Proceedings of the

23rd International Pig Veterinary Society (IPVS) Congress

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Cancun, Quintana Roo, Mexico

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Volume 1

Invited speakers, oral and corner presentations

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Preface

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

Jesús Hernández and Jeffrey Zimmerman

Acknowledgements

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

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Locations of IPVS Congresses

1969	Cambridge, UK
1972	Hannover, Germany
1974	Lyon, France
1976	Ames, USA
1978	Zagreb, Yugoslavia
1980	Copenhagen, Denmark
1982	Mexico City, Mexico
1984	Ghent, Belgium
1986	Barcelona, Spain
1988	Rio de Janeiro, Brazil
1990	Lausanne, Switzerland
1992	The Hague, Netherlands
1994	Bangkok, Thailand
1996	Bologna, Italy
1998	Birmingham, UK
2000	Melbourne, Australia
2002	Ames, USA
2004	Hamburg, Germany
2006	Copenhagen, Denmark
2008	Durban, South Africa
2010	Vancouver, Canada
2012	Jeju, Korea
2014	Cancun, Mexico

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Accountabilities in the age of transboundary and emerging porcine diseases

JCS Harding
Department Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Canada

In memory of Dr. T. J. L. Alexander

It is a great honor to present the Tom Alexander Memorial Lecture today. Few researchers have had such a profound influence on swine health. Dr. Alexander's accomplishments include fundamental breakthroughs many of us, younger by decades, take for granted: the discovery of spirochaetes causing swine dysentery(1), the epidemiology and control of *Streptococcus suis* type 2 infections(2), characterizing viral encephalomyelitis of nursery pigs in Ontario(3) now known as HEV, and fundamental theories of high health status breeding pyramids(4), specific pathogen free derivation(5) and medicated early weaning(6). A scholar and English gentleman, Dr. Alexander is an icon in the swine industry. I am proud to have helped establish the inaugural Tom Alexander Memorial Lecture at the 21st IPVS in Vancouver, and pleased the 22nd and 23rd IPVS organizing committees committed to continuing his legacy.

Never before has the risk of transboundary and emerging diseases been so real. The "perfect storm" caused by swine industry intensification, globalization, societal and environmental encroachment is having an insidious impact on the global swine industry. Many working in the swine industry have a modest understanding of the issues but continue to focus locally, rather than globally. This is understandable given that needs of local clients must be served. With the advent of PEDV in North America and ASF in Eastern Europe, attitudes however, are beginning to change.

I would like to thank Drs. Jeff Zimmerman and Jesus Hernandez for the invitation to present this lecture. My goal is to awaken swine veterinarians globally regarding the present threats, contributing factors, and necessary actions to help control catastrophic transboundary and emerging diseases. While I am at risk of sensationalizing and sounding alarmist, it is not my intent. I am passionate that we begin to heed the warnings and work towards constructive solutions.

My presentation has three parts: A) a review of some of the current transboudary and emerging disease threats, B) a summary of expert opinions pertaining to the control of catastrophic swine diseases, and C) my assessment of what should be done and who is accountable. I sincerely hope this paper provides some insight and evidence that we need to act now because the current situation will only deteriorate.

Part A: Review of transboundary and emerging pathogens and syndromes

Notifiable and transboundary animal diseases

The Food and Agriculture Organization of the United Nations (FAO) defines transboundary animal diseases as (paraphrased): *"those epidemic diseases which are highly contagious or transmissible and have the potential for very rapid spread, irrespective of national borders, causing serious socio-economic and possibly public health consequences"*. It goes on to state: *"These diseases which cause a high morbidity and mortality in susceptible animal populations, constitute a constant threat to the livelihood of livestock farmers. Furthermore, their potential consequences are of such a magnitude that their occurrence may also have a significant detrimental effect on national economies"* (7). Although many diseases common to the global swine populations could be considered "transboundary", only a few specific swine examples are listed by the FAO including African Swine Fever (ASF), Foot and Mouth Disease (FMD), Influenza A (H1N1). By contrast, the 2014 OIE-Listed diseases(8) includes 8 multiple-species and 7 swine-specific diseases or infections (Table 1), many of which are or could become transboundary. Of ~196 countries in the world, 178 are OIE member nations and are obliged to report to the OIE.

Table 1. 2014 OIE-Listed diseases and infections of relevance to pigs(8)

Diseases affecting multiple animal species	Diseases specific to pigs
Anthrax	African Swine Fever
Aujeszky's disease	Classical Swine Fever
Brucellosis (*B. suis*)	Nipah virus encephalitis
Foot and Mouth Disease	Porcine cystocercosis
Rabies	Porcine Reproductive and Respiratory Syndrome
Japanese encephalitis	Swine vesicular disease
Infection with *Trichinella* sp.	Transmissible gastroenteritis virus
Vesicular stomatitis	

Of the OIE-Listed diseases of swine relevance, few are present in every continent although the availability of diagnostic resources and the reporting policies of member nations clearly affect the official disease status reported by some countries to the OIE. Australia/New Zealand are the only continents with swine industries that report freedom from all OIE-Listed swine diseases, largely attributable to their isolation and strict trade and importation policies. Each of the OIE-Listed swine diseases has one or more continental hotspots that serve as potential reservoirs to non-infected regions (Table 2).

Table 2. Current continental hotspots for selected OIE-Listed swine diseases at greatest risk of transcontinental transmission as of March 2014(9)

Disease/pathogen	Countries reporting disease events to OIE	Reporting Period
African Swine Fever	**Europe:** Belarus[a], Poland[a], Ukraine[a], Lithuania[a] **Asia:** Russia[a] **Africa:** Chad[a], Nigeria[a], Zambia[a], Burnkina Faso[a], Togo[a], Benin[b], DR Congo[c], Mozambique[c]	Jul 2013-Mar 2014
Aujeszky's Disease	**Americas:** United States[b], Mexico[b], Argentina[d], Haiti[e] **Europe:** Spain[b], Poland[d], Ukraine[c], Croatia[c], Macedonia[c] **Asia:** Vietman[e]	Jan 2013-Dec 2013
Classical Swine Fever	**South America:** Columbia[a] **Europe:** Hungary[a], Latvia[a], **Asia:** Russia[a], S. Korea[a], Vietnam[c], Cambodia[c]	Jul 2013-Mar 2014
Foot & Mouth Disease	**Africa:** Guinea[a], Libya[a], Zimbabwe[a], Kenya[f], DR Congo[f], Central African Republic[c], Nigeria[c], Benin[f], Togo[f], Burkina Faso[c], Zimbabwe[a], Namibia[a] **Asia:** Russia[a], Mongolia[f], Vietnam[a], N. Korea[a], Cambodia[f]	Jul 2013-Mar 2014
Japanese encephalitis	**Asia:** Papua New Guinea[e]	Jan 2013-Dec 2013
PRRS	**Americas:** Canada[c], United States[c], Mexico[b], Columbia[c], Costa Rica[c], Dominican Republic[c] **Asia:** China[a], India[a], Vietman[c], Russia[b], Thailand[c], Japan[c], S. Korea[c] **Europe:** Belarus[a], Switzerland[a], UK[c], Netherlands[c], France[c], Poland[c], Spain[d], Belgium[d], Czech Republic[d], Croatia[d], Lativa[d], Hungary[b]	Jan 2013-Mar 2014
Swine Vesicular Disease	**Europe:** Italy[b], Sardinia[a]	Jan 2013-Dec 2013
Transmissible gastroenteritis	**North America:** Canada[c], United States[c], Mexico[b] **Europe:** Spain[b] **Asia:** Japan[c], S. Korea[c]	Jan 2013-Dec 2013
Vesicular stomatitis	**Americas:** Mexico[f], Nicaragua[c], Columbia[f], Ecuador[f], Peru[f], Brazil[f]	Jan 2013-Dec 2013

[a] Current disease event
[b] Disease limited to one or more zones
[c] Clinical Disease
[d] Infection
[e] Suspected
[f] Present for other serotype

Emerging human and animal diseases

Although the notifiable diseases present a formidable risk to the global swine industry, the emergence of novel diseases present an equivalent challenge. Contemporary examples include highly pathogenic PRRS, Influenza A (FLU), PCVAD, PED and ASFV. Although all of these are widely recognized as transboundary diseases, it is hit and miss whether they are notifiable to the OIE, and if not, generally fall outside national surveillance programs. As a result, little hinders the potential for global transmission. The emergence of novel zoonotic pathogens is of particular concern, and is proposed to result from a combination of human activity and demographic changes where ideal socioeconomic, environmental and ecological factors exist(10). For example, close contact with wildlife or reliance on wildlife for sustenance may facilitate transmission to humans, whereas the development of virulent human pathogens is more likely to occur where population growth and density are high. During the last 30 years, approximately 90 novel human pathogens were discovered, averaging 3 per year(11). Sixty-six percent were viruses and more than 80% of these, RNA viruses; most potentially zoonotic. Strong predictors of the emergence of zoonotic viruses are a high mutation rate and the ability to replicate in the host cell cytoplasm as opposed to the nucleus(12). As a result, RNA and single stranded DNA viruses are the most likely to emerge because of their small genome, rapid rate of replication and polymerase enzymes that lack proof-reading capability. These characteristics lead to swarms of slightly divergent genomes (known

as quasispecies(13)) that facilitate recombination, reassortment and inter-species transmission if the appropriate conditions exist(14).

Domestication and close daily contact with livestock favors inter-species disease transmission(15, 16) and suggests that the emergence of novel zoonotic pathogens will most likely occur in developing countries, particularly in SE Asia. By contrast, intensive livestock operations with a low human to animal ratio may in fact decrease inter-species disease transmission and reduce the risk of emergent zoonotic diseases(15). Importantly, intensive livestock operations with high animal density favor intra-species transmission and the evolution of host-specific pathogens. It is therefore likely that modern production systems have played a roll in the emergence of endemic pathogens such as PCV2, PRRS and FLU over the last several decades. Moreover, multiple-site units, intra- and trans-continental movement of live animals and fresh semen, and inadequate biosecurity enhance the dissemination of novel diseases within and between continents. It is most likely that the most devastating pathogens affecting the global swine industry in the last two decades, PRRSV, PCV2 and PEDV included, have evolved from pigs and will continue to evolve towards optimal levels of virulence as long as favorable conditions exist. Whereas culture and socio-economic conditions will influence the development of emergent zoonotic diseases, the design and operation of pork production systems will ultimately determine the virulence and rate at which novel pig-specific pathogens will emerge in future decades (Table 3).

Table 3. Features of the emergence of zoonotic and porcine-specific pathogens

Feature	Inter-species transmission	Intra-species transmission
Hotspots	Developing nations (SE Asia, Africa)	Developed nations (Americas, Europe)
Contact characteristics	Increased human-animal contact, wildlife	High animal density, large farms
Farm type	Mixed animal farms	Intensive livestock operations
Animal proximity and farm type	Animals and excrement in close proximity to human dwellings	Biosecure locations and farms
Pathogen emergence	Zoonotic pathogens	Host-specific pathogens
Limits to prevention	Socio-economic and cultural conditions	Motivation for higher productivity and profit
Global transmission	Transcontinental travel (humans)	Transportation and global trade (pork, animals, semen, feed ingredients)
Accountable for prevention	Society	Swine industry

Advancements in molecular diagnostics including unbiased high-throughput sequencing technologies have provided unprecedented capability to detect novel pathogens. Some pathogens may be a member of the host's microbiota of gut, skin, respiratory and reproductive systems, while others may be associated with disease. While a detailed review of all emergent viruses of pigs is beyond scope of this paper, viruses of potential importance to pig production and public health are worthy of brief review, as well as a few novel syndromes. Purposefully excluded are PRRS (HP-PRRS), FLU, CSF, FMD and other vesicular viruses. Current information on PED and ASF are discussed because of recent activity in North America and Europe, respectively.

Viruses of great industry significance

African Swine Fever Virus (ASFV): ASFV (family *Asfarviridae*) was restricted to Africa until 1957, where it is maintained in African wild pigs, many of which are asymptomatically infected(17). Several soft tick species assist in transmission, but transmission to domestic pigs in distant locations generally occurs by the illegal trade of infected pigs and pork meat(17). Since 1957, the disease has travelled around Europe, the Caribbean and Brazil, and persisted in Sardinia and Africa. It was introduced to domestic pigs in Georgia in 2007, presumably through ship waste from a vessel that docked in Poti, a seaport in the Black Sea(18). Since 2007, it has quickly spread to Armenia, Azerbaijan, Russia, and more recently Lithuania, Belarus, Ukraine, Poland. It is estimated that ASFV spreads at the rate of approximately 1 km per day in Russia(19). Eighty percent of Caucasus outbreaks involve domestic pigs, and of the 20% involving wild boars, most follow contact with domestic pigs. Although some species of soft ticks inhabit the Caucasus region, no outbreaks in eastern Europe involve soft ticks(17). Factors involved in spread in the region include high volume illegal meat trade, swill feeding, absence of veterinary services, lack of pig production infrastructure and traceability, and wild boars(17). The recent identification of ASF in the Ukraine and Poland is particularly alarming because the wild boar population is hardy and more resistant to infection than domestic pigs. Control strategies for the

wild boar population are being investigated, however hunting and trapping are deemed infeasible and may, in fact, increase transmission and the geographical spread of ASF(20). Thus, producers are advised to step up traditional measures of biosecurity.

PEDV and swine Deltacoronavirus: First identified in England 1971(21), PEDV causes acute gastroenteritis associated with high mortality in suckling piglets under 3 weeks of age. PEDV affected a number of European countries between 1978 and 2006, and became increasingly problematic in SE Asia beginning 1997(21). PEDV strains genetically similar to those identified in China in 2011 and 2012 were first introduced into the USA in April 2013(22-24). Since then, PEDV has rapidly spread over 25 US states, and into Canada (2014), Mexico and Dominican Republic (2014). At least 3 Chinese strains as well as 2 novel swine deltacoronaviruses (SwDCV), originally discovered in 2012(25), have been identified in North America. Field evidence indicates SwDCV can be, but is not always, as virulent as PEDV. PEDV and SwDCV are transmitted by the fecal-oral route, and dissemination among farm facilitated by heavy shedding, environmental contamination, transportation vehicles and other fomites. Contamination of feed ingredients, specifically plasma proteins, is proposed to be a source of infection particularly to farms in geographically isolated areas. The Canadian Food Inspection Agency (CFIA) recently determined that a specific lot of plasma protein contained live virus capable of causing disease in pigs, whereas nursery feed manufactured using the same contaminated lot did not (26). Aerosol transmission is postulated but not proven. Until such time they are widely available and proven effective, control and elimination is achieved by feed back of intestinal material or feces. Results however, are variable leading to concern of endemic infections and long-term industry exposure.

Swine hepatitis E (HEV): Discovered 1997, swine HEV (family *Hepeviridae*) has been identified in virtually all swine producing countries and is highly prevalent on commercial farms(27, 28). HEV is transmitted by fecal-oral route and replicates in gastrointestinal tissues of 2 to 4 month old pigs resulting in transient viremia before targeting the liver(29). Infection is subclinical in pigs, although microscopic hepatitis including multifocal lymphoplasmacytic hepatitis and hepatocellular necrosis occur in naturally and experimentally infected animals(30). The virus is of great industry importance due to its zoonotic potential to persons with direct pig contact as well as indirect contact via effluent(31-33). HEV remains infectious in pork products, particularly liver, but is readily inactivated by high temperature cooking, i.e. more than medium rare(34). Its zoonotic potential is documented by sporadic and cluster outbreaks occurring following consuming undercooked wild boar meat(35).

Pathogenic viruses of potential concern for commercial swine

Torque teno sus virus (TTSuV1 & 2): TTSuV (family *Anelloviridae*) is a ubiquitous organism found in healthy and diseased pigs worldwide. Following infection by either horizontal or vertical transmission, the virus is detected in multiple organs and secretions but does not necessarily associate with clinical disease(27). Dual infections with both TTSuV1 and TTSuV2 have been documented(36). In gnotobiotic pigs, TTSuV1 tissue homogenates partially contributed to the development of PDNS and PMWS(37). In naturally occurring PMWS, the involvement of TTSuV is unclear. In Spain, PMWS-affected pigs had a higher prevalence of TTSuV2 than non-affected pigs(38) whereas TTSuV levels did not statistically differ between PCVAD-affected and non-affected pigs in Korea(39). TTSuV1 & 2 are contaminants of several popular swine vaccines used in North America and Europe suggesting a possible route of global dissemination(40). While the pathogenicity of TTSuV is not clear at this time, it should be seen as a potential pathogen worthy of ongoing research.

Menangle porcine virus (MenPV): Menangle virus (family *Paramyxoviridae*) is a classic example of a virus that jumped species, in this case from fruit bats(41), to cause significant disease in pigs. It was discovered in 1997 in NSW, Australia in a single piggery experiencing reproductive disease and congenital skeletal malformations(42). MenPV is potentially zoonosis associated with influenza-like illness and rash in humans(43). Experimentally infected 6-week old pigs demonstrate viremia, shedding and viral replication in secondary lymph organs and gastrointestinal tissues (43). Although MenPV did not spread within Australia or internationally, it remains a swine industry concern due to its potential impact on production and public health.

Bungowannah virus: Bungowannah virus, an atypical pestivirus (family *Flaviviridae*), was discovered in NSW, Australia in June 2003 associated with porcine myocarditis syndrome. It was identified following an outbreak of stillborn piglets and increased pre-weaning mortality associated with multifocal non-suppurative myocarditis and myonecrosis(44, 45). Experimentally infected weaner pigs demonstrate few clinical signs in spite of shedding by oropharyngeal, nasal, and conjunctival secretions and in feces(46). As with MenPV, there is no evidence that Bungowannah virus spread domestically or internationally, and tissue samples collected from cases of abortion and respiratory disease in the USA between 2007 and 2010 were confirmed negative by PCR(47). Bungowannah virus is a pathogen of potential concern due to its association with porcine myocarditis syndrome.

Viruses of potential industry significance

Nipah virus (NiV): The most publicized outbreak of NiV (family *Paramyxoviridae*) was in Malaysia in 1998 following inter-species transmission from fruit bats to pigs. Efficiently spread amongst pigs, NiV then transmitted to pig workers(48) killing 109 of 283 infected(49) and resulting in the destruction of 1.1M pigs(50). Less well reported are the nearly annual human outbreaks of NiV in Bangladesh since 2001, as well as in neighboring India, associated with the indirect transmission from *Pteropus* bats, the natural host(51). Outbreaks are linked to the consumption of uncooked date palm sap contaminated by bat saliva(52, 53). The case fatality rate in infected humans in south Asia is ~70%(54) and over 1/3 of survivors have permanent neurologic deficits(55). Short chains of human-to-human transmission among persons who contact secretions of affected persons are reported(48) but the virus is not efficiently transmitted in the community. Pigs do not appear to be a natural host of NiV. There was no evidence of seroconversion in slaughter hogs sampled in Laos in 2008/2009(56), and only 5% of 97 serum samples tested positive for a non-neutralizing antibody to a henipavirus divergent from NiV or Hendravirus in Ghana in 2007(57). The risk to the swine industry is likely dependent on another inter-species transmission event where natural hosts co-exist with commercial hogs.

Ebola viruses (EBOV): The global swine industry is exceptionally fortunate that *Reston ebolavirus (*REBOV) is non-pathogenic to humans. If this was not the case, the 2008 outbreak in the Philippines may have elicited a completely different response and outcome. REBOV, the only Ebola virus (family *Filoviridae*) species found outside Africa, is lethal to some non-human primates but has never been linked to disease in humans despite exposure(58). The clinical signs in affected outbreak herds in the Philippines resembled highly pathogenic PRRS, which was identified as a co-infection(59). An estimated 70% of pigs in two affected farms were sero-positive(60) as well as farmers(61) demonstrating inter-species transmission. REBOV challenge in 5-week old pigs resulted in lymphoid hyperplasia and acute bronchopneumonia and shedding in oronasal secretions(62). Although the REBOV outbreak in Philippines was contained, it should be seen as a "warning shot" to justify keeping REBOV and the more virulent *Zaire* EBOV on with swine industry's radar.

Novel parvoviruses: Family *parvoviridae*, subfamily *parvovirinae* consists of 5 genera including ***Parvovirus*** (PPV) and ***Bocavirus*** (PBoV). There are 5 PPV subgroups (PPV1-PPV5), where PPV1 is the classical parvovirus of pigs. Novel PPV4 was first isolated from pigs co-infected with PCV2 in US and China(63), whereas PPV5 was identified in lung samples of nursery and grower-finish pigs suffering respiratory disease in the USA(64). PPV3, also called ***Hokovirus*** (**PHoV)** or ***Partetravirus,*** was first identified in Hong Kong and is highly prevalent and associated with high-path PRRS co-infection(65). PHoV has since been identified in the German (66) and Romanian wild boar populations(67), and in tissues of both PMWS positive and PMWS negative pigs from the UK & N. Ireland(68). **PBoV** has widespread distribution and its pathogenicity also unclear. At least 4 species designated PBoV1-4 have been identified and are highly prevalent in feces of healthy pigs in China(27, 69). In addition, a PBo-likeV was identified by PCR in over 70% of tissues collected from PMWS-positive and PMWS negative pigs in the UK and N. Ireland, with no differences in prevalence between group(70). The taxonomy and nomenclature of the parvoviruses is confusing and dynamic, but there is little conclusive evidence supporting their pathogenicity in swine at this time, except for PPV1.

Porcine enteric Caliciviruses (Sapovirus, Norovirus): Discovered 1980(71) and characterized in 1999(72), **porcine Sapovirus (PSaV) (**family *Caliciviridae*) is highly prevalent in feces of young pigs between 2 and 8 weeks of age(73). In gnotobiotic pigs, PSaV cause diarrhea and intestinal lesions(74) whereas the prevalence of PSaV did not differ between diarrheic and healthy commercial pigs in Europe(73). Although PSaV are diverse in nature and a zoonotic threat, definitive evidence of pathogenicity in pigs is lacking(27, 75). **Porcine Norovirus (PNoV)** are also highly diverse and also frequently identified in the feces of healthy animals(76, 77). Until 2012, PNoV was exclusively identified in feces of pigs without diarrhea, but a novel genotype was recently identified in feces of a piglet with diarrhea of unknown etiology in a Shanghai suburb. Subsequent experimental infection using fecal suspensions containing this recombinant strain caused acute gastroenteritis in miniature pigs(78) highlighting the potential for inter-species transmission in locations where pigs and humans co-exist.

Porcine Astrovirus (PAstV): PAstV's (family *Astroviridae*) demonstrate remarkable genetic diversity and are highly prevalent in feces of healthy pigs across all age groups from suckling to slaughter pigs(79). They have been detected in wild boars(80) and in 20% of meat samples of slaughtered pigs in Germany(76). Several studies however, report associations with diarrhea of young pigs(81-83). Although more research is required to conclusively determine if PAStV are pathogenic to commercial swine(84), the virus is clearly a zoonotic threat.

Porcine Torovirus (PToV): PToV (family *Coronaviridae*) can infect a wide range of domestic animals and humans(85) and are found commonly in many countries particularly in healthy animals (27). However, recent evidence from China indicates that 45% of surveyed farms in China were positive PToV, and the virus may be associated with piglet diarrhea(86). Passive PToV antibody levels decline by about 3 weeks of age, which is followed by seroconversion indicating infection likely occurs shortly after weaning(87). While the pathogenicity of PToV is not fully understood, the virus is an industry concern due to its zoonotic potential(27).

Aichivirus C (AiV-C; formerly porcine Kobuvirus): *Aichivirus C* (family *Picornaviridae*) was first identified in Hungary in 2008 in commercial pigs(88). Since then, the virus has been identified in Asia(89), Europe(90, 91),

USA(92) and Brazil(93) in asymptomatic pigs. In all reports, the prevalence of infection decreases with age(89). Several recent reports, however, associate AiV-C with diarrhea. Across 40 herds in Korea, the prevalence of AiV-C was two- to four-fold higher in diarrheic pigs compared to non-diarrheic pigs with ages from suckling pigs to adult sows(94). Shan *et al*. reported similar findings in a fecal virome study of pigs with and without diarrhea(95). Higher positive prevalence of AiV-C was also detected in diarrheic samples in Thailand and Japan (96, 97) and the virus was recently detected as co-infection in fecal samples of pigs infected with PEDV in China 36%(86). In spite of these reports, there is no proof that the AiV-C is causally associated with enteric disease in any age. The virus however, results in viremia(98), and therefore, may be capable of both systemic and enteric disease of pigs.

Porcine Adenovirus (PAdV): PAdV (family *Adenoviridae*) is a ubiquitous virus in which manifestation of disease depends mostly on host-related predisposing factors(99, 100). It is also potentially zoonotic, which is of primary importance for pig workers. PAdV was detected in 1-2% of European sausages at point of sale(100) highlighting concerns for pork consumers, and was also detected as a frequent contaminant of water (in 30% of 36 water samples) tested in southern Brazil)(101) proposed to be the result of swine manure contamination of watersheds. In pigs, adenovirus causes diarrhea, weight loss, and anorexia in 1- to 4-week-old pigs(102) associated with diffuse enteritis(103). Adenovirus can also result in fever and hemorrhagic enteritis of young pigs(104, 105). Although the potential pathogenicity of PAdV has been known for decades, its ubiquitous nature makes it difficult to distinguish opportunistic from pathogenic infections in commercial pigs. The virus however, has pathogenic and zoonotic potential.

