinated with a commercial non-cluster B PPV-1 vaccine, still SMEDI and/or weak piglets at birth were observed. At these farms, pooled tissue samples were collected of PPV-1 positive fetuses and mummies and subjected to PCR at different laboratories and nanopore sequencing at PathoSense.

In addition, pigs were experimentally vaccinated twice with an interval of three weeks with either one of the commercial vaccines: Reprocyc© ParvoFLEX 27a, Eryseng© Parvo NADL-2 or Porcilis© Ery/Parvo/Lepto PPV014) (2). Sera were collected 34 days (Reprocyc and Eryseng) or 41 days (Porcilis) after booster vaccination and analyzed for specific antibodies against different PPV-1 strains using (a) ImmunoPeroxidase Monolayer Assay (IPMA), (b) Haemagglutinin Inhibition (HI) and (c) Serum Neutralization (SN) assays.

Results

Recent European PPV-1 strains were mainly (73% n=72/98 PPV-1 positive cases) classified to the 27a-like European lineage (Cluster B) (Figure 1). While no significant differences in IPMA antibody titers were observed for the three vaccines, homologous PPV-1 field strains showed significantly higher neutralizing antibody titers in antisera from sows that were vaccinated with the Reprocyc© ParvoFLEX 27a subunit vaccine. Comparable, significantly higher HI antibody titers were observed for Reprocyc© ParvoFLEX 27a as compared to the other tested vaccines.

Conclusions

Over the last years, cluster B or 27a-like PPV-1 strains have become predominant in Europe. While vaccination with cluster A and F-based vaccines (Porcilis© Ery/Parvo/Lepto PPV014 and Eryseng© Parvo NADL-2, respectively) does not fully protect against heterologous infections, the Reprocyc© ParvoFLEX 27a subunit vaccine provides protection, also against heterologous infections as shown in HI antibodies (2-4). More important, due to its high mutational rate, further sequencing-basedsurveillance will be important to track further evolution of PPV-1 strains all over the world. This can also aid in fine-tuning vaccination strategies when reproductive failure is observed in vaccinatedsows.

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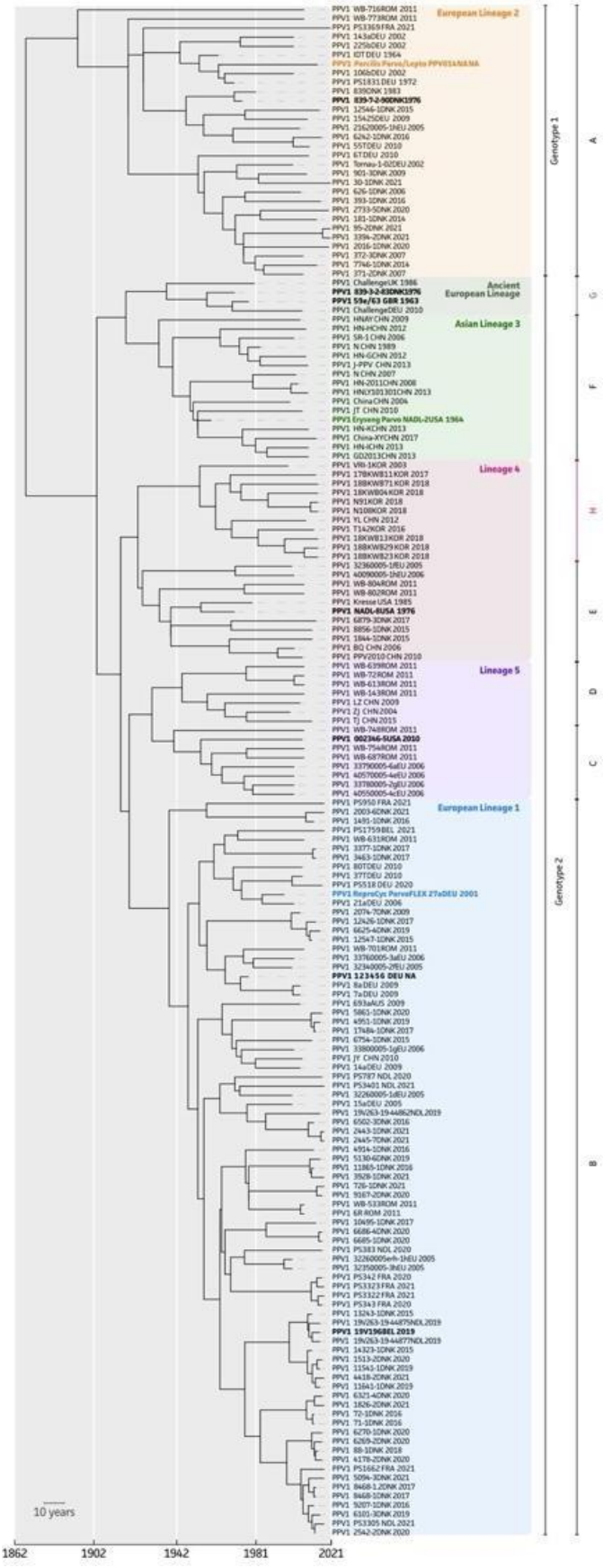

Figure 1 - Bayesian time-scaled phylogeny of PPV-1 VP1 genes

Influenza A virus detection in sow herds, a new strategy

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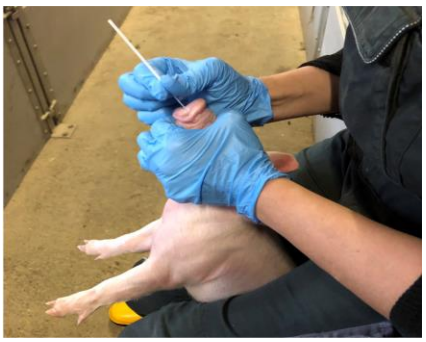
Introduction

Due to concerns in public health and its negative impact on herd health and performance, the interest in intense surveillance of swine influenza A virus (swIAV) is rising. The gold standard procedure for detecting swIAV is to sample only acutely diseased pigs. However, endemic infections with unspecific clinical signs need new approaches to detect the virus (1,2). This investigation aimed to evaluate a standardized sampling procedure for the detection of swIAV in sow herds.

Materials and Methods

Samples were collected from farms with either an acute outbreak of Influenza-like symptoms or the suspicion of an endemic course of disease. The study is multicentric, consisting of farms from 12 European countries. The sampling procedure included 2 pools/5 nasal swabs taken in suckling piglets (1-4weeks of age), weaners (approx.1week after weaning 4-6 weeks of age), and middle of nursery (7-8 weeks of age). Samples were analyzed for IAV by real-time PCR and subtyping of samples with a Ct-value below 30 was done by multiplex real-time PCR. Additionally, clinical signs of sampled animals were recorded. A three-level mixed effect logistic regression model was employed for the investigation of the association of the occurrence of a positive result with collected information on several parameters (i.e. age, course of disease, herd size, clinical signs).



Picture 1: Taking nasal swab in suckling piglet

Results

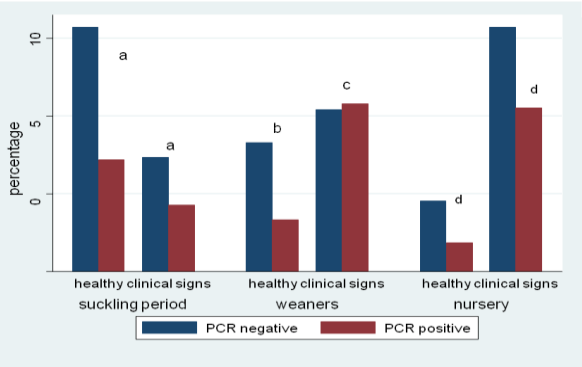
Data from 131 farms were evaluated from January to December 2021. Of these farms 78.6% (n=103) were positive for swIAV in at least one sample, and out of those 79,6% (n=82) allowed typing due to a Ct-value below 30. In 8.7% (n=9) of the positive tested farms two different subtypes were detected in different age groups. Clinical signs were significantly more often recorded in nursery pigs compared to suckling piglets and weaners ($p<0.001$) as well as significantly more often in weaners compared to suckling piglets ($p<0.001$).

Samples from weaners were 1.56 (95% CI: 1.004; 2.42) times more likely to be positive compared to samples from the nursery. Controlling for age group effect, samples collected from pools with clinical signs were three times ($p<0.001$, 95% CI: 1.82 ;4.95) more likely to be positive compared to those without clinical signs. However, when the model was run within each age group the association between positivity and clinical signs was statistically significant (OR= 7.12, 95% CI: 1.2; 42.26) only in samples from weaners (Figure 1).

Discussion and Conclusion

To obtain a complete overview of swIAV on-farm circulation, it is crucial not only to focus on the sampling of acutely diseased pigs in nursery but also to include suckling piglets and weaners. Especially in weaners the chance to receive a positive swIAV result is much higher in diseased pigs then later in nursery which can be due to the fact that later in nursery clinical signs can be already linked to secondary infections and might swIAV already have disappeared, as it is eliminated out of the lungs about 9 days after infection. In suckling piglets a high amount of samples can be positive for swIAV without animals showing clinical signs, particularly in endemic and vaccinated herds, as maternally derived antibodies can prevent suckling piglets from clinical signs, but not from an infection (Deblanc et al. 2018).

Figure 1. Percentage of positive samples per age group and clinical signs.



* Different superscripts, within each age group, indicate significant difference (p <0.05) between "healthy" and "clinical signs".

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Introductions of pre-2009 human-origin seasonal influenza A viruses in Brazilian swine herds

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Introduction

Swine H1 viruses are classified into three major genetic lineages: 1A, 1B and 1C. The 1B lineage resulted from repeated introductions of human seasonal H1 influenza A viruses (IAVs) into swine that occurred independently in Europe and America, giving rise to 1B.1 and 1B.2 virus lineages, respectively (1). IAV subtypes H1N1, H1N2 and H3N2 are endemic in Brazilian herds. Previous characterization of swIAV H1N2 from Brazil revealed that the most closely related human seasonal IAV circulated during the early 2000s (2). Since 2009 outbreaks of respiratory infections associated with H1N1pdm IAV have become frequent in Brazilian swine (3) and viral diversity increased after reassortment with co-circulating H1N1pdm virus internal genes (2). The objective of the present study was to identify and characterize 1B swine IAV isolated from pigs in Brazil.

Materials and Methods

Forty-nine IAVs of subtypes H1N2 and H1N1, previously isolated from swine, were selected for genetic characterization. Pig samples were collected between 2011 and 2020 from commercial farms located in six Brazilian states, distributed in three geographic regions. Viral RNAs were extracted and prepared for sequencing using the Ion Torrent platform. Nucleotide alignments were generated using a dataset of H1 hemagglutinin (HA) sequences of 1B lineage comprising: (a) Brazilian swIAVs sequenced for this study (n=49), (b) Brazilian swIAVs sequenced and published previously (n=6), and (c) related human and swine HA genes collected globally (n=3916). Alignment was generated using MAFFT v7.490 with default options followed by manual correction and curation using the program AliView v1.28. The final data set included a total of 3,971 H1 sequences collected globally from 1933 to 2020. A maximum-likelihood phylogenetic tree was inferred using IQ-TREE v2.1.3 following the automatic best-fit model selection process. Statistical support was assessed using ultrafast bootstrap (UFboot) and single branch tests (SH-aLRT) with 2000 replicates. The estimated time periods of human-to-swine transmission were inferred using TreeTime v.0.8.5.

Results

The H1 dataset included 56 Brazilian swIAV sequences with 49 H1N2, six H1N1, one H1Nx and two human variants (H1N2v) of swine origin. Phylogenetic analysis demonstrated three strongly-supported (UFboot ≥ 95% and/or SH-aLRT ≥ 80%) clades composed exclusively of Brazilian swine HA genes characterizing three separated introductions of the H1 segment from human seasonal influenza virus origin. New clade designations within the 1B lineage are proposed: 1B.2.3, 1B.2.4(Lopes *et al*., 2022, *unpublished*) and 1B.2.6 (this study). Clade 1B.2.3 included 19 sequences (12 H1N2, 5 H1N1, 1 H1Nx and 1 H1N2v) collected in southern, midwestern and southeastern Brazil during 2011 to 2020. Clade 1B.2.3 was closely related to human seasonal H1N1 viruses isolated during the 2006-07 human influenza season. The estimated time of human- to-swine transmission was 2003.6-2007.6. Clade 1B.2.4comprised 34 H1N2 IAVs collected in southern and southeastern Brazil from 2011 to 2020 that diverged into two statistically supported clades. One clade comprised 24 sequences (including a H1N2v), and the second clade had 10 H1N2 swIAVs. Clade 1B.2.4 was closely related to a human seasonal H1N2 virus collected in the early 2000s. The estimated time of transmission of the humanH1N2 virus into swine was 2001.4-2001.8. The newly identified clade 1B.2.6 contained six sequences (5 H1N2 and 1 H1N1) collected during 2019 in Minas Gerais state (Southeastern region) and was closely related to human seasonal H1N1 viruses from the late 1980s. The estimated time of human-to-swine transmission was approximately 1986.2-2007.5.

Conclusions and Discussion

These data demonstrate the occurrence of three separate introductions of human seasonal H1 IAVs into Brazilian swine herds leading to three different co-circulating lineages of H1N1 and H1N2 swIAVs specific to Brazil. The long branch length of clade 1B.2.6 likely represents the lack of sampling of swIAVs and only allows for timeof origin of the human-to-swine transmission event to be estimated broadly between 1986.2 and 2007.5. Future analysis of additional gene segments may indicate additional introductions and reassortment between human seasonal viruses and endemic swine IAV in Brazilian pigs. These results reinforce the significant contribution of human-to-swine IAV transmission to the genetic diversity of IAV in swine and reiterate the importance of surveillance in pigs.

Acknowledgments

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Isolation and Oral Immunogenicity of Porcine Epidemic Diarrhea Virus NH-TA2020 Strain

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Introduction

Porcine epidemic diarrhea virus (PEDV) is one of the most devastating diseases in the global pig industry due to its high mortality rate in suckling piglets. Getting maternal antibodies from sows immunized with the PEDV vaccine is an effective method for prevention of PEDV to piglets. However, antigenic variations between classical and emerging PEDV strains may result in the failure of attenuated vaccines stemming from classical strains (1). Keeping the vaccine strain up to date is important for the development of strategies to prevent and control PEDV(2).

Materials and Methods

All the diagnostic samples collected between 2017 and 2021 in China were tested for PEDV by qPCR. All the positive samples were sequenced for the S1 gene sequences. To isolate the PEDV virus, the PEDV-positive samples of intestine were processed and inoculated onto the monolayers of Vero cells until the cells developed visible cytopathic effect (CPE). The immunofluorescence assay was performed with a PEDV monoclonal antibody and an FITC-conjugated rabbit anti-mouse IgG. The PEDV IgA levels were detected by a PEDV IgA test kit according to the instructions.

Results

We first investigated the epidemic characteristics of diarrhea pig samples in China in the past four years, and found a series of viruses that differ from PEDV vaccine strains appeared (data not shown). The new emerging PEDV NH-TA2020 strain, which was grouped into an independent branch compared with thevaccine strains, was isolated (Figure 1) and its high pathogenicity to piglets was confirmed (Table 1).

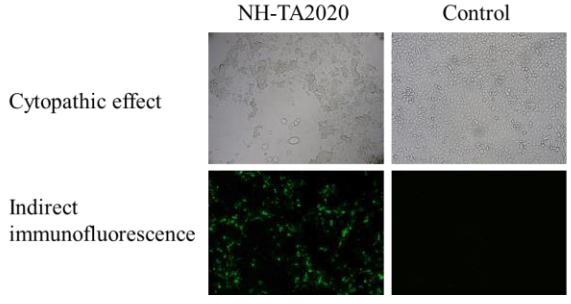

Figure 1. Cytopathic effect observation and IFA assay of NH-TA2020 (bar = 100 μm). The monolayers of Vero cells were inoculated with NH-TA2020 isolate for 24 h. Then the cells were harvested for IFA assay.

Table 1. Summary of morbidity of piglets after being challenged by PEDV NH-TA2020 strain

Item	Challenged with	Days post challenge						
		1	2	3	4	5	6	7
Clinical symptom[a]	NH-TA2020 Strain	3/5	5/5	4/4 [b]	3/4	3/4	2/4	0/4
	Control	0/5	0/5	0/4 [b]	0/4	0/4	0/4	0/4

Note: a. “Clinical symptom” refers to the appearance of vomiting, mushy or watery diarrhea; b. two piglets in the two groups (one each) were selected for IHC and HE analysis (data not shown).

We demonstrated that oral challenge by NH-TA2020 strain induced high level of PEDV IgA antibody in the milk of pregnant sows (data not shown). What's more,the clinical symptoms of piglets that challenged with Raoyang-148 strain (94% nucleotide homology with NH-TA2020) were significantly reduced after getting milk from sows which had oral administration of NH- TA2020 strain (Table 2).

Table 2. The morbidity of piglets after being challenged with PEDV NH-TA2020 strain. Pregnant sows were orally infected with NH-TA2020 strain 60 days before delivery. The piglets were challenged with PEDV Raoyang strain 3 days after the birth.

Groups	Pregnant sows challenged with	Piglets challenged with	Days post challenge										
			1	2	3	4	5	6	7	8	9	10	11
Immunized	NH-TA2020	Raoyang-148	12/35	17/35	18/35	15/35	13/35	6/35	3/35	2/35	0/35	0/35	0/35
Control	DMEM	Raoyang-148	27/33	29/29	18/18	11/11	3/3	-	-	-	-	-	-

Discussion and Conclusion

The new emerged PEDV NH-TA2020 strain was isolated and its high pathogenicity to piglets was confirmed. We demonstrated that oral challenge by NH-TA2020 strain induced high level of PEDV IgA antibody in the breast milk of pregnant sows. What's more, piglets can gain good immune protection against the heterologous PEDV strain challenge through breastfeeding. The NH-TA2020 strain has good potential as a vaccine strain.

Acknowledgments

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Live attenuated influenza A vaccine expressing an IgA-inducing protein reduces virus replication and transmission in pigs

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Introduction

The rapid evolution of influenza A viruses (IAV) complicates the control of influenza in pigs (1). Although vaccination is an effective method to control influenza, available vaccines for use in swine result in limited protection against the antigenically distinct IAV that currently co-circulate in pigs. Vaccines administered parenterally usually stimulate IgG antibodies but not a strong mucosal IgA response, which is typically more cross-reactive (2). Live attenuated influenza virus (LAIV) vaccines are known to stimulate mucosal IgA and T-cell mediated immunity, providing a more cross-protective immune response. We have developed an attenuated (*att*) virus that is safe and effective in pigs (3). We further modified this LAIV to contain an IgA-inducing protein (IGIP) as an adjuvant to enhance the mucosal IgA response (4). We tested this IGIP-LAIV vaccine in a bivalent formulation against challenge with antigenically drifted viruses in pigs.

Materials and Methods

The bivalent vaccines IGIP-LAIV and LAIV were formulated by using equal amounts of A/CA/04/2009 (CA/09) H1N1 and A/turkey/OH/313053/2004 (OH/04) H3N2 att viruses (either containing IGIP-HA or not). Four-week-old, cross-bred pigs (n=24/group) were vaccinated intranasally with each vaccine and boosted 2 weeks later. One group was not vaccinated. Three weeks post boost, pigs were challenged (n=12/group) with either heterologous H1N1 (A/sw/IA/A01778877/2016) or heterologous H3N2 (A/sw/OH/A01354299/2017) virus. At 2 days post infection (dpi), 3 pigs/group were placed in each room, without direct contact, to evaluate aerosol transmission. Sera and nasal washes were collected at 0 and 14 dpi and tested by HI and ELISA. BALF was collected at 5 and 14 dpi for virus detection or ELISA. Nasal swabs were collected at 1, 3, and 5 dpi or 3, 5, and 7 days post-contact (dpc).

Results

Both vaccines were safe in a bivalent formulation. Vaccinated pigs had increased numbers of influenza-specific IgA-secreting cells two weeks post boost. Despite low HI titers being detected in serum before challenge, titers against the vaccine antigens were boosted in vaccinated pigs after challenge. Levels of IgA were boosted after challenge in vaccinated groups. IgA levels in BALF were significantly higher in vaccinated pigs as early as 5 dpi and remained high at 14 dpi, suggesting that the challenge boosted the vaccine-specific memory IgA response.

Pigs vaccinated with both IGIP-LAIV and LAIV shed significantly less virus after challenge (Fig.1A). Both vaccines also reduced virus replication in the lungs and lung lesions after challenge. Transmission of the challenge virus to naïve, non-vaccinated respiratory contacts was confirmed in LAIV-vaccinated groups but not in contacts in the IGIP-LAIV vaccinated groups. Neither virus nor antibodies were detected in the respiratory contacts paired with IGIP-LAIV vaccinated/challenged pigs (Fig. 1A-B).

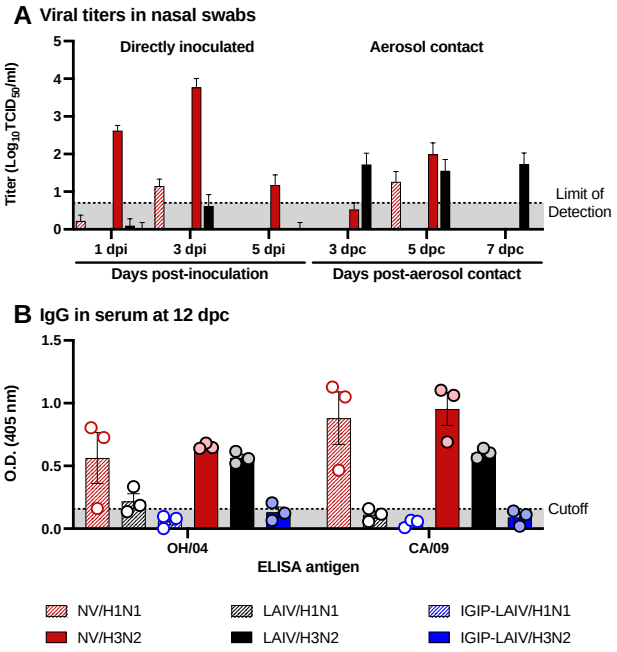

Figure 1. Viral titers in nasal swabs of directly inoculated and aerosol contact pigs (A) and IgG antibody titers in serum in aerosol contact pigs.

Discussion and Conclusion

Our results indicate that IGIP-LAIV vaccine in a bivalent formulation is as efficacious as the control LAIV against heterologous virus infection in reducing clinical signs, replication and shedding after antigenically drifted influenza virus infection. However, our results suggest that the IGIP-LAIV vaccine may be more protective against transmission, which could be a result of improved mucosal IgA response.

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Macroepidemiological aspects of Influenza virus A detection in the United States swine population between 2010-2021

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Introduction

Influenza A virus in swine (IAV-S) is an important cause of swine respiratory disease worldwide (1). An organized information hub enables a better understanding of detection and diversity for IAV-S, thus improving IAV-S strategic surveillance and control. IAV-S subtypes are classified according to the hemagglutinin (H1 and H3) and neuraminidase (N1 and N2) genes. Changes in the antigenic characteristics within subtypes pose a significant concern to animal health from geographical region to region (2). The purpose of this study was to characterize macroepidemiological aspects involving the detection of IAV-S RNA and IAV-S subtypes in swine using RT-PCR over time in porcine samples submitted to five USA National Animal Health Laboratory Network (NAHLN) level 1 accredited Veterinary Diagnostic Laboratories (VDLs), participating in the Swine Disease Reporting System (SDRS) (www.fieldepi.ogr/SDRS).