Porcine Lymphotropic Herpesviruses (PLHV): Three closely related gamma-herpesviruses (PLHV-1, -2, -3) of pigs are widely disseminated in commercial herd and feral swine populations, but are of unknown clinical significance(27). Although they are vertically and horizontally transferred, PLHV negative pigs can be produced via caesarian section(106). Co-infection with PCV2 does not lead to PMWS(107). The only evidence of pathogenicity is one report of post-transplantation lymphproliferative disease in miniature pigs following haemopoietic stem cell transplantation. Affected pigs demonstrated fever, lethargy, anorexia, high leukocyte counts, and enlarged lymph nodes(108, 109). Thus, the PLHV appear to be primarily a concern for xenotransplantation.

Picobirnavirus (PBV): These are double stranded RN viruses with a broad host range associated with diarrheic and asymptomatic infections of animals and humans especially in children under 5(110). PBV have recently been identified in feces of healthy pigs subjected to deep sequencing(111), and in the respiratory tracts of pigs from slaughterhouses in Hong Kong and Colombo, Sri Lanka(112). Their pathogenicity remains unclear but they are a potential zoonotic threat to the swine industry.

Emerging diseases and syndromes of potential industry significance

New Neonatal Porcine Diarrhoea (NNPD): Since 2008, a neonatal diarrhea that is non-responsive to antibiotics has been evident in Denmark and Sweden(113-115). Case-control studies in Europe have failed to link NNPD with common etiologies of neonatal diarrhea including ETEC *E. coli*, *Clostridium perfringens* A and C, *Clostridium difficile*, type A rotavirus, coronavirus, cryptosporidium, *Giardia*, or *Cystiospora suis* (113). In a Danish study of 51 diarrheic piglets and 50 non-diarrheic pigs 1-5 days of age, villous atrophy with crypt hyperplasia of ileum and jejunum, was more frequently noted in cases than in controls. A non-hemolytic *E. coli* 0157 was isolated more frequently in cases ($P = 0.06$) in the Danish study, but related research in Sweden ruled out any association with *E coli* with NNPD(116).

Peri-weaning failure-to-thrive syndrome (PFTS): PFTS is a relatively new syndrome associated with elevated post-weaning mortality affecting the North American swine industry. First reported in 2008, (117, 118) it has subsequently been reported in a number of American states and Canadian provinces(119) as well as outside North America(120). A case definition states that within 7 days of weaning, affected pigs become anorexic, lethargic and progress to debilitation(121). A peculiar clinical signs of PFTS is repetitive oral behavior (chomping and licking), which is observed on all farms diagnosed with PFTS, and at low frequency in most commercial farms (O'Sullivan, unpublished data). The etiology of PFTS is unknown but common swine pathogens tested to date are not likely causal(122, 123). The anatomical and clinical pathology of PFTS, most notably intestinal villus atrophy, gastritis, thymic atrophy and elevated beta-hydroxybutarate(122) are consistent across affected farms but indistinguishable from those associated with starvation. The reason why individual or groups of pigs starve after weaning in otherwise healthy farms is unknown. Profound disturbances in intestinal barrier function however, were found in PFTS affected pigs that could not be explained by anorexia alone(124). Whether or not PFTS is caused by infectious or non-infectious etiology is not known, but it is an emerging condition that can contribute to post-weaning mortality on commercial farms.

***"Brachyspira hampsonii"*:** First identified in 2010 associated with bloody and mucoid diarrhea in North American pigs(125, 126), "*B. hampsonii*" has been isolated in pure culture and used to experimentally reproduce mucohemorrhagic colitis in susceptible pigs(127, 128). In 2012, Chander *et al*. proposed a provisional name and two clades I and II(129) to honor Dr. David Hampson's lifelong contribution to *Brachyspira* research. Disease caused by clades I and II are clinically and pathologically indistinguishable. *"B. hampsonii"* was recently isolated from Lesser Snow Geese in Canadian arctic(130) and in species in Graylag Geese and Mallard Ducks in northwestern Spain(131)

indicating a number of waterfowl species are competent hosts and potential sources of transmission. However, experimental reproduction of a snow goose strain, KL180, into susceptible pigs failed to reproduce severe colitis or mucohemorrhagic diarrhea(130). Recently, "*B. hampsonii*" clade I was isolated from diarrheic gilts and grower pigs in Europe(132, 133) but presently, clade II has not been identified, nor has either clade I or II been diagnosed in cases of mucohemorrhagic diarrhea outside North America. In addition to causing potentially severe production limiting diarrhea, the swine industry should be concerned about the potential for antimicrobial resistance (AMR). The AMR profiles of "*B. hampsonii*" are not well understood, but one report found the tiamulin MIC_{90} of a select number of American isolates were higher than for *B. hyodysenterae*. Tiamulin is one of the most commonly used therapeutic drugs for this and other swine enteric diseases(134) in some countries.

Part B: A global survey of swine veterinarians to identify global threats and solutions

There is clearly no end to the threats that could potentially impact the global swine industry, and history has repeatedly demonstrated that disease events, whether notifiable, transboundary or emerging, will occur on a regular basis. **The emergence of transboundary and emerging diseases is undoubtedly a global issue that requires global solutions**. While I am familiar with hog production in many parts of the world, I am not an expert. In order to make a relevant and global contribution to this topic, it was necessary for me to seek expert opinions from every continent. In late March, I distributed a short questionnaire to about 300 swine veterinarians in the Americas, Europe, Asia, Australia/New Zealand, and South Africa. Most were emailed directly, but third party volunteers forwarded some questionnaires to veterinary colleagues in Asia, S. America and Eastern Europe. The questionnaire was translated into Chinese, Spanish and Portuguese. From March 20th to April 18th I received 162 completed surveys for an overall response rate of approximately 50%. I sincerely thank those who participated, many of which are sitting in this room today. I have collected an incredible number and assortment of comments that will help guide the swine industry. Except for S. Africa and Australia/New Zealand, respondents were reasonably well balanced by continent indicating this is a truly unique collection of ideas from the global swine veterinary community.

The first question asked respondents to ***"List the 3 most significant diseases or pathogens currently threatening the global swine industry"***. Responses were more varied than I had expected, and clustered into top, middle and lower ranked diseases of concern. Three diseases clearly stood out as the top ranked threats: PRRS (including highly pathogenic strains), PED and ASF. The first and second ranked selections by continent were: Asia (PED, PRRS), NA (ASF, PED tied with FMD), EU (ASF, PRRS), SA (PRRS, PED), Africa (PED). The middle ranked diseases of concern were FMD, CSF, FLU, PCVAD in this order. The lower ranked diseases varied widely, and generally include other production limiting, endemic diseases such as *Actinobacillus pleuropneumoniae, M. hyopnemoniae, B. hyodysenteriae, Streptococcus suis*, and others. Rarely selected, but of special interest, were emerging and novel viral pathogens including the RNA viruses noted by several North American respondents, and emergence of methicillin-resistant *Staph aureus* (MRSA), extended spectrum beta-lactamase (ESBL) and multiple drug resistant *B. hyodysenteriae* strains noted by several European respondents.

The second and third questions asked: ***"What should we "start" or "stop" doing in order to substantially decrease the risk of catastrophic emerging, trans-boundary and/or foreign animal diseases at home and globally?".*** I received over 930 open-ended comments and suggestions. While there is insufficient time or space to provide all the details, a brief overview of major themes follows.

When asked what we should "stop" doing, activities pertaining to feed and biosecurity were the most frequent and accounted for about 35% of all suggestions. Pertaining to **feed**, there was clear support for stopping the importation of ingredients from high risk countries, implementing minimum quality control standards, eliminating swill feeding in countries that permit or condone this activity, and discontinuing the feeding any rendered pigs or byproducts back to pigs. **Biosecurity** ranked second highest amongst the "stop themes", and most comments were primarily targeted at producers. Specific suggestions included measures to stop direct and indirect contact between commercial pigs and wildlife including wild boars, stop ignoring widely accepted biosecurity guidelines, stop introducing live animals into production units without quarantine, stop live pig exhibitions, and stop transmission associated with international travellers, visitors and biological souvenirs. Four other themes, **transportation, government responses, trade and industry practices,** also ranked prominently, each accounting for between 9-15% of all suggestions. The need to stop or substantially reduce inter- and trans-continental movement of animals was very clearly conveyed, as was the need to stop using recycled water and improperly cleaned transport vehicles. Similarly, respondents from all continents clearly expressed the need to stop or restrict global trade of live animals, pork meat, semen, offal and other byproducts. There was decisive support for replacing trade of live animals with germlines (embryos, semen). **Government responses** were severely criticized. In particular, there were calls to stop corruption and smuggling of live meat and animals in some continents, and in all continents, respondents expressed the need to stop government inaction on many fronts including surveillance, slow responses, ignoring high risk regions and catastrophic transboundary and emerging diseases. Pertaining specifically to foreign animal diseases, several respondents noted that governments should stop

thinking they have the tools or resources to stop an FAD outbreak or relying solely on the destruction of affected animals in the event of a FAD outbreak. Along the same vein, several respondents pointed out that the swine industry should stop relying on national governments to protect livestock industries from transboundary diseases. In general, respondents were very critical of a number of **industry practices**. Two respondent nicely summed it up by stating: *"we need to be better at stopping what doesn't work"* and *"good-enough isn't"*. Most notable, it was strongly suggested that we rethink the segregated-production paradigm, for example, decreasing the dependence on transportation and mixing, construction of large barns, ultra-high animal density, and hog dense regions. It was categorically suggested the industry should stop being solely motivated by profitability and cost control, and in contrast, develop a culture based on quality and sustainability.

When asked what we should "start" doing, approximately 490 open-ended suggestions were offered. The top ranked theme was biosecurity, accounting for 18% of all suggestions, respectively. Suggestions pertaining to **biosecurity** expectedly pertained to the implementation of improved protocols as discussed above, as well the need for more education for producers and staff. There were calls for greater **collaboration and transparency** specifically amongst governments, laboratories, universities and producers, to enable a more cohesive response to infective disease threats. Unique suggestions included the development of formal policy frameworks and input from sociologists or anthropologists to define factors impeding collaboration. One respondent summarized: "we need to work, think and share globally rather than working in regional or domestic silos". Enhancing **global and regional surveillance** systems were widely suggested, accounting for 12% of respondent comments. This included the development of integrated, active and passive systems incorporating clinical and laboratory data, premise ID, the surveillance of wild boar and animal populations, and the establishment of networks to facilitate the early detection of undiagnosed syndromes or laboratory results that reflect aberrations. It was also suggested we institute world-wide, statutory and transparent reporting of an expanded list of non-notifiable transboundary diseases to the OIE in order to better monitor threats occurring internationally. Moreover, the need for active farm-level serologic surveillance and global serum and tissue repositories was highlighted. Fifteen percent of respondents appealed for greater funding for **research and diagnostics** at regional, national and international levels. International research consortia to coordinate gaps in our understanding of pathogen evolution, genetic resistance, wild boars, epidemiology of transboundary diseases, DIVA vaccine development, and coordinated global control programs were some additional ideas proposed. One respondent recommended the development of a Center of Excellence specific to emerging diseases. Pertaining to **diagnostics**, recommendations included global standardization, enhanced global diagnostic capability especially in less developed nations, faster assay development for novel pathogens, pen-side tests, and more rapid adoption of new molecular diagnostic technologies such as next generation sequencing by commercial laboratories. **One-health** issues were not prominently raised, but several valuable suggestions were made including the need to enforce biosecurity at the inter-species interface, understanding land use and fossil fuel policies and their impact on emergent pathogens at risk of inter-species transmission, and effective public health responses to emergent diseases of swine to avoid a repeat of the "swine flu". The need for more **prudent antimicrobial usage** was also raised by some voicing concerns with continued use of growth promotants, and the relationship between commercial sales and independent veterinary advice. Finally, respondents clearly recognized the **swine industry's direct role** in the control of transboundary and emerging diseases including the need to invest in epidemiology and risk analysis to more clearly understand the most important global threats. Also recommended was the development of contingency strategies with sufficient resources to enable an immediate and substantial response. However, the overarching sentiment specific to the swine industry was the need to start building more **sustainable production systems** and a culture of **quality and accountability**. As one respondent eloquently stated: *"the industry should recognize the difference between "can't" and "won't" when it comes to (re)structuring for long-term sustainability. It is very hard for a commodity industry to operate or think beyond next quarter or next year, but the external forces including societies' constraints on license to operate, changes in consumer attitudes towards meat consumption and animal welfare, and the realities of global trade and economics will force change. Over the next two generations, pork production will need to adapt to practical and geopolitical constraints or it will be displaced to more permissive areas for production, or it will not survive"*.

Part C: Accountabilities in the age of transboundary and emerging diseases

So, what should be done and who is accountable? The purpose of my presentation today is to propose changes, controversial where necessary, that will contribute to the global control of transboundary and emerging diseases. I do not believe we can "stop the next train" but I do believe we can help "stop the next train wreck" if we choose to act (Table 4). There is however, no simple solution. In my opinion, the way we raise pigs today directly contributes to the emergence of transboundary and emerging disease. I am sure that this was not Tom Alexander's vision. Based on the questionnaire results, many believe the industry's sustainability depends on making fundamental changes to how we raise pigs, both in industrialized and in developing nations.

In theory, national governments in industrialized nations will respond vigorously to OIE-Listed diseases that are exotic or have a substantial impact on trade. Responses to OIE-Listed transboundary diseases that do not impact trade may be downloaded to other regional jurisdictions or the swine industry, as is the case with PEDV in Canada and the USA, but I suspect they will be ignored completely. The most important question for all national swine industries to answer is: ***what response would the swine industry expect from our national government if the next catastrophic transboundary disease was FMD, CSF, ASF or another exotic OIE-Listed disease*.** I have no doubt the response will be greater than that which followed the introduction of PEDV in North America, but whether or not it is cohesive and timely is another question. I therefore assign **Accountability #1** to our national governments: *to clearly state what responses would be expected in the event of an introduction of any of the exotic OIE-Listed diseases. Nothing less than a detailed response plan is acceptable. Moreover, when the time comes, do what you say and do it in a cohesive and timely manner.*

This leads me to **Accountability #2,** targeted at swine industries globally: *we must develop realistic expectations regarding government responses to an OIE-Listed exotic disease introduction, and expect "too little, too late" in the event of a non-exotic transboundary disease, unless it has a substantial impact on trade or public health.* For example, the recent decision by the USDA to consider mandatory reporting of PEDV in the US is a year late(135). Non-exotic transboundary diseases area swine industry issues that require industry-driven solutions. Whether or not we think this is right does not matter. The harsh reality is that the tax payer will not, and should not, fix the problems that we have had a hand in making.

This brings me to several more accountabilities targeted at the swine industry, including industry professionals, academics and veterinarians that have influenced its evolution. Over the last 2 decades, pork production has evolved into a mass-producing, cost-cutting, global manufacturing industry driven by managers, bean counters and the next quarterly return. We are in fact, in the food business, where quality and safety are paramount. We deal with multiple, biological systems that interact in a complex ecosystem, whether it is a barn or production flow. As veterinarians, we pretend to understand to host-pathogen interactions, promote Koch's postulates without seriously considering alternative theories of disease causation (Evans'(136), Rivers'(137) , Hill's (138) or Fredericks & Relman's postulates(139)), and work across a multitude of non-traditional disciplines in a "follow the leader" mentality. Our veterinary services are reactionary and profit-driven, often guided more by the products and services we sell than by evidence. **Accountability #3** is therefore targeted at swine veterinarians, all of us; regardless of our career or standing in the profession: *we need to deliver a lot more to solve the complex issues of the day.* We need to think independently and proactively, collaborate across discipline, step back from disciplines in which we do not have the expertise, and truly educate ourselves in the subject matters that are most essential for improving swine health and welfare. We need to think of new ways of delivering veterinary medicine motivated by quality and sustainability, not profitability. We need to think globally, and convince the swine industry to do the same.

Accountability #4 is targeted at the owners and managers of barns and systems: *to develop a more sustainable, quality-driven pork industry*. You have a huge role in preventing the "next train wreck". In my opinion and many others, this will require re-structuring the industry to reduce or eliminate the trans- and inter-continental transport of live animals. Those with past experience in FAD response scenarios will clearly understand the speed at which an infectious disease such as FMD can be transmitted to very distant locations. Yet, having known this for decades, the industrialized pig industry, particularly in North America and Europe, is very dependent on the transcontinental movement of live animals. This is neither a sustainable business practice nor considerate of animal welfare given the understanding that the next catastrophic disease is not a matter of "if" but "when". We must rethink present paradigms regarding multiple site production and the transport of finisher hogs to locations closer to feed and slaughter capacity. We must provide greater distance between units rather than concentrating hog production into geographical hotspots prone to transmission by aerosol and manure. We must help stop the inter-species transmission of pathogenic viruses by no longer raising pigs in close to other livestock, humans, wild animals or birds. While doing this, we must be considerate of cultural norms, the reliance on subsistence agriculture in some parts of the world, and the future demand for quality meat protein. The "next train is coming" whether it be a transboundary pathogen from overseas or an emerging pathogen stemming from inter- or intra-species evolution. Only the producers motivated by quality and sustainability will endure.

In North America, the feed industry has let us down by not maintaining the quality standards necessary for the maintenance of pig health. In Canada, epidemiologic data obtained from the PED outbreaks in Ontario, and subsequent testing performed by the Canadian Food Inspection Agency(140, 141) implicates contaminated plasma proteins as a potential source of PEDV. While manufacturers of plasma protein adamantly dispute this and uphold the quality of their product, the evidence is clear. I, and many others, strongly believe the practice of feeding rendered pigs and pig by-products back to pigs must be banned globally. **Accountability #5** is therefore targeted at the feed industries globally: *to discontinue the importation of animal protein destined for any livestock or pet food, and to find alternative sources of protein suitable for nursery pigs*. **Accountability #6:** *to ban the feeding of rendered pigs and pig by-products back to pigs,* is targeted at all national governments.

Global trade of live pigs is a contentious issue because to stop it would be of great detriment to both the importing and exporting industries and their respective national economies. An outright ban would have clear implications for

countries with swine industries reliant of exporting isoweans or feeder pigs, such as Canada and Denmark. However, I propose that the dissemination of non-exotic transboundary and emerging diseases cannot be stopped unless the trade of live pigs and fresh semen is banned or substantially reduced by very rigid health testing protocols. This is no simple task, but we can make progress in this area. For instance, importation of frozen rather than fresh semen could be immediately regulated by national governments and include mandatory wait periods and molecular testing for pathogens of swine industry concern. This may be inconvenient but would have little impact on the swine industry since semen can be frozen. Secondly, embryo transfer could replace the trade of live animals for breeding purposes. While not convenient with existing technology, developing commercially available cryopreservation techniques enabling embryo washing would make this a feasible alternative in the future. Ironically, I was a co-applicant on a research proposal aimed at developing such technology that was recently rejected by industry on the basis that it was not relevant to industry! **Accountability #7** is therefore targeted at the swine industry and national governments: *to ban the trade of fresh semen and live pigs for breeding purposes, and to invest in research leading to the advancement of cryopreservation techniques for embryos and semen*. Furthermore, tighter regulations must govern the importation of live pigs for feeding purposes with the primary goal of stopping the dissemination of transboundary pathogens from endemic to naïve regions and/or countries. But why stop at there? Although it is impossible to discontinue trade altogether, tighter regulations and/or selective trade restrictions, particularly for by-products from countries with endemic transboundary diseases could be mandated.

Regarding research, before the PED outbreak, it would have taken a lot of convincing and some luck to obtain substantive research funding for PEDV research in North America. Understandably, most funding agencies focus primarily on issues in their back yard. This strategy however, must change because the next intra-species emergent pathogen may be circulating within the global industry today, and the next inter-species emergent pathogen, transboundary or exotic disease may already be at the nearest international airport, seaport or imported product or ingredient. Research in foreign animal diseases is expensive and requires BSL3 facilities in countries where the pathogen is exotic. Research in emerging diseases is difficult and often results in dead ends. Multidisciplinary international teams that work collaboratively are needed to make real progress in this area. Moreover, research progress in these complex topics is measured in years and it requires multi-year funding programs, not renewable 6 to 12-month grants. **Accountability #8** is therefore targeted at research funding agencies: *to work collaboratively with other global agencies to provide multi-year (3-5y) support to inter-disciplinary, international research consortia focused on finding long-term solutions to transboundary, foreign-animal and emergent pathogens, that threaten locally and abroad*.

The need for enhanced regional and national surveillance is clear. Limitations to this are many, including lack of coordination and funding, dissimilar information technology (IT) systems, and importantly, lack of or unwillingness to be fully transparent at all levels from governments to farms. Internationally, surveillance is inseparable from politics, and there are real and perceived trade risks associated with full transparency and being the first to announce a novel or emergent transboundary disease. That being said, **Accountability #9** is targeted at national swine industries: *to work collaboratively with national governments to develop robust and meaningful surveillance systems, incorporating premise identification and mapping, with the goal of early detection and containment of transboundary and emerging diseases of industry importance. Such systems may be varied in design, but should incorporate syndromic and laboratory data, have compatible information technology (IT) systems, enable rapid national reporting and be mandatory and transparent*.

Antimicrobial growth promotants (AGP) for the purpose of improving growth and efficiency and justified on the bases of a cost:benfit ratio are no longer socially acceptable. Whether or not you believe AGP lead to antimicrobial resistance (AMR) is no longer the issue. The issue is that they may be part of the problem, and we, in addition to our human health counterparts, need to be part of the solution. Some believe we are returning to a pre-antibiotic age; *An Antibiotic Apocalypse*(142). While this may be a sensational ploy propagated by media, the number of multi-drug resistant (MDR) bacteria is increasing in human and vet medicine. In fact, the emergence of MRSA, ESBL and multiple drug resistant *B. hyodysenteriae* were raised by a number of colleagues in Europe, South America and Asia (notably, not North America). Even if AGP are never proven to be causally associated with AMR, the swine industries in my opinion, must be accountable for ensuring their actions are not contributory. I commend the national governments in Europe that have shown leadership by establishing programs to monitor defined daily doses for animals (DDDA, DADD), but hope that maintenance of animal welfare will be considered when setting lower DADD limits. **Accountability #10** is therefore targeted at the swine industries globally: *to establish credibility by voluntarily banning antimicrobial growth promotants (if not already done) and to establish voluntary DDDA programs.* If we do this proactively, swine veterinarians and producers are more likely to play a significant role in the establishment of lower DDDA limits, rather than being mandated by politicians and health care professionals who have a poor understanding of the livestock industries.

Table 4. Accountabilities to help prevent and control transboundary and merging diseases of swine

#	Who is accountable	What should be done
1	National governments	Clearly state what responses would be expected in the event of an introduction of any of the exotic OIE-Listed diseases, and when the time comes, do what you say and do it in a cohesive and timely manner.
2	National swine industries globally	Develop realistic expectations regarding government responses to an OIE-Listed exotic disease introduction, and expect "too little, too late" in the event of a non-exotic transboundary disease, unless it has a substantial impact on trade or public health.
3	Swine veterinarians	Deliver a lot more to solve the complex issues of the day.
4	Owners and managers of barns and systems	Develop a more sustainable, quality-driven pork industry.
5	Feed industries globally	Discontinue the importation of animal protein destined for any livestock or pet food, and find alternative sources of protein suitable for nursery pigs.
6	National governments globally	Ban the feeding of rendered pigs and pig by-products back to pigs.
7	Swine industry and national governments	Ban the trade of fresh semen and live pigs for breeding purposes, and invest in research leading to the advancement of cryopreservation techniques for embryos and semen.
8	Research funding agencies	Work collaboratively with other global agencies to provide multi-year support to inter-disciplinary, international research consortia focused on finding solutions to transboundary, foreign-animal and emergent pathogens, that threaten locally and abroad.
9	National swine industries globally	Work collaboratively with national governments to develop robust and meaningful surveillance systems, incorporating premise identification and mapping, with the goal of early detection and containment of transboundary and emerging diseases of industry importance.
10	Swine industries globally (where AGP are not already banned)	Establish credibility by voluntarily banning antimicrobial growth promotants (AGP), if not already done, and to establish voluntary DDDA programs.

Conclusions

This brings me to the end of a lengthy review and commentary on issues pertaining to the sustainability of the global swine industry. In conclusion, I would like to emphasize that avoiding the next "train wreck" will require a comprehensive, global and evidence-based response, and fundamental changes in the way we raise pigs. Input from all levels of government and industry will be required. However, the transboudary and emerging diseases are fundamentally an industry problem. Globalization, the present structure, and common swine industry practices have contributed to their emergence and dissemination. Although we should welcome government support, we should not expect it. It will take global leadership and commitment. Tough decisions will be needed as well as clear vision and the art of persuasion. Some will adapt and succeed, some will exit the industry, and others should. Given the breadth of training and experience and an insatiable appetite for knowledge, there is no group more suited than swine veterinarians to lead this task. Are you ready to step up?

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References

1. Taylor DJ, et al. 1971. *Br. Vet. J.* 127:58-61.
2. Clifton-Hadley FA, et al. 1981. *Pig Veterinary Society Proceedings.* 8:8-17.
3. Alexander TJL, et al. 1959. *Can. J. Comp. Med.* 23:316-319.
4. Alexander TJL. 1978. *Proceedings. 5th World International Pig Veterinary Society Congress.*:KA30.
5. Alexander TJL, et al. 1962. *Can. Vet. J.* 3:299-306.
6. Alexander TJL, et al. 1980. *Vet. Rec.* 106:114-119.
7. FAO. Available at http://www.fao.org/ag/againfo/programmes/en/empres/diseases.asp Accessed: April 10, 2014.
8. OIE. Available at http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2014/ Accessed: April 10, 2014.
9. OIE. Available at http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/Diseasedistributionmap Accessed: March 30, 2014.
10. Jones KE, et al. 2008. *Nature (London).* 451:990-993.
11. Woolhouse M, et al. 2007. *Crit. Rev. Microbiol.* 33:231-242.
12. Pulliam JRC, et al. 2009. *J. Infect. Dis.* 199:565-568.
13. Dopazo J, et al. 1988. *Proc. Natl. Acad. Sci. U. S. A.* 85:6811-6815.
14. Alexandersen S. *Proc. West. Can. Assoc. Swine Vet. Conf.* October 15-16, 2010:21-33.
15. Davies PR. 2012. *Transbound. Emerg. Dis.* 59:18-26.
16. Pearce-Duvet JMC. 2006. *Biological Reviews.* 81:369-382.
17. Sanchez-Vizcaino JM, et al. 2013. *Vet. Microbiol.* 165:45-50.
18. Beltran-Alcrudo D, et al. African Swine Fever in the Caucasus. EMPRES watch Rome. FAO.
19. Vorotnikov V. Available at http://www.globalmeatnews.com/Industry-Markets/ASF-situation-in-Russia-continues-to-worsen Accessed: March 31, 2014.
20. EuropeanFoodSafetyAuthority. 2014. *EFSA Journal.* 12(3):3616:3616/doi:3610.2903.
21. Song D, et al. 2012. *Virus Genes.* 44:167-175.
22. Chen Q, et al. 2014. *J. Clin. Microbiol.* 52:doi: 10.1128/JCM.02820-02813
23. Huang YW, et al. 2013. *mBio.* 4:e00737-00713.
24. Stevenson GW, et al. 2013. *J. Vet. Diagn. Invest.* 25:649-654.
25. Woo PCY, et al. 2012. *J. Virol.* 86:3995-4008.
26. CFIA. Available at http://www.inspection.gc.ca/animals/terrestrial-animals/diseases/other-diseases/ped/eng/1392762503272/1392762576176 Accessed: April 4, 2014.
27. Meng XJ. 2012. *Transbound. Emerg. Dis.* 59:85-102.
28. Meng XJ. Swine Hepatitis E Virus: Cross-Species Infection and Risk in Xenotransplantation. In: Salomon D and Wilson C, eds. *Xeno-transplantation* vol. 278. Springer Berlin Heidelberg, 2003:p.185-216.
29. Williams TPE, et al. 2001. *J. Clin. Microbiol.* 39:3040-3046.
30. Halbur PG, et al. 2001. *J. Clin. Microbiol.* 39:918-923.
31. Meng XJ, et al. 2002. *J. Clin. Microbiol.* 40:117-122.
32. Drobeniuc J, et al. 2001. *J. Infect. Dis.* 184:1594-1597.
33. Smith JL. 2001. *J. Food Prot.* 64:572-586.
34. Feagins AR, et al. 2008. *Int. J. Food Microbiol.* 123:32-37.
35. Li T, et al. 2005. *Emerg. Infect. Dis.* 11:1958-1960.
36. Bigarre L, et al. 2005. *J. Gen. Virol.* 86:631-635.
37. Krakowka S, et al. *Proc. Proc. Int Pig Vet Soc Cong* June 22-26, 2008 99.
38. Kekarainen T, et al. 2006. *J. Gen. Virol.* 87:833-837.
39. Lee S, et al. 2010. *J. Vet. Diagn. Invest.* 22:261-264.
40. Krakowka S, et al. 2008. *Am. J. Vet. Res.* 69:1601-1607.
41. Barr JA, et al. 2012. *J. Gen. Virol.* 93:2590-2594.
42. Love RJ, et al. 2001. *Aust. Vet. J.* 79:192-198.
43. Bowden TR, et al. 2012. *J. Gen. Virol.* 93:1007-1016.
44. Finlaison DS, et al. 2009. *Vet. Microbiol.* 136:259-265.
45. Kirkland PD, et al. 2007. *Virus Res.* 129:26-34.
46. Finlaison DS, et al. 2012. *Vet. Microbiol.* 160:245-250.
47. Abrahante JE, et al. 2012. *Transbound. Emerg. Dis.* November 27, 2012:doi:10.1111.
48. Luby SP. 2013. *Antiviral Res.* 100:38-43.
49. Chua KB. 2003. *J. Clin. Virol.* 26:265-275.
50. Kulkarni DD, et al. 2013. *Indian J. Virol.* 24:398-4508.
51. Halpin K, et al. 2011. *Am. J. Trop. Med. Hyg.* 85:946-951.
52. Rahman MA, et al. 2012. *Vector Borne Zoonotic Dis.* 12:65-72.
53. Luby SP, et al. 2006. *Emerg. Infect. Dis.* 12:1888-1894.
54. Luby SP, et al. 2009. *Emerg. Infect. Dis.* 15:1229-1235.
55. Sejvar JJ, et al. 2007. *Ann. Neurol.* 62:235-242.

56. Conlan JV, et al. 2012. *Am. J. Trop. Med. Hyg.* 86:1077-1084.
57. Hayman DTS, et al. 2011. *PLoS One.*e25256.
58. Nfon CK, et al. 2013. *PLoS One.* 8:e61904.
59. Barrette RW, et al. 2009. *Science.* 325:204-206.
60. Sayama Y, et al. 2012. *BMC Vet. Res.* 8:82.
61. Kobinger GP, et al. 2011. *J. Infect. Dis.* 204:200-208.
62. Marsh GA, et al. 2011. *J. Infect. Dis.* 204:S804-S809
63. Cheung AK, et al. 2010. *Arch. Virol.* 155:801-806.
64. Xiao CT, et al. 2013. *PLoS One.* 8:e65312.
65. Lau SKP, et al. 2008. *J. Gen. Virol.* 89:1840-1848.
66. Adlhoch C, et al. 2010. *Virol J.* 7:(25July2010).
67. Cadar D, et al. 2011. *Arch. Virol.* 156:2233-2239.
68. McQuilken A, et al. *Proc. 6th International Symposium on Emerging and Re-emerging Pig Diseases.* June 12-15, 2011
69. Yu J, et al. 2013. *Virol J.* 10:doi: 10.1186/1743-1422X-1110-1139.
70. McMenamy MJ, et al. 2013. *Vet. Microbiol.* 164:298-298.
71. Saif LJ, et al. 1980. *J. Clin. Microbiol.* 12:105-111.
72. Guo M, et al. 1999. *J. Virol.* 73:9625-9631.
73. Reuter G, et al. 2010. *J. Clin. Microbiol.* 48:363-368.
74. Guo M, et al. 2001. *J. Virol.* 75:9239-9251.
75. Bank-Wolf BR, et al. 2010. *Vet. Microbiol.* 140:204-212.
76. Machnowska P, et al. 2014. *Vet. Microbiol.* 168:60-68.
77. Scheuer KA, et al. 2013. *J. Clin. Microbiol.* 51:2344-2353.
78. Shen Q, et al. 2012. *BMC Vet. Res.* 8:doi:10.1186/1746-6148-1188-1155.
79. Luo Z, et al. 2011. *Vet. Microbiol.* 149:316-323.
80. Reuter G, et al. 2012. *Arch. Virol.* 157:1143-1147.
81. Bridger JC. 1980. *Vet. Rec.* 107:532-533.
82. Lan D, et al. 2011. *Arch. Virol.* 156:1869-1875.
83. Lager KM, et al. 2012. *J. Vet. Diagn. Invest.* 24:1177-1179.
84. Mor SK, et al. 2012. *J. Vet. Diagn. Invest.* 24:1064-1067.
85. Pignatelli J, et al. 2009. *Virus Res.* 143:33-43.
86. Ge F, et al. 2013. *Arch. Virol.* 158:2227-2231.
87. Pignatelli J, et al. 2010. *Vet. Microbiol.* 146:260-268.
88. Reuter G, et al. 2008. *Emerg. Infect. Dis.* 14:1968-1970.
89. Reuter G, et al. 2011. *Rev. Med. Virol.* 21:32-41.
90. Profio Fd, et al. 2013. *Res. Vet. Sci.* 95:782-785.
91. Dufkova L, et al. 2013. *Arch. Virol.* 158:549-558.
92. Harsha V, et al. 2013. *Virus Genes.* 46:551-553.
93. Ribeiro J, et al. 2013. *Trop. Anim. Health Prod.* 45:1757-1762.
94. Park S, et al. 2010. *Arch. Virol.* 155:1803-1811.
95. Shan TL, et al. 2011. *J. Virol.* 85:11697-11708.
96. Khamrin P, et al. 2009. *Emerg. Infect. Dis.* 15:2075-2076.
97. Okitsu S, et al. 2012. *Virus Genes.* 44:253-257.
98. Reuter G, et al. 2010. *Emerg. Infect. Dis.* 16:696-698.
99. Truszczynski M, et al. 2012. *Zycie Weterynaryjne.* 87:94-96.
100. Bartolo Id, et al. 2012. *Emerg. Infect. Dis.* 18:1282-1289.
101. Garcia LAT, et al. 2012. *Journal of Water and Health.* 10:445-452.
102. Abid HN, et al. 1984. *Vet. Med. Small Anim. Clin.* 79:105-107, 110.
103. Weikel J, et al. 2003. *Tierarztliche Praxis. Ausgabe G, Grosstiere/Nutztiere.* 31:210-213.
104. Kadoi K, et al. A porcine adenovirus isolated from young pig suffering from hemorrhagic diarrhea. Bangkok, Thailand: Faculty of Veterinary Science, Chulalongkorn University, 1994.
105. Kadoi K, et al. 1995. *J. Basic Microbiol.* 35:195-204.
106. Tucker AW, et al. 2003. *Xenotransplantation.* 10:343-348.
107. McMahon KJ, et al. 2006. *Vet. Microbiol.* 116:60-68.
108. Ehlers B, et al. The porcine lymphotropic herpesviruses: emerging pathogens in xenotransplantation? *Trends in emerging viral infections of swine*. Iowa State Press, Ames,. 2002;235-239
109. Huang CA, et al. 2001. *Blood.* 97:1467-1473.
110. Balasubramanian G, et al. 2010. *Infect. Genet. Evol.* 10:511-516.
111. Sachsenroder J, et al. 2012. *PLoS One.* 7:e34361.
112. Lau P-N, et al. 2011. *Emerg. Infect. Dis.* 17:2328-2330.
113. Kongsted H, et al. 2013. *BMC Vet. Res.* 9:doi: 10.1186/1746-6148-1189-1206.

114. Wallgren P, et al. *Proc. 22nd International Pig Veterinary Society Congress* June 10-13, 2012:75.
115. Melin L, et al. *Proc. 21st International Pig Veterinary Society Congress.* July 18-21, 2010:290.
116. Wallgren P, et al. *Proc. 22nd International Pig Veterinary Society Congress.* June 10-13, 2012:76.
117. Gauvreau H, et al. *Proc. West. Can. Assoc. Swine Vet. Conf.* October 2-3, 2008:47-54.
118. Dufresne L, et al. *Proc. Allen D. Leman Swine Conference* September 21-23, 2008:79-85.
119. O'Sullivan TL, et al. 2014. *J. Swine Health Prod.* 22:24-28.
120. Segales J, et al. 2012. *Vet. Rec.* 170:499.
121. Huang Y, et al. 2011. *J. Swine Health Prod.* 19:340-344.
122. Huang Y. Is porcine periweaning failure-to-thrive syndrome an infectious disease? [PhD Thesis]. Saskatoon, Canada: University of Saskatchewan, 2014.
123. Huang Y, et al. 2012. *J. Vet. Diagn. Invest.* 24:96-106.
124. Moeser AJ, et al. 2012. *Res. Vet. Sci.* 93:975-982.
125. Harding JCS, et al. *Proc. Int. Pig Vet. Soc. Congr.* July 18-21, 2010:740.
126. Primus A, et al. *Proc. Amer. Assoc. Swine Vet. Conf.* March 5-8, 2011:109-110.
127. Burrough ER, et al. 2012. *J. Vet. Diagn. Invest.* 24:1025-1034.
128. Rubin JE, et al. 2013. *PLoS One.* 8:e57146.
129. Chander Y, et al. 2012. *J. Vet. Diagn. Invest.* 24:903-910.
130. Rubin JE, et al. 2013. *Microb. Ecol.* 66:813-822.
131. Martínez-Lobo FJ, et al. 2013. *PLoS One.* 8:e82626.
132. Mahu M, et al. 2013. *Vet. Rec.*:in press.
133. Rohde J, et al. 2014. *Vet. Microbiol.* 168:432-435.
134. Clothier KA, et al. *J. Vet. Diagn. Invest.* 23:1140-1145.
135. Polansek T, et al. Available at http://uk.reuters.com/article/2014/04/14/us-usa-hogs-virus-idUKBREA3D1SI20140414 Accessed: April 18, 2014.
136. Evans AS. 1976. *Yale J. Biol. Med.* 49:175-195.
137. Rivers TM. 1937. *J. Bacteriol.* 33:1-12.
138. Hill AB. 1965. *Proc. R. Soc. Med.* 58:295-300.
139. Fredericks DN, et al. 1996. *Clin. Microbiol. Rev.* 9:18-33.
140. CFIA. Available at http://www.inspection.gc.ca/animals/terrestrial-animals/diseases/other-diseases/ped/2014-03-03/eng/1393891410882/1393891411866 Accessed: April 5, 2014.
141. CFIA. Available at http://www.inspection.gc.ca/animals/terrestrial-animals/diseases/other-diseases/ped/2014-02-18/eng/1392762739620/1392762820068 Accessed: April 5, 2014.
142. Ash C. 1996. *Trends Microbiol.* 4:371-372.

Genetic diversity of *M. hyopneumoniae* isolates of abattoir pigs

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Mycoplasma hyopneumoniae is present in the majority of swine herds around the world (Kobisch and Friis, 1996). It is the primary agent involved in porcine enzootic pneumonia (EP). This condition is associated with respiratory disease and reduced productivity in pigs causing severe economic losses to the swine industry. The importance of *M. hyopneumoniae* is also linked to its ability to increase the severity of infections caused by viruses (Opriessnig et al., 2004), as well as bacteria (Marois et al., 2009; Palzer et al., 2008). When these pathogens are in co-infection with *M. hyopneumoniae*, the severity of the respiratory lesions is increased. Moreover, *M. hyopneumoniae* can enhance the quantity and the persistence of PCV2 antigens and can increase the incidence of postweaning multisystemic wasting syndrome (PMWS) in swine (Opriessnig et al., 2004; Thacker et al., 2001).

Isolation of *M. hyopneumoniae* is known to be fastidious due to the long incubation period needed for its culture (Friis, 1975; Marois *et al*., 2007) and to the frequent co-isolation of *Mycoplasma hyorhinis*, a normal flora inhabitant of the upper respiratory tract of young pigs (Kobisch and Friis, 1996). *M. hyorhinis* has also been involved in a variety of diseases in swine including enzootic pneumonia and respiratory disease in general (Kawashima et al., 1996; Kobisch and Friis, 1996; Lin et al., 2006).

At the genomic level, high heterogeneity has been demonstrated between *M. hyopneumoniae* isolates throughout the world using various typing techniques such as random amplified polymorphic DNA (RAPD) (Artiushin and Minion, 1996), amplified fragment length polymorphism (AFLP) (Kokotovic *et al*., 1999) and pulsed-field gel electrophoresis (PFGE) (Stakenborg et al., 2005). However, the RAPD technique and the analysis of polyserine repeats have weak reproducibility rates among different laboratories, and the AFLP and PFGE techniques are considered fastidious. Thus, new techniques based on DNA amplification have been developed in the last few years. The multiple loci variable number of tandem repeats (VNTR) analysis (MLVA) and the PCR combined with restricted fragments length polymorphism (PCR-RFLP) are two methods that can be easily performed, are reproducible and have a high discriminatory power (Marois-Créhan et al., 2012; Stakenborg et al., 2006; Vranckx et al., 2011). Recently, a MLVA assay was described as a tool to differentiate *M. hyopneumoniae* strains in samples from the respiratory tract without prior cultivation (Vranckx *et al*., 2011). Previous studies have shown genetic heterogeneity between isolates from different farms (Mayor et al., 2007; Nathues et al., 2011; Stakenborg et al., 2005). However, other reports have shown both genetic heterogeneity and homogeneity between isolates from the same herds (Maes *et al*., 2008; Marois-Créhan *et al*., 2012). Field isolates of *M. hyopneumoniae* have also shown virulence variability (Vicca *et al*., 2003).

Actually, little is known about *M. hyopneumoniae* isolates found in Canada. The aim of this study was to evaluate the genetic diversity of *M. hyopneumoniae* isolated from single or mixed infections from abattoir pigs. A total of 160 swine lungs with lesions suggestive of enzootic pneumonia originating from 48 different farms were recovered from two slaughterhouses and submitted for gross pathology. The pneumonic lesion scores ranged from 2% to 84%. Eighty nine percent of the lungs (143/160) were positive for *M. hyopneumoniae* by realtime PCR whereas 10% (16/160) and 8.8% (14/160) were positive by PCR for *M. hyorhinis* and *M. flocculare*, respectively. By culture, only 6% of the samples were positive for *M. hyopneumoniae* (10/160). The *M. hyopneumoniae* isolate and mixed cultures of *M. hyorhinis* and *M. hyopneumoniae* showed relatively low minimal inhibitory concentrations against the antibiotics tested (Table 1). No resistance genes or point mutations were found in isolates with slightly higher MICs. This is likely indicative of an absence of acquired antibiotic resistance.

Among the selected *M. hyopneumoniae*-positive lungs (n = 25), 9 lungs were co-infected with *M. hyorhinis*, 9 lungs with PCV2, 2 lungs with PRRSV, 12 lungs with *S. suis* and 10 lungs with *P. multocida* (Table 2). MLVA and PCR-RFLP clustering of *M. hyopneumoniae* revealed that analyzed strains were distributed among three and five clusters respectively, regardless of severity of lesions, indicating that no cluster is associated with virulence. However, strains missing a specific MLVA locus showed significantly less severe lesions and lower numbers of bacteria. MLVA and PCR-RFLP analyses also showed a high diversity among field isolates of *M. hyopneumoniae* with a greater homogeneity within the same herd. Almost half of the field isolates presented less than 55% homology with selected vaccine and reference strains.

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Table 1. MICs of *M. hyorhinis* and *M. hyopneumoniae* cultures

Antimicrobial agent	Number of cultures with a MIC (µg/ml) of:																						
	≤ 0.125	**≤ 0.25**	**0.25**	**≤ 0.5**	**0.5**	**≤ 1**	**1**	**> 1**	**2**	**> 2**	**≤ 4**	**4**	**≤ 8**	**8**	**> 8**	**16**	**> 16**	**32**	**> 32**	**≤ 2/38**	**> 2/38**	**64**	**> 64**	**≤ 256**	**> 256**
Ampicillin																	**10**								
Ceftiofur															**10**										
Chlortetracycline				3			**2**		4			1													
Clindamycin		**10**																							
Danofloxacin	**10**																								
Enrofloxacin	**10**																								
Florfenicol		**4**			6																				
Gentamicin						**5**			5																
Neomycin											**5**			2		3									
Oxytetracycline				**8**			2																		
Penicillin															**10**										
Spectinomycin													**10**												
Sulfadimethoxine																									**10**
Tiamulin				**10**																					
Tilmicosin											**10**														
Trimethoprim/Sulfas.																					**10**				
Tulathromycine							**10**																		
Tylosine				**8**			1		1																

Numbers in bold represents the MICs of the pure culture of *M. hyopneumoniae*
Gray areas, concentrations not tested

Table 2. Severity of lesions, quantification of *M. hyopneumoniae* in lungs with lesions suggestive of EP with or without other pathogens in abattoir pigs

Lung identification number	Severity of lesions (%)	*M. hyopneumoniae* culture [a]	*M. hyopneumoniae* quantification (genome/mL)	*M. hyorhinis*	PRRSV	PCV 2	*S. suis*	*P. multucida*	*H. parasuis*	*APP*	*A. suis*
#101	69	*Mhp/Mhr*	$9.20x10^6$	+	-	-	+	+	-	-	-
#105	60	*Mhp/Mhr*	$1.20x10^9$	+	+	-	-	-	-	-	-
#112	57	*Mhp/Mhr*	$4.16x10^8$	+	-	-	+	+	-	-	-
#122	41	*Mhp*	$3.01x10^8$	-	-	-	-	+	-	-	-
#119	20	*Mhr*	$1.14x10^6$	-	-	-	+	+	-	-	-
#120	5	*Mhr*	$1.41x10^6$	-	-	-	+	-	-	-	-
#123	17	*Mhp/Mhr*	$3.25x10^7$	+	-	-	+	+	-	-	-
#125	24	*Mhp/Mhr*	$1.59x10^9$	+	-	-	+	-	-	-	-
#127	23	*Mhp/Mhr*	$9.87x10^7$	+	-	-	-	+	-	-	-
#132	14	*Mhp/Mhr*	$1.90x10^8$	+	-	+	+	-	-	-	-
#135	22	*Mhp/Mhr*	$9.45x10^8$	+	-	+	-	-	-	-	-
#149	17	*Mhp/Mhr*	$5.49x10^8$	+	-	+	-	+	-	-	-
#007	14	-	$3.01x10^7$	-	-	-	-	-	-	-	-
#014	44	-	$1.82x10^6$	-	-	-	-	-	-	-	-
#021	25	-	$6.74x10^7$	-	-	+	-	-	-	-	-
#028	33	-	$3.16x10^8$	-	-	+	+	+	-	-	-
#035	22	-	$1.26x10^7$	-	-	-	+	-	-	-	-
#042	27	-	$5.09x10^7$	-	-	+	-	-	-	-	-
#049	22	-	$1.02x10^8$	-	-	-	+	-	-	-	-
#056	45	-	$1.03x10^8$	-	+	+	-	+	-	-	-
#063	8	-	$4.40x10^8$	-	-	-	-	-	-	-	-
#070	42	-	$4.62x10^8$	-	-	+	-	+	-	-	-
#077	25	-	$2.02x10^6$	-	-	+	+	-	-	-	-
#084	62	-	$1.12x10^8$	-	-	-	-	-	-	-	-
#091	48	-	$2.10x10^8$	-	-	-	+	-	-	-	-

Results for *M. hyorhinis*, PRRSV and PCV2 are from PCR testing whereas those for *S. suis*, *H. parasuis*, *P. multocida*, *A. suis* and *A. pleuropneumoniae* are from traditional bacteriological culture.
[a] *Mhp*: *M. hyopneumoniae; Mhr*: *M. hyorhinis;* shifted cultures were confirmed by multiplex PCR
PRRSV : Porcine reproductive and respiratory syndrome virus; PCV2 : Porcine circovirus type 2; *APP* : *Actinobacillus pleuropneumoniae*

References

- Artiushin, s., minion, f.c., 1996. Arbitrarily primed pcr analysis of mycoplasma hyopneumoniae field isolates demonstrates genetic heterogeneity. Int j syst bacteriol 46, 324-328.
- Friis, n.f., 1975. Some recommendations concerning primary isolation of mycoplasma suipneumoniae and mycoplasma flocculare a survey. Nord vet med 27, 337-339.
- Kawashima, k., yamada, s., kobayashi, h., narita, m., 1996. Detection of porcine reproductive and respiratory syndrome virus and mycoplasma hyorhinis antigens in pulmonary lesions of pigs suffering from respiratory distress. J comp pathol 114, 315-323.
- Kobisch, m., friis, n.f., 1996. Swine mycoplasmoses. Rev sci tech 15, 1569-1605.
- Kokotovic, b., friis, n.f., jensen, j.s., ahrens, p., 1999. Amplified-fragment length polymorphism fingerprinting of mycoplasma species. Journal of clinical microbiology 37, 3300-3307.
- Lin, j.h., chen, s.p., yeh, k.s., weng, c.n., 2006. Mycoplasma hyorhinis in taiwan: diagnosis and isolation of swine pneumonia pathogen. Vet microbiol 115, 111-116.
- Maes, d., segales, j., meyns, t., sibila, m., pieters, m., haesebrouck, f., 2008. Control of mycoplasma hyopneumoniae infections in pigs. Vet microbiol 126, 297-309.
- Marois, c., gottschalk, m., morvan, h., fablet, c., madec, f., kobisch, m., 2009. Experimental infection of spf pigs with actinobacillus pleuropneumoniae serotype 9 alone or in association with mycoplasma hyopneumoniae. Vet microbiol 135, 283-291.