Materials and Methods

Results from IAV-S RT-PCR screening and subtyping along with submission metadata were obtained from diagnostic submissions from January 2010 to December 2021 from the Iowa State University VDL, University of Minnesota VDL, Kansas State University VDL, South Dakota State University Animal Disease Research and Diagnostic Laboratory, and the Ohio Animal Disease Diagnostic Laboratory.

RT-PCR screening data was collated and organized at the submission level into an inter-VDL standardized format. Variables included date, geographic region (state), RT-PCR test result, age category, specimen, and sample. The IAV subtype data was organized at the sample level considering the same variables.

Results

From the total of samples submitted for testing, 32.85% were RT-PCR-positive for IAV-S RNA. Five specimens accounted for 96.43% of total samples submitted: oral fluid (47.84%), tissue-lung (34.26%), nasal swab (3.43%), and oropharyngeal swab (1.43%), and 6.30% of the submissions had multiple specimens. The grow-finish age category represented the largest number of submissions (29.07%), and nursery was the second age category with the number of submissions (15.00%). A bi-seasonal pattern of IAV-S detection with increased detection during Spring (March-May) and Fall (September-November) was revealed.

The four most frequent specimens tested by PCR for subtyping were: oral fluid (44.36%), lung (27.53%), nasal swab (16.35%), and virus isolates (7.68%). Samples identified from the grow-to-finish age categorywere the most frequent with subtyping requests (36.81%). The most frequent complete subtypes detected were H1N1 (31.42%), H1N2 (27.33%), and H3N2 (23.59%). Surprisingly, 2.13% of the samples had both H and N subtypes (H1H3N1N2) detected fromindividual samples demonstrating the presence of genetic material from more than one IAV-S subtype in a single sample. Some samples had mixed subtype detections of H and N genes: H1N1N2 (1.96%), H1H3N2 (1.47%), H1H3N1 (0.23%), H3N1 (0.22%), H1H3 (0.12%), H3N1N2 (0.12%), and N1N2 (0.05%).

Samples with detection of either the H or N genes included: H1 (3.53%), H3 (2.11%), N1 (1.62%), and N2 (4.09%).

Discussion and Conclusion

This study described macroepidemiological aspects of IAV-S RNA detection and its distribution by age category, specimen, and season. Additionally, this study described the diversity of IAV-S subtypes over time and confirmed that IAV-S seasonality trends are bimodal every year around the same time, Spring and Fall. This study demonstrated the capability of monitoring IAV-S and subtypes in a standardized method using SDRS, thus enabling timely surveillance and detection of emergent IAV-S subtypes in the field.

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Medium chain fatty acids show potential to mitigate ASFv in feed

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Introduction

It was demonstrated that several viral pathogens, amongst which ASFv, can survive in feed ingredients and raw materials (1). Moreover, researchers showed that plant-based feed materials can promote ASFv stability and survival (2). As feed delivery is a recurrent event and there is a high risk of viral transmission via the feed, conclusion can be made that a considerable amount of pig farms can be infected via this route. Furthermore, it has been shown before that ASFv can be transmitted orally via contaminated feed and cause infection (3). As there is an important need for improved global biosecurity via the feed we developed a synergistic mixture of medium chain fatty acids (MCFA, (C6:C8:C10) as antiviral agent. In a recently published study we successfully showed that this product inhibit against a field ASFv strain in an *in vitro* feed model (4).To bring inside into the effect on piglets, the present *in vivo* study was conducted.

Materials and Methods

In total 15 pigs were randomly distributed to the trial groups: negative control (n=5), positive control (n=5) and treatment group (n=5). Complete swine feed was used as a negative control, whereas in the positive control the feed was inoculated with ASFv to a final concentration of 10^5 HAD50/g. The treatment group was treated with MCFA (C6:C8:C10) at a dose of 0.5% and afterwards spiked with ASFv. The feed spiked with ASFv was fed to the piglets for 5 days, afterwards all piglets were exposed to the respective diets without ASFv until the end of the experiment. At 14days after the start of the experiment, all piglets were euthanized and ASFv presence was determined in the spleen via real-time-PCR.

Results

As expected, in none of the piglets of the negative control group, ASFv could be detected. In the positive control group, we observed 40% mortality (i.e. 2 piglets died during the course of the experiment, one piglet at day 6 and one at day 7). In both piglets, next to detection of viral DNA, clear clinical signs and pathological findings for the presence of ASFv were seen. The remaining piglets euthanized at day 14 were tested negative for ASFv, obtaining a total infection rate of 40%. On the contrary, in the piglets receiving the diet with 0.5% MCFA, no mortality could be observed and none of the piglets euthanized were tested positive for ASFv.

Discussion and Conclusion

Interestingly, in our previously published in vitro experiment, we observed a strong anti-ASFv effect of MCFA (C6:C8:C10) inclusion in the diet, with siginifcant increase in Cq value at doses of 0.125% to0.5% (4). As a follow-up study, an *in vivo* piglet trial was performed in which it was shown that the protocol and dose of 10^5 HAD/g ASFv applied in this study was sufficient to cause ASFv infection in piglets, which is in accordance to previous observations (3). In this piglet study, we tested the effect of 0.5% inclusion of the same MCFA product, in which we have shown a potential *in vivo* effect to reduce the risk of ASFv contaminated complete feed via oral route. All in all, these results indicate an important role for MCFA as feed desinfectants and in the prevention of ASFv transmission via the feed globally.

Acknowledgments

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Modelling the role of mortality-based response triggers on the effectiveness of African swine fever control strategies in Brazil

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Introduction

The introduction of African swine fever (ASF) into commercial pig farms often results in epidemics, which can spread quickly, mainly by the transportation of pigs between production phases. The main modes of ASF transmission are direct contact with infected animals, ingestion of infected pork products and or residuals, and contact with contaminated fomites [1]. Even though mathematical models have been proposed to estimate epidemiological consequences of ASF introduction in European countries [2], [3], there are gaps in such models mainly because known variations on ASF clinical presentation and mortality patterns were not considered.

Here we developed a nested multiscale model for the transmission of ASF, combining spatially explicit network model of animal movements with a deterministic compartmental model for the dynamics of two ASF strains within-pixels of 3 km x 3 km, amongst the pig population in one Brazilian state. The model outcomes are epidemic duration, number of secondary infected farms and pigs, and distance of ASF spread. The model also shows the spatial distribution of ASF epidemics. We analyzed quarantine-based control interventions in the context of mortality trigger thresholds for the deployment of control strategies.

Materials and Methods

The study area comprises the swine population of Rio Grande do Sul, Brazil. We developed a network model to simulate ASF transmission on a pixel-level directed temporal (daily) network of animal movements. Each pixel (1:4367) can be in one of four health states: susceptible (S), exposed (E), infectious (I), and (Q) quarantined. The pixel, the index case, immediately transitions to the infectious state (I). Transmission is then driven by outgoing animal movement between- farms among pixels (between-farm pig movements derived from the SEAPI-RS database (3 years of data).

Four delayed ASF control triggers based on mortality were simulated individually for each ASF strain (highly and moderately virulent), and later combined with quarantine base control scenarios.

Each simulation produced three outputs: i) pixel state variables: Susceptible, Exposed, Infectious, and Quarantined, at the final time step; ii) maximum transmission distance and epidemic duration; and iii) the observed transmission and quarantine networks (i.e., who infected or triggered quarantine for whom, and at what time step). As measures of potential severity, we calculated the duration in days, spread capacity in kilometers, and secondary attack rates of ASF epidemics prior to enacted quarantines at the pixellevel.

Results

The mean epidemic duration of a moderately virulent strain was 11.2 days assuming the first infection is detected (best-case scenario) and 15.9 days when detection is triggered at 10 % mortality. Under the best-case scenario, we projected an average number of infected farms of 23.77 farms and 18.8 farms for the moderate and highly virulent strains, respectively. At 10% mortality-trigger, the predicted number of infected farms was on average 46.27 farms and 42.96 farms, respectively. We also demonstrated that the establishment of ring quarantine zones regardless of size (i.e., 5 km, 15 km) was outperformed by backward animal movement tracking (Figure 1).

Discussion and Conclusion

The proposed modeling framework provides an evaluation of ASF epidemic potential, providing a ranking of quarantine-based control strategies that could assist animal health authorities in planning the national preparedness and response plan. Our model predicted that delaying disease control response under a 10% mortality trigger resulted in an epidemic duration and number of secondary cases that were more than double that of the "optimistic scenario" in which disease control started on the same day that the first dead pig was identified. The most effective simulated intervention strategy, backwards contact tracing and quarantining previous 15 or 30 days had nearly the same contribution in reducing ASF propagation.

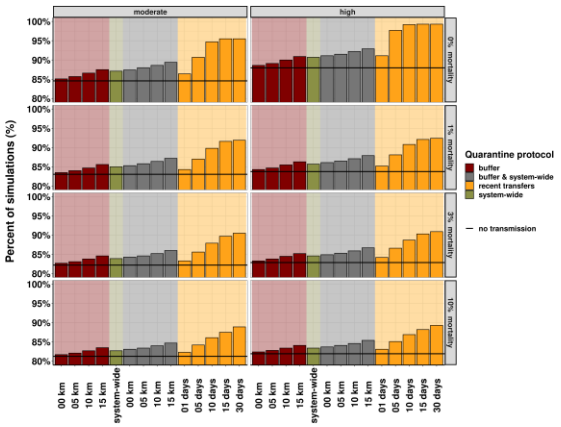

Figure 1 *The percent of ASF epidemics successfully halted by mortality-trigger and virulence combinations*

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Molecular epidemiology of PRRS virus in 4 pyramidal production systems in Spain

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Introduction

Biosecurity and monitoring are key to reduce the risk of PRRS virus (PRRSV) introduction and dissemination in the farm. Usually, the prioritisation of biosecurity measures is mostly based on the general knowledge about how the disease is transmitted, on common sense and on the previous professional experiences. The implementation of a diagnostic and monitoring scheme - including sequencing - may help to see how often new PRRSV strains are introduced in a farm. If sequencing is performed systematically, and regional sequences are available, the source of new infections can be potentially traced. However, as PRRSV may recombine relatively frequently (1) whole genome sequences would be preferable over ORF5 sequences.

The aim of the present study was to characterize the PRRSV transmission flows among farms of pyramidal systems using whole genome sequences obtained by NGS.

Materials and Methods

A longitudinal study was undertaken in 4 production pyramids, that were composed by 4 selection sites, 7 breeding farms and 17 nurseries. Monthly for an 8 months period, 30 samples were collected from weaners of selection sites and breeding farms. Sera were also collected from 10 animals at 6 and 9 weeks of age in the nurseries.

Samples were pooled in groups of five. RNA was extracted and evaluated by means of a commercial RT-qPCR (VETMAX EUNA 2.0) targeting PRRSV nucleocapsid. When a sample tested positive, individual samples were recovered and analysed. Those showing the lowest Ct-value were used for isolation of the virus in porcine alveolar macrophages (PAMs). When cytopathic effect was observed in >70% of the PAMs, the supernatant was collected and the RNA was extracted using the TRIzol Reagent.

Whole genome sequences were obtained directly from the RNA following the procedures depicted by Cortey et al. (2) using MiSeq System Illumina platform. A phylogenetic analysis of each sequence was constructed with the complete genome, as well as with each protein, using Bioedit and MEGA X software. Transmission chains (namely, strains belonging to a same clade circulating in different farms from one or more pyramids for at least 6 months) were constructed using the sequencing data and was compared with the flow of animals within the pyramid. Finally, recombination analysis was performed with RDP 5 software and GARD package (www.datamonkey.org/GARD).

Results, Discussion and Conclusion

PRRSV was detected in 2/4 selection sites, 4/7 breeding farms and in 12/17 nurseries. In total, 49 PRRSV isolates were detected and sequenced from all 4 production pyramids.

The whole genome sequence analysis allowed to determine 8 different transmission chains sustained for at least 6 months. In 4 of them, an atypical virulent PRRSV1 strain related to the Italian PR40/2014 (3) wasfound. The genetic drift of the virus observed among the transmission chains of the virulent Italian-like strain was in average seven times higher than the drift of the virus in the pyramids with local resident strains. At the third month of the study, two nurseries switched the breeding origin. In both, the transmission chain changed.In the first, the PRRSV isolate previously detected on that nursery was replaced for the one detected in the new donor breeding farm. In the second, the virus was not related with neither the new nor the old donor breeding farm. This second result suggested a lateral introductionprobably unrelated to the changes in the pig flow.

Recombination analyses pointed to the existence of two recombination events, both involving the atypical virulent strain. The first event, included 6 isolates detected in a single transmission chain. In this case, a segment of 700 nucleotides including the beginning of the NSP9 of an undetermined PRRSV1 subtype 1 isolate was detected as the minor parental. The atypical virulent PRRSV strain was detected in the rest of the genome. The second event involved only one isolate in one of the nurseries. In this case, both the major (a conventional PRRSV subtype 1 isolate) and the minor parental (the atypical virulent strain) were detected in the same farm in previous samplings. The recombinant isolate included also a segment of 700 nucleotides in the NSP9of the minor parent. It would be interesting to analyse infurther studies if this change in the NSP9 (the replicaseof the virus) affected to the kinetic of replication.

In conclusion, the analysis at whole genome level of the isolates retrieved from these production pyramids allowed to determine in depth the origin of the infections and evolutionary patterns that would be undetected only by analysing the ORF5 sequences.

Acknowledgments

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Monitoring PRRSV vaccination and field infection in nursery pigs

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) continues to be one of the most economically significant diseases affecting the swine industry worldwide. In the US, its impact has been estimated at $4.67 for every pig marketed (1). Novel strategies are needed for an optimized control of PRRSV in the herds, including disease and vaccination monitoring programs. This study reports on the use of an oral fluid ELISA to identify groups of nursery pigs at high risk of PRRSV infection and monitor vaccination.

Materials and Methods

A total of 26 wean-to-finish pig farms sourcing three-week-old pigs from two different breeding herds were included in the study. The breeding herds were considered PRRS stable (2) with uncertain shedding status at the time of the study. Pigs were injected with a PRRSV vaccine (Ingelvac PRRS MLV, Boehringer Ingelheim) within one week after placement. Six pen-based oral fluid samples were collected per barn at the time of vaccination and 3 weeks after.

Samples were tested using an ELISA for detection of antibodies against PRRSV in oral fluids [PRRS Oral Fluid Ab Test, IDEXX] following the manufacturer's instructions and analyzed by PRRSV qPCR at Iowa State University Veterinary Diagnostic Laboratory. RFLP patterns were obtained from qPCR positive samples and used to differentiate between the vaccine strain and wild-type PRRSV. In addition, production parameters (feed conversion ratio [FCR], average daily weight gain [AWDG] and mortality) were recorded for each farm.

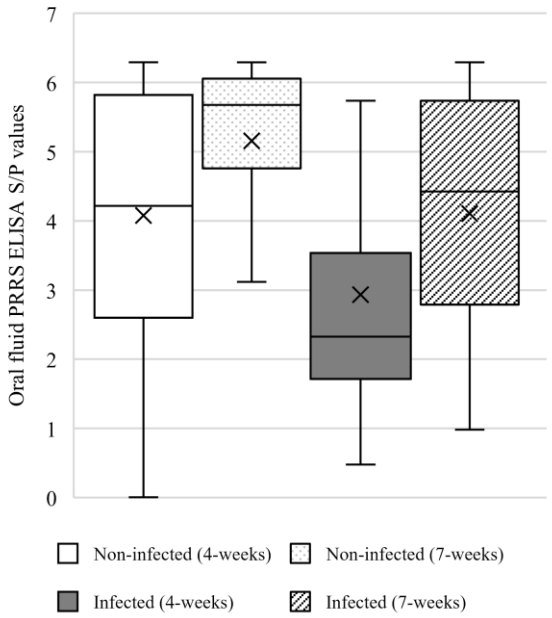
Results

At the time of the first sampling, a higher ELISA mean S/P value was shown in non-infected nurseries compared with wild-type PRRSV infected groups (Mean S/P = 4.08 and 2.93, respectively. Figure 1). Non-infected farms presented a more homogenous distribution in S/P values, with a lower coefficient of variation compared to infected farms (CV% = 43 and 63, respectively).

Following vaccination, the mean S/P value increased to 5.16 in the non-infected groups, with a marked reduction of variation (CV% = 26). Similarly, the mean S/P value increased in the infected farms following vaccination (Mean S/P = 4.11). However, the high variability persisted (CV% = 46).

Wean-to-finish average mortality increased to 5.02% in the farms with wild-type PRRSV infection at the time of vaccination, compared with 3.85% in the non-infected groups. No differences were recorded in FCR or ADWG between infected and non-infected groups.

Figure 1. Box plots identifying differences in oral fluid PRRS ELISA S/P values in wild-type PRRSV infected and non-infected groups of pigs at the time of PRRSV vaccination (4-week) and three weeks later (7-weeks).



Discussion and conclusion

Non-infected nurseries showed a higher ELISA mean S/P value at the time of the first sampling. Despite the oral fluid ELISA detecting IgG, this finding suggests higher titers of maternally derived neutralizing antibodies in the non-infected groups having a protective effect.

In the PRRSV infected nurseries, the low homogeneity in S/P values 3 weeks post-vaccination is indicative of an impaired immune response to vaccination, probably caused by the active infection at the time of vaccination.

Routine monitoring using an oral fluid antibody test can help to differentiate groups of pigs at high risk of PRRSV infection post-weaning (low mean S/P value and high variability) and evaluate successful PRRSV vaccination (increased mean S/P value and reduced variability three weeks post vaccination) for an optimized management of PRRSV infection. This study also suggests that monitoring PRRSV oral fluid antibodies can be a valuable tool to identify groups with wild type infection at placement, allowing a more appropriate disease control strategy.

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N-glycosylation patterns of epidemic PRRSV2 sub-lineages in the U.S.

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Introduction

N-glycosylation is a form of posttranslational modification of proteins commonly reported on the GP5 protein of PRRSV2 [1] that can determine its structural conformation, thus potentially modulating immune recognition [2]. Implications for immune recognition are particularly relevant in PRRSV2 in the U.S due to the periodic emergence and co-circulation of numerous genetic variants through time [3,4]. Describing the patterns of N-glycosylation found in PRRSV2sequences over time may reveal insights into immune- mediated evolution and competition amongst strains and increase understanding of drivers for multi-strain dynamics of PRRSV2.

Materials and Methods

18,659 PRRSV2 ORF5 sequences from the UMN VDL were classified into lineage and sub-lineage and the translated GP5 protein sequences were evaluated *in silico* for potential N-glycosylated sites (i.e., with N-X-S/T motif). Two PRRSV2 sub-lineages, 1A and 1C, responsible for recent epidemics in the U.S. were chosen for illustration. The frequency in which different glycosylation patterns were identified was summarized over time.

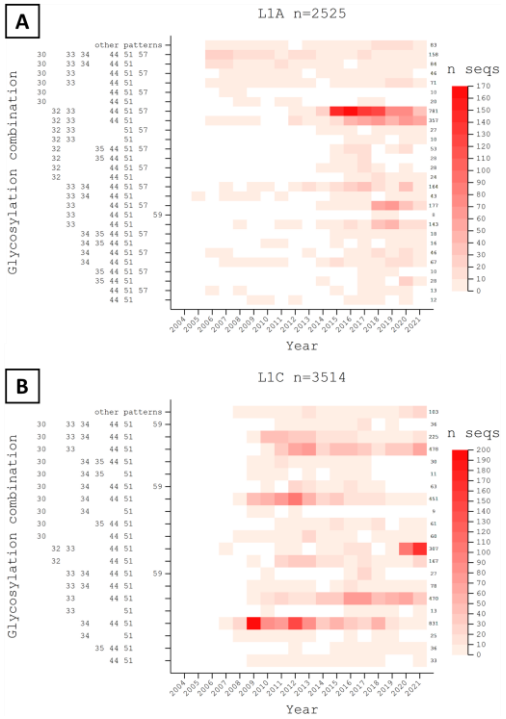
Results

Sublineage 1A occurs in the UMN VDL since 2005 and no characteristic glycosylation pattern was found until 2015, when a pattern of N-glycosylated sequences at sites 32-33-44-51-57 emerged and was responsible for 62%, 52% and 40% of the L1A sequences in 2015, 2016 and 2017, respectively (Fig. 1A), which corresponds to the rapid spread of this sub-lineage. Sublineage 1C is a genetically diverse sublineage [3] that peaked in 2013-14, though a rapidly spreading L1C variant re-emerged in 2020 [4]. The period between 2009 and 2018 is marked mostly by the occurrence of sequences with N-glycosylation at sites 34-44-51. However, beginning in 2020, the occurrence of a relatively rare pattern (32-33-44-51), responsible for <5% of the L1C sequences prior to 2020, sharply increased to >45% (Fig. 1B).

Discussion and Conclusion

The emergence of distinctive N-glycosylation patterns correspond with epidemics of both Lineage 1A and Lineage 1C viruses. The L1A sequences from 2015 onward correspond with the emergence of the 1-7-4 RFLP type. The L1C sequences with the pattern 32-33-44-51 correspond to the newly emerging L1C 1-4-4 variant [4]. Glycosylation in this region of the GP5 protein potentially changes PRRSV2 epitopes, which are targets of the host's neutralizing and non-neutralizing immune response [5]. Changes in the N-glycosylation patterns of the GP5 protein may thus potentially allow the virus to escape host immunity. As an observational study, we can not distinguish if the change in the N-glycosylation pattern had causal effects on the epidemic dynamics or if the N-glycosylation pattern represent an artifact of a "founder effect" or "hitchhiker" mutation that does not impact fitness. Documenting changes in the frequency of N- glycosylated sited in large-scale field data sets has important implications for understanding possible drivers of emergence of new PRRSV variants in the U.S., and for new insights into multi-lineage ecological theory.

Figure 1. Frequency of GP5 glycosylation patterns byamino acid site of L1A (Fig. 1A) and L1C (Fig. 1B) sequences from 2004-2021.



Acknowledgments

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Natural variation in the host genome impacts response to PRRS challenge

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Introduction

Porcine reproductive and respiratory syndrome (**PRRS**) was first detected in the U.S. in the late 1980s (1) and has caused major production and economic disruption ever since. This is due, in part, to the high mutation rate of the virus, resulting in a high degree of genetic and antigenic variation. As a result, numerous strains of the virus exist (2). Differences in virulence and pathogenicity have been detected among PRRS virus (**PRRSV**) strains, suggesting that the genome of the virus impacts host response to challenge (3). Likewise, there is increasing evidence that the genome of the host also impacts response to challenge (4,5). Therefore, the objective of this study was to evaluate differences between and within sire lines in response to PRRSV-challenge.

Materials and Methods

Animals. Two PRRS challenge trials were conducted over the course of two years. For Trial 1, Topigs Norsvin parent females were bred to a Topigs Norsvin terminal line (TN Line), or to boars from a competing genetic supplier (Line A) to produce two groups of 730 pigs each. Ear tissue was collected from each full-program Topigs Norsvin piglet at birth and used for genotyping. Pigs in Trial 2 were produced by mating Topigs Norsvin parent females to either TN Line boars or boars from competitor lines B or C to produce three groups of 702 pigs each.