- Marois, c., le carrou, j., kobisch, m., gautier-bouchardon, a.v., 2007. Isolation of mycoplasma hyopneumoniae from different sampling sites in experimentally infected and contact spf piglets. Vet microbiol 120, 96-104.
- Marois-crehan, c., fablet, c., devendec, l.l., poëzevara, t., tocqueville, v., gautier-bouchardon, a.v., madec, f., kobisch, m. 2012. Molecular typing of 241 mycoplasma hyopneumoniae strains isolated in france by real-time taqman pcr assay, pulsed-field gel electrophoresis and multiple-locus variable-number tandem-repeat analysis. In: 19th congress of the international organisation of mycoplasmology, toulouse, france.
- Mayor, d., zeeh, f., frey, j., kuhnert, p., 2007. Diversity of mycoplasma hyopneumoniae in pig farms revealed by direct molecular typing of clinical material. Vet res 38, 391-398.
- Nathues, h., beilage, e.g., kreienbrock, l., rosengarten, r., spergser, j., 2011. Rapd and vntr analyses demonstrate genotypic heterogeneity of mycoplasma hyopneumoniae isolates from pigs housed in a region with high pig density. Vet microbiol 152, 338-345.
- Opriessnig, t., thacker, e.l., yu, s., fenaux, m., meng, x.j., halbur, p.g., 2004. Experimental reproduction of postweaning multisystemic wasting syndrome in pigs by dual infection with mycoplasma hyopneumoniae and porcine circovirus type 2. Vet pathol 41, 624-640.
- Palzer, a., ritzmann, m., wolf, g., heinritzi, k., 2008. Associations between pathogens in healthy pigs and pigs with pneumonia. Vet rec 162, 267-271.
- Stakenborg, t., vicca, j., butaye, p., maes, d., peeters, j., de kruif, a., haesebrouck, f., 2005. The diversity of mycoplasma hyopneumoniae within and between herds using pulsed-field gel electrophoresis. Vet microbiol 109, 29-36.
- Stakenborg, t., vicca, j., maes, d., peeters, j., de kruif, a., haesebrouck, f., butaye, p., 2006. Comparison of molecular techniques for the typing of mycoplasma hyopneumoniae isolates. J microbiol methods 66, 263-275.
- Thacker, e.l., thacker, b.j., janke, b.h., 2001. Interaction between mycoplasma hyopneumoniae and swine influenza virus. J clin microbiol 39, 2525-2530.
- Vicca, j., stakenborg, t., maes, d., butaye, p., peeters, j., de kruif, a., haesebrouck, f., 2003. Evaluation of virulence of mycoplasma hyopneumoniae field isolates. Vet microbiol 97, 177-190.
- Vranckx, k., maes, d., calus, d., villarreal, i., pasmans, f., haesebrouck, f., 2011. Multiple-locus variable-number tandem-repeat analysis is a suitable tool for differentiation of mycoplasma hyopneumoniae strains without cultivation. J clin microbiol 49, 2020-2023.

Transmission of influenza A virus in swine populations

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Introduction

Influenza A virus (IAV) remains one of the most important respiratory infectious diseases in humans and animals. In swine, IAV causes respiratory disease characterized by anorexia, fever, sneezing, coughing, rhinorrhea and lethargy and the febrile state in pregnant animals can lead to abortions (Karasin et al., 2000, Olsen et al., 2006). The disease is characterized by low mortality but high morbidity and decreased growth performance which results in increased pig weight variation. Besides the effects on animal health, IAV is an important zoonotic pathogen and pigs can be a reservoir and a source of novel reassortants (Ma et al., 2009), including viruses of pandemic potential. Therefore IAV has implications for both animal and public health, and understanding transmission of IAV in animal populations is crucial to prevent zoonotic infections.

Transmission of IAV is complex to say the least. Influenza virus was first recognized as a viral agent causing respiratory disease in pigs in 1918 (Koen 1919, Shope 1931). For many years, swine influenza viruses in the USA remained relatively stable until 1998. Subsequently, new strains, new subtypes and multiple reassortant viruses have been identified in pigs in North America (Olsen et al., 2002; Webby et al., 2004). The new reassortant viruses contained genetic components derived from both human and avian species, which resulted in new strains that are difficult to control. Unfortunately, the detection of novel strains continues today, and with it the challenges to control influenza.

Transmission of influenza in pigs

The general routes of influenza virus transmission include aerosol, large droplet, and direct contact with secretions of infected individuals or contaminated fomites (Tellier, 2006). Influenza virus transmission via direct contact with infected pigs has been observed in many experimental studies and is thought to be a major transmission route (Shope, 1931; Brookes et al., 2009; Lange et al., 2009). Both sick and subclinically infected pigs likely play a large role in the transmission of influenza virus within and between swine herds, highlighting the importance of controlled animal movement and practices to minimize the transmission of infectious agents. Influenza virus in pigs is not transmitted through semen.

Influenza indirect transmission

Indirect transmission of influenza virus is also assumed to take place in field settings. Water contaminated with bird feces has been implicated as a source of influenza virus in several swine outbreaks involving avian origin viruses (Karasin et al., 2000; Karasin et al., 2004; Ma et al., 2007). Routes of infection in wild pigs are not well known but exposure is likely related to contact with either wild bird droppings, contaminated water or access to commercial pigs. Transmission of influenza virus via other indirect routes such as aerosols and fomites have only had limited study in pig settings.

Influenza virus has been detected in air samples from rooms of experimentally infected pigs (Loeffen et al., 2011; Corzo et al., 2012; Corzo et al., 2013), in the exhaust air from infected farms and in air samples collected at one mile from an infected farm (Corzo et al., 2013b) highlighting the potential for aerosol transmission in pigs and between farms. IAV has also been detected and isolated from air samples collected at swine predominant live animal markets and agricultural fairs in North America (Torremorell personal communication). Pig farm proximity to turkey flocks has been associated with turkey flock seropositivity to swine-origin influenza virus (Corzo et al., 2012b). Additionally, IAV has also been detected in aerosols from experimentally infected pigs with maternal immunity suggesting that aerosol transmission within farrowing rooms could be a possibility (Corzo et al., 2012). In humans, mathematical models have suggested that the airborne route may be the dominant route of influenza transmission (Atkinson and Wein, 2008).

Transmission via fomites also plays a role in the spread of influenza virus. Recently, Allerson et al., (2013c) showed transmission of influenza virus between an infected population and a population of naïve pigs when study personnel moved between rooms even after following biosecurity procedures including hand sanitation, change of coveralls and change of boots. IAV could be transmitted in both low and medium biosecurity settings.

Transmission of influenza virus through pig transport has not been granted much attention until recently. Long distance pig movement has been implicated in the spatial dissemination of influenza viruses of human origin from swine production areas into the Midwest US swine population (Nelson et al., 2011). Additionally, pig transport has been shown to be responsible for the transfer of infected pigs from breeding herds to weaning sites contributing to the movement of influenza virus between different production sites (Allerson et al., 2012).

Influenza virus population dynamics and diversity

For the most part, IAV is considered widespread in pig populations. Estimates at the herd level indicate that IAV infections are common with a seroprevalence of 83% in sow herds in Ontario, Canada and over 90% in sow herds in Belgium, Germany, and Spain (Poljak et al., 2008; Van Reeth et al., 2008).

Swine production systems have changed significantly during the last 20 years and most pigs today are reared in well circumscribed populations. Infection and transmission dynamics in large populations can differ significantly from the dynamics observed at the individual animal level or in small groups. In individual pigs, flu infections are self-limiting with an average duration of infection of 5 to 7 days. In contrast, flu infections in populations can be maintained for longer periods of time ranging from weeks to years (Brown, 2000; Allerson et al., 2013). There are many factors (known or suspected) that contribute to the maintenance of influenza infections in populations including the infusion of animals, varying levels of immunity, and the various routes of virus spread within populations. However, how these factors interact to affect virus introduction and virus maintenance is not well understood or well known.

Endemically infected herds are common and it is now clear that there are more subclinical flu infections in pigs than previously thought. In a longitudinal active surveillance study, Corzo et al., (2013c) reported that approximately 90% of herds surveyed tested positive for influenza at least once. Influenza circulation was detected throughout the year and showed less seasonality than inferred previously from diagnostic laboratory submissions. In addition, IAV was detected in both vaccinated and non-vaccinated herds and in animals without clinical signs. The profile of strains was dynamic in many herds, where both pre-existing "resident" and new swine strains were detected, as well as new reassortants not previously identified. In addition, the introduction of 2009 H1N1 pandemic virus into pig populations has altered the dynamics of endemic viruses and has resulted yet again in the emergence of novel reassortants of unknown consequences for pigs and people. Therefore, endemically infected herds represent a reservoir for flu viruses that may infect other pigs, other animal species and, more importantly, humans. IAV infections can also be prevalent in breeding herds. Multiple subtypes and strains were detected co-existing in 5 breeding herds monitored over a one year period (Diaz et al., 2014).

In another study, Allerson et al., (2013) documented the role of the neonatal pig as a reservoir of influenza virus for both the maintenance of enzootic infections in breeding herds and the introduction of virus to weaned pig populations. Neonatal pigs in the absence of positive sows in breeding herds were also observed to potentially serve as the maintenance host over time in a Danish sow herd (Larsen et al., 2010). Neonatal pigs obtain passive immunity from their mothers at birth and remain suckling until weaning at about 21 days of age. During this period maternal immunity slowly wanes while pigs may become exposed to flu virus shed from the older piglets or from other sources of infection present in the herd. In turn at weaning, a small but significant proportion of pigs is infected which serve as the source of the infection for the rest of the pigs during the growing stages. As maternal immunity wanes, virus disseminates within the population often with lower transmission rates compared to those in naïve populations (Allerson et al., 2013b). As a result, viruses may be maintained longer than expected in growing pig populations, which has been reflected in results of monitoring populations using sampling techniques such as oral fluids. Allerson et al., (2013) showed that influenza virus could be detected for up to 70 days in growing pigs housed in a finisher where no additional animals were introduced, suggesting that viruses could be maintained in populations longer than previously considered.

Prevalence estimates from samples collected at slaughter also suggest that virus can be maintained in growing pig populations throughout a prolonged period. In several studies the prevalence of influenza virus at slaughter ranged from 2 to 4% (Peiris et al., 2009; Olsen et al., 2000; Smith et al (2009); Vijaykrishna et al., 2010). Even though influenza cannot be transmitted through eating pork (Vincent et al., 2009), this information suggests that growing pigs can remain as a source of infection for prolonged periods of time or alternatively if those are recent infections, it emphasizes that pigs of all ages can be infected.

Immunity can also influence transmission dynamics in populations. Influenza virus transmission was recently quantified in non-vaccinated and vaccinated pig populations with a reproduction ratio estimate of 10.66 in non-vaccinated pigs and reproduction ratio estimates of 1 and 0 for pigs vaccinated with heterologous and homologous inactivated vaccines, respectively (Romagosa et al., 2011). A follow-up transmission study identified a similar reproduction ratio estimate in non-vaccinated pigs and a reduction in transmission parameters in pig populations with homologous maternal immunity (Allerson et al., 2013b). Overall, these studies indicate that immunity may mitigate transmission and reduce the burden of influenza virus in pig populations.

Recent surveillance in breeding herds also indicates that other subpopulations of animals such as replacement gilts can play a role in the introduction of viruses in breeding herds (Diaz et al., 2014). The exact role of whether these animals alter the landscape of existing virus in herds needs to be further studied.

One aspect of influenza infections that also contributes to transmission and diversity of influenza viruses in pigs is the introduction of viruses of human origin. Nelson et al (2012) recently documented 49 human to swine transmission events of pH1N1 and 23 seasonal H1& H3 introductions concluding that humans contribute substantially to the

influenza virus diversity found in pigs. Therefore, efforts to decrease the introduction of human origin viruses should also be taken in consideration.

Summary

In summary, influenza transmission in endemically infected herds appears to be very dynamic. IAV can be found in the various subpopulations found in breeding herds (piglets, gilts, and young gilts) with piglets representing a major source for virus dissemination. Newborn piglets are naïve at birth to IAV and they can become a source of IAV for other pigs at weaning. Replacement animals can also be a source of new viruses and can become reservoirs for endemic viruses in the breeding herd. In addition, transmission dynamics are affected by immunity, both active and passive. Overall, swine populations are potential mixing vessels for different viruses if a mixed infection takes place. Further research is needed in order to fully understand the critical control points of influenza transmission and how to prevent the introduction of new strains in herds.

References

- Allerson M, Davies PR, Gramer M, Torremorell M (2013). Infection dynamics of pandemic 2009 H1N1 influenza virus in a two-site swine herd. Transboundary and Emerging Diseases. Doi:10.111/tbed.12053.
- Allerson M, Deen J, Detmer S, Gramer M, Joo HS, Romagosa A, Torremorell M. (2013b) The impact of maternally derived immunity on influenza A virus transmission in neonatal pig populations. Vaccine. doi:pii: S0264-410X(12)01616-7. 10.1016/j.vaccine.2012.11.023.
- Allerson M, Cardona C, Torremorell M (2013c). Indirect transmission of influenza A virus between pig populations under two different biosecurity settings. PLoS ONE 8(6): e67293. doi:10.1371/journal.pone.0067293.
- Atkinson, M. P., and L. M. Wein, 2008: Quantifying the routes of transmission for pandemic influenza. *Bull. Math. Biol.* 70, 820-867.
- Brookes, S. M., R. M. Irvine, A. Nunez, D. Clifford, S. Essen, I. H. Brown., K. Van Reeth, G. Kuntz-Simon, W. Loeffen, E. Foni, L. Larsen, M. Matrosovich, M. Bublot, J. Maldonado, M. Beer and G. Cattoli, 2009: Influenza A (H1N1) infection in pigs. *Vet. Rec.* 164, 760-761.
- Brown, IH (2000). The epidemiology and evolution of influenza viruses in pigs. Vet. Microbiol. 74:29-46.
- Corzo CA, Allerson M, Gramer M, Morrison RB, Torremorell M (2012). Detection of airborne influenza A virus in experimentally infected pigs with maternally derived antibodies. Transboundary and Emerging Diseases. Doi:10,1111/j.1865-1682.2012.01367.x
- Corzo CA, Gramer M, Lauer D, Davies PR. (2012b) Prevalence and risk factors for H1N1 and H3N2 influenza A virus infections in minnesota turkey premises. Avian Dis. 56(3):488-93.
- Corzo C, Romagosa A, Gramer M, Dee S, Morrison R, Torremorell M (2013). Relationship between airborne detection of influenza A virus and the number of infected pigs. The Veterinary Journal.196(2):171-175.
- Corzo C, Gramer M, Dee S, Morrison R, Torremorell M (2013b). Airborne detection and quantification of swine influenza A virus in air samples collected inside, outside and downwind from swine barns. PLoS One. 2013 Aug 8;8(8):e71444. doi: 10.1371/journal.pone.0071444. eCollection 2013.
- Corzo C, Culhane M, Juleen K, Stigger-Rosser E, Ducatez MF, Webby RJ, Lowe JF (2013c). Active surveillance for influenza A virus among swine, midwestern United States, 2009-2011. Emerg Infect Dis. 2013 Jun;19(6):954-60. doi: 10.3201/eid1906.121637.
- Diaz CA, Diaz A, Enomoto S, Corzo C, Culhane M, Sreevatsan S, Torremorell M (2014). Influenza A virus infection and diversity in commercial pig herds. Proc Am Assoc Swine Vet, Dallas, USA, p:40.
- Karasin, A. I., I. H. Brown, S. Carman, and C. W. Olsen, 2000: Isolation and characterization of H4N6 avian influenza viruses from pigs with pneumonia in Canada. *J. Virol.* 74, 9322-9327.
- Karasin, A. I., S. Carman, and C. W. Olsen, 2006: Identification of human H1N2 and human-swine reassortant H1N2 and H1N1 influenza A viruses among pigs in Ontario, Canada (2003 to 2005). *J. Clin. Microbiol.* 44, 1123-1126.
- Karasin AI, Olsen CW, Anderson GA (2000) Genetic characterization of an H1N2 influenza virus isolated from a pig in indiana. J Clin Microbiol 38(6): 2453-2456.
- Koen, J. S. (1919). A practical method for field diagnosis of swine diseases. American Journal of Veterinary Medicine. 14:468-470.
- Lange, E., D. Kalthoff, U. Blohm, J. P. Teifke, A. Breithaupt, C. Maresch, E. Starick, S. Fereidouni, B. Hoffmann, T.C. Mettenleiter, M. Beer, T.W. Vahlenkamp, 2009: Pathogenesis and transmission of the novel swine origin influenza virus A/H1N1 after experimental infection of pigs. *J. Gen. Virol.* 90, 2119-2123.
- Larsen, L. E., N. K. Christian, S. Aakerblom, C. K. Hjulsager, J. P. Nielsen, H. H. Stege, C. S. Kristensen, A. Elvstrom, and L. Lau, 2010: Dynamics of swine influenza infections in the farrowing unit of a Danish sow herd. *Proceedings of the 21st IPVS Congress*. pg. 80.
- Loeffen, W. L., N. Stockhofe, E. Weesendorp, D. van Zoelen-Bos, R. Heutink, S. Quak, D. Goovaerts, J. G. Heldens, R. Maas, R. J. Moormann, and G. Koch, 2011: Efficacy of a pandemic (H1N1) 2009 virus vaccine in pigs

against the pandemic influenza virus is superior to commercially available swine influenza vaccines. *Vet. Microbiol.* 152, 304-314.

- Ma, W., A. L. Vincent, M. R. Gramer, C. B. Brockwell, K. M. Lager, B. H. Janke, P. C. Gauger, D. P. Patnayak, R. J. Webby, and J. A. Richt, 2007: Identification of H2N3 influenza A viruses from swine in the united states. *Proc. Natl. Acad. Sci. U. S. A.* 104, 20949-20954.
- Ma W, Lager KM, Vincent AL, Janke BH, Gramer MR, et al. (2009) The role of swine in the generation of novel influenza viruses. Zoonoses Public Health. 56(6-7): 326-337.
- Nelson MI, Gramer MR, Vincent AL, Holmes EC (2012). Global transmission of influenza viruses from humans to swine. J Gen Virol 2012: 2195-203. doi: : 10.1099/vir.0.044974-0. Epub 2012 Jul 12.
- Nelson MI, Lemey P, Tan Y, Vincent A, Lam TT-Y, et al (2011). Spatial dynamics of human-origin H1 influenza A virus in North American Swine. PLoS Pathog 7(6):e1002077.doi:10.1371/journal.ppat.1002077
- Olsen C., et al (2002). The emergence of novel swine influenza viruses in North America. Virus Res 85:199-211.
- Olsen, C. W., S. Carey, L. Hinshaw, and A. I. Karasin, 2000: Virologic and serologic surveillance for human, swine and avian influenza virus infections among pigs in the north-central United States. *Arch. Virol.* 145, 1399-1419.
- Olsen CW, Brown IH, Easterday BC, Van Reeth K (2006) Swine influenza. In: Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ, editors. Ames, Iowa: Blackwell Publishing. pp. 469-482.
- Peiris JSM, Guan Y et al. (2001) Cocirculation of avian H9N2 and contemporary “human” H3N2 influenza A viruses in pigs in southeastern China: Potential for genetic reassortment? J Virol 75:9679-9686
- Poljak, Z., C. E. Dewey, S. W. Martin, J. Christensen, S. Carman, and R. M. Friendship, 2008a: Prevalence of and risk factors for influenza in southern Ontario swine herds in 2001 and 2003. *Can. J. Vet. Res.* 72, 7-17.
- Romagosa A, Allerson M, Gramer M, Joo HS, Deen J, Detmer, Torremorell M (2011). Vaccination of influenza A virus decreases transmission rates in pigs. Vet Res. 2011 Dec 20;42(1):120
- Shope, R. E. (1931). Swine Influenza : Iii. Filtration Experiments and Etiology. J. Exp. Med. 54:373-385**.**
- Smith GJD, Vijaykrishna D et al. (2009) Origins and evolutionary genomics of the 2009 swine origin H1N1 influenza A epidemic. Nature 459:1122-1125.
- Tellier, R. 2006: Review of aerosol transmission of influenza A virus. *Emerg. Infect. Dis*. 12, 1657-1662.
- Van Reeth, K., I. H. Brown, R. Durrwald, E. Foni, G. Labarque, P. Lenihan, J. Maldonado, I. Markowska-Daniel, M. Pensaert, Z. Pospisil, and G. Koch, 2008: Seroprevalence of H1N1, H3N2 and H1N2 influenza viruses in pigs in seven European countries in 2002-2003. *Influenza Other Respi Viruses.* 2, 99-105.
- Vijaykrishna D, Poon LLM, Zhu HC et al. (2010) Reassortment of pandemic H1N1/2009 in-fluenza A virus in swine. Science 328:1529).
- Webby RJ, Rossow K, Erickson G, et al. Multiple lineages of antigenically and genetically diverse influenza A virus co-circulate in the United States swine population. *Virus Res* 2004;103(1-2):67-73.

Changing receptor use of PRRSV leads to different virological, immunological and clinical outcome - impact on diagnosis and control

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A new disease characterized by reproductive and respiratory problems emerged in Northern America and Western Europe in the late eighties, early nineties. It was caused by a porcine arterivirus, which based on the symptoms was called porcine reproductive and respiratory syndrome virus (PRRSV)(Meulenberg et al., 1993). On the two continents, two clearly different genetic/antigenic viruses were circulating: an American type (amPRRSV) and a European type (euPRRSV). Based on serological examinations, it was shown that amPRRSV circulated already earlier in Northern America and by genetic analysis, more genetic variation was detected for amPRRSV in Northern America than for euPRRSV in Western Europe. This latter finding could be attributed to the earlier circulation and/or multiple introductions of the virus in the American pig population. Western Europe was confronted with a single introduction, starting in Western Germany. As a consequence, the early euPRRSV strains were genetically closely related (Stadejek et al., 2002). In the early nineties, amPRRSV was proven to be more pathogenic than euPRRSV. Indeed, whereas both virus types had the same power to give reproductive problems upon infection during late gestation, amPRRSV was giving more general clinical signs (fever, anorexia) and respiratory problems than euPRRSV. Only in combinations with other pathogens/toxins euPRRSV was able to induce overt general and respiratory clinical signs (Van Reeth et al., 1996). By recombination and genetic drift, American strains evolved fast, giving rise to new strains that were even more virulent and extremely difficult to control by commercial vaccines (atypical PRRSV, Sow Abortion and Mortality Syndrome (SAMS))(Mengeling et al., 1998). In 2006, extremely aggressive variants of amPRRSV appeared in China, which are now damaging the whole Asian pig population (long lasting high fever, respiratory and reproductive problems, high mortality) and which represent a real threat to other continents (Tian et al., 2007). In Eastern Europe, a surprisingly large variation was found for euPRRSV isolates leading to the identification of new subtypes (2, 3 and 4) that were quite different from subtype 1 (Stadejek et al., 2006). At present, it is hypothesized that euPRRSV was circulating in Eastern Europe a long time before the entrance of subtype 1 in Western Europe. When the whole group of Western and Eastern European euPRRSV strains are considered, a genetic variation of euPRRSV was found that was even larger than the one found with amPRRSV in the US, leading to a very complex genetic world picture of PRRSV. Eastern European PRRSV strains of subtype 2 (prototype Bor) and 3 (prototype Lena) have been shown to be more virulent than the Western European strains (subtype 1 (prototype Lelystad))(Karniychuk et al., 2009). The euPRRSV strain Lena is even as virulent and pathogenic as the high fever disease amPRRSV in Asia. Over the years in Western Europe, euPRRSV remained mainly linked with reproductive problems. Fever and respiratory problems were absent upon experimental single inoculations. However, starting from mid 2013, PRRSV is responsible for flu-like problems in nurseries in Belgium and most probably also neighboring countries (unpublished data). Upon experimental inoculation with one of these isolates, Flanders 13, fever and respiratory problems were reproduced. Genetically, this virus is quite different from other circulating PRRS viruses.