For both trials, pigs were transported to a commercial research barn at weaning and placed into pens with a stocking density of 27 pigs per pen. Post-arrival, each pig was vaccinated for PRRS using a PRRS modified live virus vaccine. Four weeks later, all pigs in Trial 1 were experimentally inoculated with 1 x $10^{3.5}$ TCID50 of PRRSV 1-7-4, whereas only three pigs per pen were inoculated with PRRSV for Trial 2.

Traits. Body weight and feed intake (measured on a pen basis) were recorded at the start and end of test and used to calculate wean-to-finish average daily gain (**ADG**) and feed conversion ratio (**FCR**). Mortality was recorded for each individual as a binary variable (0/1) from inoculation to market. Individual body weight measurements were also available for pigs from Trial 1.

Statistical analyses. A fixed effects model was used to estimate the effect of sire line on each trait by analyzing Trials 1 and 2 separately. Genomic information, obtained in Trial 1, was used to facilitate genetic parameter estimation for each trait recorded at the individual level for all full-program Topigs Norsvin animals.

Results

Significantly fewer mortalities were detected among TN Line-sired pigs than pigs sired by Lines A, B, or C ($P < 0.0001$) (**Tables 1 and 2**). TN Line-sired pigs also had significantly greater ADG than pigs sired by Line A ($P < 0.0001$) (**Table 1**) or Line C ($P = 0.007$), but was not different from Line B ($P = 0.07$) (**Table 2**). TN Line-sired pigs had significantly lower FCR than pigs sired by Line A (0.0003) (**Table 1**), but significantly higher FCR than pigs sired by Line C ($P < 0.0001$) (**Table 2**). Based on results of genetic parameter estimation, heritability was estimated at 0.25 (0.05) for ADG and 0.10 (0.07) for mortality, when analyzed using a LOGIT model.

Table 1. Least squares means and *P*-values for the effectof sire line on each trait for Trial 1.

Sire line	TN	A	*P*-value
ADG (g/d)	771	717	<0.0001
FCR (live)	2.39	2.45	0.0003
Mortality (%)	13.0	22.0	<0.0001

Table 2. Least squares means and *P*-values for the effect of sire line on each trait for Trial 2.

Sire line	TN	B	C	*P*-value
ADG (g/d)	767^{a}	753^{ab}	748^{b}	0.02
FCR (live)	2.52^{a}	2.50^{a}	2.44^{b}	0.0001
Mortality (%)	7.6^{c}	16.2^{a}	10.7^{b}	<0.0001

Discussion and Conclusion

Results obtained from this study provide strong evidence that variation in the host genome impacts response to PRRS challenge. Significant differences in performance and mortality rate post-challenge were detected between sire lines, whether evaluated using an experimental or natural PRRS challenge model. Further, evidence of variation within a sire line in response to challenge was also detected among TN Line-sired pigs. Together, these results indicate that, while the TN Line-sired progeny outperformed the progeny of three major competitor sire lines following PRRS challenge, substantial variation among TN Line-sired progeny was also detected. Therefore, breeding pigs for improved host response to PRRS challenge by capitalizing on existing variation within the host genome, is a promising, viable PRRS control strategy.

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Newly emerged PRRSv Lineage 1C RFLP 144 variant in breeding herds

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Introduction

The Morrison Swine Health Monitoring Project (MSHMP) is a national voluntary initiative currently monitoring PRRSv in ~50% of the U.S. sow population (1). MSHMP monitors weekly sow farm PRRSv status and also ORF5 sequences, when available. Here we describe how the newly emerged lineage 1C RFLP 144 (L1C144) variant (2) has affected the monitored breeding herds.

Materials and Methods

The PRRSv L1C144 variant is being monitoredamongst MSHMP participating systems through their sequence submissions to tree main veterinary diagnostic laboratories. Submissions were linked to a participant site using premises ID, farm name, production system, and/or location. Date of the first detected L1C144 sequence was linked to the site's reported PRRS status if the reported change to a new break occurred within ±30 days of the sequence detection. Whether sites were able to move to a different PRRS status or continued to be affected by this variant according to season in which they broke was compared through Cox proportional hazards model, taking time in a status 1 category (active break) and production system into account. Sequencing of the ORF5 region of the PRRSv genome from additional historical samples from one site was performed.

Results

As of December 2021, 492 sequences characterized as the L1C144 were identified, of which one-fourth (123/492) originated from breeding herds. 93 of these were linked to a site, corresponding to 52 different sites. However, sequence detection was only linked to a change in the reported PRRSv status for 44 sites. Of those, 12 (27%) changed status during the follow-up period: six to 1.2 (another PRRS strain was detected), two to status 2, two to status 2fvi, and two did depopulation. The remaining 32 sites (73%) were still positive. One of these sites initially detected the L1C144 outbreak in November 2020 and reported a new PRRSv strain introduction in September 2021, which corresponded to a related L1C144 variant (<98% identical to the initial strain identified). The phylogenetic tree of the additional historical sequences from this site suggests two co-occurring clades with <2% nucleotide difference amongst proximate nodes but >2% nucleotide difference between the two most contemporary nodes (Figure 1).

Sites that changed statuses during the studied period were affected by this variant for a median of 161 days (max. 329). Sites that remained affected throughout the studied period stayed in an active break status for a median of 226 days (max 443), censoring at January 07, 2022. Season in which sites broke was not associated with sites being able to change statuses throughout the study period.

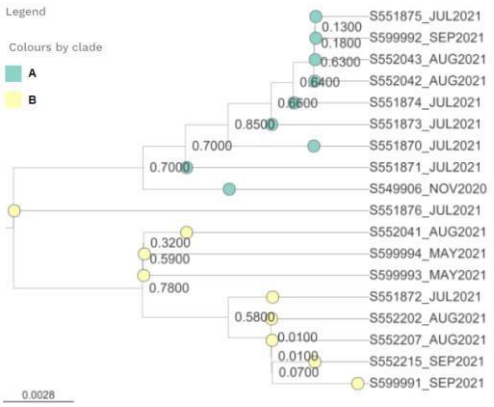

Figure 1. Maximum likelihood tree of historical ORF5 sequences collected from a single site during a L1C144 outbreak.

Discussion and Conclusion

The median time affected by this variant is smaller than previously described time to stability (~41 weeks)(3), however, most affected breeding sites have remained positive with this variant throughout the studied period. Although the report of a new L1C144 strain in the longitudinally sequenced farm might be perceived as a rebreak, historical phylogenetic investigation suggests the new strain was already present before its initial detection and might have originated from natural evolution within the site. Further investigations are needed, but this finding warrants caution in interpreting rebreaks of this particular variant and highlights the importance of maintaining historical samples even during the course of an outbreak to fully inderstand the epidemiology of this new variant.

Acknowledgments

Authors would like to acknowledge MSHMP participants, collaborating VDLs. The project was funded by the Swine Health Information Center (SHIC), and by the joint NIFA-NSF-NIH Ecology and Evolution of Infectious Disease.

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Occurrence of antibodies against Swine Influenza Virus subtypes in the South and Southeast regions of Brazil in 2021

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Introduction

Influenza Viruses A (IVA) are important pathogens in public and animal health, due to their zoonotic potential and continuous genetic and antigenic evolution. The constant genetic variability of IVA is a result of antigenic drift (mutations) and antigenic shift (reassortment), especially in its surface proteins - hemagglutinin (HA) and neuraminidase (NA), which impacts the ability of the host's antibodies efficiently defeat the infection by neutralizing these proteins. In this context, pigs have an important epidemiological role because a single animal cell can be infected by multiple strains from human, avian and swine IVA subtypes (1). This phenomenon led the pigs to be considered "mixing vessels" of human, avian and swine IVA. The swine influenza virus A (SIV) induces an acute respiratory disease, with high morbidity, leading to important economic losses worldwide, both because of its inflammatory reaction and also in co-infections with other viruses and bacteria. Since the 2009 pandemic, SIV has become endemic in Brazilian herds and there are four circulating subtypes: pH1N1, H1hu, H1N2 e H3N2. Only a monovalent pH1N1 vaccine against SIV has been available in Brazil since 2014, even though cross-protection against heterologous viruses is limited (2). In this context, the aim of this study was to assess the occurrence of SIV subtypes from routine samples, collected between March to December 2021 from swine herds of the South and Southeast regions of Brazil.

Materials and Methods

A total of 1,874 serum samples received in 2021 at the Laboratório de Pesquisa em Virologia Animal (LPVA), Veterinary School, UFMG, from non-vaccinated herds, were processed to analyze the presence of antibodies against pH1N1, H3N2 and H1hu by the hemagglutination inhibition test (HI).

Results

Results from the HI test showed that 77.5% of the samples were positive for the presence of SIV antibodies, with 9,2% positive for pH1N1, 3.8% for H3N2 and 15.3% for H1hu. Co-infection between pH1N1+H1hu were observed in 9.6% of the samples, H1hu+H3N2 in 4.4%; pH1N1+H3N2 in 11.3%, and the three subtypes in 24% of the samples.

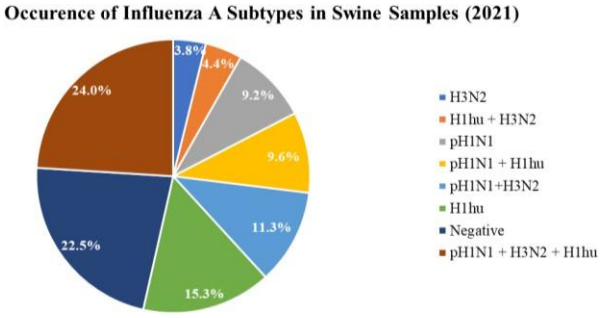

Figure 1: Percentage of occurrence of antibodies against SIV subtypes from Brazilian herds from the South and Southeast regions in 2021.

Discussion and Conclusion

Considering that respiratory diseases are one of the most important causes of economic losses in the swine industry, the monitoring of their etiologic agents is an essential measure to guide preventive actions and reduce negative impacts inside the farms (3). In this context, the study reinforced that SIV still had a high percentage of occurrence in pigs from Brazilian herds and antibodies against the three subtypes could be found in a single sample. This result showed that more than one subtype can be circulating within the farms, even though there is only one vaccine against pH1N1 available, and it cannot protect efficiently against heterologous infections. It is a complicated situation that leads the farmers without an efficient vaccination protocol to follow in order to prevent SIV infections. Regarding the occurrence of each subtype individually, H1hu had the higher percentage, and this result is alarming, considering that its occurrence in humans is low, which brings some risks to public health in case of zoonotic transmission to people in direct contact with infected pigs. In conclusion, continuous surveillance of SIV subtypes is also essential to reduce risks of transmission in both human and swine populations. According to this study, a high occurrence of SIV antibodies was observed in routine samples, with a high percentage of H1hu and co-infection of the three subtypes.

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One four-step procedure to resume pig production after precision removal of ASFV-positive pigs in farms in China

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Introduction

African swine fever virus (ASFV), a devastating virus in pigs, has broken out in China for more than four years (1,2). Previously, culling the whole swine herd was a common strategy for eliminating ASFV. Since 2018, a new strategy, precision removal, has been developed in China and shown to preserve non-infected pigs to the greatest extent possible (3). However, how to avoid secondary breaking out and resuming pig production as soon as possible after the precision removal process is still a problem. From cases in four large-scale farms, a mature and effective procedure to resume production involving four steps has been summarized and developed.

Materials and Methods

The procedure mainly included four steps. First, before the resumption of pig production, the ASFV test was done in the whole farm, and then electronic maps bearing ASFV positive information were drawn, including the whole farm, production area, area outside of the production area within 1km and living area. The ASFV-positive sites were marked in red on these maps (shown in **Figure1**). Second, disinfection measures were taken in ASFV-positive areas, and the qPCR test was taken to evaluate the disinfection effect. In addition, different disinfectants and disinfection methods were used according to the size of the contaminated area and the type of objects. Third, the internal environment samples of farms were also collected and tested by qPCR, and the positive area were further cleaned and disinfected. Last, the equipment, facilities and material modules were upgraded and maintained to meet the standard of biosecurity.

All the data were collected from four farms, and then TTNH (time to negative herds, in which clinically normal pigs, clinically abnormal pigs and environment were all negative), and the time to basic production (TTBP, the production level was evaluated in terms of service rate) were calculated. Farm 0 was cited as the control group which didn't use the four-step procedure, and farm 1 to 3 were as the experimental groups.

Results

The TTNH in farm 1 to 3 were three weeks, five weeks and six weeks respectively, whereas that in farm 0 was sixteen weeks (shown in **Figure2**). The TTBP in farm 1 to 3 were two weeks, three weeks and four weeks, whereas farm 0 was twenty-five weeks (shown in **Figure3**). These data showed that the four-step procedure spent less time for resumption which meant less production cost and less management cost.

Discussion and Conclusion

The four-step resumption procedure is a new anti-ASFV strategy which was developed from filed experiences, which takes into account swine production process and management process. Hopefully this procedure could provide some ideas to other swine companies around the world in the fight against ASFV and promoting the elimination of ASFv from swine herds.

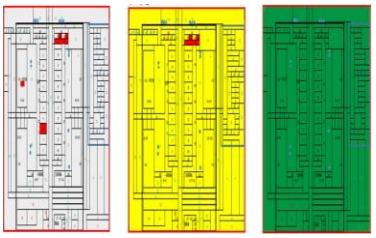

Figure1. The electronic map with ASFV-positiveinformation

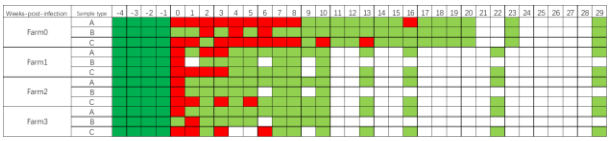

Figure2. TTNH in four farms. A, B and C mean samples of clinically abnormal pig, clinically normal pigs, environment respectively; red, green and white mean positive status, negative status and not tested.

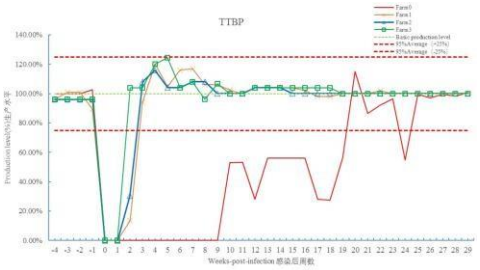

Figure3. TTBP in four farms.

Acknowledgments

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Optimisation of Influenza A Virus detection by PCR in oral fluids samples by using Real Time Respiratory Health Status (ReHS) monitoring to determine time of sampling

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Introduction

Accurately determining the timing of infections in herds impacted by Swine Influenza A Virus (swIAV) is important for the implementation of cost-effective vaccination and preventative therapeutics (e.g. anti-pyretic/anti-inflammatory/antibiotic treatments). The objective of this study was to demonstrate the value of using SoundTalks® (ST) 24-hour real time auditory monitoring alarms to determine the optimal timing of diagnostic sampling and intervention pograms in the face of swIAV outbreaks. . The sensor based and algorithm calculated ReHS emits a green light (healthy/ReHS-) that changes to yellow or red alarms with increasing cough (ReHS+).

Material and methods

During a 6-month period a site nursery containing 8 rooms, weekly filled with 900 4-week-old pigs were evaluated. The rooms were equipped with 2 ST monitors/room. Every week, half of the rooms were tested for IAV by AniCon GmbH, using 2 oral fluids (OF)/room, placed below ST monitor. A Chi-square (non-parametric) test was used to study whether there was a statistically significant difference between the observed and the expected number of positive/negative results with or without ReHS alarms.

Results

A total of 136 rooms were OF sampled during the 6-month period (June – early November) in 2021. In 29of the rooms the ST monitor showed a Yellow warning or Red alarm signal, meaning Respiratory Health Status problem (ReHS+) the day of sampling; 107 rooms showed a green (Healthy) signal (ReHS-) indicating no respiratory health issues. swIAV was detected in 22/29 ReHS+ rooms (detection rate 82%) and in 31/107 ReHS- rooms (detection rate 29%). Results indicated that the ReHS+ was significantly associated with an increase in swIAV PCR-positive OFs (p-value <2.2e-16 Pearson's Chi-squared). Fig 1.

Positive swIAV samples were evenly distributed by month except for September when no alarms were detected Fig 2. Highest frequency of swIAV were detected during week 5th and 6th after placement in the nursery. Fig 3. Coughing events were distributed evenly between rooms. Sequencing of some of swIAV positive low Ct-value samples showed that a mix of swH1N1, and H1N1 pdm091 could be present at the same time.

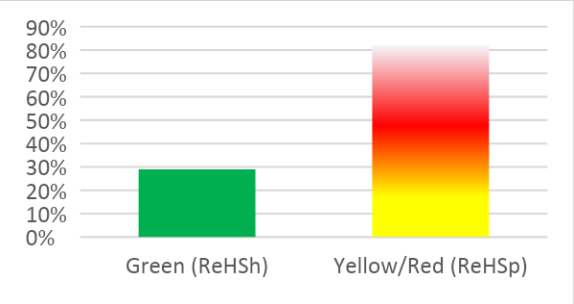

Figure 1. Proportion of positive swIAV OF PCR inrelation to ST monitor color. Green bar =ReHS-, Yellow/Red bar = ReHS+

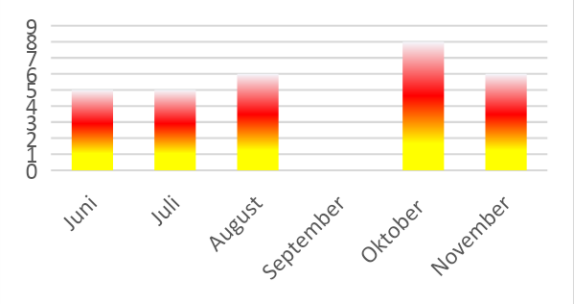

Figure 2. Distribution of ST ReHS+ alarms by month

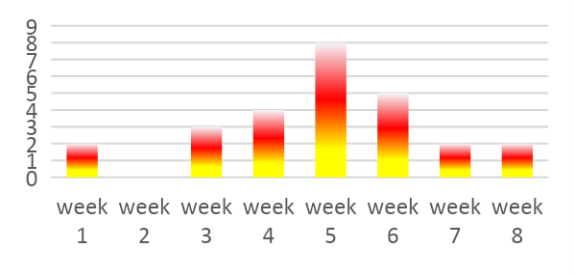

Figure 3. Distribution of ReHS+ ST alarms by week after placement in ordinary nursery rooms

Conclusions

SoundTalks® was able to identify multiple episodes of cough in a nursery facility and was a helpful surveillance tool to determine the most optimal time for OF sampling in order to detect swIAV. Although IAV was detectable in 29 % of rooms without any respiratory sign, the 82% success rate in ReHSp alerts saved the farmer significant diagnostic costs and allowed him to be more precise in his intervention.

Passive and Active Surveillance of African Swine Fever in Wild Boars in Poland in the years 2014-2021

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Introduction

African swine fever (ASF) is a severe, highly contagious, hemorrhagic disease, which since its first introduction to Europe continues to pose a serious danger not only for European but also international production and trade of swine (1,2,3). First case of ASF in Poland was confirmed in 2014 in a wild boar, on the eastern border of the country. Since that time the National Veterinary Research Institute in Pulawy (NVRI) conducts monitoring studies on wild boar samples (1). This research is a part of passive and active surveillance program, introduced by EU in response to progressive ASF epidemic. Despite the use of preventive measures, the epidemic continues tospread to another areas of Poland (4).

Materials and Methods

All environmental samples, including wild boar's blood and tissues, were collected from ASF restricted zones 0, I, II and III, by the workers of theVeterinary Inspectorate as a part of ASF monitoring program in Poland. The zones were designated in accordance to 2014/709/EU decision and updated with new legislation of 21 April 2021 (2021/605/EU) (4). All stages of the laboratory studies, *i.e.* analysis for the presence of ASFV DNA and antibodies against ASFV were carried out in the biosafety level 3 (BSL-3) of the National Reference Laboratory for ASF in Poland. For this purpose molecular (real-time PCR) and serological (ELISA and immunoperoxidase assay IPT) methods were used (2,3,4).

Results

In this study were r the data from different periods of time (2014-2021), with particular emphasis on the II and III ASF restricted zones were analysed. Between 2017 and 2021, in the whole country, a total of 442947 wild boars were tested, with division into three groups: found dead - 31042, road-killed - 35842 and hunted - 376063. The results showed a total of 20191 (4.56%) ASF positive and 422756 (95.44%) negative wild boars. Most of the positive results were observed in the group of animals found dead. Between 2014 and 2021, within zones II and III, 23571 wild boars were found dead , of which 16258 (68.97%) were ASF positive. In the same period of time, a total of 5822 road-killed wild boars were analysed. In this group, a positive results were obtained in 243 (4.17%)animals (Table 1). In active surveillance, since 2015 inASF zones II and III, a total of 230216 hunted wild boars were analyzed. In that group 3280 (1.42%) wereASF positive (Table 2). As a result of the activities carried out as part of passive and active surveillance, the number of tested wild boars was increasing every year. The number of ASF positive animals also increased accordingly.

For example, in 2021 within II and III zones, the number of ASF positive wild boars found dead, was almost 80 times higher than in 2014.In the case of hunted wild boars, the number of ASF confirmed animals was 73 times higher compared to 2015. In the road-killed wild boars this number was 20times higher between 2016 and 2021. For about five years, the stability in the percentage ratio of ASF positive wild boars to the total number of tested animals found dead and hunted has been confirmed. Inthe group of road-killed animals, similar phenomenonwas not observed.

Table 1. Passive surveillance: the number of tested wild boars and the number (%) of positive wild boars in 2014-2021 in zones II-III

Year	II and III zones					
	found dead wild boar			road-killed wild boar		
	tested	ASF(+)	% ASF(+)	tested	ASF(+)	% ASF(+)
2014	115	46	40,00%	68	0	0%
2015	130	67	51,00%	53	0	0%
2016	149	63	42,00%	95	3	3,15%
2017	1241	879	70,80%	137	6	4,38%
2018	4732	3453	72,97%	709	63	8,89%
2019	4699	3065	65,23%	1384	36	2,60%
2020	7156	5014	70,07%	1846	74	4,01%
2021	5349	3671	68,63%	1530	61	3,99%

Table 2. Active surveillance: the number of tested wild boars and the number (%) of positive wild boars in 2015-2021 in zones II-III

Year	II and III zones (hunted wild boar)		
	tested	ASF(+)	% ASF(+)
2015	3387	14	0,41%
2016	4221	24	0,56%
2017	6016	117	1,95%
2018	20590	303	1,47%
2019	41758	612	1,47%
2020	83962	1184	1,41%
2021	70282	1026	1,46%

Conclusion and Discussion

The analysis of the presented data clearly shows a significant role of dead, ASF positive wild boars as a main source of ASF in Poland. It confirms the importance of actions based on intensive carcass removal, preventing further spillover of the virus. The effectiveness of such measures has already been confirmed in the Czech Republic and Belgium (4).

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Porcine Deltacoronavirus occurrence in the United States breeding herds

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Introduction

Since first reported in the US in 2014, the Porcine Deltacoronavirus (PDCoV) became a recognized enteric pathogenic cause of diarrhea in suckling pigs (1, 2). Breeding herd cases of PDCoV have been increasing in the past years raising concern to the industry. Even though some epidemiological research has been done when the disease was first reported (2014-2015), there is a lack of understanding of the spatiotemporal patterns at the regional level since then, the factors associated with incidence over time, and the capacity of monitoring this non-reportable disease. In this study, we performed a formal epidemiological assessment of PDCoV occurrence and spread dynamics between 2015 and2021 in the Midwest and Southeast regions of the country. These regions are where the highest US swine density is located, and where the majority of the Morrison Swine Health Monitor Project (MSHMP) participating farms operate (3).

Materials and Methods

We utilized location and outbreak dates to describe and analyze the spatiotemporal trend of PDCoV from reports of more than 1300 farms from 38 systems. We assessed for global and local spatial and spatiotemporal clustering of MSHMP-reported PDCoV cases between January 2015 and July 2021. We applied the Cuzick-and-Edwards nearest neighbor test (4), directionality test, and Knox test (5) for global clustering and the permutation model of spatial scan statistics (6) to identify the location of the clusters (local clusters).

Results

There was a total of 163 cases reported in 15 states, with the majority of them (70%) reported in the last couple of years (2019-2021), and over winter time (93/163), when risk was 3.43 times higher (95%CI: 2.07 – 5.67) related to the lowest incidence season (summer). Astrong (p-value <0.01) spatio-temporal clustering was detected in the study area, with an increased risk (1.5 times) of disease at <5 km distances of farms infected within 35 days. Three significant (P-value<0.05) clusters were observed in different regions in 2020, and one in the northwest of the Midwest area during the lastsemester of 2015. However, when adjusting by thesystem only one cluster (P-value<0.05) was observed between March and May 2020.

Discussion and Conclusion

Results obtained here highlight a steady increase in cases over the years. The areas and periods of disease aggregation suggest that local transmission from infected to susceptible neighboring farms is happeningand likely serving as sources of virus maintaining the disease in the area. Results here show that PDCoV whole-system monitoring is still important, and controls measures need to be strengthened to limit the spread and impact of the disease.

Acknowledgments

Participants from Morrison Swine Health Monitoring Project, Funding from Swine Health Information Center.

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Porcine epidemic diarrhea: retrospective survey of clinical signs and management for farms in South Korea

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Background

Porcine epidemic diarrhea virus (PEDV) is a highly contagious coronavirus disease that causes vomiting, diarrhea, dehydration in neonatal piglets. PEDV can also c ause diarrhea, agalactia, and abnormal reproductive cycles in pregnant sows. PEDV was first reported in Korea in 1992. After the o utbreak of the PED in 2003, it gradually declined. But it has been showing a high incidence of diseases again s ince 2014, and in recent years, it has tended to occur throughout the year regardless of the season. Thus, we described the clinical sings and management methods of PEDV in South Korea between May to July in 2020.

Materials and Methods

PED occurred in 6 farms from May to July 2020, and the outbreak area is a pig farm complex, with more than10 farms located very close. The information and immunization methods were Table 1.

The 6 farms testedwere A to F in the order of the outbreak date. Clinical signs were classified from mild to severe based on the vomiting, diarrhea and mortality rate of suckling piglets. There were 2 immunized methods, which were live vaccine(oral, vaccine 2ml+milk 3ml, $1\times10^{5.3}$ TCID50/dose, CAVAC, Korea) and feedback(oral, use10 suckling piglet intestines per 100 sows) accompanied with inactived vaccine(IM, $10^{6.5}$ TCID50/dose, CAVAC, Korea). In all farms, feces and sludge were collected and genotype analysis was performed.

Results

The results showed as Table 2.

On farms A, C and D, severe watery diarrhea and vo miting were observed in suckling piglets, and anorexia and diarrhea were observed in most sows.In the case of farms B, E and F, watery diarrhea and v omiting in suckling piglets were observed lower than those of other farms.

The mortality rate in live vaccination groups was significantly lower than that of in feedback groups. The duration of clinical signs in the live vaccination groups observation was shorter than in the feedback groups. As a result of the genotype analysis of A to F farms, i t was confirmed as G2b, which was popular in 2014 in South Korea.

Table 1. Information and immunization methods of farms

Farm	Size of farm(sow)	Methods of immunization	Vaccination history before infection
A	200	Feedback+Inactivated vaccine	None
B	400	Live vaccine+Inactivated vaccine	Inactivated vaccine
C	200	Feedback+Inactivated vaccine	None
D	70	Feedback+Inactivated vaccine	None
E	350	Feedback+Inactivated vaccine	None
F	100	Live vaccine+Inactivated vaccine	Inactivated vaccine

Table 2. Clinical signs, mortality rate and genotype

Farm	Clinical sign	Duration of clinical sign	Mortality rate of piglets	Genotype
A	Severe	2 weeks	100%	G2b
B	Mild	2 weeks	40%	G2b
C	Severe	3 weeks	90%	G2b
D	Severe	3 weeks	100%	G2b
E	Moderate	3 weeks	100%	G2b
F	Mild	1 weeks	30%	G2b

※ Mortality rate(including culling)

Discussion

As a result of using live vaccines against PEDVon farms, it is considered that live vaccines can replace feedback treatment. And PED vaccination consistently in the farms will prevent PED outbreak and cut PEDV circulation on farms.

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IPVS2022
26th international pig veterinary society congress – rio de janeiro – brazil

Potential relationship of emerging Porcine parvovirus with Porcine Circovirus reproductive failure in Mexico

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Introduction

Worldwide, emerging porcine parvovirus (PPVs) have been linked to both porcine circovirus associated disease (PCVAD) and reproductive failure (RF) in sows but their potential participation remains elusive (1,2,3,4). In Mexico, the prevalence and concurrent infection of PPV2-6 was found markedly higher than that reported in other countries and a significant relationship of PPV5 and PPV6 with PCVAD was described (5). The aim of the present work was to evaluate the relationship of PPVs with reproductive failure in sows.

Materials and Methods

One hundred thirty paraffin- embedded fetal hearts were selected to perform a nested PCR specific for PPV2, PPV3, PPV5, and PPV6 which were composed of 56 cases of PCV2$^+$-RF (as determined by clinical signs, non-suppurative myocarditis, and PCV2+ *in situ hybridization*), 44 cases of PCV2$^-$-RF, and 30 non-affected fetal hearts from slaughterhouse. In addition, the cases were grouped based on the farm PCV2 statuses as described (5) to compared with the abattoir frequency. The data were analyzed by Ji2test ($\alpha < 0.01$).

Results

The prevalence of PPVs is shown in table 1. The PPV5 prevalence in the abattoir cases was markedly lower (3.3%) than that of the RF cases whereas the PPV6 frequency in the abattoir cases was higher (80.0%) compared to RF cases. In these species, the prevalence was higher in cases of PCV2$^+$-RF than that of the PCV2$^-$-RF. PPV5 showed statistically significant relationship with both PCV2$^+$-RF and PCV2$^-$-RF (Table 2). Regarding the PCV2 farm status, PPV5 and PPV6 depicted significant relationship to PCV2$^+$-RF and PCV2$^-$-RF, respectively (Table 3). No significant interspecies association was revealed for abattoir cases.

Discussion and Conclusion

In this work, the selected tissues fulfilled diagnostic criteria for PCVAD to estimate the relationship while most studies did not comply with all of them (1,3). Although significant interspecies associations were reported in Mexico, no significant relationship with RF in sows was found (5). Herein, PPV5 showed a statistically significant relationship with PCV2$^+$-RF and PCV2$^-$-RF as well as in PCV2-affected farms, despite a minor prevalence for PPV5 has been reported in fetal samples elsewhere (3,4). Also, the PPV6 prevalence was 62.3% that is closed to that reported in China (50%) from aborted fetuses (8), displaying a significant relationship in the PCV2 unaffected farms. Therefore, it is likely that PPV6 may contribute to RF as suggested, since its detection in fetal tissues from abortions was unrelated to other reproductive pathogens (3,8). Overall, PPV5 and PPV6 may have a potential involvement in RF. PPV2 was the most prevalent species as in previous reports (2,3,4,6,7,8) but no significant association with RF was found. Further prospective studies must be done to ascertain the PPV5 and PPV6 participation in RF.

Table 1. Prevalence of PPVs in fetal hearts.

CASES	PPV2	PPV3	PPV5	PPV6
PCV2$^+$-RF	54/56	21/56	18/56	35/56
%	96.4	37.5	32.1	62.5
PCV2$^-$-RF	41/44	17/44	9/44	22/44
%	93.2	38.6	20.5	50.0
Abattoir	28/30	11/30	1/30	24/30
%	93.3	36.6	3.3	80
Total	123/130	49/130	28/130	81/130
%	94.6	37.7	21.5	62.3

Table 2. Relationship of PPVs with RF

CASES	PPV2		PPV3		PPV5		PPV6	
	+	–	+	–	+	–	+	–
[a] PCV2$^+$-RF	54	2	21	35	18	38	35	21
Abattoir	28	2	11	19	1	29	24	6
Total	82	4	32	54	19	67	59	27
[b]PCV2$^-$-RF	41	3	17	27	9	35	22	22
Abattoir	28	2	11	19	1	29	24	6
Total	69	5	28	46	11	63	45	29

[a] Cases of reproductive failure associated to PCV2 complied with diagnostic criteria (clinical signs, non-suppurative myocarditis, PCV2+ in situ hybridization), [b] Cases of reproductive failures negative to PCV2. □Statistically significant (P=0.01)

Table 3. Relationship of PPVs conformed to farm PCV2 status

CASES	PPV2		PPV3		PPV5		PPV6	
	+	–	+	–	+	–	+	–
[a] PCV2-affected	53	2	16	39	20	35	34	21
Abattoir	28	2	11	19	1	29	24	6
Total	81	4	27	58	21	64	58	27
[b]PCV2$^-$-unaffected	19	1	11	9	1	19	7	13
Abattoir	28	2	11	19	1	29	24	6
Total	47	3	22	28	2	48	31	19

[a] At least 5 submitted tissues per farm with > 70% positivity to PCV2, [b] At least 5 submitted tissues per farm with > 70% negativity to PCV2. □ Statistically significant (P=0.01)

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Practical use and interpretation of PRRSV ORF5 sequencing in vaccinated pigs in the U.S.

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Introduction

The use of modified live vaccines (MLVs) to control PRRS in breeding herds and growing pigs is a common practice worldwide. MLV vaccines help mitigate the clinical impact and losses due to PRRS.[1,2,3.] Given that MLV vaccines contain replicating viruses, results from surveillance and diagnostic investigations that target the identification of wild-type (WT) PRRS viruses in vaccinated populations are difficult to interpret. Phylogenetic analysis using reference sequences has been used to evaluate relatedness between viruses using gene ORF5 as this encodes the major envelope glycoprotein and shows high genetic diversity. Historically, values of genetic homology of >97% similarity of ORF5 have been used to indicate close genetic relationships[4,5,6]. The objective of this study was to evaluate whether a cut-off of 2% heterology between ORF5 sequences obtained from vaccinated growing pigs could be used to differentiate vaccine from WT-PRRS strains.

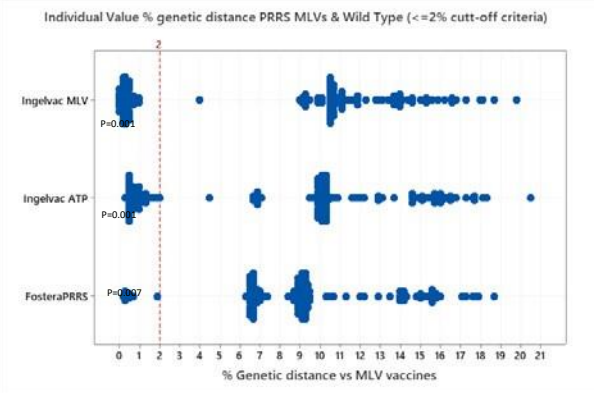
Materials and Methods

One-hundred and thirty-two sequences were analyzed from an observational prospective cohort study involving 60 wean to finish sites located in the Midwest U.S. Herds we located in medium to high pig dense areas. Eight oral fluid samples were collected from each group of wean-to-finish pigs every 4 weeks, from approximately weaning to market, using fixed spatial sampling. RT-PCR was run in 2,475 samples and positive samples with the lowest cycle threshold (Ct) value of $\leq$ 33 were characterized by sequencing of the ORF-5 region (Sanger sequencing UofM D-lab, Alonso et al, 2013). Sequences were aligned using MegAlign from DNASTAR software (version 15.1.0) usingClustal W and compared with reference MLV PRRS virus strains from MLV vaccines used in the herds part of the study (Ingelvac PRRS MLV Accession # AF066183, Ingevalc ATP Acc # DQ988080 and FosteraPRRS, Acc # JB398244). Sequences were classified as WT-PRRSV if they had equal or more than 2% of nucleotide differences from those of any of the vaccine strain sequences. An individual plot value distribution of the genetic distance between ORF5 sequences and each reference vaccine sequence was created (Figure 1). Wilcoxon signed rank test was used to determine whether the percent homology value from strains classified as MLV was less than 2% (Figure 1). In addition, genetic distances between reference vaccine sequences and sequences obtained in the study classified as MLV-like were analyzed at different sampling points(3, 8, 12, 16, 20 and 25 weeks post-placement).

Results

Distribution of genetic distances for sequences classified as MLV clustered together with sequences grouping within 2% genetic distance from each of thereference MLV sequences.

Figure.1 Plot of genetic distances between PRRS MLV and wild-type PRRSV sequences.



In addition, we observed a statistically significant association ($p<0.001$, Rsq 30.21%) and a positive correlation ($r=0.55$) between genetic distance of PRRS MLV sequences and time from vaccination. Genetic distance of MLV-like sequences in vaccinated pigs increased with age of the growing pigs.

Discussion and Conclusion

Our study provides supporting evidence that a 2% cut- off can be used to diferentiate wild-type PRRS viruses from MLVs strains in vaccinated herds. It is important to mention that the use of this criteria is merely for epidemiological purposes, and should not be considereda metric for predicting vaccine cross protective immunity. Interestingly, we also observed that genetic distance of vaccine-like viruses increased in relation to time from vaccination. Additional research is needed to better understand how PRRSV evolves in growing pig populations.

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Prevalence and distribution of porcine rotavirus (RV) group and type in suckling piglets in Canada between July 2019 and July 2021

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Introduction

Rotavirus (RV) is a diarrhea-causing pathogen well stablished in the swine industry (1). Older animals become resistant to disease caused by the virus as they develop post-exposure immunity to it, coupled to maturation of the gut physiology and overall immunity (1). RV A, B, and C have been demonstrated to cause disease in swine and are identified by the viral protein 6 (VP6) antigenicity, while other structural proteins, VP4 and VP7, are employed in further typing the strains into P or G type based on antibody neutralization (1). Buchan and colleagues (2) summarized three years of diagnostic reports involving diarrhea presentation in Ontario (ON), Canada, during the lactation. RV A was detected in 69% of the cases of diarrhea in suckling piglets, RV C in 37% of the cases, and RV B in 13% of the cases. Observing the need of better data across Canada to aid in informed decisions, the objective of this study was to determine the prevalence of RV groups and types on suckling pigs from different Canadian provinces (AB, BC, MB, NB, ON, QB, SK).

Material & Methods

Canadian swine veterinarians submitted samples (fresh tissues, fecal swabs, or fecal material) from perinatal diarrhea cases to the Animal Health Lab at the University of Guelph (AHL) for RV confirmation, type identification, and VP7 sequencing. Analysis of the VP7 was performed using Merck Animal Health Sequivity Dashboard (3). Descriptive analysis was employed.

Results

RV positive samples from 245 diarrheic were sequenced. RV C was present in 46.5%, RV A in 40.8%, and RV B in 12.6%. Individual RV infection summed 148 cases (60.41%), while 44 cases were co-infections. RV A was present in 84% (37/44) of these cases, followed by RV C in 81.8% (36/44), and RV B in 40.9% (18/44). Sixteen different group types were identified by sequencing of the VP7 protein (5 RV As, 7 RV Bs, and 4 RV Cs). Table 1 presents each RV group/type found per province. Some RV types were specific to a certain region or province. Only ON observed RV B G18, MB a RV B G25, RV A G11 was only found in AB, RV B G12 was found in AB and SK only, and RV B G16 in MB and ON.

Discussion & Conclusion

Rotavirus-related diarrhea in suckling piglets is still a concern for swine industry due to its perinatal damages. Similar to other studies, suckling piglets were mostly infected by only one RV, although co-infections were common. RV B was the least prevalent strain, however, the most diverse group. RV C G6 was the most prevalent RV type in Canada (except ON) in this study.

Table 1: Distribution of RV A, C, and B types by Canadian provinces.

RV type	AB	BC	MB	NB	ON	QC	SK	Total
A	27		25		40	1	7	100
A G11	1							1
A G3	4		2		2		1	9
A G4	1		1		4			6
A G5	15		3		6	1	2	27
A G9	6		19		28		4	57
B	7		5		6	1	12	31
B G12	2						1	3
B G14	2		1				2	5
B G16			3		2			5
B G17					2		8	10
B G18					1			1
B G20	3				1	1	1	6
B G25			1					1
C	28	3	33	1	25	9	15	114
C G1	6		3		5	1	2	17
C G5			3		1		4	8
C G6	22	3	26	1	14	6	8	80
C G9			1		5	2	1	9
Total	62	3	63	1	71	11	34	245

AB: Alberta; BC: British Columbia; MB: Manitoba; NB: New Brunswick; ON: Ontario; QC: Quebec; SK: Saskatchewan.

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Prevalence and seasonal variation of different respiratory pathogens in post-weaned pigs with signs of clinical respiratory disease using TBS sampling technique: an update

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Introduction

Besides *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*), many other viruses and bacteria can concurrently be present in pigs (1,2). These pathogens can provoke clinical signs, known as porcine respiratory disease complex (PRDC). A sampling technique on live animals, namely tracheobronchial swab (TBS) sampling, was applied to detect the major PRDC pathogens in pigs using PCR (3,4). The objective was to determine prevalence of different PRDC pathogens and their seasonal variations in Belgium and the Netherlands.

Materials and Methods

A total of 600 pig farms in Belgium (n = 248) and the Netherlands (n = 352) and 9,000 post-weaned piglets were sampled using TBS over a 4-year period. TBS samples were analyzed using mPCR for *M. hyopneumoniae*, PRRSV, IAV-S and PCV-2. Results were categorized and analyzed according to the season of sampling.

Results

In Belgium, 53.8% of the sampled farms were PRRSV-positive, followed by *M. hyopneumoniae* (48.8%) and IAV-S (40.3%), whereas only 20.6% of the farms were detected PCV-2-positive. In the Netherlands, a similar percentage of farms were detected positive for PRRSV (51.4%) and IAV-S (49.1%), whereas a lower percentage of farms was *M. hyopneumoniae*-positive (32.4%) and only 8.0% was detected PCV-2-positive. Combined infections consisted of *M. hyopneumoniae* – PRRSV, PRRSV – IAV-S, and *M. hyopneumoniae* – PRRSV – IAV-S in both countries. In Belgium, the combination of *M. hyopneumoniae* – PRRSV – PCV-2 also had a relevant prevalence.

Figure 1. Prevalence (expressed as % positive farms) of different single PRDC pathogens in post-weaned pigs (3-12 weeks of age) at farm level. In total, 9,000 pigs were sampled in 600 different farms distributed throughout Belgium (n = 248) and the Netherlands (n = 352) for diagnostic purposes of clinical respiratory disease.

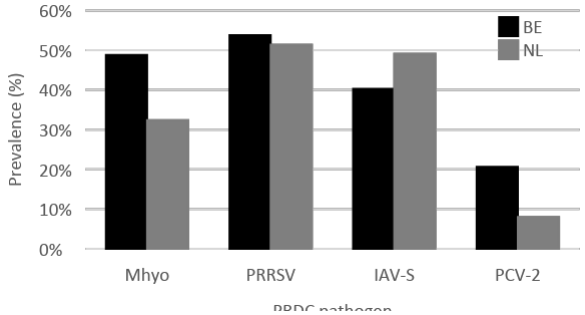

Table 1. Prevalence (expressed as % positive farms) of different double, triple and multiple PRDC pathogen interactions in post-weaned pigs (3-12 weeks of age) at farm level. In total, 9,000 pigs were sampled in 600 different farms distributed throughout Belgium (n = 248) and the Netherlands (n = 352) for diagnostic purposes of clinical respiratory disease.

	Belgium	Netherlands
Double infections		
M. hyo – PRRSV	10.9%	11.1%
M. hyo – IAV-S	4.0%	5.1%
M. hyo – PCV-2	3.6%	0.0%
PRRSV – IAV-S	11.3%	18.2%
PRRSV – PCV-2	1.2%	3.1%
IAV-S – PCV-2	0.4%	1.1%
Triple infections		
M. hyo – PRRSV – IAV-S	6.9%	6.3%
M. hyo – PRRSV – PCV-2	7.3%	0.6%
PRRSV - IAV-S – PCV-2	1.2%	2.3%
Multiple infections		
M. hyo – PRRSV – IAV-S – PCV-2	4.0%	0.6%

Discussion and Conclusion

The present study clearly shows that several viral and bacterial pathogens responsible for PRDC may be present during the post-weaning period. Following analysis of seasonal variation, it can be concluded most pathogens show seasonal patterns with a higher percentage of farms positive during autumn and winter, which has more favorable conditions for longer survival and better transmission of pathogens between animals and farms in our region. In conclusion, the present study showed that many respiratory pathogens are present during the post-weaning period, which may complicate the clinical picture of respiratory disease.

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Prevalence of Porcine Circovirus type 2 in intensive swine farms with routine vaccinationagainst PCV2 in Colombia

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Introduction

Porcine Circovirus Type2 (PCV2) has been associated with various pathological conditions grouped under PCAVD (Porcine Circovirus -associated diseases) (1). The virus has evolved rapidly, and a classification system based on the DNA sequence of the ORF2 gene of the virus has identified eight different genotypes (PCV2a through PCV2h); being PCV2a, PCV2b, and PCV2d the most prevalent genotypes (2). Even among vaccinated populations, the virus is still circulating despite the use of vaccines (3) and virological and immunological data suggest that cross-protection of current vaccines containing genotype a may not be complete. Poor cross-protection may explain the PCV2 genotype shifts observed over time, as well as the impossibility of preventing subclinical presentations (4). Therefore, this study aimed to establish the prevalence of PCV2 in Colombia in vaccinated farms.

Materials and Methods

Eighty-six farms from different swine production regions in Colombia were selected with different production systems and vaccination protocols forPCV2 control. One hundred sixty-six cases with signs suggesting PCV2 were collected from these farms. Samples included blood, lymph nodes, spleen, and thymus. DNA was extracted from all samples using a commercial kit. Then, conventional PCR was performed using primers that amplify the PCV2 region of 550 bp, which detects all PCV2 genotypes. We determined the PCV2 viral load through a quantitative multiplex PCR that detects PCV2a and PCV2b/d. A viral load was calculated using a standard curve developed for qPCR where pigs with high viral load (Ct<26) were positive. Sections of lymph nodes, tonsils, thymus, lung, heart, spleen, and liver were fixed in neutral buffered formalin and sent to the laboratory for histopathologic examination.

Results

Most of the examined animals had respiratory signs (coughing, sneezing, respiratory distress), someshowed wasting, and some of them had a reproductive failure or skin lesions (PDNS) (Figure 1).