The pathogenesis of PRRSV is fully determined by differentiated macrophages. During the past 20 years, the euPRRSV strains of subtype 1 (prototype Lelystad) replicated very similarly in a pig (Duan et al., 1993). Targets are differentiated macrophages that are carrying the sialoadhesin receptor (Duan et al., Vanderheijden et al., 2010, Karniychuk et al., 2013). These cells can easily be found in tonsils and lungs, lymph nodes, spleen, maternal endometrium and fetal placenta and at lower levels in all other tissues of the pig (Karniychuk et al. 2009). Because the virus does not replicate well in the upper respiratory tract, the virus is difficult to isolate from nasal swabs and the virus does not spread fast between pigs (Albina, 1997). Due to the rather restricted number of differentiated cells that are infected, virus titers of 10^2-10^4 $TCID_{50}$/ml are generally found in serum. EuPRRSV strains of subtype 3 (prototype Lena) differ from LV-like strains because they are able to infect a new subset of differentiated macrophages that do not possess the sialoadhesin receptor (Frydas et al. 2013). An additional receptor is most probably responsible for this. By experiments in nasal mucosa explants it was found that the additional subset is present at high concentrations in and under the respiratory epithelial cells of the upper respiratory tract, allowing a much stronger replication in respiratory tissues (up to 10-100x higher) and giving rise to a strong viral shedding and a fulminant viremia (100x higher; virus titers up to 10^4-10^6 $TCID_{50}$/ml). Based on the localisation of this new subset of susceptible macrophages, it is hypothesized that they represent nasal macrophages. These cells are forming a dense network and are taking care of the first line of defense against pathogens (Vareille et al., 2011). Destroying both nasal and alveolar lung macrophages is most probably the reason why euPRRSV Lena has been associated with secondary bacterial infections and sepsis (Karniychuk et al., 2010). The new Flanders 13-like strains are euPRRSV subtype 1 strains that are also evolving in the same direction as Lena. By using the nasal mucosa explants, this virus also replicated in non-sialoadhesin positive macrophages. The virus titers in nasal secretions from euPRRSV infected animals are in line with the replication of the virus in nasal mucosa explants. Whereas it is difficult to detect LV-like euPRRSV subtype 1 in nasal secretions, it is very easy to do so with the more virulent euPRRSV subtype 3 and Flanders 13-like strains. Transmission experiments with these different strains are ongoing in order to find out if the power of the virus to replicate in the nasal macrophages may be

related to its aerogenic spread. The increase of macrophage subsets that are infected with euPRRSV in time is a dangerous evolution. A ten- to hundredfold increase of replication gives rise to viral mutants with the same magnitude. Knowing that mutagenesis is helping the virus to escape from immunity and is increasing the risk that highly virulent strains emerge is bringing Europe in a very dangerous situation. In addition, because of the close relationship of the porcine receptors sialoadhesin and CD163 with their human homologs, one should consider the risk of a species jump to humans (Van Breedam et al., 2013).

With these interesting findings with euPRRSV strains, we have recently performed experiments with amPRRSV strains in nasal mucosa explants. It was found that both old (VR2332) and more recent amPRRSV strains (SDSU-73, NADC, MN-184) from the US easily replicate to high levels in both sialoadhesin positive and sialoadhesin negative macrophages in the nasal mucosa (unpublished data). This finding is explaining several things. AmPRRSV behaved differently from euPRRSV from the very beginning; it replicated in more subsets of macrophages than the old subtype 1 euPRRSV LV-like strains. Its high replication in the upper respiratory tract differs from the low level of replication of the old subtype 1 euPRRSV strains. This also explains why it was and still is very easy for amPRRSV to spread via airborne transmission (Otake et al., 2010). In addition, the higher number of macrophage subsets that are infected with amPRRSV explains why it is more virulent/pathogenic than the old subtype 1 euPRRSV strains and give more rise to secondary infections. It also explains why amPRRSV is able to replicate in sialoadhesin-negative pigs (Prather et al., 2013).

All these findings are very important in function of the diagnosis. An etiological diagnosis of PRRS during reproductive failure is straightforward for all PRRSV strains. PRRSV is replicating in the macrophages of the fetal placenta when they become sialoadhesin positive at late gestation (Karniychuk et al., 2009). This results in a severe placentitis and viral spread to the fetus. The placentitis is the main cause of fetal pathology and death (Karniychuk et al., 2013). Due to the huge size of the placenta and the regularly localized PRRSV replication, it is difficult to make the diagnosis from placental tissues (which part to take?). Because fetuses do not have the time to develop antibodies before they die, it is impossible to diagnose PRRS by serological examinations on fetal fluids. Taking all these pathogenetic aspects into account, it is advised to do the diagnosis of PRRS during reproductive problems by qRT-PCR on umbilical cords (connected with fetal placenta) and organ pools (lungs/spleen) of aborted fetuses. Diagnosis of euPRRS in the context of respiratory problems is very difficult with LV-like euPRRSV strains. They are always causing long-lasting infections in young piglets, independent of their health status (diseased or healthy). Therefore, what is the meaning of a positive result (demonstrating the presence of the virus in lungs or blood). In the same context, it is difficult to interpret a seroconversion. It is not because an animal is seroconverting to LV-like euPRRSV strains that the virus is responsible for problems. This diagnostic problem is one of the main reasons why most farmers did not vaccinate their piglets up till now in Western Europe. However, in pigs with flu-like problems caused by amPRRSV, subtype 2 and 3 euPRRSV and the new Flanders 13-like euPRRSV, it is easy to demonstrate the high replication of PRRSV in the upper respiratory tract by taking nasal swabs and blood and quantitating the high viral load (up to 10^5 $TCID_{50}$/ml in nasal secretions and 10^{4-6} $TCID_{50}$/ml in blood) by virus titration or qRT-PCR.

PRRSV is a difficult target for the immunity. Several branches of the immunity have been shown not to be induced or not to be functional. Low levels of interferons are induced (Van Reeth et al., 1999). Antibodies are raised starting from 8 days post infection, but it takes several weeks before a weak neutralization can be demonstrated (Labarque et al., 2000). Natural killer cells and cytotoxic T-lymphocytes are not sufficiently effective (Cao et al., 2013; Costers et al., 2009). Only neutralizing antibodies, which appear after one month and at low levels together with a not yet identified porcine killer cell are the two branches that still can do the job and should be activated by vaccination. The drift of the virus makes it a moving target and complicates the whole vaccination strategy (Labarque et al, 2004). In the near future, it is important to have access to vaccines that are adaptable, enclosing strains that are closely related to the strains circulating in the field (Nauwynck et al., 2012). The technologies for making effective adaptable inactivated (Geldhof et al., 2012) and attenuated vaccines (unpublished data) became available and should be urgently implemented in the field. The ultimate dream is the development of an adaptable marker vector vaccine (cassette system) that induces a local immunity in the respiratory tract. As long as PRRSV does not persist such as its sister-arterivirus, lactate dehydrogenase elevating virus (LDV), we should keep on going with the investment to develop vaccines.

In conclusion, it is extremely important to better control PRRS in the near future and not to wait till a complete catastrophe occurs. There is an urgent need for adaptable inactivated and attenuated marker vaccines and improved biosafety measures in order to fully control PRRSV circulation. Pig producers, PRRS researchers and pharmaceutical companies should take their responsibility and join forces to come up with solutions to eradicate this ever-changing enemy that may turn into a real nightmare. In this context, funding PRRS research should be prioritized by all agencies all over the world. Not doing this is an unforgivable error not only for animal health but possibly also for human health.

References

- Albina E. (1997). Epidemiology of porcine reproductive and respiratory syndrome (PRRS): an overview. Veterinary Microbiology 55, 309-316.
- Cao J., Grauwet K., Vermeulen B., Devriendt B., Jiang P., Favoreel H. and Nauwynck H.J. (2013). Suppression of NK Cell-mediated cytotoxicity against PRRSV-infected porcine alveolar macrophages in vitro. Veterinary Microbiology 164, 261-269, doi:10.1016/j.vetmic.2013.03.001.
- Costers S., Lefebvre D.J., Goddeeris B., Delputte P. and Nauwynck H.J. (2009). Functional impairment of PRRSV-specific peripheral CD3+CD8high cells. Veterinary Research 40, 46 (doi: 10.1051/vetres/2009029).
- Duan X., Nauwynck H.J. and Pensaert M.B. (1997). Virus quantification and identification of cellular targets in the lungs and lymphoid tissues of pigs at different time intervals after inoculation with porcine reproductive and respiratory syndrome virus (PRRSV). Veterinary Microbiology, 56, 9-19.
- Duan X., Nauwynck H.J., Favoreel H.W. and Pensaert M.B. (1998) Identification of a putative receptor for porcine reproductive and respiratory syndrome virus (PRRSV) on porcine alveolar macrophages. Journal of Virology 72, 4520-4523.
- Geldhof M.F., Vanhee M., Van Breedam W., Van Doorsselaere J., Karniychuk U.U. and Nauwynck H.J. (2012). Comparison of the efficacy of autogenous inactivated porcine reproductive and respiratory syndrome virus (PRRSV) vaccines with that of commercial vaccines against homologous and heterologous challenges. BMC Veterinary Research 8, 182 (doi:10.1186/1746-6148-8-182).
- Frydas I., Verbeeck M., Cao J. and Nauwynck H.J. (2013). Replication characteristics of porcine reproductive and respiratory virus (PRRSV) European subtype 1 (Lelystad) and subtype 3 (Lena) strains in nasal mucosa and cells of the monocytic lineage: indications for the use of new receptors of PRRSV (Lena). Veterinary Research 44, 73 (doi:10.1186/1297-9716-44-73).
- Meulenberg J.J., Hulst M.M., de Meijer E.J., Moonen P.L., den Besten A., de Kluyver E.P., Wensvoort G., and Moormann R.J. (1993) Lelystad virus, the causative agent of porcine epidemic abortion and respiratory syndrome (PEARS), is related to LDV and EAV. Virology 192, 62-72.
- Nauwynck H., Van Gorp H., Vanhee M., Karniychuk U., Geldhof M., Cao A., Verbeeck M. and Van Breedam W. (2012). Microdissecting the pathogenesis and immune response of PRRSV infection paves the way for more efficient PRRSV vaccines. Transboundary and Emerging Diseases 59, 50-54 (doi: 10.1111/j.1865-1682.2011.01292.x).
- Karniychuk U.U. and Nauwynck H.J. (2009). Quantitative changes of sialoadhesin and CD163 positive macrophages in the implantation sites and organs of porcine embryos/fetuses during gestation. Placenta 30, 497-500.
- Karniychuk U.U., Geldhof M., Vanhee M., Van Doorsselaere J., Saveleva T.A. and Nauwynck H.J. (2010). Pathogenesis and antigenic characterization of a new East European subtype 3 porcine reproductive and respiratory syndrome virus isolate. BMC Veterinary Research 6, 30 (doi: 10.1186/1746-6148-6-30).
- Karniychuk U.U. and Nauwynck H.J. (2013). Pathogenesis and prevention of placental and transplacental porcine reproductive and respiratory syndrome virus infection. Veterinary Research 44(1):95 (doi:10.1186/1297-9716-44-95).
- Labarque G.G., Nauwynck H.J., Van Reeth K. and Pensaert M.B. (2000). Effect of cellular changes and onset of humoral immunity on the replication of porcine reproductive and respiratory syndrome virus in the lungs of pigs. Journal of General Virology 81, 1327-1334.
- Mengeling W.L., Lager K.M. and Vorwald A.C. (1998). Clinical consequences of exposing pregnant gilts to strains of porcine reproductive and respiratory syndrome (PRRS) virus isolated from field cases of ”atypical” PRRS. American Journal of Veterinary Research 59, 1540-1544.
- Otake S., Dee S., Corzo C., Oliveira S. and Deen J. (2010). Long-distance airborne transport of infectious PRRSV and Mycoplasma hyopneumoniae from a swine population infected with multiple viral variants. Veterinary Microbiology 145, 198-208.
- Prather R.S., Rowland R.R., Ewen C., Tribel B., Kerrigan M., Bawa B., Teson J.M., Mao J., Lee K., Samuel M.S., Whitworth K.M., Murphy C.N., Egen T. and Green J.A. (2013). An intact sialoadhesin (Sn/SIGLEC1/CD169) is not required for attachment/internalization of the porcine reproductive and respiratory syndrome virus. Journal of Virolgoy 87, 9538-9546.
- Stadejek T., Stankevicius A., Storgaard T., Oleksiewicz M.B., Belak S., Drew T.W. and Pejsak Z. (2002). Identification of radically different variants of porcine reproductive and respiratory syndrome virus in Eastern Europe: towards a common ancestor for European and American viruses. Journal of General Virology 83, 1861-1873.
- Stadejek T. Oleksiewicz M.B., Potapchuk D. and Podgorska K. (2006). Porcine reproductive and respiratory syncrome virus strains of exceptional diversity in eastern Europe support the definition of new genetic subtypes. Journal of General Virology 87, 1835-1841.
- Tian K., Yu X., Zhao T., Feng Y., Cao Z., Wang C., Hu Y., Chen X., Hu D., Tian X., Liu D., Zhang S., Deng X., Ding Y., Yang L., Zhang Y., Xiao H., Qiao M., Wang B., Hou L., Wang X., Yang X., Kang L., Sun M., Jin P.,

Wang S., Kitamura Y., Yan J. and Gao G.F. (2007). Emergence of fatal PRRSV variants: unparalleled outbreaks of atypical PRRS in China and molecular dissection of the unique hallmark. PLoS One 2, e526.

- Van Breedam W., Verbeeck M., Christiaens I., Van Gorp H. and Nauwynck H.J. (2013). Porcine, murine and human sialoadhesin (Sn/Siglec-1/CD169): portals for porcine reproductive and respiratory syndrome virus (PRRSV) entry into target cells. Journal of General Virology 94, 1955-1960.
- Vanderheijden N., Delputte P.L., Favoreel H.W., Vanderkerckhove J., van Damme J., van Voensel P.A. and Nauwynck H.J. (2003). Involvement of sialoadhesin in entry of porcine reproductive and respiratory virus into porcine alveolar macrophages. Journal of Virology 77:8207-8215.
- Van Reeth K., Nauwynck H. and Pensaert M. (1996). Dual infections of feeder pigs with porcine reproductive and respiratory syndrome virus followed by porcine respiratory coronavirus or swine influenza virus: a clinical and virological study. Veterinary Microbiology 48, 325-335.
- Van Reeth K., Labarque G.G., Nauwynck H.J. and Pensaert M.B. (1999). Differential production of proinflammatory cytokines in the pig lung during different respiratory virus infections: correlations with pathogenicity. Research in Veterinary Science 67, 47-52.
- Vareille M., Kieninger E., Edwards R.M. and Regamey N. (2011). The airway epithelium: soldier in the fight against respiratory viruses. Clinical Microbiology Reviews 24, 210-229.

Assessing on farm pig welfare

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Introduction

Animal welfare can be defined in a number of different ways, but there is a growing consensus that whatever the definition, it has to include three elements: the emotional state of the animal, its biological functioning and its ability to show normal patterns of behaviour *(*Manteca, Velarde & Jones, 2009). These three elements are by no means contradictory; in fact they are closely interrelated. The Five Freedoms developed by the Farm Animal Welfare Council (1992) combine elements from the three approaches to welfare explained above and are a very useful framework to identify the main welfare problems. These freedoms, which represent ideal states rather than actual standards for animal welfare, include: freedom from hunger and thirst, freedom from discomfort, freedom from pain, injury and disease, freedom to express normal behaviour, and freedom from fear and distress.

The objectives of this paper are to discuss some of the main welfare problems in intensive pig production and to introduce the Welfare Quality® protocol for pigs.

Welfare quality assessment

Welfare Quality® (www.welfarequality.net) was an integrated research project co-financed by the European Commission, which ran from May 2004 to December 2009. The project was designed to integrate farm animal welfare into the food chain and the main aims were to develop a standardised system for assessing the welfare of animals kept on farms that are scientifically sound and feasible (Blokhuis, Jones, Geers, Miele & Veissier, 2003).

The awareness that welfare is multidimensional and that its overall assessment requires a multicriteria evaluation resulted in the decision to base the Welfare Quality® assessment system on four main principles according to how they are experienced by animals: good feeding, good housing, good health, and appropriate behaviour (Blokhuis Keeling, Gavinelli & Serratosa, 2008). Within these principles, the project highlighted twelve distinct but complementary animal welfare criteria. These twelve animal welfare criteria provide a very useful framework for understanding the components of animal welfare.

1. Good feeding includes two criteria: absence of prolonged hunger and absence of prolonged thirst. Hunger may result from malnutrition, undernutrition or both. Malnutrition occurs when nutrients are not balanced, whereas undernutrition reflects insufficient supply. Malnutrition and undernutrition cause stress and, if sufficiently prolonged or severe, this can lead to debilitation, loss of body condition, immunosuppression and disease. Chronic hunger in pregnant sows is the main welfare problem related to this criterion.

Prolonged thirst causes stress and, if long-lasting or severe, leads to debilitation, loss of body condition and disease. For example, low water intake in pregnant sows may cause urinary infections. Thirst also reduces food intake which, in turn, may lead to the welfare problems associated with prolonged hunger. Prolonged thirst can occur when animals are given water of poor quality or when drinking facilities are insufficient or inadequate, mainly due to neglect or poor husbandry. The latter condition is exacerbated when there is competition with conspecifics.

2. Good housing includes comfort around resting, thermal comfort and ease of movement. Lack of comfort around resting may be a consequence of an excessive stocking density or inadequate housing facilities. To satisfy its need for comfort around resting, each animal should have enough space to stand up, lie down and turn around. For instance, pigs prefer to separate their dunging area from their lying area. If the stocking density is too high, some pigs may have to lie in the dunging area, among faeces and urine.

Farm animals are homoeothermic, which means they are able to maintain a relatively constant deep body temperature that differs from the environmental temperature within certain limits. A relatively constant deep body temperature means that heat production and heat loss are equal. Lower environmental temperature leads to higher heat losses, which have to be compensated by a higher heat production. Temperatures which are too low or too high cause cold and heat stress, respectively. Cold stress is a particular hazard for newborn animals and, together with starvation, plays an important role in neonatal mortality in piglets (Mellor and Stafford, 2004). On the other hand, pigs have great difficulty in losing heat and may therefore suffer heat stress at ambient temperatures close to the upper limit of their thermoneutral zone and at high humidity. Heat stress may result from poor ventilation, inadequate housing and an overly high stocking density.

Ease of movement, i.e. the ability of animals to turn round, get up and lie down has long been considered a basic requisite for good welfare (Brambell Committee, 1965). Too high stocking densities or inadequate design of housing

facilities may prevent animals from moving normally. Lack of comfort around resting can be detected by several indicators such as shoulder sores in farrowing sows mainly caused by an inappropriate size of the crates.

3. Good health is an important component of animal welfare and it can be defined as the absence of injuries, disease and pain. These negative states can have many causes, including certain management procedures. Injuries can cause acute and/or chronic pain. Pain is defined as an aversive emotional experience and is therefore a welfare problem. The legs and the feet are the parts of the body that are most frequently injured in farm animals. These injuries interfere with normal behaviour and locomotion, and may have a debilitating effect by preventing the animal from feeding normally. Wounds can become infected and, under some circumstances, may lead to systemic disease. Infectious, systemic diseases secondary to injuries as well as the debilitating effect of some injuries may result in the animals being culled. Fighting with other animals can also cause injury; this is more common when animals are mixed with unacquainted individuals and when animals have to compete for access to feed, water or resting space (Velarde, 2007). Tail-biting is also an important cause of injuries. According to the most widely accepted hypothesis, tail biting is a form of redirected behaviour derived from the thwarting of normal exploratory motivations. It can also derived from situations of competition (e.g. for food and drinkers), frustration and stress (such as thermal stress). Tail-biting is a welfare problem because of the pain and suffering experienced by the bitten animal (not only due to the biting but also to secondary infections), the restlessness caused to the group, and the likely discomfort of the biter animal. As for other behaviour problems in intensive pig production, tail biting is a multi-factorial problem involving both internal and environmental risk factors; these include genetic background, sex, age, health status, diet, feeding management and different characteristics of the pen (SchrØder-Petersen *et al*., 2004). Some types of tail biting have similar risk factors than vulva biting observed in sows.

Absence of disease is a basic requisite for good welfare. Diseases can cause pain and may interfere with normal behaviour. Chronic diseases often have a debilitating effect on the animal and may lead to it being culled. Some of the diseases that are more relevant from an animal welfare standpoint are called “multifactorial diseases”, meaning that they are caused by the interplay of several factors. Diseases can be classified in five main categories: respiratory (e.g. coughing, sneezing, labour breathing or twisted snouts as symptoms observed on farm or the presence of pneumonia or pleurisy when lungs are evaluated at the slaughterhouse), digestive (i.e. diarrhoea, solid faeces, rectal prolapse), reproductive (such as mastitis, metritis, uterine prolapse), neurological (tremor, splay leg) and those affecting the skin (i.e. mange).

Several management procedures that are routinely carried out in farm animals can cause pain. These include tail docking, castration, teeth clipping and nose ringing in outdoor pigs. The pain associated with these procedures normally lasts a few days, but in some cases such as tail docking, there may additionally originate chronic pain. Usually, castration of male pigs is performed surgically without anaesthesia or post-operative analgesia despite evidence that castration at any age is painful and may have a detrimental influence on health (Prunier at al., 2006).

4. Appropriate behaviour includes the expression of social behaviour, expression of other behaviours, good human-animal relationship and a positive emotional state. All farm species are social animals and as such are strongly motivated to have contact with conspecifics. Disruption of social groups (through mixing of unacquainted animals, for example) may lead to an increase in aggressive behaviour and a reduction in positive social interactions. Mixing of unfamiliar animals (often with a change of physical environment) is a common practice in pig husbandry and often happens at weaning, at the beginning of the growing-finishing period and during transport to slaughter. Mixing of unacquainted pigs has adverse effects on welfare and production, mainly because pigs fight in order to establish dominance relationships. Housing conditions that result in increased competition for resources may increase the number of negative social interactions. This may happen when stocking density is too high or when access to resources is insufficient, for example when feeding space is limited. High stocking densities also increase aggression because the easy escape of attacked individuals is thwarted and may cause the dominance hierarchy to be less successful (Ewbank and Bryant, 1969).

Animals are strongly motivated to perform particular behaviour patterns. This is the case, for example, with exploration, rooting, and nest building in farrowing sows. In some circumstances, the inability to perform such behaviour patterns may cause distress and lead to the development of abnormal behaviours such as tail biting. A poor human-animal relationship results in the animals being fearful of the stockpersons and other humans. Poor stockmanship is considered the main cause of bad human-animal relationships. The term “stockmanship” covers the way that animals are handled, the quality of their daily management and health care, and how well problems other than disease are recognised and solved (Waibingler and Spoolder, 2007).

Fear and anxiety are two emotional states induced by the perception of a danger or a potential danger, respectively, that threaten the integrity of the animal (Boissy, 1995). Fear and anxiety both involve physiological and behavioural changes that prepare the animal to cope with the danger. Although fear and anxiety have not always been clearly differentiated, fear can be operationally defined as states of apprehension focusing on isolated and recognisable dangers while anxieties are diffuse states of tension that magnify the illusion of unseen dangers (Rowan, 1988). General fear becomes a problem particularly when animals encounter new or unexpected stimuli, (e.g. a sudden noise or movement,

an unfamiliar animal), or situations, e.g. a new housing facility, transportation. This has important implications for animal housing and management.

Animal welfare measures

For each one of these criteria potential measures were identified and evaluated for inclusion in the pig welfare assessment on farm based on their validity, reliability and feasibility. Validity was the main criterion used and was defined as the extent to which the measure is meaningful in terms of providing information on the welfare of an animal or a group of animals (Winckler, Capdeville, Gebresenget, Hörning & Roiha, 2003). Validation of the measures was based on scientific bibliographies or on research studies carried out during the project. Only those measures with high validity were selected for the operational protocols. Reliability assessment included: 1) inter-observer reliability, which refers to agreement between two or more observers after they have received reasonable training (Dalmau, Geverink, Van Nuffel, Van Steenbergen, Van Reenen, Hautekiet, Vermeulen, Velarde & Tuyttens, 2010); 2) intra-observer reliability which requires that results are largely the same when the same observer repeats assessments (e.g. using video-clips or pictures); 3) test-retest reliability to assess the robustness of the measure to external factors, such as time of day or weather conditions (i.e. repeated tests with the same subjects yield similar data). This means that results must be representative of the longer-term farm situation and not too sensitive to changes in the farm conditions or the internal states of the animals as long as the situation has not changed significantly. At the same time, a measure should be sensitive enough to detect variations in the welfare state of the animals between farms and across periods. Infrequent welfare problems might result in weak test-retest reliability. Feasibility means the possibility to carry out the protocol under practical conditions. For this purpose, issues such as time or equipment needed to take the measure were taken into account. These requirements excluded some physiological parameters that need experimental equipment (e.g. heart rate recordings) or laboratory analyses as well as complex behavioural tests that could not be integrated into the farm routine (e.g. cortisol analyses and open-field tests, respectively). In terms of the feasibility of the whole assessment protocol, it should be possible for a single observer to carry out a farm assessment during a one-day visit.

Previous monitoring systems and legislation largely rely on examination of inputs, ‘what’ or ‘how much’ of different resources are given to animals. These parameters are easy to define, to measure and have a high inter and intra reliability. However, these measures have often been criticized for potentially low validity due to their indirect nature and complex interactions with other resource and management conditions (Waiblinger, Knierim & Winckler, 2001). Thus, input measures are a poor guarantee for good animal welfare, as animals may experience the same situation or handling procedure differently depending of their genetic background, temperament, or previous experiences.

Since welfare is a condition of the individual animal, wherever possible, the Welfare Quality assessment system places its emphasis on animal-based measures (also called „outcome‟ measures) rather than on the resource and management in an attempt to estimate the actual welfare state of the animals. Such physiological, health, performance and behavioural measures have inherent advantages over input measures. The first advantage is clearly that, since welfare is a condition of the animal, outcomes measures are likely to be the most direct reflection of their actual welfare state. It permits to evaluate the welfare by directly observing the animal, regardless of how and where it is kept. Secondly, as it is applicable to all farms, animal-based measures permit to compare the welfare of animals from different farms, and remain more transparent to stakeholders.