PCV2-DNA was detected by conventional PCR in 54.8% (91/166) of cases and 74.1% (123/166) of cases by qPCR. These differences could be explained that the samples with 10^2 are not detected by conventional PCR.

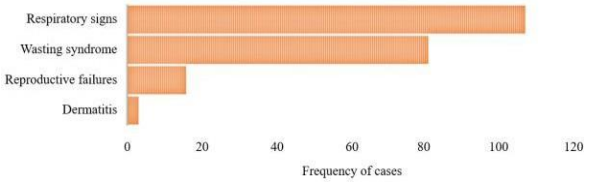

Figure 1. Main clinical signs observed in pigs studied

PCV2 DNA was found in all the evaluated regionswhere PCV2 circulation was detected in 87.2% (75/86)of the evaluated farms. Nursery pigs had the highest positive percentage (58%) from all the cases. In addition, 9% of the mummies evaluated were positive but with medium to low viral load. The PCV2 viralload in the positive samples was, in general, low. In contrast, only 6% of the samples tested had high viral loads. The most common histopathological lesions were detected in the lymphoid tissues and consisted of mild to moderate lymphoid depletion, with some histiocytic infiltrate. Most of the cases had interstitial lung pneumonia with moderate to severe thickening of alveolar septa.

Discussion

Despite the routine use of vaccination to control PCAVD, this virus is still circulating in the vaccinated farms enrolled in this study across main pig production regions in colombia, and clinical and subclinical signs may be encountered.

According to this study, the nursery pigs between 3 – 7 weeks of age were the most positive, regardless of the vaccination protocol?. These findings may suggest insufficient vaccine protection because of the viral genetic shifts in the pigs' population. In the vaccinated farms studied, the highest viral loads were associated with pigs showing respiratory signs and wasting insome cases. Pathological lesions suggest the presence of the subclinical disease.

Acknowledgments

Zoetis Colombian Swine Veterinary Team

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Recombinant protein based CSFV E2 vaccine induced long lasting high neutralizing antibody responses in pigs

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Introduction

Classical swine fever (CSF) is caused by classical swine fever virus (CSFV) which causes vasculitis, clinically manifested as hyperemia and cyanosis of the skin and other problems, resulting in devastating consequences on swine industry. To prevent CSF, several countries execute eradication programs, but sporadic outbreaks continue to occur in most major pig-producing countries. Vaccination is regarded as one of a most effective tools to prevent and control CSF. This study was performed to evaluate the immunogenicity and duration of the humoral immunity of recombinant protein based CSF E2 vaccine (HIMMVAC, Donoban-E2(CSF), KBNP, South Korea) in conventional pig farms on Jeju Island, South Korea, where recently affected by modified live vaccine virus (LOM strain) (1).

Materials and Methods

This study was conducted in three conventional CSF-naïve pig farms on Jeju Island, South Korea. A total 108 of forty days old (do) piglets were used, 36 were administered with HIMMVAC Donoban-E2(CSF) recombinant E2 vaccine (A group), 36 were administered BYOVAC CSF E2 vaccine (B group), and 36 were control group without vaccination. Pigs in A group and B group were vaccinated at 40do and 61do. For serum neutralization assay, pigs were bled 5 times; at 40do, 61do, 102do, 124do, and 152do, respectively. Serum was mixed with same volume of 200 TCID 50/100ul of LOM strain and added to 96-well plate with PK15 cell monolayers. After incubation, the Neutralizing Peroxidase-Linked Assay (NPLA) was used to determine the neutralizing antibody titer.

Results

The percentage of pigs showing CSF neutralizing antibody titer >32 is shown in Figure 1. In A group, percentage of titer >32 was increased at 61do, maintained 100% until 124do, and slightly decreased at 152do. In B group, the percentage was increased at 61do, increased to 100% at 102do and gradually decreased until 152do (Figure 1). The percentage of pigs showing CSF neutralizing antibody titer >8 is shown in Figure 2. In A group, the percentage of titer >8 was 100% at all experimental periods after vaccination. In B group, the percentage was 88% and 85% at 61do and 112do, while 100% at 102do and 124do, respectively (Figure 2). All animals in control groups remain negative to CSFV throughout experimental periods.

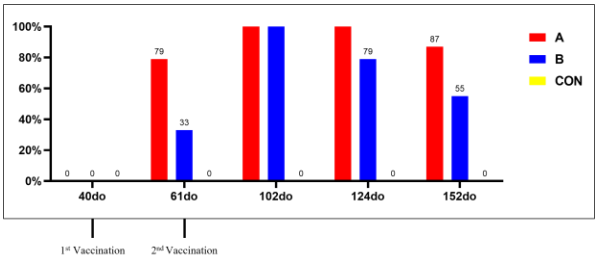

Figure 1. The percentage of pigs showing CSF neutralizing antibodies titer >32 (%)

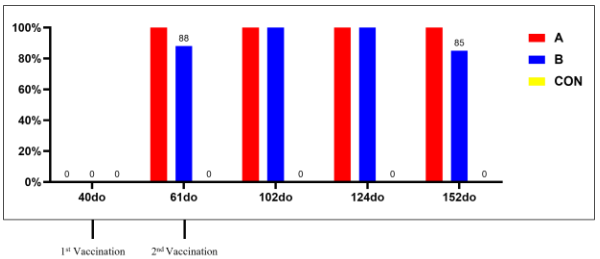

Figure 2. The percentage of pigs showing CSF neutralizing antibodies titer >8 (%)

A, Himmvac Donoban-E2(CSF) recombinant E2 vaccine group; B, Byovac CSF E2 vaccine group; CON, control group

Discussion and Conclusion

The CSFV neutralizing antibody titer >32 regarded as efficacy of vaccine to prevent clinical signs, shedding, and transmission of filed strain of CSFV and the titer >8 is a titer could prevent LOM strain of CSFV (2) in host animals. In this study, the vaccine of A group induced long lasting high neturalizing antibody titer until slaughter, it is most likely expected to successfully prevent the field and LOM strain of CSFV. In conclusion, the recombianat protein based CSF E2 vaccine (HIMMVAC Donoban-E2(CSF), KBNP, South Korea) can be one of an effective options to control CSFV for pigs where CSFV need to be controlled by inducing long lasting high neutralizing antibody response.

Acknowledgments

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Relationship between number of PRRSV strains, presence of recombination events and breeding herd production performance

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Introduction

Porcine reproductive and respiratory syndrome (PRRS)is one of the most economically important global swine diseases. Comparison of PRRS virus (PRRSV) strains has largely been based on the ORF5 gene representing approximately 4% of the PRRSV-2 genome. ORF5 sequence is often determined by the Sanger technique, which rarely detects more than one PRRSV strain if present in the sample. Next- generation sequencing (NGS) may provide a more appropriate method of detecting multiple PRRSV strains when present in the sample. Processing fluid (PF), the serosanguineous sample recovered from testicles and tails at processing time,[1] a population- based sample type, has been described and validated for PRRSV surveillance and monitoring and is currently primarily used for PRRSV surveillance/monitoring in breeding herds in the United States. [2]

This work assessed the effect of PRRSV genetic variability, number of PRRSV strains, and presence of PRRSV recombination events on the time to low prevalence, time to baseline production, and total losses in breeding herds undergoing PRRSV elimination.

Materials and Methods

This was a prospective cohort study following 20breeding herds that reported a PRRSV outbreak and adopted measures to eliminate the virus from the herd.Serum, lung, or live virus inoculation materialcollected within 3 weeks of the reported outbreak datewere submitted for PRRSV NGS. Subsequently, PF was collected on day of processing and pooled byweek for PRRSV monitoring by RT-qPCR. Based ontime post-PRRSV outbreak and RT-qPCR Ct values,six processing fluid samples per breeding herd wereselected and submitted for NGS: a) two samplesrecently after the outbreak (week 1-4); b) two samplesaround ten weeks after the outbreak (week 8-12); c)post week 12. All samples were tested for PRRSV and stored at -80ºC at the Iowa State University Veterinary Diagnostic Laboratory. After the farm achieved low prevalence, the last two PF samples with a Ct < 30were retrospectively selected and submitted forPRRSV NGS. Low prevalence was defined as eightconsecutive weeks with negative RT-qPCR results for PRRSV. Whole-PRRSV-genome or partial sequences recovered through NGS were used to characterizewithin and between herd PRRSV genetic variability. Kaplan-Meier survival analysis[3] was used to comparetime to low prevalence and time to baseline productionacross group. Time to baseline production wasmeasured as the time in weeks it took for the farms torecover the levels of pigs weaned per week as prior tothe outbreak based on statistical process control analysis. Total losses were calculated as the cumulative sum of the total number of pigs not weaned per 1,000 sows from the PRRSV outbreak to when theherd returned to baseline production and compared across different groups using a generalized linear mixed model.

Results and Discussion

The near complete-PRRSV genome was recovered in5/6 (83.3%) lung, 16/22 (72.73%) serum, and in 5/95 (5.26%) PF samples. Whole-PRRSV genomesrecovered from serum or lung were used as farmreferent strains in 16/20 (80%) farms. In 4 farms, only partial genome sequences were recovered and used as farm referent strains.

The genetic comparison revealed the presence of at least two wild-type PRRSV strains circulating simultaneously in 18/20 (90%) farms and at least one vaccine-like strain co-circulating in 8/20 (40%) farms. PRRSV recombination events were detected in 11 farms (55%), with 9/11 occurring between wild-type strains and 2/11 between wild-type and vaccine-like strains.

Farms having relatively higher genetic variability, i.e., ≥3 PRRSV, had a 12-week increase in the median time to achieve low prevalence compared with herds with ≤ 2 strains detected. Farms with ≤ 2 PRRSV strains detected (n = 10) had 1,837 fewer piglet losses,and farms with no recombination events detected (n =8) had 1,827 fewer piglet losses per 1,000 sows compared to farms with ≥3 PRRSV strains (n = 8) ordetected recombination events (n = 10), respectively. No differences in time to low prevalence or time to baseline production were found according to the number of PRRSV strains or the presence of recombination events.

Conclusions

This study reports the detection of multiple PRRSV strains co-circulating in a breeding herd at a single sampling point. Utilizing the near complete-PRRSV genome and novel methods to visualize NGS outcomes provided more thorough insight into the PRRSV dynamics, genetic variability, and detection of multiple strains co-circulating in breeding herds and helped establish practical guidelines for using PRRSVNGS outputs.

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Retrospective survey of rotavirus species (A, B, C, and H) in fecal samples from diarrheic suckling piglets from rotavirus-vaccinated Brazilian pig herds

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Introduction

The main viral etiology associated with severe diarrhea in newborn piglets is porcine rotavirus (RV). Rotaviruses belong to the *Reoviridae* family and *Rotavirus* genus. Nine RV species are recognized, named RVA to RVD and RVF to RVJ (1). Among these RV species, the most frequently associated with outbreaks of diarrhea in suckling piglets is the RVA followed by RVB and RVC, and sporadically, RVH is detected in diarrheic piglets (2,3). However, the diarrheal outbreaks caused by RVB and RVC have become increasingly frequent and may occur in singular infections or associates with other species of the genus. This study aimed to evaluate the frequency of RV species infection associated with diarrhea in suckling piglets from regularly RVA-vaccinated Brazilian herds.

Materials and Methods

From January/2015 to December/2021 were evaluated 511 diarrheic fecal samples from suckling piglets aged up to 4 weeks: 0-7 days (*n*=323), 8-14 days (*n*=120), 15-21 days (*n*=48), and 22-28 days (*n*=20). These fecal samples were obtained from 112 pig farms located in the three main pig production Brazilian geographic regions (South, Southeast, and Midwest). All the pig herds used commercial vaccines for RVA (G4P[6] and/or G5P[7]). The nucleic acid from diarrheic fecal samples was extracted and subjected to investigation of RVA, RVC, and RVH by RT-PCR assays (4,5,6,7) and RVB by semi-nested-RT-PCR assay (8).

Results

Of the 511 diarrheic fecal samples analyzed, 221 (43.2%) were positive for at least one of the RV species. Regarding the RV species distribution among the positive fecal samples from diarrheic suckling piglets with singular infections, 99/511 (19.4%) were positive for RVB, 63/511 (12.3%) for RVC, and 45/511 (8.8%) for RVA. RVH was not identified in singular infection. Mixed infections were identified in 14/511 of the RV-positive piglets, representing 2.7% of the positive fecal samples. The mixed infections identified were RVB+RVH in 11 (2.1%) samples, followed by RVA+RVB in 1 (0.2%), RVA+RVC in 1 (0.2%), and RVB+RVC in 1 (0.2%) fecal sample. The diarrheic fecal samples were categorized into four piglets age groups. Table 1 shows the distribution of RV species according to age groups. In relation to pig farms, in 75/112 (67%) at least one fecal sample of the total evaluated was RV-positive.

Table 1. Frequency of occurrence in the detection of infection by rotavirus of different species observed by age groups.

RV species	Molecular detection (%)				Total (*n*=511)
	0-7 (*n*=323)	8-14 (*n*=120)	15-21 (*n*=48)	22-28 (*n* =20)	
RVA	22 (6.8)	14 (11.7)	4 (8.3)	5 (25.0)	45 (8.8)
RVB	80 (24.8)	12 (10.0)	4 (8.3)	3 (15.0)	99 (19.4)
RVC	47 (14.5)	14 (11.7)	1 (2.1)	1 (5.0)	63 (12.3)
MI[1]	12 (3.7)	2 (1.6)	-	-	14 (2.7)
TOTAL	161 (49.8)	42 (35.0)	9 (18.8)	9 (45)	221 (43.2)

[1]M.I.: Mixed infection (0-7 days: RVA+RVB *n*=1; RVB+RVH *n*= 11; 8-14 days: RVA+RVC *n*= 1; RVB+RVC *n*= 1).

Discussion and Conclusion

High rates of RVA detection are reported in neonatal diarrhea in pig herds around the world. The frequency of RVA detection is higher than other RV species, such as RVB, RVC, and RVH, both in Brazil and in other countries (2,3,9). In this study on the frequency of diagnostic of RV species in diarrheic piglets show a significant increase in RVB and RVC infections in suckling piglets. Currently, RVB and RVC infections are widely disseminated in the pig herds of the main Brazilian pork production geographical regions. Immune pressure promoted by RVA vaccination may favors an increase in diarrhea outbreaks caused by other RV species. Finally, this study allowed to characterize the importance that other RV species, than RVA, have in the etiology of neonatal diarrhea in piglets. Additionally, these data highlight the importance of using effective prevention and control measures to minimize production losses resulting from RV infection in pig herds.

Acknowledgments

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Shedding patterns and virus diversification of heterosubtypic influenza infection in pigs

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Introduction

Influenza is one of the top diseases in the US swine industry and influenza A virus (IAV) is hard to control due to its rapid spread and abundant genetic diversity found in swine populations (1). Pigs can replicate IAVs from various hosts, and the co-circulation of different subtypes and strains of IAVs is common in swine herds, which facilitates virus evolution and makes vaccination difficult (2). However, how a co-infection with a heterosubtypic IAV impacts virus shedding and overall diversity at the individual pig level is poorly understood. In this study, we assessed the shedding patterns of IAV infected pigs upon a secondary infection of IAV of a distinct subtype and we evaluated its impact on virus diversity.

Materials and Methods

Fourteen naive pigs were inoculated with either an H1N1 or an H3N2 IAV, and distributed in 7 rooms with one pig of each subtype housed together. Nasal swabs were taken daily, and bronchoalveolar lavage fluid (BALF) samples were collected during necropsy at seven days post-contact. Samples were tested by matrix and HA subtyping real-time PCR. Any nasal swabs or BALF samples with Ct values under 35 were quantified for viable virus (TCID50) and whole genome sequenced by Illumina Nextseq platform. Forty plaques were isolated from three BALF samples to identify virusreassortment in pigs with a confirmed heterosubtypic IAV secondary infection.

Results

During the 7-day observation, we found 43% of pigs (6/14) with a heterosubtypic IAV secondary infection in the lungs or nasal cavities. Among these pigs, five pigs apparently cleared their primary IAV infection and at necropsy (7 dpc) they had mainly the subtype of the other pig. Of importance is that the simultaneous shedding of both challenge viruses was detected through the upper respiratory tract in one pig at 2 to 5 dpc, a second pig at 3 to 5 dpc, and a third pig at 2 to 3 dpc. We observed similar genetic variation patterns between the primary and secondary IAV infections even though we found a positive selection on H1 and M1 IAV proteins in pigs that had a primary H1N1 infection. Purified (negative) selection and antigenic drift were the primary selection forces shaping IAV within-host diversity regardless of infection statuses. About 10% (4/40) of plaques isolated from 3 pigs (BALF samples) were identified as reassortants which resulted in 2 distinct genotypes. We detected different selection trajectories of specific amino acid changes among three pigs with different shedding intensity of secondary infected virus. As IAV reassortants emerged in two of these three pigs, we speculate that the introduction of a distinct subtype may have flipped the genetic background of IAV quasispecies affecting the genetic interactions within the virus genome between the multiple mutations (epistasis) possibly through reassortment, which may change the fitness landscape for the selection of viral variants at specific amino acid sites.

Discussion and Conclusion

Our study demonstrated that pigs that become infected consecutively by multiple subtypes of IAVs can have extended IAV shedding patterns over time which may affect virus diversity in the pigs. The results of this study extended our understanding of the relationship between quasispecies and epistasis and how they contribute to the complex evolutionary process of the influenza viruses. More research is needed to validate these results under field conditions.

Acknowledgments

The authors gratefully acknowledged the funding from Zoetis.

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Sow herd stabilization using a PRRS modified live virus vaccine against 2 Type PRRSV isolates in aThai swine farm

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Introduction

The 5-step process of PRRS Control has been demonstrated initially in Thailand[1].

It has shown to systematically control PRRS given under the farm's production system, co-infections and the presence of the pathogenic variants. Co-existence ofboth Type I and Type II PRRSV Type in a herd has been reported[2]. The objective of the study is to evaluatethe time laps it takes to stabilize the sow herd infected with Type I and Type II PRRSV , using a modified live virus vaccine (MLV) and holistic management.

Materials and Methods

A two-site, farrow-to-nursery with an inventory of 1,700sows, located in central of Thailand experienced a PRRSType II outbreak in the November 2019. The previous outbreak, the farm was vaccinated quarterly with PRRS Type I MLV and the clinical related was shown obviously in the grower-finisher site. Moreover, the farm was introduced to a big amount of replacement gilts during the Sep-Oct 2019. Two months later, the abortion was slightly drop combination with high rate of mummification, high preweaning mortality. The diagnostic confirmed that there were both Type I and Type II PRRSV strains in the farm by the serum PCR. Subsequently the PRRS control by 5-Steps was established, the sow herd was mass-vaccinated with the Ingelvac PRRS MLV (Type I) and re-vaccinated 4 weekslater, followed by quarterly mass vaccinations to maintain the herd immunity and minimize the non- immune subpopulation. Piglet vaccination was also conducted at 14 days of age since the sow mass vaccination started. During the week of implementing MLV vaccination, the sow herd also received a supportive treatment by anti-pyretic and antibiotic as theusual farm's program. The sow herd performances were computerized as usual. To evaluate the efficacy of Ingelvac PRRS MLV, the comparative between before and after implementation demonstrated by moving rage chart, Minitab, LLC USA. The stabilized status was monitored by PRRSV RT-PCR based on 4 consecutive samples of umbilical cord (PUCS) from 30 offspring. Thereplacement gilts were checked for PRRSV shedding status by oral fluid PCR every quarter before entering theherd.

Results and Discussion

Figure 1. Summarizes sow performance for each period (Before vs After), Reproductive performances that were improved in many sows such as farrowing rate, low % of mummified & pre-weaning mortality , higher % weaned and the number of pigs weaned per sow per year. Moreover, the non- reproductive sow days were decreased from 51 to 44 days which also relevant of farm economic in the term of maximize utility of feed use. The stabilize status has been shown 8 months after implementation of mass vaccination by PCR on the umbilical cord.

Figure 1
Pictures of sow performance before and after implementing Ingelvac PRRS MLV

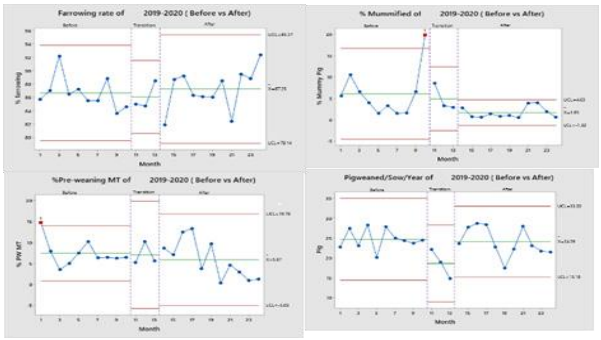

Figure 2
Pictures of piglets before and during PRRS Control

Conclusion

The 5-step process has proven again, that systematic PRRS control is probably the only way to control PRRS especially in Thailand. In this case, holistic approach, combining immunization with pig flow management and having 2 sites can help break the PRRS circulation in a production system.

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Surveillance of PRRSV infection in growing pig herds

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Introduction

Characterizing the source, incidence and spatial clustering of porcine reproductive and respiratory syndrome virus (PRRSV) in growing pig populations is critical to the control and elimination of the disease in breeding herds at both, the individual herd and regional levels (1). While the goal of PRRS control ina breeding herd is to return to a negative status after an outbreak, the ability to maintain the PRRS negative status has proven difficult (2). It has been hypothesized that nursery and grow-finish populations can serve as amplifiers and potential source of virus infection to breeding herds, thus, posing important reinfection risks. However, little information has been reported in the literature to support the above- mentioned hypothesis. Therefore, the objectives of this study were to determine the frequency of infectionand time of first detection of PRRSV in grow-finish pigs, and to analyze the spatial clustering of PRRSV in a production system.

Material and Methods

The study was performed in a >60K-sow swine production company in the Midwest USA. Seventy-four non-PRRSV vaccinated grow-finish herds were conveniently selected for this investigation based on their known PRRSV negative status at placement. Oral fluid samples were collected randomly from twelve pens per air space. The first sample collection occurred at placement into the finishing phase and monthly thereafter, until marketing or upon observation of clinical signs suggestive of PRRSV infection. Samples were stored at -20C until selection and submission for individual PRRS RT-qPCR testing. Of the 74 herds enrolled in the study, a subset of 21 was randomly selected for PRRSV PCR test at each collection time, to determine first PRRSV detection and thus positive status. PRRSV PCR, and ELISA upon a negative PCR result, were completed on the last sample collected for the remaining of the herds. Sequencing of PRRSV was attempted on PCR positive samples with a sufficiently high concentration of genetic material. Odds ratios of PRRSV infection were compared between pigs sourced from PRRSV naïve herds and herds in which PRRS elimination programs had been completed. Spatial clustering analyses were completed (4,5).

Results

Fifty of 74 sites enrolled in the study (67.5%) were detected PRRSV PCR or ELISA positive at somepoint between placement and marketing. Of positive or suspect sites in the subset group, seven of 16 (43.7%) were positive or suspect at the first collection. Nine PRRSV sequences were obtained out of 14 attempts. A similar PRRSV strain (strain 1-4-4 1C) was identified in all sequences. Ct values of positive PCRs ranged from 25.9 to 36.7. Odds ratio confidenceinterval of PRRSV positive status of pigs sourced from naïve sow herds compared to PRRS eliminated sow herds indicated no statistical difference. Spatialanalyses of PRRS incidence identified one low- incidence cluster and two high-incidence clusters.

Discussion and Conclusions

This study suggests that both a large sample size and frequent sampling are necessary for an accurate identification of the onset of PRRSV infection in a population, as a high proportion of pigs assumed negative to PRRSV resulted positive at first sampling. Spatial clustering analysis suggested regional differences in the incidence of PRRSV in growing pig populations. A reduction of the incidence of PRRSV infections in growing pigs is necessary to reduce the risk of PRRS outbreaks in breeding herds.

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Swine influenza prevalence and subtype variance on swine farms in Central EuropeA field study

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Introduction

Swine influenza virus is an important player in the PRDC of the swine farm industry.
The aim of this study was to get more information about how many farms in Hungary are infected with influenza, and how the presence of 4 different subtypes differ between the sampled farms.

Materials and Methods

We made the surveys on 25 Hungarian farms in 2019-2021. We used the same protocol for blood sampling:4-8-12-16-20-24 weeks old pigs, and fatteners were sampled, and 14 blood samples were taken from every age group. We used the IDEXX ELISA for Influenza-A antibody search, and HI tests for the subtypes (H1N1, pandemic H1N1, H1N2, H3N2).

Results

We found that 84% of the farms were positive with IDEXX ELISA for Influenza-A.
60% of the farms were positive for H1N1, 65% for pandemic H1N1, 65% for H1N2, and 65% for H3N2.
Variance between the subtypes:

Conclusions and Discussion

We found that 84% of the farms were positive with IDEXX ELISA for Influenza-A.
60% of the farms were positive for H1N1, 65% for pandemic H1N1, 65% for H1N2, and 65% for H3N2.If we want to vaccinate against influenza on our farm,we have to know which subtypes are present on the farm. Commercial vaccines sometimes can't protect against all the subtypes what are inside the farm, so maybe in the future there will be a need for farm specific virus-vaccines. It is very important tocontinuously monitor the pressure of the virus, the level of the protection, and the circulation.

Acknowledgements

MSD AH CER SBU HU
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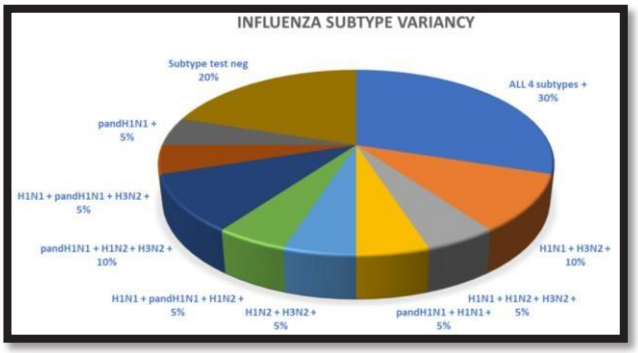
3. Disease of swine 11th edition



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Targeting porcine viral pathogens using a bait-capture method followed by long-read sequencing

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Introduction

Viral diseases are one of the most important health challenges in swine production, leading to significant economic losses worldwide. Sequencing of viral genomes from field samples can provide an increased understanding of the ecology, evolution, and pathogenesis of viruses within swine populations (1). However, low viral titers and high levels of host genomic material are some of the technical challenges when applying sequencing to field samples. Furthermore, methods such as PCR amplicon sequencing usually target only single viruses, which limits the ability to understand viral co-infection and co-circulation dynamics. Finally, viral culture is often needed to enrich genomic material prior to sequencing, which can introduce bias into the resulting genomic sequences. We hypothesized that these limitations could be overcome by a "bait capture and enrichment" method, combined with real-time long-read sequencing. This approach is based on hybridization of pre-designed cDNA biotinylated "baits" to target and capture viral pathogen genomes, followed by enrichment and sequencing (2). Therefore, the aim of this study was to design a custom bait panel for swine pathogens and employ it on field samples.

Materials and Methods

Based on expert input, a list of 31 swine viruses was compiled. Full-length genomes were identified using the NCBI Complete Genomes for Viruses. All genomeswith nucleotide completeness were downloaded as input into bioinformatic bait design. For influenza A, B, and C, nucleotide sequences were downloaded from theNCBI Influenza Virus Resource, selecting only those identifying the host as swine and only those with complete sets of full-length genome segments. Bait design was conducted using a custom bioinformatic algorithm, with the following parameters: length of probe = 120bp; number of potential mismatchesbetween probe and target = 30bp; coverage of targets genomes = 100%.

Four nasal swabs were used to pilot the novelenrichment assay: 2 influenza A (IAV) qPCR positive and 2 IAV qPCR negative swabs obtained from weanedpigs. IAV RNA were extracted by QIAamp® ViralRNA Mini Kit, followed by complementary DNA synthesis with SuperScript IV Reverse Transcriptase and NEBNext® Ultra™ II Non-Directional RNA Second Strand Synthesis Module. Sequence library preparation, hybridization, and subsequent enrichment were performed on all samples using the SureSelect XTHS2 DNA System (Agilent Technologies).Subsequently, a PCR barcoding kit (SQK-PB004) was performed prior to loading the samples in the minION flow cell (Oxford Nanopore technology).

Results

The number of accessions with complete genomic sequences varied widely, with a low of just 1 sequence for classical swine fever virus to a high of 5,995 for IAV. In total, 10,672 genomes across all 31 viruses were included in the panel design. The final bait set was comprised of 19,957 unique baits, which together covered 100% of each of the input genomes. When the designed baits were applied to IAV positive field samples, the target enrichment increased the percentage of on-target IAV reads from 0.1% to 62% and 49% (Table 1) without enriching for the IAV qPCR negative samples.

Discussion and Conclusion

Using available algorithmic approaches, we were able to design a custom panel of cDNA biotinylated baits that covers 100% of all genomic sequences for 31 important viruses of swine. This design easily fits within current manufacturing constraints and can be obtained through existing commercial offerings. When the baits were applied to field samples of known positive influenza status, IAV was enriched, providing unbiased full-genome sequences. Nevertheless, the representation of viral genomes within current databases was highly variable, which may bias the performance of the bait capture approach. Therefore, validation of the bait capture method with additional field samples of known status for other viruses needs to be further investigated. In conclusion, the bait capture method followed by real-time long-read sequencing is an innovative approach that allows for robust identification and characterization of dozens of viral genomes simultaneously. The method can effectively deplete host genome without affecting viral load, while also generating long-read sequences within one day. This method thus shows promise for enhancing our ability to understand the dynamics of viral co-infections within swine populations.

Table 1. Percentage of reads for IAV cDNA samples (not enriched) vs IAV enriched samples.

Sample	IAV status (By qPCR)	Percentage of reads	
		cDNA	Enriched
1	Positive	0.1%	62%
2		0.1%	49%
3	Negative	0.063%	0.035%
4		0.043%	0.011%

Acknowledgments

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The mice as an animal model to study swine influenza virus infection

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Introduction

Although mice are not a natural host for influenza viruses, this species is one of the most used to study the replication, pathogenesis and immune response of many subtypes of influenza viruses and may be considered a first-line preclinical model to study vaccines and antiviral therapies (1). In Brazil, at least four lineages of swine influenza A virus (SIV) circulate in pig population: H1N1 from the 2009 pandemic origin, H3N2 and H1N1 and H1N2 from human seasonal lineages (2). Given some difficulties imposed to study these viruses in a swine model, especially when performing vaccine immunization experiments with subsequent challenge using wild viruses, the mice as an animal model may be an accessible option for primary research before performing experiments in the swine target species. The objective of this study was to access the morbidity and mortality of Brazilian circulating swine influenza viruses in a mice model.

Materials and Methods

C57BL/6 mice with six weeks of age were intranasally inoculated with three wild influenza A viruses of swine origin: 2019 pandemic H1N1 (H1N1pdm19); 2019 human seasonal H1N1 (H1N1hu19) and 2018 H3N2 (H3N218). The mice were randomly distributed into 10 groups, with eight animals each. Titers of 10^6, 10^5, 10^4 PFU/mL were inoculated for each viral subtype, except for H1N1pdm19 that the highest analyzed titer was 7,6 x 10^5 followed by 1x10^5 and 1x10^4 PFU/mL. A control group, inoculated with PBS, was evaluated as well as an ambient group which did not receive any treatment. Animals were daily monitored for weight loss, clinical signs, and mortality for 21 days. Humane endpoint euthanasia was realized in animals that lost 25% of their weight. On the 21st day after inoculation, blood was collected from surviving mice in other to obtain their blood serum and perform serological analyzes by the hemagglutination inhibition (HI) for the detection of neutralizing antibodies.

Results

Morbidity rates are presented in the Figure 1. Weight loss was observed in animals challenged with H1N1pdm19 and H1N1hu19, and the higher the inoculated viral titer, the greater the weight loss observed in the animals. Animals challenged with H3N218 virus did not lose weight during the analyzes, even when inoculated with the highest viral titer. Mortality was observed only at the H1N1pdm19 group. Except for the PBS and ambient groups, all the animals from the study seroconverted and showed neutralizing antibodies against the viruses that they had been challenged.

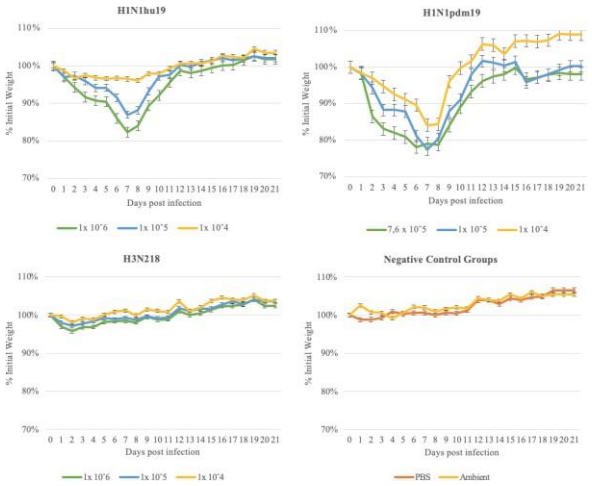

Figure 1. Body weight changes in mice inoculated with different swine influenza virus titers

Discussion and Conclusion

In swine, both α2,6 and α2,3 linked sialic acid, which are cellular receptors for influenza A viruses are presentat the respiratory tract (3), which may allow the species to be infected with human and avian influenza viruses lineages, respectively. In mice, the α2,3-linked sialic acid receptor is predominantly found (4), and because of the relative lower concentration or lack of α2,6 receptorin the murine respiratory tract, many influenza viruses are poorly or not infectious for the species. In this studyit was possible to reproduce the morbidity of SIV infection in a murine model by the infection of swine H1N1pdm19 and H1N1hu19 viruses. H1N1 viruses are capable to bind both types of sialic acid receptors. whileH3N2 viruses are more adapted to bind to α2,6 receptors (5), which might explain the fact that this strain did not cause morbidity in mice in the present study [5]. For this lineage, it may be necessary the adaptation of the virus in the mice model. It was possible to reproduce the morbidity of SIV infection with the lineages H1N1pdm19 and H1N1hu19 in a mice model. Using the murine model, as a non-target but a primary species, will greatly contribute for preliminary studies involving SIV,such as vaccine and challenge experiments.

Acknowledgments

CNPq and FAPEMIG.

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Transmission of human influenza A virus in pigs selectsfor mutations on the HA gene segment

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Introduction

Interspecies transmission of influenza A viruses (IAV) from humans to pigs is relatively common and has led to many human-origin influenza viruses becoming endemic in pigs, including the contribution of genes to the triple reassortant internal gene (TRIG) constellation that is widespread in North American pigs (1). These human-to-swine spillover events have significantly affected the epidemiology and the resulting diversity imposes a major challenge to disease prevention and control. Interestingly, experimental infection of pigs with human viruses often results in low replication and rare transmission among animals (2). Little is known about the evolutionary processes that occur at the human-swine interface that allow viruses to adapt and efficiently replicate and transmit within the new host. The objective of this study was to evaluate the evolutionary dynamics of a human IAV during transmission in pigs.

Materials and Methods

To test the evolution of human seasonal surface genes, we generated a reassortant virus containing human HA and NA (from A/Victoria/361/2011 H3N2; VIC11) with the 2009 pandemic lineage matrix gene and remaining genes of the TRIG lineage (the internal gene constellation that predominated in North American swine until recently). Four-week-old, cross-bred naïve pigs (n=10/group) were challenged with the reassortant virus (VIC11pTRIG), the wild-type VIC11,or a control swine-adpated strain (A/sw/MO/A01410819/2014 H3N1; MO14). At 2 days post infection (dpi), 5 naïve pigs were placed in the same room as each group, without direct contact, to evaluate aerosol transmission. Nasal swab sampleswere collected at 1, 3, and 5 dpi and at 5, 7, and 9 dayspost-contact (dpc) from respiratory contact pigs. Swab samples were submitted to next generation sequencing(NGS) using a high-throughput Illumina MiSeq sequencing platform to evaluate virus evolution.

Results

Our results show that the reassortant virus (VIC11pTRIG) resulted in replication and transmission in pigs (Fig. 1A) while the wild-type VIC11 did not, indicating that the TRIG constellation favors replication and transmission of human IAV surface gene segments in pigs.

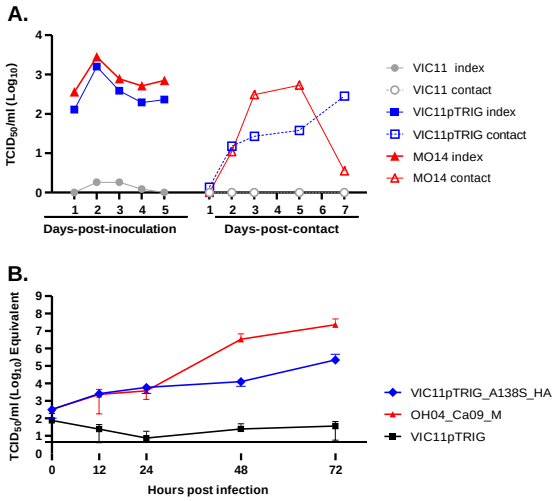

Figure 1. The reassortant VIC11pTRIG virus showed efficient replication in pigs and transmitted to respiratory contacts (A). The A138S mutation detected in all respiratory contacts shows improved replication compared to the human VIC11 virus (B).

Although the viral population consensus remained invariant for the majority of directly inoculated animals throughout infection, a minor variant in the HA (A138S; H3 numbering) was observed at 3 dpi in two animals and became fixed in one animal at 5 dpi. This mutation was fixed in respiratory contact pigs starting at 5 dpc The mutation is located in the HA1 near the region of the H3 antigenic sites. Viral growth kinetic studies in swine tracheal epithelial cells (STEC) showed that the A138S mutant has a fitness advantagecompared to VIC11pTRIG (Fig. 1B).

Conclusions and Discussion

Determining the evolutionary basis of cross-species transmission is key to understanding the mechanisms that control the emergence of new influenza viruses. Our results show that minor variants are selected quickly after replication and transmission of human HA in pigs, resulting in selection of more fit variants.

Acknowledgments

Funding was provided from ARS-USDA, APHIS-USDA, and University of Gerogia OVPR.

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Use of an endogenous reference control in a commercial PRRSV RT-qPCR

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Introduction

Real-time polymerase chain reaction (qPCR) performance is affected by testing procedures, i.e., extraction and/or amplification steps (1), but detection can also be affected by sample handling, e.g., whether the sample was chilled after collection or whether it went through multiple freeze-thaw cycles. Ideally, a qPCR procedure would include an indicator of sample quality. In basic research, endogenous reference controls (ERC) provide assurance that the test was conducted correctly and that the sample was of good quality (2). However, ERCs are used infrequently in veterinary diagnostics and, where used, there is little information regarding the interpretation of ERC responses.

The commercial assay evaluated in this project (RealPCR*NA PRRS Types1-2 RNA Mix, IDEXX Laboratories, Inc., Westbrook, Maine, USA) includes an ERC; in this case, designated by the manufacturer as the "internal sample control" (ISC). The genetargeted is proprietary, but the ISC is specific for swine nucleic acids. Thus, each sample tested using this assay reports a Cq for PRRSV RNA and a Cq for the ISC.

The objective of this study was to characterize the variation in ISC responses within and between pigs before and after PRRSV vaccination.

Materials and Methods

Animals (n = 12) were individually housed under experimental conditions and vaccinated with a PRRSV MLV (Ingelvac® PRRS MLV, Boehringer Ingelheim Vetmedica, Inc., Duluth, Georgia). Serum (n = 132) and oral fluids (n = 130) were collected prior to and after PRRSV vaccination (-7 to 42 DPV). Samples were randomly ordered (within specimen type) and tested for PRRSV and ISC nucleic acid.

Reference intervals were calculated from the ISC data using R 4.1.0 (R core team, 2020). Reference intervals refer to a set of values for a particular measurement in which, the 95% of a “healthy” population would fall. Reference intervals were then used to compare ISC Cq responses within and between pigs over DPV.

Results

All serum and oral fluid samples were negative for PRRSV RNA at -7 and 0 DPV, with the first PRRSV qPCR-positive samples at 3 DPV (both sample types).

The ISC was detected in all serum and oral fluidsamples (n = 262). Data for the ISC in oral fluids was normally distributed (Shapiro-Wilk normality test, p =0.584), and the 95% reference interval was estimated as Cqs between 23.1 to 30.1 (90% CI), with 124 of 130 oral fluids within the reference interval.

For serum data, the normality assumption was rejected(p = 0.031) and attempts to transform the data were unsuccessful. Therefore, the 95% reference interval (Cqs 23.9 to 29.4, 90% CI) was obtained using the non-parametric percentile method, with 128 of 132 sera within the reference interval. ISC Cqs were not affected by PRRSV vaccination, i.e., values were within the established reference intervals.

Discussion and Conclusion

This experiment represents our initial effort tocharacterize the swine ISC response in typical diagnostic specimens. Understanding the ISC response is necessary in order to establish its role in the verification of sample quality and testing. In this study, ISC Cqs in samples collected in the best-case scenario, i.e., samples from pigs under experimental conditions and immediately stored, were uniform over time and were unaffected by PRRSV vaccination. The use of an ISC could be an important addition to quality management in routine PRRSV RT-qPCR testing.

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Using animal movement data to understand the spread and evolution of PRRSV

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Introduction

Although the critical role of pig movements in the spatial spread of porcine reproductive andrespiratory syndrome virus (PRRSV) has been reported previously [1], [2], understanding the relative contribution of different types of pig movements to the spread and evolution of PRRSV can help us focus disease management efforts for cost-effective interventions during outbreaks. Here, we applied evolutionary models to explain the geographical spread and evolution of a rapidly spreading strain of PRRSV-type 2 (sub-lineage L1A) which predominantly correlates to the RFLP type 1-7-4.

Materials and methods

We combined animal movement data, spatial, environmental data and ORF-5 PRRSV-L1A sequences collected from farms in a swine dense production region in the US between 2014 and 2017. We grouped animal movement data by age-group of animals moved: wean movements (3-4 weeks), feeder movements (8-25 weeks), breeding movements(≥21 weeks). The study area (~ 85,000 mi2) was also divided into 13 sectors, and model predictors were summarized over each sector for analysis.

Results

We observed that the between-sector spread of L1A was positively associated with three main factors: movement of feeder pigs, the spatial adjacency of sectors, and farm density (Figure 1). Compared to movements of weaned and breeding pigs, transportation of feeder pigs was much more strongly associated with higher viral dispersal rates between sectors. The sublineage was likely introduced in the region in the first quarter of 2013. The viral population first peaked in the summer of 2015 and subsequently fluctuated seasonally, with peaks in the summer of 2016 and 2017.

Discussion and Conclusion

This suggests that nurseries may be acting as amplifiers for viral population, perhaps related to waning maternal antibodies and mixing of weaned pigs from multiple sources. Also, unlike breeding farms, biosecurity measures in nurseries (or trucks originating from them) tend to be more relaxed, which may influence the risk of onward transmission of the virus. L1A spread amongst adjacent sectors could also have been through mechanical spread-especially in areas with shared service providers and infrastructure. As such, for effective disease management, biosecurity threats inadjacent sectors should be considered when designing intervention measures in nearby regions.

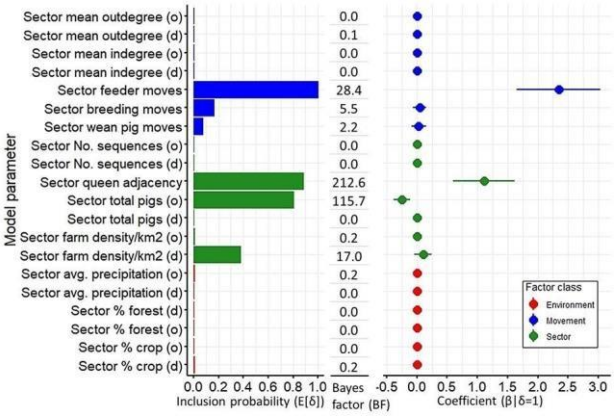

Figure 1. Predictors of the spatial diffusion of PRRSV sub-lineage 1A (L1A) between geographic sectors in a swine-dense production area in the USA.

High farm density has been associated with PRRS spread and maintenance [3], [4]. Our findings underscore the risk associated with high farm density. Thus, considerations for enhanced biosecurity measures for nurseries and farms in high-density production areas are necessary.

Acknowledgements

We thank the MSHMP team and participating pig production systems for data sharing and curation. This study was funded by the Swine Health Information Center (SHIC), US-UK NIFA-NSF NIH-BBSRC and USDA.

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Vaccination decreases the risk of influenza A virus reassortment but not of genetic variation in pigs

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Introduction

Influenza A viruses (IAV) are critical zoonotic pathogens and one of the most prevalent respiratory pathogens that cause substantial production losses in pigs. Although influenza vaccination is widely used in pigs to control the impact of the disease, IAV continues to evolve rapidly, and has resulted in the emergence of multiple H1 and H3 IAV lineages in pigs in the last 20 years (1). The high diversity of IAVs found in pigs due in part to the ability of the virus to mutate and reassort, can result in novel variants with antigenic characteristics that facilitate virus escape from the host immunity posing a challenge for vaccine development (2). To our knowledge there are no studies that investigate how vaccination affects IAV mutation and reassortment simultaneously in pigs.

Materials and Methods

We performed IAV whole-genome sequencing directly on 28 bronchoalveolar lavage fluid (BALF) samples from pigs receiving distinct vaccination protocols including a) prime boost, b) single live attenuated influenza vaccine (LAIV), or c) no vaccine. After vaccination, pigs were challenged with an H1N1 and an H3N2 virus simultaneously using a seeder pig model. Plaque assays to identify IAV reassortants in pigs were performed in 13 BALF samples yielding a total of 202 IAV plaques recovered from 13 pigs.

Results

Approximately, 27% (54/202) of the plaques were identified as reassortants grouped in 16 distinct genotypes and 18 mixed genotypes. The prime-boost vaccination protocol significantly reduced the emergence of new reassortant viruses in pig lungs compared with nonvaccinated pigs ($p = 0.022$). Reassortant viruses were detected in 6 of 13 pigs, and 85% (46/54) of reassortants originated from just 3 pigs (23%). Besides, we found the duration of co-infection with the H1 and H3 subtypes was positively correlated with the proportion of reassortant viruses generated in the pigs ($p = 0.0046$). In contrast, vaccination did not appear to affect IAV HA diversity, evolutionary rates, and nucleotide polymorphisms in the swine lower respiratory tracts. Even though there were abundant functional relevant amino acid changes in the H1 and H3 subtypes recovered from pigs regardless of vaccination statuses, none of the changes were associated with HA antigenic or receptor binding properties. Moreover, there was limited shared amino acid changes in H1N1 and H3N2 viruses in pigs from all three groups.