In Welfare Quality, resource- or management-based measures were therefore only taken into account to complement the animal-based ones or as a substitute when there were no promising animal-based measures available (Botreau at al., 2007). For example, no valid, reliable and feasible animal based measure was found for the evaluation of prolonged thirst. In this case, it was necessary to include resource based measures as the presence, number, cleanliness and functioning of drinkers. Resource and management based measures can also be used to identify risks to animal welfare and identify causes of poor welfare so that improvement strategies can be implemented.

The measures that met the requirements for validity, reliability and feasibility, were combined and integrated into the welfare assessment protocol. Table 1 shows the final lists of measures included in the ‘operational’ protocols for growing pigs on farm.

An important consideration to increase the repeatability and reliability of the assessment is that the measures should be simple to collect and scored in a way that maximises reliability. . For this purpose, most of the measures are scored according to a three-point scale ranging from 0 to 2. So that a score 0 is awarded where welfare is good, a score 1 is awarded where there has been some compromise on welfare, and a score 2 is awarded where welfare is poor and unacceptable. In some cases a binary (0/2 or Yes/No) or a cardinal scale (e.g. cm or m^2) is used.

Table 1. Collection of data for growing pigs on farm

	Welfare criteria		Measures
Good feeding	1	Absence of prolonged hunger	Body condition score
	2	Absence of prolonged thirst	Water supply
Good housing	3	Comfort around resting	Bursitis, absence of manure on the body
	4	Thermal comfort	Shivering, panting, huddling
	5	Ease of movement	Space allowance
Good health	6	Absence of injuries	Lameness, wounds on the body, tail biting
	7	Absence of disease	Mortality, coughing, sneezing, pumping, twisted snouts, rectal prolapse, scouring, skin condition, hernias
	8	Absence of pain induced by management procedures	Castration, tail docking
Appropriate behaviour	9	Expression of social behaviours	Social behaviour
	10	Expression of other behaviours	Exploratory behaviour
	11	Good human–animal relationship	Fear of humans
	12	Positive emotional state	Qualitative Behaviour Assessment (QBA)

Organisation of the welfare assessment

At the beginning of the visit, general information related to the farm is recorded by means of a questionnaire answered by the farmer as well as by visual inspection. The questionnaire records information on management, prevention of diseases, feeding, hygiene management, temperature regulation, castration routine, euthanasia criteria, and production and mortality records.

In the second stage, the assessment involves collecting data on the animals and the resources. A specific order in which the measures to be taken on farms is provided in the protocol for each animal type. In general, the animal-based assessment starts with measures recorded from outside the pen and by observing the whole group. In sows and growing pigs for example, the measures recorded from outside the pen consist of those related to the positive emotional state criterion (by means of the Qualitative Behavioural Assessment, QBA), the expression of social and other behaviours (by means of a scan sampling), and the presence of stereotypies, respiratory problems (coughing and sneezing), and thermal comfort measures (shivering, panting, huddling). Afterwards, the assessor enters the pen to assess the human–animal relationship and other animal-based measures related to the welfare principles of good feeding, housing, and health. Animals are individually scored for body condition, bursitis, shoulder sores, dirtiness (or presence of manure on the body), wounds on the body, tail biting, vulva lesions, lameness, pumping (heavy and laboured breathing), twisted snouts, rectal prolapse, uterine prolapse, skin condition, constipation, scouring, metritis, mastitis, local infections, tremor, splay leg and hernias. These measures are taken in approximately 30 pregnant sows, in 10 lactating sows and their litters, and/or in 150 growing pigs from 10 different pens. Some measures will require sampling of animals at specific stages of pregnancy (early, mid and late gestation) or at different stages of the growing/fattening period (at the beginning of the period but at least one week after mixing to avoid effects of the hierarchy formation, and at the end when space allowance is lower). The stage of pregnancy or growth are not considered likely to affect other measures. However, ensuring a representative sample simplifies the selection process. On many farms, animals in different stages may be housed within the same building (or even room), and are likely to be distributed equally across the building/room. However, if there are many small pens within a building or room those at either end of the building (and

in the middle if necessary) should be selected. On farms where animals at the same biological stage are housed in different buildings it is important to sample animals from all the different types of buildings.

To facilitate the use of the welfare assessment system by third parties, the Welfare Quality project edited a book for pigs with a standardized description of the measures, data collection, sample size, evaluation methodology and the calculation of welfare scores (Welfare Quality, 2009). Once the protocols are generated a critical component to obtain objective and repeatable assessments is their uniform interpretation and application by the assessors, particularly for the animal based parameters assessed by direct observation, so a previous standardized training process is recommended.

Practical testing of the protocols

One of the main objectives of the welfare assessment protocols is to be applicable to most farming conditions. This was evaluated through surveys on representative samples of different rearing systems and local conditions (e.g. climate) found in Europe and Latin America. Farms were selected for the survey on the basis of management practices, farm size, and veterinary records.

The protocols were tested on 190 farms (90 for sows and 100 for growing pigs). The sow farms examined used extensive outdoors or indoor husbandry systems with different types of floor, bedding material, and feeding system. Similarly, the sample of farms with growing-finishing pigs included outdoor and indoor units with o without bedding material. It was concluded that many animal-based measures in the Welfare Quality assessment protocols are sufficiently sensitive to show high variability and thereby allow discrimination between the farms (Temple et al., 2011). The mean time taken to perform the full protocol in growing pigs at farm is 6 h and 20 min (± 51 min) per visit, ranging from 5h15 to 9h30 min (Temple et al., 2011). The interview, the only part of the protocol that requires farmer participation, takes approximately 40 min (ranging from 25 to 60 min) depending on the farmer's level of interest. Furthermore, the time taken to record general information varies according to the size of the farm (as much as 200 min on the largest farm). Farm size and the distance between buildings also affected the time required for QBA, scan sampling of behaviours, and the recording of good feeding, housing, and health measures. The number of animals per pen, the density, the fear level of the animals, the dirtiness of pigs, and the light intensity within the buildings are other factors that might influence the time needed to record feeding, housing, and health measures.

Conclusion

In general, the protocols developed in Welfare Quality® to assess animal welfare on farm seem to work well and they are feasible. The responses obtained from farmers at the end of a visit have been encouraging. Most were pleasantly surprised that the protocol involved little input from their part in data collection (thus not taking up their time) and that none of the measures were invasive or involved to move animals or. Perhaps even more important was the high level of interest they showed in animal-based parameters as they were usually not provided with this type of information.

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References

- Blokhuis, H.J., Jones, R.B., Geers, R., Miele, M., & Veissier, I. (2003). Measuring and monitoring animal welfare: Transparency in the food product quality chain. *Animal Welfare, 12*, 445-455.
- Blokhuis H., Keeling L., Gavinelli A. & Serratosa J. (2008). - Animal welfare's impact on the food chain. *Trends in Food Science and Technology*, 19, 75-83.
- Boissy, A. (1995). Fear and fearfulness in animals. *Quartery Review of Biology, 70, 165-191.*
- Botreau, R., Bonde, M., Butterworth, A., Perny, P., Bracke, M.B.M., Capdeville, J., & Veissier, I. (2007). Aggregation of measures to produce an overall assessment of animal welfare: Part 1 - A review of existing methods. *Animal, 1*, 1179-1187.
- Brambell Committee, 1965. Report of the Technical Committee to Enquire into the Welfare of Animals kepth under Intensive Livestock Husbandry Systems. Command Reprot 2836, London: Her Majesty's Stationery Office.
- Dalmau, A., Geverink, N. A., Van Nuffel, A., van Steenbergen, L., Van Reenen, K., Hautekiet, V., Vermeulen, K., Velarde, A., & Tuyttens, F. A. M. (2010). Repeatability of lameness, fear and slipping scores to assess animal welfare upon arrival in pig slaughterhouses. *Animal, 4(5)*, 804–809.

- Ewbank, R. and Bryant, M.J., 1969. The effects of population density upon the behaviour and economic performance of fattening pigs. Farm Building Progress 18, 14-15.
- Farm Animal Welfare Council (FAWC) updates the five freedoms. (1992). *The Veterinary Record*, 17, 357.
- Manteca, X., Velarde, A., & Jones, B. (2009). Animal Welfare Components. In F. Smulders, & B. Algers (Eds.), *Welfare of production animals: assessment and management of risks* (pp. 61-77). The Netherlands: Wageningen Academic Publishers.
- Mellor, D.J. and Stafford, K.J., 2004. Animal welfare implications of neonatal mortality and morbidity in farm animals. The Veterinary Journal 168, 118-133.
- Prunier, A., Bonneau, M., Von Borell, E.H., Cinotti, S., Gunn, M., Fredriksen, B., Giersing, M., Morton, D.B., Tuyttens, F.A.M., Velarde, A., 2006. A review of the welfare consequences of surgical castration in piglets and the evaluation of non-surgical methods. Animal Welfare 15, 277-289.
- Rowan, A.N., 1988. Animal anxiety and animal suffering. Applied Animal Behaviour Science 20, 135-142.
- Schrøder-Petersen, D.L., Heiskanen, T. and Ersboll, A.K. 2004. Tail-in-mouth behaviour in slaughter pigs, in relation to internal factors such as: Age, size, gender, and motivational background. Acta Agriculturae Scandinavica. Section A. Animal Science 54, 159-166.
- Temple, D., Dalmau, A., Ruiz de la Torre, JL., Manteca, X., Velarde, A., 2011. Application of the Welfare Quality® protocol to assess growing pigs kept under intensive conditions in Spain. Journal of Veterinary Behavior, 6, 138-149.
- Velarde, A. (2007). Skin lesions. In A. Velarde & R. Geers (Eds.) *On farm monitoring of pig welfare* (pp.79-83). The Netherlands: Wageningen Academic Publishers.
- Waiblinger, S. and Spoolder, H., 2007. Quality of stockpersonship. In Geers, R. and Velarde, A. (Eds.). On farm monitoring of pig welfare. Wageningen Academic Publishers, Wageningen, pp 159-166.
- Waiblinger, S., Knierim, U., & Winckler, C. (2001). The development of an epidemiologically based on-farm welfare assessment system for use with dairy cows. *Acta Agriculturae Scandinavica Section a-Animal Science suppl.* 30, 73-77.
- Welfare Quality®, 2009. Welfare Quality® assessment protocol for pigs (sows and piglets, growing and finishing pigs). Welfare Quality® Consortium, Lelystad, The Netherlands.
- Winckler, C., Capdeville, J., Gebresenget, G., Hörning, B., & Roiha, U. (2003). Selection of parameters for on-farm welfare-assessment protocols in cattle and buffalo. *Animal Welfare, 12*, 619-624.

Variation in growth: Causes, measurement, and management

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Introduction

The growth performance of pigs under commercial conditions is considerably below the animal's genetic potential. This is illustrated by comparing typical growth rates of grow-finish pigs on commercial facilities, which are commonly around 800 g/day, with those observed in controlled research studies where average growth rates in excess of 1000 g/day are normal and as high as 1500 g/day are achieved. In addition to the wide disparity between potential and realized growth, there is also considerable pig-to-pig variation in growth rate within populations that results in wide variation in live weight of pigs of similar age (weight for age) at the end of finishing. This variation is problematic for the producer when supplying pigs to markets with increasingly narrow carcass weight specifications. This is particularly the case for all-in/all-out systems that are the norm in many industries today where the lightest pigs in the population can delay the emptying of facilities with a consequent increase in costs. However, this variation in weight within populations of pigs is not entirely negative and can present opportunities to differentially manage subpopulations of animals to maximize system output and efficiency. This paper will describe the typical variation in weight within populations of pigs of similar age that has been observed in research studies carried out on commercial wean-to-finish facilities of The Maschhoffs, LLC. Also, the potential causes of this variation in weight for age and potential approaches to minimizing and/or managing this under commercial conditions will be reviewed.

Describing within-population variation in growth rate and weight for age

Over the last 15 years, we have weighed thousands of pigs on commercial facilities in the wean-to-finish period as part of on-farm research protocols. Consequently, we have built up a substantial data base on variation in weight within populations of pigs and, also, on some of the factors that can influence this variation. Interestingly, our data would suggest that there are some consistent aspects to weight for age that can be used to predict what is happening on commercial facilities. Firstly, the frequency distribution of weight for age generally conforms to a normal distribution as illustrated in Fig. 1. The two distributions in this figure relate to the same population of barrows at week 6 [mean weight ~25kg; standard deviation (SD) of weight 3.95 kg] and week 20 (mean weight ~110kg; SD 10.67 kg) post-weaning. If we have estimates of the mean and standard deviation of weight for a population we can predict the weight of all pigs in the population based on the properties of the normal distribution. It is, generally, relatively straightforward to estimate the mean weight of pigs in a pen or barn but estimating the variation (SD) of live weight is more problematic.

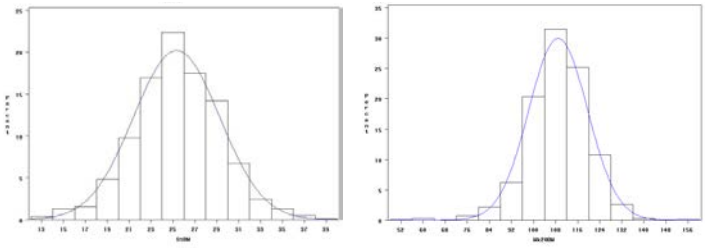

Figure 1. Distribution of live weight within a population of barrows at week 6 (left figure) and week 20 (right figure) post-weaning

Fortunately, variation in weight for age within a population of pigs appears to change in a predictable manner as pigs grow. This is illustrated by the data presented in Fig. 2 which shows the changes in the within-pen coefficient of variation (CV) of live weight as the mean live weight increases over the wean-to-finish period. The data in Fig. 2 are from a number of studies that were conducted in the early part of our research program about 15 years ago and are based on mixed-gender pens.

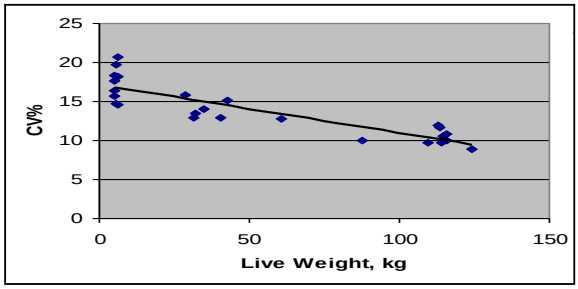

Figure 2. Changes in the CV of live weight within a population with increasing mean live weight

The linear regression equation that gave the best fit to the data in Fig. 2 was: CV = 17.15 – (0.062 × Live Weight); (R^2 = 0.77).

In a more recent study (1), changes in the mean and the within-pen CV of live weight for barrows and gilts (penned separately) were monitored from 40 to 140 kg live weight. The relationship between these two measurements is illustrated in Fig. 3 and the linear regression equations for the two genders were as follows.

Barrows: CV = 21.0 - (0.083× Live Weight); (R^2 = 0.95)

Gilts: CV = 18.7 – (0.065× Live Weight); (R^2 = 0.96)

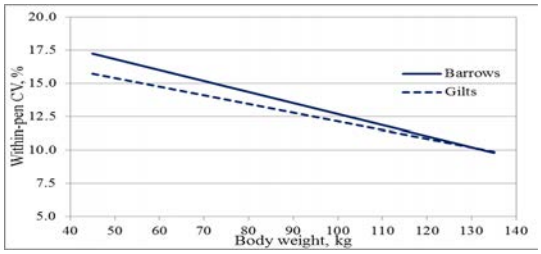

Figure 3. Changes in the CV of live weight within a population with increasing mean live weight

A number of conclusions can be drawn from the relationships presented in Fig. 2 and 3 as follows:

- The initial CV was much greater for the population described in Fig. 3 (~20%) than in Fig. 1 (~17%). In fact, the CV at weaning for the population in Fig. 3 was greater than 20% (data not shown). Interestingly, the CV at weaning for 2 of the populations included in Fig. 2 was also around 20%. The reason(s) for this difference between populations in initial CV are not clear but may be related to the range in ages in the various populations at weaning.
- Despite differences in initial CV, the final CV at harvest weight (110 to 120 kg in Fig. 2 and 130 to 140 kg in Fig. 3) was very similar (~10%) in both examples.
- The initial CV at ~40 kg live weight was greater for barrows than gilts (Fig 3); however, the rate of decrease in CV with weight was also greater for barrows than gilts resulting in similar CV (~10%) for the two genders at the end of the growth period (140kg; Fig. 3). Further research is needed to determine if there is an innate difference in weight variability between barrows and gilts.

Based on these data, variation in weight for age is relatively predictable with CV increasing by ~0.7% for each 10kg increase in live weight. The caveat is that the data used in developing these relationships is from one system only and needs to be validated in other populations.

The above discussion is obviously based on changes in an entire population of pigs over the growth period. We have little understanding of the relative growth of individual pigs that are in the various parts of the weight distribution. Do light pigs at the start of the growth period remain in the lightest part of the weight distribution at the end of the growth period? Caleb Shull (1) tried to answer this question as part of his PhD program by evaluating the relationships between individual pig ranking for weight at various times in the growth period using the data from the population that was used to develop Fig. 3. Surprisingly, the correlations between the animal's weight rank within the population at various times during the growth period were relatively low. For example, the correlation between weight rank at birth and weight rank at either weaning, 10 week, or 22 week post weaning were 0.61, 0.57, and 0.50, respectively.

Similarly, the correlations between weight rank at weaning and at week 10 and 20 post weaning were 0.60 and 0.51, respectively. These relatively low correlations could have resulted from relatively modest changes in the weight rank of a large number of pigs in the population. To address this, the population of pigs was divided into weight quartiles and the movement of pigs between these quartiles over the growth period was evaluated. Although some animals remained in their initial weight quartile throughout the growth period, a substantial number moved between quartiles. For example, at week 22 post weaning the distribution of pigs that were in the lightest weight quartile at birth across the 4 weight quartiles (lightest to heaviest) was 49, 24, 21, and 5%, respectively. In other words, 26% of pigs in the lightest weight quartile at birth ended up in the 2 heaviest weight quartiles by the time the pigs approached harvest weight. As expected, the closer together the time points under consideration, the less movement of pigs between weight quartiles that occurred. The distribution of the pigs that were in the lightest quartile at weaning across the 4 weight quartiles at week 22 post weaning (lightest to heaviest) was 53, 29, 14, and 4%, respectively. However, there were still 18% of pigs that were in the lightest quartile at weaning that ended up in the heaviest 2 quartiles by the time the population reached harvest weight. Obviously, the situation regarding the relative growth of individual pigs within the population is complex and our understanding of this area is limited. However, the major conclusion from these data is that live weights early in the growth period are relatively poor predictors of subsequent growth rates.

Causes of variation in growth rate and weight for age in populations of pigs

The starting point for any consideration of approaches to minimize variation in weight for age within a contemporary group of pigs is to identify the causal factors involved. Unfortunately, our understanding of all of the causes of variation in and the extent of their influence on growth rate and, therefore, on variation in weight within a group of pigs is far from complete. However, we are aware of some of the primary factors involved, if not always the extent of their involvement. These can be considered as either animal related factors or those associated with the environment that the pigs experience.

Animal Factors: The major animal factors influencing the relative growth rates of pigs in a group include:

Genotype: There is considerable genetic variation in growth rate as evidenced by estimates of both the heritability for this trait, which are generally in the range 0.20 to 0.30, and also of heterosis for growth rate which are generally positive (2), suggesting that genetic differences in growth rate between pigs are a fundamental cause of a significant proportion of the variation in weight observed in commercial populations.

Gender: Differences between genders for growth rate are well established. Most commercial swine industries use barrows and gilts, however, there is increased interest in using entire males rather than surgical castrates, particularly as strategies to control boar taint such as immuno-castration gain broader industry acceptance. Rearing pigs in mixed- rather than single-gender populations obviously contributes to variation in growth rates and weight for age.

Age: The greater the range of ages within a population, the greater will be the range in weights. The range in ages within a population will largely be dictated by management practices. However, it is not uncommon in commercial finishing facilities to have a range of ages of at least 7 days which will contribute to the weight variation in such populations.

Gestation Length: There is evidence that piglets from litters with shorter than average gestations lengths, either because of a naturally early farrowing (3) or because of early induction of parturition using exogenous hormones (4,5, 6), can have reduced pre-weaning growth rates which could contribute to variation in weight within a population of animals weaned at the same time. However, the impact of gestation length on the subsequent performance of pigs to harvest weight has not been clearly established and is worthy of study.

Birth Weight: The impact of birth weight on growth performance to harvest has been the subject of considerable research. As previously discussed, the relationship between the pig's weight at birth and at subsequent times through to the time of harvest is positive but not necessarily strong. There is no doubt that light birth-weight pigs grow slower, on average, than heavy birth-weight animals. What has not been clearly established is whether this relationship is linear across the range of birth weights or if it has a plateau. Studies carried out by Dr. Beau Peterson at the University of Illinois (8) illustrated in Fig. 4 would suggest that the later is the case. These data suggest that as birth weight decreased below ~1.5 kg there was a linear reduction in birth-to-finish growth rate; however, above this weight there was no effect of birth weight on subsequent growth rate. However, Fig. 4 is based on a relatively small number of pigs and was carried out under research conditions; further research carried out under commercial conditions is needed to clearly establish the relationship between birth weight and subsequent growth performance.

Environmental Factors: The major environmental factors that potentially could influence the relative growth rate of pigs within a population would include:

Nutrition: The nutritional program can contribute to variation in weight via its effect on the relative growth rates of pigs in the population. In practice, only one diet is fed during a specific period of growth and this will meet the nutrient requirements of only a small proportion of the population. By definition the majority of the pigs will either be under or

over supplied with nutrients. In either case, pigs will grow at below their potential with the extent of the reduction being proportional to the degree to which the nutrient supply is above or below each animal's requirements.

Health Status: In most situations, the major reason that pigs grow at below their potentials is because of the diseases to which they are exposed. Pigs within a contemporary group can be differentially affected by a health challenge which will increase variation in growth rates within the group and contribute to variation in weight.

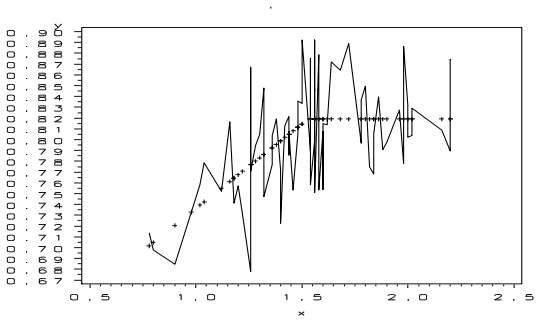

Figure 4. Broken line regression analysis of average daily gain from birth to 145 kg BW on birth weight (Peterson, 2008)

Facility Environment: There are a wide range of factors that contribute to the environment that a pig is exposed to within the facility. These include the climate (temperature, humidity, air movement), the physical environment (pen design, floor space, feeder design and space), and the social environment (group size, mixing, pig removal, stock person interactions). Pigs can be exposed to widely different environments even within the same commercial production system due to variation between and within facilities in aspects such as the climate and access to resources such as feeder and floor space. It is very difficult, if not impossible, to provide an optimum environment for every pig in a population and the different environments that animals experience will contribute to differential growth rates.

Minimizing variation in growth rate and weight

Approaches to minimizing variation within a population, obviously, need to address the principal causes described above. From the standpoint of minimizing genetic variation (in the absence of commercially viable approaches to produce pigs with identical genotypes) the only thing that a producer can do is to minimize the number of sires used to produce the pigs. The use of artificial insemination can obviously help in that regard as will advances in reproductive technologies that minimize the number of sperm needed per insemination dose. There is interest in genetic selection for reduced variation in birth weight within a litter which, in theory, should reduce in variation in weight throughout the wean-to-finish period. However, this approach is unproven and it is unclear if and when such genetic lines would become available to the industry. Consequently, there is little that producers can do at the present time to reduce variation in birth weight or to minimize its impact on subsequent growth rates.

Separating the genders into two subpopulations that can be managed independently combined with nutritional programs specific for each gender can reduce variation in weight as will minimizing the range of ages within commercial groups of pigs. Technologies that synchronize both the time of mating and of farrowing can help in this regard. From a nutritional standpoint, having accurate estimates of the pig's nutrient requirements combined with increasing the number of dietary phases used should reduce the extent of over or under supplying nutrients and reduce variation in growth rate within the population to some degree.

Ensuring that all pigs experience the same or very similar environments should reduce variation in growth rates and weight for age between and within populations in the same production system. Standardizing the number of pigs within a facility as well as providing a consistent rearing environment are important considerations to minimize variation in growth rate. Taking appropriate steps to maintain a high health status in the population in question should reduce variation in growth rate between animals and in weight within the population.

Obviously, there may be practical and/or economic limitations to the extent to which producers can reduce variation in commercial populations.

In addition, it should be borne in mind that the impact of a number of the factors discussed above on growth rates and variation in weight within a population of pigs has not been fully evaluated. This is illustrated by studies that have evaluated the impact of floor space on growth performance. Reducing the floor space at which pigs are kept below a certain level will reduce average growth rates but can increase facility output. However, studies carried out under

commercial conditions have shown no effect of floor space on variation in weight within the population (8). It would appear that the reduction in growth rate resulting from low floor space is relatively the same across all pigs in the population.

This unexpected finding highlights the need to base decisions on changes in management on the results of research carried out under the relevant commercial conditions.

Managing variation in growth rate and weight

Even if it were possible to optimize all of the factors discussed above, there will still be substantial, unavoidable, variation in growth rate and weight for age within a population of pigs. As such, the only option available to producers for dealing with this variation is to manage it and the major potential approaches to achieve this are discussed below.

Sorting of Pigs: One approach that has been proposed to reduce variation in weight within a group is to sort pigs into groups of a similar weight early in the growing period in the belief that this reduced variation will be maintained through to harvest weight. Wolter et al. (9) looked at the effect of sorting groups of 108 pigs at ~30 kg live weight either into two groups of 54 with the same mean and variation in weight as the original group (average weight groups) or into a heavy and a light group each of 54 pigs. After sorting, the light and heavy groups had reduced weight variation compared to the groups of average weight (Table 2). However, by the end of the study period at average live weight of 112kg there was no difference in the CV between the different live weight groups. The CV for weight in the light and heavy groups increased during the study period whereas that for the average weight groups decreased. On the basis of these results and those from other similar studies, sorting pigs on the basis of weight early in the growth period will not reduce variation within the population at harvest weight.

Table 1. Effect of sorting pigs by weight on subsequent Perfomance (Wolter et al., 2002)

	Weight after sorting		
	Average	Light	Heavy
Live weight, kg			
Start (after sorting)	31.1	28.6	34.2
End	112.0	112.8	112.3
Coefficient of variation of live weight, %			
Start (after sorting)	10.7	7.6	6.3
End	9.3	8.5	9.2

One strategy that offers some potential to reduce the variation in weight of the pigs marketed from a population is to remove the heaviest pigs from the population when they reach the target live weight window for the market outlet in question. This not only maximizes the proportion of the population in the target harvest weight window but also results in increased growth performance of the pigs that remain in the pens. The results of a number of studies that we have carried out on commercial facilities investigating the effects of removing a proportion of the pigs from the pen on the performance of the remaining animals are summarized in Table 3 (10). These studies differed in a number of important respects such as the proportion of pigs removed, the timing of removal, and the time period post-removal over which performance was measured. However, all of the studies showed a consistent and large improvement in not only growth rate but also feed efficiency for the pigs that remained in the pen after removal compared to those kept in intact pens (Table 3). Subsequent research has suggested that the increase in growth performance when pigs are removed from the pen results largely from the extra floor space that becomes available to the remaining animals. Consequently, the increase in growth performance after pig removal is likely to be greatest in pigs that were previously kept at floor spaces low enough to restrict growth and is likely to be minimal in pigs that previously had adequate floor space.

Table 2. Effect of removing pigs from a group on the performance of the remaining pigs (summary of 13 studies; DeDecker, 2006)

	Response, %[1]		
	Day 0 to 7	Day 7 to end	Overall
ADG	+10.4	+12.3	+11.3
ADFI	+5.7	+5.5	+5.6
G:F ratio	+6.5	+8.0	+7.2

[1]Response = Percentage difference in performance between pens with pigs removed and intact pens (no pigs removed)

Increasing Growth Rates in Late Finishing: There is some debate over whether increasing the growth rates of populations of pigs throughout the wean-to-finish period will result in less pig-to-pig variation in growth rate and, therefore, weight for age within the population. Unfortunately, research has not generally focused on the impact of growth performance *per se* on variation in weight within populations. As discussed previously, our floor space research would suggest that low growth rates resulting from low floor space do not increase the weight variation within the population. However, it would be wrong to conclude that the lack of a relationship between growth rate and weight variation is proven based on this very limited amount of data. Although it is not clear if increasing the growth rate of pigs at any stage during the growing period directly reduces variation, it should increase the numbers of pigs that reach the target live weight window before the building has to be emptied. There is evidence that increased early growth can be lost later in the growth period (11) and, on this basis, emphasizing increasing growth rates in late finishing has merit.

Fortunately, we have approaches and technologies available that could substantially boost growth rates in late finishing thereby increasing the number pigs in the target weight window and/or speeding up the process of emptying the barn. These include:

Ractopamine: Feeding ractopamine in late finishing has been proven to substantially increase growth rate, feed efficiency, and carcass leanness and this can be a useful tool to reduce the number of light weight pigs at the end of the finishing phase.

Immuno-castration: The major reason for interest in immuno-castration is to allow producers to exploit the production advantages of the entire male compared to the surgical castrate whilst minimizing the risk of producing pork with boar taint. In addition, immuno-castrates exhibit a substantial increase in feed intake and an associated increase in growth rate following the second injection of the vaccine (which effectively castrates the male) which could also aid with management of weight variation in late finishing (12).

Combining Technologies: A number of the approaches to managing weight variation previously discussed can in practice be combined. For example, combining the feeding of ractopamine to immuno-castrates with pig removal is likely to result in a substantial boost in growth performance of the pigs remaining after removal which will considerably increase the proportion of the lighter pigs reaching acceptable harvest weights. However, there has been limited research evaluating the optimum strategy for pig removal (e.g., the frequency and timing of removal and the proportion of pigs to remove) in immuno-castrates fed ractopamine.

Mixing of Pigs: Managing variation in weight within a population can be facilitated by the mixing of pigs at critical times in the production process. For instance, moving the lightest pigs in pens at the end of finishing to another facility could speed up the process of emptying barns. One of the concerns is that the mixing of unfamiliar pigs could have a major negative impact. Luis Ochoa (13) evaluated the effect of mixing of pigs under commercial conditions as part of his PhD program. In general, the results of several of his studies suggested that mixing of pigs had only a small and transitory effect on growth with no effect on longer term performance levels. However, in one study there was evidence of reduced growth performance in pigs that were mixed on two occasions compared to mixing once and in another study the response to mixing was dependent on the weight of the pig, with heavier animals showing a negative response whereas lighter pigs and those of intermediate weight showed no response. On the basis of these studies, the growth response of pigs to being mixed is relatively complex and further research is needed to provide an understanding of the situations in which negative effects can occur.

Conclusions

Variation in growth rates and live weights within a group of contemporary growing pigs has potential advantages but also considerable disadvantages, particularly for managing the marketing of animals at the end of the finishing period in all in/all out systems. This review has attempted to identify the sources of variation and to highlight approaches to minimizing variation. However, even in situations where every practical step has been taken to minimize variation within a population of pigs there will still be a substantial, as yet not quantified, amount of variation remaining. Fortunately, there are tools available to producers, such as the use of ractopamine and immuno-castration, and managing how pigs are removed from the pen when they reach harvest weight, which can be used to manage this variation and increase the proportion of pigs that hit the target weight window before the building has to be emptied. However, the relationship between average growth rates of a group and variation in weight within the group is complex. Further research is needed to clearly understand how variation within a group of pigs is influenced by the whole range of factors that can impact the growth of pigs under commercial conditions. This research is not easy to conduct because it requires large-scale controlled studies in commercial barns and involves the frequent individual weighing of very large numbers of animals. It is unlikely that major progress will be made in our understanding of how pigs grow in commercial units and how the environment that they are exposed to influences the rate of and variation in

that growth until reliable systems become available to automatically capture the weight of pigs in groups without impacting their behavior or performance.

References

1. Shull, 2013. PhD Dissertation, University of Illinois.
2. Cassady et al., 2002. J. Anim. Sci. 80:2286-2302.
3. Mota-Rojas et al., 2014. Anim. Prod. Sci. (in press).
4. Diehl et al., 1977. J. Anim. Sci. 44:89-94.
5. Gunvaldsen et al., 2007. J. Swine Health Prod. 15:84-91.
6. Nguyen et al., 2011. J. Swine Health Prod. 19:214-217.
7. Peterson, 2008. PhD Dissertation, University of Illinois.
8. Shull, 2010. MS Dissertation, University of Illinois.
9. Wolter et al., 2002. JAS 80:2241-2246.
10. DeDecker, 2006. PhD Dissertation, University of Illinois.
11. Wolter et al., 2003. JAS 81:836-842.
12. Puls, 2014. PhD Dissertation, University of Illinois.
13. Ochoa, 2014. PhD Dissertation, University of Illinois

Brachyspiral colitis: An evolving problem

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Background

The name "Brachyspiral colitis" recently was introduced into the literature on swine diseases to describe the situation where colitis, diarrhea and/or dysentery occur in pigs infected with one or more pathogenic *Brachyspira* species (Hampson, 2012). The term was created to emphasise an increasing understanding of the diversity of anaerobic intestinal spirochetes in the genus *Brachyspira* and the fact that a number of different species may have a role in inducing inflammation in the large intestine.

Until only a few years ago most veterinarians would have felt comfortable with the concept of there being two pathogenic *Brachyspira* species capable of causing disease in pigs, each associated with a distinct named disease. The first was the strongly hemolytic *Brachyspira hyodysenteriae* causing swine dysentery, a severe mucohemorrhagic colitis seen mainly in grower and finisher pigs. The second was the weakly hemolytic *Brachyspira pilosicoli* causing porcine intestinal spirochetosis (porcine colonic spirochetosis), a milder form of colitis seen mainly in weaner and grower pigs. Other weakly hemolytic species colonising pigs generally were thought of as being commensals.

Now it is known that there are at least three strongly hemolytic pathogenic *Brachyspira* species that may infect swine and cause a swine dysentery-like disease; furthermore, there is increasing evidence that one or more of the other weakly hemolytic species besides *B. pilosicoli* also have pathogenic potential in swine and other species. Specifically, the weakly hemolytic *Brachyspira murdochii* is increasingly being implicated as an occasional cause of mild colitis in pigs (Jensen et al., 2000; Weissenböck et al., 2005; Komarek et al., 2009; Osorio et al., 2013). It is also clear that individual pigs on different farms may be colonised with more than one *Brachyspira* species and/or strains that may contribute towards causing disease symptoms.

In and interesting parallel, the ceca and colon of adult chickens and other poultry species may be colonised by a number of *Brachyspira* species that can induce inflammation, wet feces and reduced egg production. This disease complex (most commonly is associated with *B. intermedia* and/or *B. pilosicoli*) currently is known as "Avian Intestinal "Spirochetosis" (Hampson, 2013), but for clarity and consistency with the revised disease nomenclature in pigs it might be better if it was to be called "Brachyspiral typhlitis".

Novel strongly hemolytic *Brachyspira* species

Besides *B. hyodysenteriae*, the two provisionally named species "*Brachyspira suanatina*" (Råsbäck et al., 2007a) and *"Brachyspira hampsonii"* (Chander et al., 2012) are both strongly hemolytic and appear to cause a disease that is indistinguishable from swine dysentery (Råsbäck et al., 2007a; Burrough et al., 2012; Rubin et al., 2013). Consequently the definition of the swine dysentery

should be extended to include a mucohemorrhagic colitis caused by any strongly hemolytic *Brachyspira* species, with this disease being part of the Brachyspiral colitis complex.

To date "*B. suanatina*" has only been described in feral mallards and in pigs in Scandinavia (Råsbäck et al., 2007a). In contrast "*B. hampsonii*" has been found to be widespread in pig farms in Canada and parts of the USA (Chander et al., 2012; Rubin et al., 2013a), and more recently has been described in pigs in continental Europe (Mahu et al., 2013; Rhode et al., 2013). The first known isolate of "*B. hampsonii*" (strain P280/1) was recovered from a pig in the UK in the 1980s and later was identified as belonging to a new species (Atyeo et al. 1999). This isolate earlier had been shown to be pathogenic in experimentally infected gnotobiotic pigs (Neef et al., 1994). Another strongly hemolytic spirochete that was distinct from *B. hyodysenteriae* has been recovered from an Australian pig with swine dysentery (Phillips et al., 2007), although its species identity is not yet entirely clear. Further studies are required to determine whether *"B. suanatina"*, *"B. hampsonii"* and other novel strongly hemolytic *Brachyspira* species also occur in other regions of the world and are responsible for causing a swine dysentery-like disease.

Importantly, *"B. hampsonii"* has been isolated from migratory snow geese in Canada (Rubin et al., 2013b) and in migratory waterfowl in Spain (Martínez-Lobo et al., 2013). Migratory waterfowl likely represent natural reservoirs of these *Brachyspira* species, and hence pose a risk of transmitting these bacteria into and between pig farms. Some of these birds may travel long distances and settle on ponds and lagoons in pig farms. Other less migratory species of waterfowl also could act as a source of local transmission between farms, with pigs that are raised in outside lots likely being at greatest risk of exposure to infection. *B. hyodysenteriae* and *B. pilosicoli* also have been isolated from feral waterfowl on occasion, and hence may be transmitted in the same way (Oxberry et al., 1998; Jansson et al., 2011).

Isolates of "*B. hampsonii*" have been described as belonging to two clades (or sub-species), both of which have been identified in North America and in Europe. There has been speculation as to whether the two clades may actually

represent different closely-related species, but given their very similar strongly hemolytic and indole-negative phenotypes, shared reservoir hosts and capacity to cause disease, there does not seem to be any need to consider dividing them further at this time.

These important new findings have required a change in diagnostic procedures. In recent years some diagnostic laboratories have stopped growing *Brachyspira* species from clinical samples, and have relied on using molecular methods for detecting pathogenic species. Unfortunately PCR methods based on the *nox* gene sequence and other sequences that are commonly used for detecting *B. hyodysenteriae* do not detect "*B. suanatina*" or "*B. hampsonii*", and cross-reactions with these species can occur with the *B. intermedia nox* PCRs. Consequently it remains important to culture specimens to look for growth of strongly hemolytic spirochetes. Where these spirochetes are negative in *B. hyodysenteriae* PCRs, it is recommended that their *nox* gene be amplified, sequenced, and the sequence aligned with that of the known *Brachyspira* species (Chander et al., 2012; Osorio et al. 2013; Rhode et al., 2013). Challenges still exist in isolating *Brachyspira* species from clinical samples: they grow slowly and require specialised culture conditions; more than one species or strain may be present; and obtaining pure cultures is problematic as they do not readily form colonies. The latter difficulty also has impeded analysis of individual spirochete strains in cultures that have been subjected to genetic manipulation.

Genomic sequencing as a springboard for discovery

Interest in *Brachyspira* infections of pigs has intensified in the last few years. Reasons for this include the increased occurrence of tiamulin-resistant and multiple drug resistant strains of *B. hyodysenteriae*, recorded particularly in the European region (Duinhof et al., 2008; Sperling et al., 2011); the recent re-emergence of *B. hyodysenteriae* infections in pigs in North America and Brazil; the emergence of "*B. hampsonii*" in north America and Europe; and, importantly, an enhanced understanding of the biology of these bacteria following the publication of genome sequences and the application of new molecular technologies. Detailed information about these *Brachyspira* species still lags behind that available for many other bacterial pathogens of pigs, but the knowledge gap is being reduced and a more contemporary approach to their study is developing.

The genome sequence of *B. hyodysenteriae* strain WA1 was first published five years ago (Bellgard et al., 2009), and since then genome sequences of a number of strains of other *Brachyspira* species have been published. These data have provided new opportunities for undertaking investigations into potential disease mechanisms and identification of virulence factors - for example by comparing gene content and gene expression in pathogenic and non-pathogenic species, and in virulent and non-virulent strains of a species. A list of genome sequences for different *Brachyspira* species available in our laboratory at the time of writing is shown in **Table 1**. The large range of genome sizes that exists between species and even between strains of a single species is an interesting feature, and previously has been described for three strains of *B. pilosicoli* (Mappley et al., 2012). This finding helps to re-emphasise the extensive genomic plasticity of these spirochetes, and the fact that there is considerable redundancy in the genomes of the species – including pathogenic species. Comparative analysis of these data should provide new insights into the evolution and biology of the genus. As with other bacteria, at present many of the genes that have been identified in the sequenced genomes have no known function. Unfortunately at present easy methods for genetic manipulation of *Brachyspira* species are still not available, including being able to undertake gene transfer and gene inactivation experiments that are required to allow a better understanding of gene function.

Vaccines: The availability of the genome sequence of *B. hyodysenteriae* WA1 and other genomes has provided opportunities for new practical applications. For example genome sequence data has been used to broaden the approach to vaccine development through the application of the "reverse vaccinology" approach, where the genes encoding large numbers of predicted surface-exposed proteins or lipoproteins were identified from the *B. hyodysenteriae* genome sequence, screened for distribution amongst different strains, produced as recombinant proteins and tested as vaccine candidates in pigs. A combination of four recombinant proteins that were first identified using reverse vaccinology has given useful levels of protection against swine dysentery in experimentally infected pigs (Song et al., 2009). It is anticipated that a new generation of commercial vaccines for *B. hyodysenteriae* and other *Brachyspira* species that are based on this new approach will become available in the future.

Table 1. Genome sizes of sequenced strains of *Brachyspira* species

Species	No. of strains sequenced	Genome size range (Mb)
B. hyodysenteriae	21	2.92 - 3.85
"B. hampsonii"	5	2.94 - 3.35
B. pilosicoli	4	2.56 – 2.98
B. intermedia	2	3.30 – 3.51
B. innocens	1	3.85
B. murdochii	1	3.24
B. alvinipulli	1	3.36
B. aalborgi	1	2.51

Serological tests: Other predicted surface-exposed proteins of *B. hyodysenteriae* have been identified from the genome sequences, and after extensive testing a number of these proteins that have been expressed in recombinant form and developed as potential antigens for use in a serological ELISA for detection of herds infected with *B. hyodysenteriae*. Such a test has the potential to be a useful adjunct to herd diagnosis and also can be used for disease monitoring, especially as large numbers of samples from individual pigs can be examined regularly in ELISA systems at a relatively low cost. Meat juice samples also can be used as a convenient alternative source of antibodies for this testing (Song et al., 2012).

Plasmid: An unanticipated finding resulting from the sequencing of the genome of *B. hyodysenteriae* strain WA1 was the identification of a previously unrecognised 36 kb plasmid: this contained 31 genes, including six *rfbA-D* genes that were predicted to be involved with rhamnose biosynthesis, and hence lipooligosachharide (LOS) structure, as well as glycosyltransferase genes associated with protein glycosylation (Bellgard et al., 2009). Subsequently a set of PCRs was developed to amplify the plasmid genes, and when applied to DNA extracted from virulent strain B204 this generated the expected product (La et al., 2011). Unexpectedly, however, no PCR products were generated with DNA from avirulent strain A1. Analysis of the DNA using pulsed field gel electrophoresis confirmed the presence of a plasmid band in virulent *B. hyodysenteriae* strains WA1 and B204, but not in the avirulent strain A1. These results suggested that the lack of the plasmid might explain why strain A1 is avirulent. Subsequently 264 Australian field isolates of *B. hyodysenteriae* were tested, and only one was found to lack the plasmid. This strain was predicted to have reduced virulence, and when used experimentally to infect pigs significantly fewer became colonised and developed swine dysentery compared to pigs infected with a control strain containing the plasmid. The results support the likelihood that plasmid-encoded genes of *B. hyodysenteriae* are involved in colonisation and/or in disease expression. Strains of *B. hyodysenteriae* that lack this newly described plasmid (and/or the associated genes) are predicted to have a reduced capacity to colonise pigs. Although such strains appear to be uncommon, where they do occur they are unlikely to induce significant levels of disease. From a practical perspective such strain differences may explain difference in disease severity seen in the field.

New methods for molecular epidemiology

In recent years additional strain typing methods have been developed to assist with understanding and monitoring the molecular epidemiology of B. hyodysenteriae and other Brachyspira species strains on a broad scale. These include multiple-locus variable-number tandem repeats analysis (MLVA) (Hidalgo et al., 2010; Neo et al., 2013a) and multilocus sequence typing (MLST) (Råsbäck et al., 2007b; La et al., 2009b; Phillips et al., 2010; Osorio et al., 2012). MLVA is a rapid and discriminating technique, and is particularly suited to local epidemiological investigations. Although more complex and time consuming, MLST has the advantage that the sequence data can be stored in a public database (PubMLST), and can be added to with time as new sequence types are discovered. Hence MLST has the potential to provide a global picture of strain dissemination and diversity. Both methods have been used to identify the clonal nature of B. hyodysenteriae, the existence of considerable genetic diversity, and the transnational spread of specific clonal groups, including groups with reduced susceptibility to antimicrobials (Hidalgo et al., 2010; Osorio et al., 2012). On the other hand, use of these methods has confirmed that B. pilosicoli has a recombinant population structure and extensive strain variation (Neo et al., 2013a; 2013b).

Pathogenesis

As part of their colonisation process ingested *Brachyspira* cells move through the mucus overlying the epithelium of the large intestine, with their corkscrew-like motility being an important virulence attribute that allows them to penetrate the mucus. In the case of *B. pilosicoli*, this spirochete shows increased motility under viscous conditions (Nakamaru et al., 2006), including mucin concentrations of 6% that are equivalent to those found in the lumen of the porcine colon (Naresh and Hampson, 2010). In addition to their motility, the cells of different *Brachyspira* species demonstrate a

chemotactic attraction to colonic mucin. Comparison of genome sequences has shown that *B. pilosicoli* strain 95/1000 has fewer methyl-accepting chemotaxis genes than *B. hyodysenteriae* strain WA1, and completely lacks *mcpC* genes; hence these species are predicted to have different chemotactic responses, and this in turn may help to explain their different host ranges and colonisation sites in the large intestine (Wanchanthuek et al., 2010). Experimentally, strains of *B. intermedia* and *B. innocens* have been shown to be less attracted to mucin than virulent strains of *B. hyodysenteriae* (Milner and Sellwood, 1994). On the other hand, while cells of both *B. hyodysenteriae* and *B. pilosicoli* were attracted to and entered mucin solutions, at mucin concentrations above 6% this attraction was reduced for *B. hyodysenteriae* but not for *B. pilosicoli* (Naresh and Hampson, 2010). Hence the two species respond differently in different viscous environments. Even within a species there are substantial strain differences: for example, different *B. pilosicoli* strains vary in their motility and chemotactic responses to mucin (Naresh and Hampson, 2010), and two avirulent strains of *B. hyodysenteriae* were less attracted to mucin than were virulent strains tested under the same conditions (Milner and Sellwood, 1994).

Recently an *in vitro* study using colonic-origin Caco-2 cell monolayers has provided some insights into how *B. pilosicoli* interacts with colonic enterocytes to cause disease (Naresh et al., 2009). Similar detailed studies are still required for *B. hyodysenteriae* and other *Brachyspira* species. In the study the Caco-2 cell junctions were shown to be the initial targets of attachment by *B. pilosicoli.* Colonised monolayers then demonstrated a time-dependent series of changes over six hours, including accumulation of actin at the cell junctions, loss of tight junction integrity and condensation and fragmentation of nuclear material consistent with the occurrence of apoptosis induced by the spirochete. Using quantitative reverse transcription PCR, the colonised monolayers exposed to live spirochete cells or sonicates demonstrated a significant up-regulation of interleukin-1ß (IL-1ß) and IL-8 expression, whilst culture supernatants of *B. pilosicoli* and sonicates of non-pathogenic *B. innocens* did not alter cytokine expression. These cytokines/chemokines are likely to be responsible for attracting inflammatory cells to the colonisation site, and causing localizsed colitis. Potential mechanisms for inducing such cellular damage include the biological activity of LOS and/or the action of membrane proteases.

Another likely virulence determinant in *B. hyodysenteriae* and the newly described species is their strong hemolytic activity. A number of studies on *B. hyodysenteriae* molecules with hemolytic activity have been conducted over the years. Currently eight genes encoding proteins with predicted hemolytic activity have been described in *B. hyodysenteriae*, and all but one of these appears to be present in *B. pilosicoli* strain 95/1000 (Bellgard et al., 2009; Wanchanthuek et al., 2010). If these genes really do encode proteins with hemolytic properties, these may not be expressed in *B. pilosicoli* or may not be assembled and/or secreted in a functional form. Further work is required to investigate the functional significance of the one gene that is present in *B. hyodysenteriae* but not in *B. pilosicoli*. In addition, the genetic basis of the strong hemolysis produced by "*B. suanatina*", "*B. hampsonii*" and other non-*B. hyodysenteriae* strains, and the potential contribution of this activity to virulence in these species requires investigation. Currently, the detection of strongly hemolytic *Brachyspira* strains is a good indicator that they are likely to be pathogenic.

Influences of stress hormones on Brachyspiral colitis

A recent interesting finding was that *in vitro* exposure of *B. pilosicoli* to the stress hormone norepinephrine increased growth of the spirochete, attraction to mucin and attachment to Caco-2 cells (Naresh and Hampson, 2011). Norepinephrine is released into the gut following stress, and is likely to have a similar *in vivo* stimulatory affect on *B. pilosicoli* (and other *Brachyspira* species). This finding provides an additional theoretical basis to advise that environmental and social stresses on pigs be minimized as far as possible in order to reduce their susceptibility to infection.

Influences of diet on Brachyspiral colitis

For *Brachyspira* species to induce disease they need to colonise the large intestine and to proliferate to large numbers. Their anaerobic metabolism and use of substrates has been tuned to allow them to thrive in the environment of the large intestine. Nevertheless, there are complex physical and chemical interactions that occur between components of the diet and the normal microbiota in the pig colon, and these can profoundly influence the environment. It has become clear that the resultant conditions can affect colonisation by the spirochetes.