Discussion and Conclusion

Our study suggests that vaccination should be explored as a measure to mitigate the emergence of IAV reassortment in pigs. However, more research is needed to assess the impact of vaccination on the emergence of reassortant viruses under field conditions.

Acknowledgments

The authors gratefully acknowledged the funding from Zoetis.

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WELFARE

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Assessment of pig oxidative stress model

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Introduction

During their production cycle, pigs are subjected to different challenges which may induce oxidative stress. The accumulation of free radicals in the gut is associated to a compromised intestinal function, decreasing nutrient transport, and increasing susceptibility for gastro-intestinal disorders (1,2). Current models to investigate the effects of oxidative stress in pigs seriously impact the physiological condition of the animals, due to the severe oxidative challenges used (80-100µg lipopolysaccharide (LPS)/Kg BW) to unbalance the oxidative status of the animals. Moreover, remains unclear the most suitable period to measure this response. Therefore, the present study aims to compare the piglets' response against oxidative stress after an immune challenge mediated by LPS at a lower dose than normally described in the literature, on days 28 and 41 post-weaning. The ultimate goal of this study is todevelop an oxidative stress model with the fewer negative impacts possible on the welfare of the animals.

Materials and Methods

Piglets weaned at 25.7 ± 4 days of age (5.64 ± 0.73 Kg BW) were randomly assigned either to a control (saline solution, Crtl) or a challenged (LPS challenge, LPS) group. Challenged animals were intraperitoneally (i.p.) injected with 25µg/Kg BW of LPS on day 28 or day 41 after weaning. The dosage of LPS was lower than the ones normally reported in the literature (3, 4). All piglets received the same diet and husbandry management untilthe day of the challenge. Samples of blood and intestinal tissues (colon, ileum, and jejunum) were collected 4 h post-challenge. Analysis of glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD) activity in plasma and tissue homogenates were conducted using available commercial kits (Abcam), following procedures described by the manufacturer. Statistical analyses wereperformed by T-test within each sampling day (SAS, version 9.4).

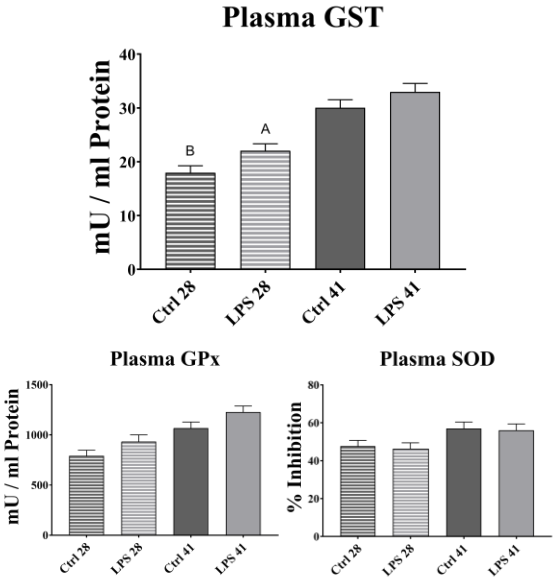
Results

Animal performance (growth and feed intake) was not different between treatment groups at the times of the oxidative challenges.
There were significant differences between groups Crtl and LPS in the antioxidant activity in plasma on day 28 in GST (P=0.012), and a statistical trend in GPx (P = 0.062), but no differences were observed in the plasmatic SOD or the antioxidant markers in the intestinal tissues. The activity of main oxidative stress- protecting enzymes in plasma on day 28 is shown in Figure 1.
On the other hand, no significant differences were observed between Crtl and LPS groups on challenge day 41, neither in plasma nor in the intestinal tissues.

Discussion and Conclusion

The magnitude of a biological response to an immunological challenge depends on its severity and the maturity of the immunological system of the animal, among other factors. The lack of significant differenceson day 41 between treatment groups at the sampled tissues in our model denoted that the dose of LPS was insufficient to generate an elevated oxidative stress situation enough acute to activate the natural mechanisms of protection against oxidative damage. Whereas some changes were observed in plasma on day28 even at this very low i.p. LPS dose. The matureness of an animal increases its robustness to face immune-related challenges (5,6).
Furthermore, the observation of changes only in plasma, even applying an i.p. LPS injection, suggested that blood sampling may be enough to look for parameters enabling the evaluation of the oxidative stress response of piglets under a mild immunological challenge. Thus, sacrifice procedures to sample tissues may not be needed to observe the effects of the oxidative stressor in the physiology of the animal.

Figure 1. Mean of the oxidative biomarkers' activity in plasma of Control (Ctrl) and LPS challenge groups on day 28 and 41.



Research animal models must ensure welfare conditions, as well as researchers and producers' expectations while maintaining a high accuracy and feasibility of the studies. The optimization and development of less-invasive and/or less-stressful study models would fulfill the refinement concept from the "three R's" principle (*Replacement, Reduction, Refinement*) (7). Even though the described model was designed to maximize welfare conditions by minimizing the exposure of the animals to LPS, the mild response obtained suggests looking for a model based on an LPS challenge with a slightly higher dose, in animals at a younger stage (day 28) and focused on the evaluation of oxidative stress parameters at the systemic level (plasma).

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Contrasting costs of pig systems: co-benefits and tradeoffs in yield, greenhouse gas emissions, animal welfare and antibiotic use

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Introduction

Food production has a greater impact on biodiversity and land use than any other human activity. Livestock production is rapidly growing, with particular growth in the pig sector. Pork is now the most eaten meat globally (1), and demand is growing rapidly (2). How we meet rising livestock product demand will be pivotal for environmental sustainability and human and animal wellbeing.

Growing empirical evidence suggests the impacts of farming on biodiversity might best be limited through land sparing (boosting yields on farmland whilst conserving natural habitats; 3), but high-yield systems are perceived to have other negative costs, including greater greenhouse gas emissions, higher antibiotic use and poorer animal welfare. However, until now systematic quantification of these costs per unit of production had not yet been attempted. This could identify systems that combine high yields with low impacts in other domains, as well as help flag less favourable systems that carry high impacts in several domains.

Balmford et al., (4) developed a framework to address and quantify the extent of potential trade-offs in externalities by comparing costs per unit production of systems in biophysically comparable contexts. Balmford et al., (4) found that trade-offs among these may be less common than typically perceived – when assessed per unit production systems that take up less land often generated fewer externalities. However, data availability was a key limitation; there were sufficient data for only a few systems and externalities so the application of this framework to date has been limited by data availability. Monogastric systems, antibiotic use and animal welfare were among those systems and externalities where suitable information was extremely sparse.

Materials and Methods

To help to fill this gap, we recruited and visited over 100 pig farms in the UK and Brazil, ranging from indoor fully slatted systems to free-range and those certified as Organic. We used Life Cycle Assessment methods to quantify yields, carbon footprints and antibiotic use, and developed new methods to quantify animal welfare – considering both the quality and quantity of years of life affected.

We conducted statistical analyses to determine the differences between different categories of production system (using ANOVAs and Tukey post-hoc analyses) and Spearman Rank correlations to evaluate the strength and significance of relationships among externalities.

Results

We found that there was considerable overlap in the impacts of systems considered to be very different, for example those with different labelling or certification (e.g. in the UK, Organic, RSPCA assured and Red Tractor).

We found a positive relationship between greenhouse gas emissions and land use – those systems that used less land emitted fewer greenhouse gases. We found no clear pattern between antibiotic use and land use and a general tradeoff between animal welfare and land use. We found that, when examining specific systems, tradeoffs are not inevitable and importantly, some systems appear to provide opportunities for "win-win" outcomes – that are low impact in multiple domains.

Discussion and Conclusion

The framework and methodology we developed enabled and will continue to enable the explicit concurrent consideration of several externalities of alterantive production systems.

We explored tradeoffs and synergies between an array of outcomes of societal concern, including yield, greenhouse gas emissions, antibiotic use and animal welfare. From that we could identify livestock production systems which offer most promise in these several domains.

Acknowledgments

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Effect of different vaccination protocols against *L. intracellullaris* on the performance and welfare of piglets in the nursery phase in Brazil

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Introduction

Ileitis is a disease caused by *Lawsonia intracellularis* (LI), responsible for economic damage for pig farmers and health problems in pigs. Currently, there are two vaccines commercially available in Brazil for the prevention on ileitis. The objective of this study was to measure the impact of two LI vaccines on zootechnical and animal welfare indices in the nursery phase.

Materials and Methods

This study was carried out in a farm in Brazil. One hundred and eighty pigs were evaluated during the nursery phase (22 to 64 days of life). Three vaccination protocols were investigated with different combinations of Porcine Circovirus type 2 (PCV2), *Mycoplasma hyopneumoniae* (Mhyo) and LI vaccines. (Table 1).

Table 1: Vaccine protocols by group: A (live oral vaccine, LI), B (injectable vaccine, LI), C (no vaccine for LI)

	A	B	C
Day 0 – nursery (beginning of the study) – 22 days of age			
Vaccine:	PCV2 (1mL)	PCV2	PCV2 (1mL)
PCV2 and Mhyo	Mhyo (1mL) [2]	Mhyo (RTU)	Mhyo (1mL) [1]
Frequency, dose, route	Single dose: 2mL – IM	Single dose: 2mL – IM	Single dose: 2 mL - IM
Adjuvant	ImpranFLEX®	Mineral oil	-
Day 8 – 29 days of age			
Vaccine: *LI*	Live	Dead bacterin	Saline solution 0,9%
Frequency, dose, route	Single dose (2ml) Oral	Single dose (2ml) IM	Single dose (2ml) IM

[1]Vaccine PCV2 and Mhyo were mixed at the time of application, as recommended by the manufacturer.

Rectal temperature was measured at the following times: pre-vaccination (D7), post-vaccination D8 (+8 h) and at D8 (+24 h). The welfare assessment was performed by analyzing the behavior of the animals in all pens, according to Weimer (1).

Results

The mean rectal temperature of the pigs from treatment B, 8 hours post vaccination, was 1.31°C higher (P<0.05) than that of the pigs from treatment A (Table 2). The pigs from treatment B showed 4.6 and 1.7 times more individuals lying down compared to those from treatment, at 4 and 12 hours after vaccination respectively (P <0.05; Figure1).

Table 2. Rectal temperature (°C) of pigs from groups, one hour before vaccination for *Lawsonia*.

Time	A	B	C
-1h D0	39.11	39.19	39.15
D0 + 8h	39.59[b]	40.90[a]	39.51[b]
D1	39.44[b]	39.69[ab]	39.47[b]

a-b: different letters in the lines represent a statistically significant difference (P<0.05)

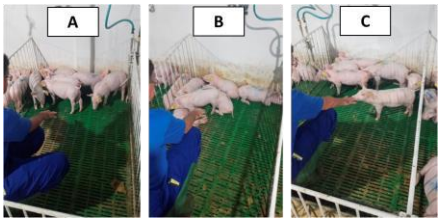

Figure 1. Pig's behavior 12 hours post vaccination by group.

Pigs from Group A were heavier (528 grams) in the final weight of the nursery phase and had 12 grams higher ADWG in this phase (Table 3).

Table 3. Animal's zootechnical performance in the nursery phase: weight (Kg) and ADWG (gram/day)

Parameters	A	B	C
Average initial weight (22d)	6.344	6.342	6.345
Average final weight (63d)	24.769	24.241	24.976
ADWG	438	426	443

Discussion and Conclusion

The oral live attenuated LI vaccine in combination with a watery based IM PCV2 and Mhyo combination, did not harm the animals' well-being and performance. On the other hand, the injectable vaccine for LI in combination with a mineral oil based PCV2 and Mhyo combination, caused systemic adverse reactions, like an increased rectal temperature, with a negative impact on animal welfare and zootechnical indices (lower ADWG).When evaluating vaccine interventions, differences in adjuvant platform and its direct effect on pigs should betaken in account for a sustainable way of pig vaccination.

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Evaluation of the effect of Meloxicam on Hypogalactia Syndrome in Colombia.

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Introduction

Pain and inflammation control at farrowing becomes a matter of not only animal welfare but of economic importance due to the detrimental effects on the sow´s and piglets' productive performance. Mastitis, metritis and agalactia, commonly referred to as MMA, is a complex syndrome seen in sows leading to increased piglet mortality and reduced weaning weights. This syndrome usually occurs within three days of farrowing, although hypogalactia is the most consistent sign, mastitis, fever, vaginal discharge, listlessness, weakness, anorexia, sternal recumbency and refusal by the dam to permit nursing seem to be commonly present in affected sows. The effects of MMA can be reduced when appropriate treatment (non-steroidal anti-inflammatory drug) are implemented and accompanied by proper husbandry measures. The benefits of the treatment can be observed even when no evident MMA syndrome is in place, the wellbeing of the postpartum sow will come back as less hypogalactia and better performance of the progeny (1,2). In this field trial performed under tropical conditions on a farm suffering from subclinical hypogalactia in Colombia, the effects of meloxicam (Metacam®) on post-farrowing behavior of sows and piglets' productive parameters were investigated

Materials and Methods

A total of 96 pre-farrow sows where randomly assigned to two groups. Metacam was applied by the intramuscular route immediately after farrowing at the manufacturer recommended dose (5ml /20mg per ml) in one group, the other group remained as untreated control. Productive parameters linked to the sows and piglet's performance were recorded and compared among groups. For the sows: lactation duration, body condition after lactation and the weaning to service interval, whereas for the piglets the percentage of mortality, the number of weaned piglets, the weight at weaning and the lactation weight were recorded. The statistical analysis considered a significance of $P<0.05$ using T Student andFisher tests for the quantitative and qualitative variables comparison, respectively.

Results

The comparison data is summarized in Table 1 where a statistically significant difference was observed for the lactation length, 21 days for the untreated sows and 18,7 days for the Metacam treated group. The corporal condition after weaning was improved in the sows treated with the non-steroidal drug. Additionally, a numerically important decrease in the number of days in the weaning to service parameter was observed. The offspring results were favored by the treatment showing decreased mortality 4.5% vs 4.8% in the treated and untreated groups, respectively. The total of weaned piglets is a very important parameter and showed a numerical difference in favor of the treated group 11,5 vs 11.1.

As shown in the table, the overall weight performance difference gave the treated group the advantage with gain during lactation of 4,22 kg vs 3,95 kg. Figure 1. provides a graphic view of the progeny performance and the advantages of managing the pain and inflammation to achieve all the piglet's genetic potential.

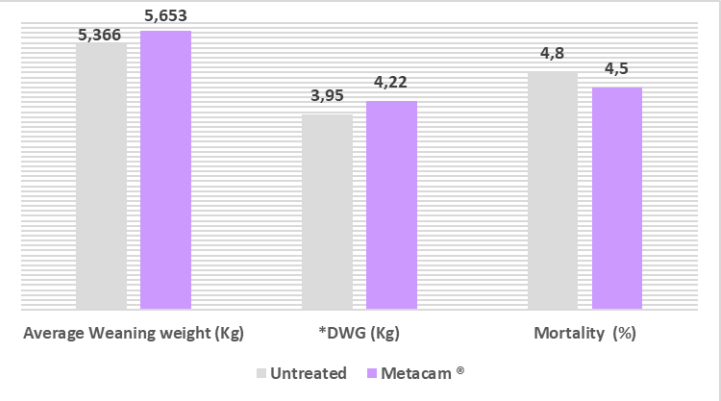

Fig 1. Piglets' performance comparison.

Discussion and Conclusion

Pain and inflammation management post farrowing positive effects on productive performance in the swine industry have been previously documented (1). The mastitis, often clinically undetected, is responsible for many cases of hypogalactia, the use of non-steroidal drugs aid in the resolution of this clinical or subclinical set up is a sound option (2).

These results show that the use of a long action non-steroidal anti-inflammatory as Metacam, allows for a higher milk production, as suggested by the progeny weight gain and overall number of weaned piglets. The results show a tendency towards significance, but a higher number of sows would be needed to confirm these results under the Colombia tropical conditions. The difference in the weaning to service interval has revealed to be highly correlated to success in the farrowing unit and subsequent lifetime reproductive productivity. The incidence of MMA can be controlled with the use of modern sanitary, slatted flooring, better management, sanitation, sow's exercise, nutrition, and body condition. Overall, the results from this field trial suggest that the use of Metacam at farrowing can be a suitable complement for the above-mentioned husbandry practices, showing an important positive effect on the productive parameters and the farmers income.

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Table 1. Productive parameters comparison

Group	#	Mortality (%)	Weaned piglets	Birth Weight (Kg)	Individual Weaning Weight (Kg)	Lactation Weight Gain (Kg)	Litter Weight Weaning (Kg)	Lactation (Days)	Condition (Caliper)	Weaning to service interval (days)
Untreated	n=48	4,8	11,1	1,41	5,37	3,95	59,7	21,0	12,4	5,2
Metacam ®	n=48	4,5	11,5	1,36	5,58	4,22	64,4	18,7	12,8	4,1
p-value		0,422	0,153	0,2	0,182	0,096	0,067	<0,001	0,265	0,546

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Impact of transdermal flunixin on prostaglandin E_2 and cortisol concentrations in piglets following castration

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Introduction

Castration poses an animal welfare challenge as this procedure inflicts tissue damage and inflammation, resulting in pain experienced by the piglet (1). Flunixin meglumine is a promising solution to control pain and inflammation on-farm, given it is relatively available and accessible to livestock veterinarians, can be economically feasible to implement on a large scale (2,3) and provide flexibility in regards to administration route. Therefore, the objective of this study was to assess the efficacy of transdermal flunixin (TDF; Banamine transdermal, Merck Animal Health, Germany) administration on prostaglandin E_2 (PGE_2) and cortisol concentrations in piglets undergoing castration.

Materials and Methods

Litters were randomly assigned to receive one of two treatment regimens 24h before castration: TDF applied topically (3.33 mg/kg; CF = 28) or physiological saline applied topically (equivalent volume; C = 24). Blood samples were collected from a subset of piglets at -24h, 1h and 25h relative to castration procedure for PGE_2 analysis and at -24h, 1h, 4h and 25h relative to castration procedure for cortisol analysis.

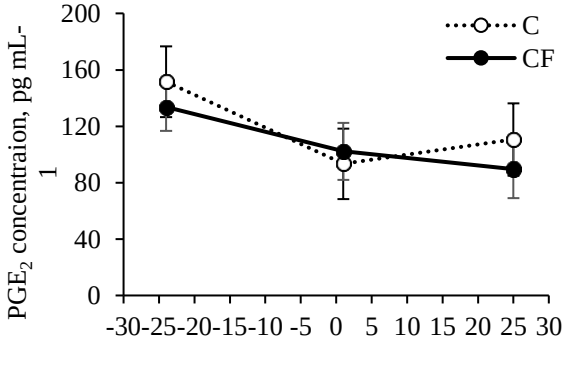
Data were analyzed using generalized mixed linear regression models with fixed effects for drug, time, their interactions, and potential confounding variables, and random effects for litter and piglet (4).

Results

Plasma PGE_2 concentrations are presented in Figure 1. The PGE_2 concentration of piglets enrolled in the C and CF groups did not ($P \geq 0.43$) differ at any timepoint.

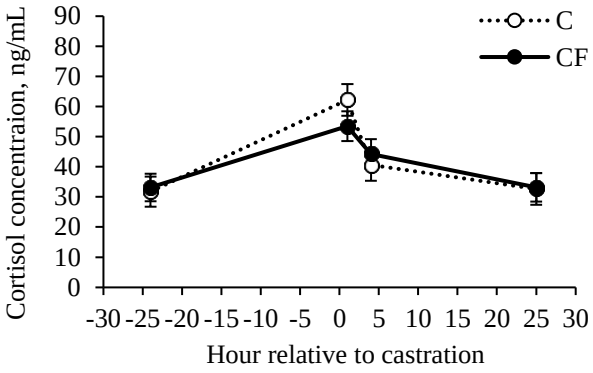
Plasma cortisol concentrations are presented in Figure 2. Similarly, cortisol concentrations of piglets enrolled in the C and CF groups did not ($P \geq 0.23$) differ at any timepoint.

Figure 1.



Mean PGE_2 concentrations (±SEM) for 25h for flunixin or physiological saline treated litters following surgical castration. CF = castrated and flunixin treated (n = 53 piglets); C = castrated and saline treated (n = 35 piglets).

Figure 2.



Mean cortisol concentrations (±SEM) for 25h for flunixin or physiological saline treated litters following surgical castration.CF = castrated and flunixin treated (n = 688 piglets); C = castrated and saline treated (n = 516 piglets).

Discussion and Conclusion

When evaluating drug efficacy in mitigating inflammation and stress associated with castration, PGE_2 or cortisol concentrations did not differ between castrated piglets that received TDF compared to those receiving saline. The data suggested that transdermally applied flunixin at 3.33 mg/kg was not effective in decreasing PGE_2 and cortisol concentrations in castrated piglets and further studies are needed to explore optimal dosing regimens including effective dosage, administration frequency and pharmacokinetic profile of transdermal flunixin applied in castrated piglets.

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Intradermal needle-free vaccination in piglets: effects on welfare and nursery performance

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Introduction

A common procedure for vaccine administration for pigs has been the needle-syringe device, with intramuscular administration. However, this method is considered to be potentially painful to animals (1, 2). As a consequence of a painful vaccination process, the animals can display similar physiological alterations to a febrile response and sickness behaviours such as lethargy, decreased appetite and thirst, huddling, shivering, sleepiness, reduced groomimg and exploration, uncoordinated body movements and an increase in pain sensitivity (3,4) resulting in a reduced production performance. The aim of the present study was to compare intramuscular injection with a needle and an intradermal needle-free vaccination process in piglets at 21 days of age by studying temperature and initial starter feeding after weaning.

Materials and Methods

A total of 500 piglets, intact males and females, were individually weighed at weaning at 21 days of age, identified with ear tags and distributed between two groups according to sex and weight: intramuscular vaccination (IM- 2 ml) and intradermal needle-free vaccination (ID- 0.2 ml). After weaning, they were assigned to the nursery phase, and vaccinated against Mycoplasma hyopneumoniae and Porcine circovirus type 2 associated diseases either via the intramuscular route (IM) (n=250) or with Mhyosphere® PCV ID (n=250), an intradermal (ID) vaccine administered using Hipradermic® 3.0. The body temperature was measured at 0 (time of vaccination), 6 and 24 hours after vaccination. In order to evaluate the starter feed consumption, animals were supplied with the pre- initial feed, an iron oxide 1% as a red faecal marker. At6- and 24-hours post-vaccination, the faecal colour was evaluated by rectal swab to identify the piglets consuming the feed (those with a red-coloured swab). The piglets were individually weighed at 7 days and 42 days after weaning to assess weight gain in the nursery phase.

Results

Body temperature after vaccination was affected by the administration route and the vaccine used. It was observed a significant increase in body temperature in those animals that were vaccinated by IM route in contrast to the ones that received the vaccine by ID route.

The percentage of piglets that started feed intake at 6 h after vaccination was also affected by the administration route. Piglets in ID group started feed consumption after weaning well before the piglets in the IM group (55.0% vs. 0%, P<0.001). During the first week after weaning, nursery performance was also affected by the vaccination, the IM animals having a lower performance (Body weight [BW] and Average Daily Weight Gain [ADWG]) than the ID piglets (Table 1)

Table 1. Temperature, feed consumption and nursery performance.

Temperature after vaccination	Intradermal	Intramuscular	*p-value*
0h	40.01	39.95	-
6h	41.05	41.23	< 0.001
Temp. Increase 6h	1.04	1.28	< 0.001
24h	39.59	39.63	0.36
Temp. Increase 24h	-0.42	-0.32	0.36
Feed intake 6h	55%	0	< 0.001
Feed intake 24h	93%	80%	0.25
Weaning weight	6.48Kg	6.49Kg	-
Body weight 7d	6.33Kg	6.06Kg	< 0.001
ADWG 7d	0.021	-0.006	< 0.001
Body weight 42d	21.57Kg	20.51Kg	< 0.001
ADWG 42d	0.359	0.334	< 0.001

Conclusions and Discussion

The present study confirms the administration route effect on pig behaviour and performance, together withthe safety effect of the vaccine. The percentage of pigs in the intramuscular vaccine group that showed an increase in body temperature was higher than the percentage in the animals in the intradermal needle- free vaccination group. As a consequence of the high increase in body temperature and the painful process that intramuscular vaccination causes, the animals in this group were lethargic after vaccination. The lethargic behaviour and pain had a negative effect on starter feed intake after weaning. In contrast, the intradermal needle-free piglets started feed intake 24h earlier than the intramuscular group. Low feed intake after weaning is a factor that negatively contributes to performance during the nursery phase because piglets that take more time to start feeding are more likely to lose weight in the first week after weaning and to show underdevelopment (5,6). The advantages associated with intradermal needle-free vaccination and Mhyosphere® PCV ID were proven in this study, because the benefit in terms of animal welfare was reflected in performance in the first week after weaning and consequently in nursery performance.

Acknowledgments

The authors thank the following Brazilian partner company in the field experiments: Capal Cooperativa Agroindustrial.

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On-farm euthanasia decision tool for pre-weaning piglets

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Introduction

During the pig production cycle, there are inevitable situations that require animals to be humanely euthanized. On commercial pig units, it is impractical and financially unfeasible for a veterinarian to perform every emergency killing (1). Therefore, the responsibility of euthanasia decision-making and execution relies on the caretakers, under the guidance of veterinarians. Their willingness to perform the act, however, is a commonly overlooked factor about timely and humane euthanasia (2,3). Some barriers linked to good practices and effective training regarding pig euthanasia are stock peoples attributes such as empathy, confidence, and sourcing advice (4). Veterinary training for making timely euthanasia decisions can help to bridge the gap between existing euthanasia-specific training resources and needed on-farm education for caretakers (5). The pre-weaning period is one of the production phases in which piglets are more fragile and likely to be crushed by the sows, besides being the first moment in which anatomical alterations incompatible with life are detected (6). Plus, their lower economic value and shorter intervals between batches lead to less attention to the implementation of timely euthanasia as a welfare measure for pre-weaning piglets (1, 6). The objective of this study was to develop an on-farm euthanasia decision tool to avoid the insecurity and lack of sourcing advice for caretakers.

Method

Literature on the good practices for on-farm pig euthanasia was reviewed between September to December 2021. This survey started searching papers published in English in peer-reviewed journals that were identified using the keyword combinations: pig, piglet, or swine, plus on-farm, and euthanasia. The online electronic databases PubMed and Scopus were searched with a restriction: the paper must have been published from 2010 until 2021. Additional sources were searched through the Brazilian Federal Council of Veterinary Medicine and Ministry of Agriculture, Livestock and Supply electronic handbook databases. We did consult, as a focal group, with scientists, who published in euthanasia, and with field veterinarians regarding their experience with euthanasia of piglets and the usefulness of the decision-making tool. The material was compiled and then used to develop a methodology for euthanasia decision-making and a technical paper (Figure 1).

Results and Discussion

Our search found that many articles on the humane euthanasia methods and their decision impact on people involved have been published in the United States and Australia (2, 3, 4, 6). But only a few are dedicated to exploring the situation in Brazil (1). The Brazilian Ministry of Agriculture, Livestock and Supply has published the handbook "On-farm pig euthanasia" which includes a euthanasia decision-making tool. However, it covers decisions that are mainly applicable for adult animals, ignoring the recommended actions that need to be performed for injured nursery piglets. The decision-making tool was reviewed favorably by field veterinarians associated with a Cooperative.

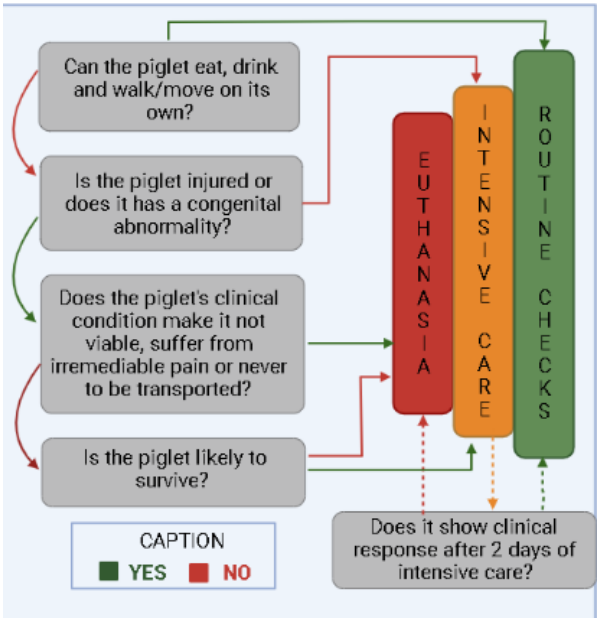

Figure 1. Decision-making tool for nursery piglets' euthanasia

Conclusion

We anticipate that a decision-making tool could empower caretakers and reduce the effects of depression and remorse reported by stock people who need to perform these practices, which may be even worse in piglets due to the greater need for culling (1, 7).

Further research is needed to achieve a fully Brazilian methodology to assist the decision-making process to ensure that the welfare of piglets is not compromised. Our goal is to apply this preliminary model to Brazilian farms.

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Pathogenesis of porcine ear necrosis in nursery piglets.

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Introduction

Porcine ear necrosis (PEN) is characterized by uni- or bilateral lesions of the ear tip or margin followed by necrosis (1). Lesions mostly appear in piglets between the sixth and eighth week of life (2). Many different risk factors have been described such as infections, ear-biting, high stocking density, poor ventilation, mycotoxins in the feed or insufficient enrichment of the environment (1-4). However, three main hypotheses have been proposed in the literature (5):

(I) skin damage caused by exfoliative toxins from *Staphylococc*i, with damage of the epidermis (6)
(II) small blood ear vessels occlusion with antigens and agglutinins forming complexes due to infection with *Mycoplasma suis* (7)
(III) external damage due to e.g. ear biting and subsequent infections with hemolytic *Streptococci* (6)

However, ear lesions and pathogens involved are poorly characterized. The present study investigated the progression of the lesions and associated viruses and bacteria in affected pigs from commercial farms.

Materials and Methods

Three farms with PEN problems in nursery piglets have been selected. On each farm, PEN prevalence was assessed and the severity of lesions was scored during the nursery period. In the last two weeks of the nursery, affected and non-affected animals were blood sampled (n=90 animals) for analysis of serum and plasma. Swabs (n=30) were collected for microbiological aerobic and anaerobic culture, whereas scrapings (n=30) were used for viral and bacterial metagenomics using nanopore-sequencing. Biopsies were taken (n=89) for histological examination.

Results

On all three farms, problems with ear lesions started to occur at the 4th week of the nursery and reached he highest prevalence 33-48%. Microbiological culturing resulted in growth of *Staphylococcus aureus and hyicus, Streptococcus suis and S. dysgalactiae* as well as *Escherichia coli* in both, PEN affected and non-affected pigs. The nanopore-sequencing results showed the presence of more than twenty different viruses and bacteria, of which *Streptococcus, Staphylococcus, Fusobacterium* and *Mycoplasma* appeared most frequent and relevant.

Of these, only *Mycoplasma hyopharyngis* was present in 78% of lesions, and absent in all control animals with unaffected ears. Histopathology results of affected tissues revealed epidermal hyperplasia (100%), hyperkeratosis (100%) and ulcerations (64%), with only few samples with vasculitis (6%) or thrombosis (3%).

Discussion and Conclusions

A broad variety of environmental and physiological germs were found on the ears of affected and healthy piglets. The role of *Mycoplasma hyopharyngis*, which is part of the microbiota of the upper respiratory tract and pharynx, warrants investigation. However, its presence on affected ears only may indicate the contact between the mouth and ear tips. The histopathology results suggest that the skin alternations may be due to bacterial toxins. Nanopore-sequencing results and pigs behavior (ear chewing/biting) during the visits also suggest that ear biting & bacteria may play a role in the pathogenesisof PEN in present three farms. Further research is morethan desirable, as it could indicate the most likely pathogenesis of PEN, leading to the establishment of effective control measures.

Acknowledgments

The authors are grateful to Veepeiler Varken for financial support, to the colleagues of the Unit of Porcine Health Management for practical help during the study, and to the farmer families for collaboration.

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Probiotics supplementation improve sow's farrowing process.

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Introduction

Gestation and lactation phases can be very stressful for sows (1). The long-term stress can affect reproductive efficiency and impact the vitality of newborn piglets (2). Probiotics modulate the intestinal microbiota, which can affect physiological and behavioral processes through the gut-microbiota-brain axis (GMB). Such changes are directly favorable to animal welfare (3, 4). This is a trending topic in animal nutrition, but there are still few studies dealing with the potential improvement of sow welfare through probiotic supplementation. Thus, this study was developed to evaluate the effects of dietary supplementation of gestation-lactation feeds on sow welfare and piglets' vitality.

Materials and Methods

Two treatments were tested: control (CON, n = 17), without supplementation; and probiotic (PRO, n = 18), in which sows received a probiotic composed by *Bacillus subtillis* and *Bacillus licheniformis* (Bioplus® 2B) during gestation and lactation phase. Interval time, farrowing time and number of interventions were evaluated. In addition, piglet vitality was assessed by meconium presence, umbilical cord rupture, blood glucose, blood pH, blood oxygen saturation and heart rate. Salivar levels of cortisol were evaluated five days after farrowing in the sows. Data was analyzed by ANOVA using PROC GLIMMIX (SAS 9.3 software) and interpreted at 5 and 10% significance levels (5).

Results

The supplemented treatment tended to reduce birth interval (P = 0.069), farrowing duration (P = 0.072), and number of interventions per litter (P = 0.090).

In addition, the PRO group had 45% less frequency of long labors (more than 300 minutes) than the CON group.

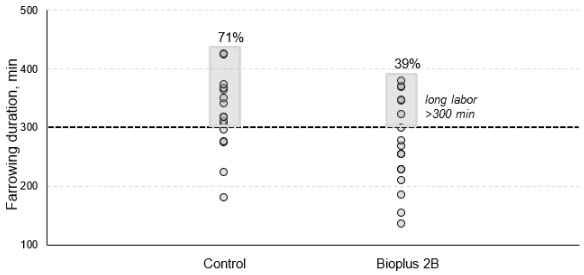
In the PRO sows, the presence of meconium, umbilical cord rupture, blood glucose level, blood pH, blood oxygenation, heart rate and number of interventions (CON= 4.07 ± 0.8; PRO = 2.28 ± 0.6), did not differ between treatments.

The level of salivar cortisol showed a tendency to decrease, at 6% of significance.

Discussion and Conclusion

The frustration and stress of the sows may have been mitigated through the action of probiotic microorganisms. The action pathways were probably neurobiological in the transport of serotonin, for example, or by the action of metabolites in the hypothalamic-pituitary-adrenal axis, positively affecting the physiology of stress and improving the welfare of sows.

Graph 1. Effect of probiotic supplementation to gestating-lactating sows on the farrowing duration and frequency of sows with long labor



It is understood that the improvement in welfare probably conditions the sow in a state of greater relaxation and tranquility, making the delivery process more efficient, reducing the time of expulsion of the piglet and, consequently, leading to a shorter delivery time. Piglets that spend less time undergoing uterine contractions tend to result in more active and vigorous piglets being born. However, this was not observed in this study and need to be further evaluated in future projects.

A better understanding of the gut-microbiota-brain axis (GMB) can be the key to improving the pig welfare.

Acknowledgments

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Reduction of aggression by supplementation of plant extracts

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Introduction

Animal welfare is increasingly becoming a major concern in industrially kept pigs worldwide. A trial was set up to investigate the efficacy of a plant based complementary feed for the reduction of aggression and tail biting. Sedoline® is composed out of a well balanced ixture of 5 plant extracts: *Humulus lupulus*, *Melissa officinalis*, *Crataegus monogyna*, *Valerina officinalis* and *Passiflora incarnata* and magnesium (1).

Materials and Methods

Healthy pigs (n=272, 0.24 m²/pig) with long, uncut tails and teeth cut on day 1 after birth were allocated in 2 groups of 136 pigs (16 to 18 pigs per pen) at the French Zootests Institute (2). The first group was supplemented with Sedoline® at 1 kg/tonne pelleted feed for 42 days: from weaning at day 28 (7-8 kg) till day 70 (25-29 kg) after birth. The second group was a negative control group. Two parameters were weekly (day 36, 42, 49, 56, 63 and 70) assessed by the same person. Firstly, the number of fightings was reported by a 15 minutes observation per pen. Secondly, the tail lesions of every pig were scored for 3 parameters: damages, length and blood freshness. Score '0' indicated no impact, score '3' was assigned in case of great impact.

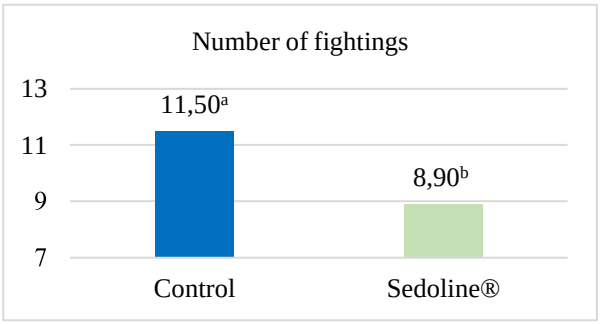
Table 1. Scoring grid for each of the 3 tested parameters

	Tail lesions		
	Damages	Length	Blood freshness
0	no damage	intact	no blood
1	hair removed/ bite marks	Outer part is missing	dried blood
2	small wound	>50% is missing	sticky blood
3	big wound	<1cm left	fresh blood

Results

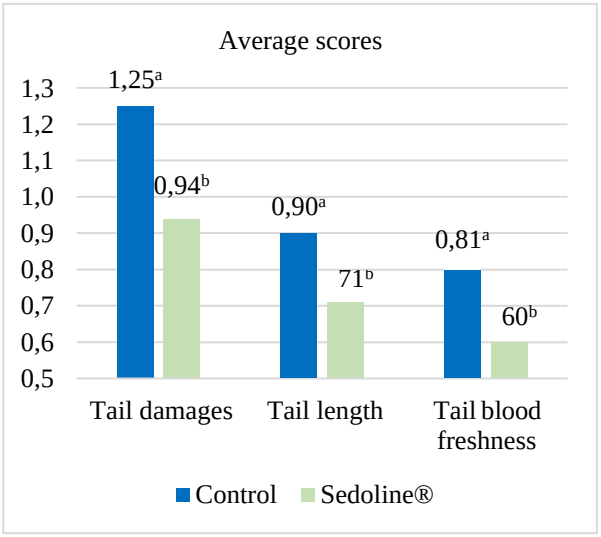
A significant effect was noted on the average number of fightings per pen (p 0.008), which was 2.6 less in the supplemented group in comparison to the control group (8.90 vs 11.50, resp.). The supplementation also resulted in a significant reduction of the severeness of the 3 parameters evaluated for the tails (p<0.001). When comparing the average scores of the tail lesions of the supplemented group to the control group, the score reduction of the tail damages was 0.31 (0.94 vs 1.25, resp.), the average score for the tail length was 0.19 lower (0.71 vs 0.90, resp.) whilst the blood freshness score also demonstrated a decrease of 0.21 (0.60 vs 0.81, resp.).

Figure 1. Number of fightings in the supplemented and control group



a,b Different superscripts indicate significant difference (p 0.008)

Figure 2. Average scores of tail lesions in the supplemented and control group



a,b Different superscripts indicate significant difference (p<0.001)

Discussion and Conclusion

A significant reduction of aggression and severeness of lesions caused by tail biting was achieved by the supplementation of this mixture of plant extracts in feed, contributing to animal welfare.

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Validating behavioral endpoints for pain research

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Introduction

To accurately assess pain and support broadly-based analgesic protocols to mitigate swine pain, it is imperative to develop and validate a species-specific pain scale. The objective of this study was to investigate the clinical validity and reliability of an acute pain scale (UPAPS) adapted for newborn piglets undergoing castration (Table 1).

Materials and Methods

Forty-nine piglets (n=39 castrated) five days of age, 1.62 ± 0.23 kg BW, were enrolled in the study. One- hour post-procedure, all castrated piglets received rescue analgesia (flunixin meglumine 2.2 mg/kg IM). Behavior of each piglet was video recorded continuously for 1 h at four recording periods (24 h pre-procedure, 15min post-procedure, 3 and 24 h post- procedure). An individual, not involved in the behavioral analysis, downloaded videos to obtain 4-minunedited video clips from each recording period. Behavior was assessed by two trained blinded observers. Statistical analysis was performed using R software (1) and differences were considered significant when $p < 0.05$.

Results and Discussion

The scale was unidimensional based on the principal component analysis, all items except for nursing were representative ($r_s \geq 0.74$) and had excellent internal consistency (Cronbach's alpha ≥0.85). The sum of scores were higher in castrated piglets' post procedure than pre-procedure, confirming construct validity and responsiveness. Scale sensitivity when piglets were awake was 92.9% and specificity was moderate (78.6%). The scale had excellent discriminatory ability (area under the curve > 0.92) and the optimal cut-off sum for analgesia was 4 out of 15.

Table 1. Adapted from the UNESP-Botucatu composite pain scale for assessing postoperative pain in piglets (UPAPS)

Score/Criterion
(0) Normal (any position) or sleeping (2)
(1) Changes posture, with discomfort
(2) Changes posture, and protects the affected area
(3) Tense, and back arched
(0) Interacts with others; interested in the surroundings or sleeping
(1) Only interacts if stimulated by other animals; interested in the surroundings
(2) Occasionally moves away from the other animals, but accepts approaches, little interest in the surroundings
(3) Does not allow approaches; disinterested in the surroundings
(0) Moves normally or sleeping
(1) Moves with less frequency
(2) Moves constantly, restless
(3) Reluctant to move or does not move
(A) Elevates pelvic limb or alternates the support of the pelvic limb
(B) Scratches or rubs the painful area
(C) Moves or runs away or jumps after injury of the affected area
(D) Sits with difficulty
(0) All the above behaviors are absent
(1) Presence of one of the above behaviors
(2) Presence of two of the above behaviors
(3) Presence of three or all the above behaviors
(A) Wags tail continuously and intensely
(B) Bites the bars or objects
(C) The head is below the line of the spinal column
(D) Presents difficulty in overcoming obstacles
(0) All the above behaviors are absent
(1) Presence of one of the above behaviors
(2) Presence of two of the above behaviors
(3) Presence of three or all the above behaviors

2 Criterion in bold indicate the adaptation of the original pain scale developed by Luna et al(2020) to account for normal behavioral repertoire of pre-weaned piglets (sleeping).
3 Nursing behavior was observed and analyzed in the study but was excluded from the finalpain scale table as it did not meet any of the validation criteria (principal component analysis, specificity, internal consistency, item-total correlation, and responsiveness)

Conclusion

The UPAPS scale is a valid and reliable clinical tool to assess acute pain in castrated piglets.

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Validation of the effectiveness of electric stunning for euthanasia of mature swine *(Sus scrofa domesticus)*

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Introduction

Euthanasia remains a moral obligation of all individuals working in the swine industry. While a majority of acceptable methods of humane euthanasia have been validated for market weight pigs, considerably less attention has focused on heavy- weight mature animals. Euthanasia of heavy-weight mature boars and sows has its unique challenges due to distinct age and gender specific anatomical features of the *suid* family (1,2). Currently, frontal placement of either a gunshot or penetrating captive bolt gun (PCBG) and head-to-heart electrocution represent the most common techniques performed on-farm for mature swine (3,4). Kramer et al. (2021) recently demonstrated the efficacy of a heavy-duty cylinder style PCBG for use in both mature boars and sows
>200 kg (5). The objectives of the current study were to evaluate the efficacy of a two-stage mobile electric stunning process and to assess euthanasia outcomes compared with those using a frontal placement of a heavy duty PCBG in heavy weight (>200 kg), mature boars and sows.

Materials and Methods

Effectiveness of an E-STUN (TBG 96/N mobile E-STUN, Hubert HAAS, Neuler, Germany) and PCBG (Super Heavy-Duty Jarvis In-line Cylinder Style Power Actuated Stunner, BUNZL Processor Division/Koch Supplies, Riverside, MO) were evaluated first in unconscious anesthetized mature swine (n = 7 boars and sows per treatment; average weight 282 ± 48 kg, n=28) and then in conscious mature swine (n = 3 boars and sows per treatment; average weight 282 ± 63 kg, n=12). Data from both stages were combined for analyses. Treatment efficacy was defined as any pig that achieved cardiac and respiratory arrest within 10 min after treatment application. Basic descriptive statistics were used to describe demographics for the study population and frequency distributions for categorical variables (6).

Results

Animals were rapidly rendered insensible with either the E-STUN or PCBG. Both treatments were 100%effective in meeting the 10 min endpoint confirmation of death as verified by cardiac and respiratory arrest. When evaluating time to last heartbeat, there was a significant interaction between sex and treatment. Boarseuthanized via E-STUN had 346.8 s decrease in time to last heartbeat compared to boars euthanized via PCBG ($P < 0.001$) and females euthanized via E-STUN had a 479.3 s decrease in time to last heartbeat compared to females euthanized via PCBG ($P < 0.001$).

Discussion and Conclusion

This study confirmed that E-STUN for on-farm euthanasia of mature swine (>200 kg) using a two-step process (head-only/head-to-heart) and supplying a maximum of 2.4 A is as effective as the use of a heavy duty PCBG considering the E-STUN technique reliably and rapidly induced insensibility, cardiac arrestand death. The data presented in this study is in agreement with Vogel et al. (2011) who reported that, in a slaughter setting (< 130 kg pigs), a two-stage electrical stunning method supplying a mean current of
2.3 A using a standard head-only stun immediately followed by stunning wand application to the cardiac region of the chest, ensured insensibility and abolishment of heartbeat, rhythmic breathing, natural blinking, eye tracking to a moving object, and rightingreflex in pigs. These results justify a two-step E-STUNas an effective and reliable technique for on-farm euthanasia for heavy weight (>200 kg) mature swine. This technique is as effective as other common euthanasia techniques currently used on farm and has the ability to safeguard animal welfare by eliminating pain and suffering in compromised pigs that warrant euthanasia.

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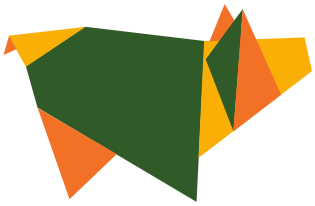

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