Colonisation of pigs by *B. hyodysenteriae* has been shown to be inhibited by feeding a highly digestible cooked-rice based diet that results in dry pellet-like feces and minimal large intestinal contents and mucus, which presumably is an environment in which the spirochete cannot easily survive (Pluske et al., 1996). Addition of rapidly fermentable fibre sources to this diet returns the contents and consistency of the large intestine to its normal appearance, and reinstated susceptibility to swine dysentery (Pluske et al., 1998). Inhibition of spirochete growth also can be induced in a different approach: the addition of highly fermentable chicory root (containing fructans, especially inulin) and lupins (containing galactans) to a pig diet has been shown to reduce the susceptibility of the animals to experimental swine dysentery

(Thomsen et al., 2007). Subsequently this protective effect was replicated (Hansen et al., 2010), although it was found that the diet needed to contain 80g/kg of inulin to achieve high levels of protection (Hansen et al., 2011). The means by which protection occurs is unclear, but the composition of the diet is known to influence the colonic microbiota of the pig (Leser et al., 2000), and in the case of the diet containing chicory root and lupin it was suggested that increased numbers of *Bifidobacterium thermacidophilum* and *Megasphaera elsdenii* might have inhibited growth of the spirochete (Mølbak et al., 2007). On the other hand, some species of anaerobic bacteria that form part of the normal intestinal microbiota can facilitate *B. hyodysenteriae* colonisation and augment inflammation and lesion production (Whipp et al., 1979). It is likely that inhibition of such members of the microbiota may be one of the ways that ionophores such as monensin and salinomycin can help reduce the expression of swine dysentery. Overall it may be difficult to devise a diet to obtain an appropriate stable composition and balance of species in the colonic microbiota to inhibit spirochete colonisation and/or growth. Furthermore the experimental diets that have been used to reduce susceptibility to swine dysentery to date have been impractical for general commercial use due to their high cost and limited availability. If there were a better understanding of which components of the microbiota were most important for inhibiting *B. hyodysenteriae* and other pathogenic *Brachyspira* species, and how they did this, it might be possible to develop other means to achieve the same end. From a practical perspective, however, these observations suggest that some of the variation in disease severity that occurs between infected herds could be associated with the composition of the normal intestinal microbiota in pigs on the farm, and this itself could be influenced by the diet used.

Colonisation and/or disease expression associated with *B. pilosicoli* also is influenced by diet, and hence dietary manipulation may assist with control of this infection. For example, an analysis of risk factors on farms showed that using home-mixed and/or non-pelleted diets was associated with a reduced prevalence of *B. pilosicoli* infection (Stege et al., 2001). When carboxymethylcellulose was added to an experimental pig diet it resulted in an increased viscosity of the intestinal contents, and enhanced colonisation with *B. pilosicoli* (Hopwood et al., 2002). High levels of soluble non-starch polysaccharide in grains like barley and rye may also increase viscosity, and therefore enhance *B. pilosicoli* colonisation. Consistent with this, pigs fed diets based on cooked white rice (highly-digestible and low in soluble fiber) have shown reduced colonisation with *B. pilosicoli* compared to pigs fed conventional diets (Hampson et al., 2000; Lindecrona et al., 2004). Feeding a pelleted diet rather than meal increased the risk of colonisation, but fermented liquid feed or lactic acid had no influence on colonisation (Lindecrona et al., 2004).

Although it is likely that newly emerging pathogenic species such as "*B. hampsonii*" may respond to dietary changes in similar ways, this has yet to be determined.

Summary

The last few years has witnessed an evolution in understanding of diversity and disease associations of *Brachyspira* species, with, for example, several new pathogenic species being recognised in pigs. In turn this has challenged diagnostic laboratories to develop new methods to identify them, and has emphasised the need to understand their epidemiology and best methods for control. Concurrently, with the recent availability of *Brachyspira* genome sequences and development of new molecular technologies, better insights are emerging into questions such as the growth requirements of the *Brachyspira* species and the pathogenic mechanisms involved in disease causation. This information is of direct benefit for control, since, for example, information about growth and colonisation requirements derived from metabolic reconstructions of the spirochetes can help to predict what changes in the colonic environment are likely to reduce their growth (Mappley et al., 2012). Further detailed studies are needed to determine how the porcine colonic microbiota is influenced by different dietary substrates, and how this impacts on colonisation by different *Brachyspira* species. The sequence data also has allowed the use of a reverse vaccinology approach to vaccine development, and has facilitated the identification of new diagnostic antigens. In the future disease control in infected herds may be achieved using recombinant vaccines, perhaps in conjunction with feeding selected appropriately priced dietary ingredients or additives to modulated the colonic microbiota. This combination is likely to result in reduced spirochete colonisation and minimisation of lesion development.

References

- Atyeo R.F., Stanton T.B., Jensen N.S., Suriyaarachichi D.S. and Hampson D.J. (1999). Differentiation of *Serpulina* species by NADH oxidase (*nox*) gene comparisons and *nox*-based polymerase chain reaction tests. Vet Microbiol 67: 49-62.
- Bellgard M.I., Wanchanthuek P., La T., Ryan K., Moolhuijzen P., et al. (2009). Genome sequence of the pathogenic intestinal spirochete *Brachyspira hyodysenteriae* reveals adaptations to its lifestyle in the porcine large intestine. PLoS ONE 4(3): e4641.
- Burrough E.R., Strait E.L., Kinyon J.M., Bower L.P., Madson D.M., et al. (2012) Comparative virulence of clinical *Brachyspira* spp. isolates in inoculated pigs. J Vet Diagn Invest 24: 1025-1034.

- Chander Y., Primus A., Oliveira S. and Gebhart, C.J. (2012) Phenotypic and molecular characterization of a novel strongly hemolytic *Brachyspira* species, provisionally designated "*Brachyspira hampsonii*". J Vet Diagn Invest 24: 903-910.
- Duinhof T. F., Dierikx C.M., Koene M.G., van Bergen M.A., Mevius D.D., et al. (2008) [Multiresistant *Brachyspira hyodysenteriae* in a Dutch sow herd]. Tijdschr Diergeneeskd 133: 604-608.
- Hampson D.J. (2012). Brachyspiral colitis. Chapter 50. In: *Diseases of Swine* 10th Ed. (Eds: Zimmerman, J.J., Karriker L.A., Ramirez, A., Schwartz K.J., and Stevenson G.W.). Wiley-Blackwell, Iowa, USA. pp. 680-696.
- Hampson D.J. (2013). Avian Intestinal Spirochetosis. Chapter 23. In: *Diseases of Poultry* 13th Ed. (Eds. Swayne D.E., Glisson J.R., McDougald L.R., Nolan L.K, Suarez D.L. and Nair V.) Wiley-Blackwell, Iowa, USA. pp. 995-1007.
- Hampson D.J., Robertson I.D., La T., Oxberry S.L, and Pethick D.W. (2000) Influences of diet and vaccination on colonisation of pigs with the intestinal spirochaete *Brachyspira (Serpulina) pilosicoli.* Vet Microbiol 73: 75-84.
- Hansen C.F., Phillips N.D., La T., Hernandez A., Mansfield J., et al. (2010) Diets containing inulin but not lupins help to prevent swine dysentery in experimentally infected pigs. J Anim Sci 88: 3327-3336.
- Hansen C.F., Hernández A., Mansfield J., Hidalgo Á, La T., et al. (2011) A high dietary concentration of inulin is necessary to reduce the incidence of swine dysentery in pigs experimentally challenged with *Brachyspira hyodysenteriae.* Br J Nutr 106: 1506-1513.
- Hidalgo Á., Carvajal A., La T., Naharro G., Rubio P., Phillips N.D. and Hampson D.J. (2010) A multiple-locus variable-number tandem repeats analysis of the swine dysentery pathogen, *Brachyspira hyodysenteriae.* J Clin Microbiol 48: 2859-2865.
- Hopwood D.E., Pethick D.W. and Hampson D.J. (2002) Increasing the viscosity of the intestinal contents stimulates proliferation of enterotoxigenic *Escherichia coli* and *Brachyspira pilosicoli* in weaner pigs. Brit J Nutr 88: 523-532.
- Jansson D.S., Persson M., Zimmerman U. and Johansson K.E. (2011) Phenotypic and genetic diversity among intestinal spirochaetes (genus *Brachyspira*) in free-living wild mallards (*Anas platyrhynchos*) sampled in southern Sweden. Syst Appl Microbiol 34: 566-575.
- Jensen T.K., Christensen A.S. and Boye M. (2010) *Brachyspira murdochii* colitis in pigs. Vet Pathol 47:334-385.
- Komarek V., Maderner A., Spergser J., and Weissenböck H. (2009) Infection with weakly haemolytic *Brachyspira* species in pigs with miscellaneous chronic diseases. Vet Microbiol 134:311-317.
- La T., Phillips N.D., Harland B.L., Wanchanthuek P., Bellgard M.I. and Hampson D.J. (2009) Multilocus sequence typing as a tool for studying the molecular epidemiology and population structure of *Brachyspira hyodysenteriae*. Vet Microbiol 138: 330-338.
- La T., Phillips N.D., Wanchanthuek P., Bellgard M.I., O'Hara A.J. and Hampson D.J. (2011) Evidence that the 36kb plasmid of *Brachyspira hyodysenteriae* contributes to virulence. Vet Microbiol 153: 150-155.
- Leser T.D., Lindecrona R.H., Jensen T.K., Jensen B.B. and Møller K. (2000) Changes in bacterial community structure in the colon of pigs fed different experimental diets and after infection with *Brachyspira hyodysenteriae*. Appl Environ Microbiol 66: 3290-3296.
- Lindecrona R.H., Jensen T.K. and Møller K. (2004) Influence of diet on the experimental infection of pigs with *Brachyspira pilosicoli*. Vet Rec 154: 264-267.
- Mahu M., de Jong E., De Pauw N., Vande Maele L., Vandenbroucke V., et al. (2013) First isolation of "*Brachyspira hampsonii*" from pigs in Europe. Vet Rec 2013 Dec 11.
- Mappley L.J., Black M.L., Abuoun M., Darby A.C., Woodward M.J., et al. (2012). Comparative genomics of *Brachyspira pilosicoli* strains: extensive genome rearrangements and reductions, and correlation of genetic compliment with phenotypic diversity. BMC Genomics 2012 Sep 5;13(1):454.
- Martínez-Lobo F.J., Hidalgo A., García M., Argüello H., Naharro G., et al. (2013) First identification of "*Brachyspira hampsonii*" in wild European waterfowl. PLoS One. 2013 Dec 4;8(12):e82626.
- Milner J.A. and Sellwood R. (1994) Chemotactic response to mucin by *Serpulina hyodysenteriae* and other porcine spirochetes: Potential role in intestinal colonisation. Infect Immun 62: 4095–4099.
- Mølbak L., Thomsen L.E., Jensen T.K., Bach Knudsen K.E. and Boye M. (2007) Increased amount of *Bifidobacterium thermacidophilum* and *Megasphaera elsdenii* in the colonic microbiota of pigs fed a swine dysentery preventive diet containing chicory roots and sweet lupine. J Appl Microbiol 103: 1853-1867.
- Nakamura S., Adachi Y., Goto T. and Magariyama Y. (2006) Improvement in motion efficiency of the spirochete *Brachyspira pilosicoli* in viscous environments. Biophys J 90: 3019-3026.
- Naresh R. and Hampson D.J. (2010) Attraction of *Brachyspira pilosicoli* to mucin. Microbiology 156: 191-197.
- Naresh R. and Hampson D.J. (2011) Exposure to norepinephrine enhances *Brachyspira pilosicoli* growth, attraction to mucin and attachment to Caco-2 cells. Microbiology 157: 543-547.
- Naresh R., Song Y. and Hampson D.J. (2009) The intestinal spirochete *Brachyspira pilosicoli* attaches to cultured Caco-2 cells and induces pathological changes. PLoS ONE 4(12): e8352.
- Neef N., Lysons R.J., Trott D.J., Hampson D.J., Jones P.W. and Morgan J.H. (1994) Pathogenicity of porcine intestinal spirochetes in gnotobiotic pigs. Infect Immun 62: 2395–2403

- Neo E., La T., Phillips N.D. and Hampson D.J. (2013a) Multiple locus variable number tandem repeat analysis (MLVA) of the pathogenic intestinal spirochaete *Brachyspira pilosicoli.* Vet Micro 163: 299-304.
- Neo E., La T., Phillips N.D., Alikhani Y. and Hampson D.J. (2013b) The pathogenic intestinal spirochaete *Brachyspira pilosicoli* forms a diverse recombinant species demonstrating some local clustering of related strains and potential for zoonotic spread. Gut Pathogens 2013 5:24.
- Osorio J., Carvajal A., Naharro G., La T., Phillips N.D., et al. (2012) Dissemination of clonal groups of *Brachyspira hyodysenteriae* between pig farms in Spain, and their relationships to isolates from other countries. PLoS ONE 7(6): e39082.
- Osorio J., Carvajal A., Naharro G., Rubio P., La T., et al. (2013) Identification of weakly haemolytic *Brachyspira* isolates recovered from pigs with diarrhoea in Spain and Portugal. Res Vet Sci 95: 861-869.
- Oxberry S.L., Trott D.J. and Hampson D.J. (1998) *Serpulina pilosicoli*, water birds and water: potential sources of infection for humans and other animals. Epidemiol Infect 121: 219-225.
- Phillips N.D., La T., Dunlop H. and Hampson D.J. (2007) An unusual strongly haemolytic spirochaete isolated from an Australian herd with suspected swine dysentery. Proceedings of the Fourth International Conference on Colonic Spirochaetal Infections of Animals and Humans. Prague, Czech Republic. Abstract 19.
- Phillips N.D., La T., Amin M.A. and Hampson D.J. (2010) *Brachyspira intermedia* strain diversity and relationships to other indole-positive *Brachyspira* species. Vet Microbiol 143:246-254.
- Pluske J.R., Siba P.M., Pethick D.W., Durmic Z. Mullan B.P. and Hampson D.J. (1996) The incidence of swine dysentery in pigs can be reduced by feeding diets that limit fermentation in the large intestine. J Nutr 126: 2920-2933.
- Pluske J.R., Durmic Z., Pethick D.W., Mullan B.P. and Hampson D.J. (1998) Confirmation of the role of rapidly fermentable carbohydrates in the expression of swine dysentery in pigs after experimental infection. J Nutr 128: 1737-1744.
- Råsbäck T., Jansson D.S., Johansson K.E. and Fellström C. (2007a) A novel enteropathogenic, strongly haemolytic spirochaete isolated from pig and mallard, provisionally designated '*Brachyspira suanatina*' sp. nov. Environ Microbiol 9: 983-991.
- Råsbäck T., Johansson K-E., Jansson, D.S., Fellström, C. Alikhani, Y., et al.. (2007b) Development of a multilocus sequence typing scheme for intestinal spirochaetes of the genus *Brachyspira*. Microbiology 153: 4074-4087.
- Rohde J., Habighorst-Blome K. and Seehusen F. (2013) "*Brachyspira hampsonii*" clade I isolated from Belgian pigs imported to Germany. Vet Microbiol 2013 Nov 22.
- Rubin J.E., Costa M.O., Hill J.E., Kittrell H.E., Fernando C., et al. (2013a) Reproduction of mucohaemorrhagic diarrhea and colitis indistinguishable from swine dysentery following experimental inoculation with "*Brachyspira hampsonii*" strain 30446. PLoS One. 2013a;8(2):e57146.
- Rubin J.E., Harms N.J., Fernando C., Soos C., Detmer S.E., et al. (2013b) Isolation and characterization of *Brachyspira* spp. including "*Brachyspira hampsonii*" from lesser snow geese (*Chen caerulescens caerulescens*) in the Canadian Arctic. Microb Ecol 66: 813-822.
- Song Y., La T., Phillips N.D., Bellgard M.I. and Hampson D.J. (2009) A reverse vaccinology approach to swine dysentery vaccine development. Vet Microbiol 137: 111-119.
- Song Y., Frey B. and Hampson D.J. (2012) The use of ELISAs for monitoring exposure of pig herds to *Brachyspira hyodysenteriae.* BMC Vet Res 2012 Jan 17;8:6.
- Sperling D., Smola J. and Cízek A. (2011) Characterisation of multiresistant *Brachyspira hyodysenteriae* isolates from Czech pig farms. Vet Rec 168: 215.
- Stege H., Jensen T.K., Møller K. Baekbo P. and Jorsal S.E. (2001) Risk factors for intestinal pathogens in Danish finishing pig herds. Prev Vet Med 50: 153-164.
- Thomsen L.E., Knudsen, K.E.B., Jensen, T.K., Christensen, A.S., Møller, K. and Roepstorff, A. (2006) The effect of fermentable carbohydrates on experimental infections with *Brachyspira hyodysenteriae* and *Trichuris suis* in pigs. Vet Microbiol 142: 112–122.
- Wanchanthuek P., Bellgard, M.I., La T., Ryan K., Moolhuijzen P., et al. (2010) The complete genome sequence of the pathogenic intestinal spirochete *Brachyspira pilosicoli* and comparison with other *Brachyspira* genomes. PLoS ONE 5(7): e11455.
- Weissenböck H., Maderner A., Herzog A.M., Lussy H. and Nowotny N. (2005) Amplification and sequencing of *Brachyspira* spp. specific portions of *nox* using paraffin-embedded tissue samples from clinical colitis in Austrian pigs shows frequent solitary presence of *Brachyspira murdochii*. Vet Microbiol 111: 67-75.
- Whipp S.C., Robinson I.M., Harris D.L., Glock R.D., Matthews P.J. and Alexander T.J. (1979) Pathogenic synergism between *Treponema hyodysenteriae* and other selected anaerobes in gnotobiotic pigs. Infect Immun 26: 1042-1047.

Innate and adaptive immunity responses of swine to PRRSV

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The vexing problem with PRRS immunity

Porcine reproductive and respiratory syndrome (PRRS) is an economically important disease of swine characterized by abortion, stillbirth and weak-born pigs. In its non-reproductive form, this syndrome affects younger pigs more severely than older animals, which clinically manifested by reduced growth rate, feed efficiency and pneumonia that can be made more severe by co-infection with other pathogens (Thacker, 2004; Klinge et al., 2009). The etiologic agent for this disease is an RNA virus (PRRSV) that belongs to the family *Arteriviridae*, which targets macrophages for infection (Wensvoort et al., 1991; Collins et al., 1992). PRRSV exhibits a significant degree of genetic diversification (Murtaugh et al., 1998; Key et al., 2001; Goldberg et al., 2003). There are two genotypes of PRRSV, denoted Types 1 (European type) and 2 [North American (NA) type]. In North America, Type 2 PRRSV is the dominant genotype in the field, which based on a comprehensive collection of ORF5 sequences, Type 2 PRRSV has been classified into nine distinct lineages (Shi et al., 2010). Notably, over the last 10 years a major shift in the genetic composition of Type 2 PRRSV has occurred in the Midwest USA as a result of multiple introductions of virus from Canada (Shi et al., 2013). These viruses are now endemic and have gradually replaced the original local virus populations that belong lineages 6 to 9 (USA-like), and have been replaced predominantly by lineage 1 (Canadian-like) viruses. Included in lineage 1 are the notorious PRRS viruses with an RFLP 1-8-4 and 1-22-2 types, which, as compared to the original USA-like lineages 6-9, appear to be more virulent, grow to larger titers in pigs and are more readily shed in aerosols (Shi et al., 2013).

Under experimental conditions, the currently available modified live virus (MLV) vaccine against this pathogen has been shown to provide immunized pigs adequate protection from subsequent challenge with a non-genetically divergent (homologous) strain (Lager et al., 1997a, b), and partial protection against infection by a genetically divergent (heterologous) strains (Osorio et al., 1998; Lager et al., 1999; Mengeling et al., 2003a, b). Based on the steady increase in the prevalence of PRRS in the commercial swine operations in the United States as reported by the USDA APHIS as well as the results from experimental vaccination and challenge studies (Halbur, 2003), it can be reasonably stated that the overall level of protective immunity provided to swine by currently available PRRS modified live virus vaccines in commercial settings can be considered inadequate. This situation can be attributed to the apparent lack of development of sufficiently strong levels of protective immune effector mechansism elicited by either wild-type and modified live virus vaccines. This situation is evidenced by the fact that the infection of pigs with wild type PRRSV (Nelson et al., 1994; Loemba et al., 1996; Vezina et al., 1996; Yoon et al., 1995; Albina et al., 1998b; Gonin et al., 1999) or their vaccination with a live attenuated form of this virus (Labarque et al., 2000; Ostrowski et al., 2002) elicits an exuberant production of non-neutralizing antibodies. In contrast, a transient T cell mediated PRRSV-specific lymphoproliferative response is detected at 4 weeks post infection and lasts an additional 9 (Bautista and Molitor, 1997) to 14 weeks (Lopez-Fuertes et al., 1999). Moreover, during this time interval, limited quantities of IFN–γ secreting cells (SC) are generated (Meier et al., 2003; Xiao et al., 2004). Interestingly, in the absence of additional antigenic stimulation this polarity reverses within the ensuing 5 months, as manifested by a decreasing antibody response and a gradual increase in the intensity of the IFN–γ response (Meier et al., 2003). The initial antibody-dominated immune response is not the result of insufficient antigenic stimulation, since neither the inclusion of a commercial adjuvant during primary vaccination (Meier et al., 2003) nor booster immunizations of previously heavily vaccinated pigs (Bassaganya-Riera et al., 2004) enhances virus-specific cell-mediated immunity (CMI). Thus, PRRSV seems to inherently stimulate an imbalanced immune response characterized by an abundant virus-specific non-neutralizing antibody response and a limited, but potentially protective, T helper (Th) 1–like IFN–γ response (Murtaugh et al., 2002). Remarkably, a significant variability within the swine population in regards to their innate and adaptive immune responses to PRRSV has been observed (Xiao et al., 2004; Royaee et al., 2004). This variability is likely responsible for the inconsistency of the clinical outcomes seen upon challenging either naïve or previously immunized pigs with virulent PRRSV (Labarque et al., 2003; Mengeling et al., 2003a,b). Thus, the ultimate outcome of the interaction between this virus and its host will be determined by the ability of the host to overcome the inherent PRRS virus propensity to prevent the timely development of protective innate or adaptive immunity capable of inhibiting its infectious process.

Definition of PRRSV protective immunity

For a clinician the raison d'être for studies on the innate and adaptive immune response to a virus is develop strategies to elicit protective immune responses. In the case of PRRSV, the identification of the immunologic mechanism(s) responsible for mediating protective immunity against PRRSV poses a significant challenge for several reasons. First, while vaccination can decrease the duration and magnitude of viremia following an experimental challenge (van Woensel et al., 1998; Verheije et al., 2003), the reduction in viremia is not necessarily associated with a commensurate

amelioration in the severity of other clinical parameters associated with PRRSV infection, such as a lessened rate of weight gain, fever, respiratory distress or virus transmission to sentinel pigs (Nodelijk et al, 2001; Labarque et al., 2003; Mengeling et al., 2003a). In studies conducted by van der Linden et al. (2003), a similar lack of correlation between viremia and clinical signs was also noted when two different age groups of non-immune pigs were infected with PRRSV. In theses studies a greater frequency of viremia with an accompanying higher virus titer was found in younger animals (2 months of age) as compared to older pigs (6 months of age), while the later exhibited more severe clinical signs. The difficulty of deciphering PRRSV biology is further revealed by the marked degree of variability and irreproducibility of consecutive trials conducted by the same investigators (Labarque et al., 2003; Mengeling et al., 2003a,b).

In the case of pseudorabies virus a positive association between protection from disease and the intensity of the IFN–γ response as well as virus neutralizing antibodies was established (Zuckermann et al., 1998; 1999; van Rooij et al., 2004). However, in the case of PRRSV, the association between protective immunity and humoral and/or cellular immune adaptive immune responses elicited by vaccination has been more difficult to establish in the respiratory form of the disease a similar relationship concerning PRRSV may or may not be observed (Meier et al., 2004). Thus, the identification of immunologic mechanism(s) responsible for mediating protective immunity against PRRSV will require studies that monitor both the humoral and cellular immune response to vaccination (Meier et al, 2003, 2004), and measure the extent of reproductive failure and pneumonia caused by this virus as a measurement of the degree of protection (Lager et al., 1999). Notably, in recent, related field studies we have noticed a positive correlation between the reduction of abortion/still births in sows and the relative frequency of PRRSV-specific IFN–γ SC in their blood (Lowe et al., 2004), although this correlation is not always evident (Lowe et al., 2005)

Studies by Osorio et al. (2002) have already shown that virus-neutralizing antibodies are capable of providing sterilizing immunity against PRRSV-induced reproductive failure. Strategies designed to shift the bias of the initial reaction to PRRSV from the strong elicitation of non-neutralizing antibody production towards a greater Th1-like immune response including the development of virus neutralizing antibodies are worth exploring since they could conceivably lead to the development of an improved vaccine against this pathogen. The need to develop such methodology is made palpable by the unusual kinetics of the immune response to this virus, as evidenced by the lack of a marked increase in the cell-mediated immune response upon vaccination and subsequent challenge with virulent virus. The IFN–γ response to PRRSV therefore appears to be determined at the time of the first exposure to this pathogen and is only minimally affected by re-exposure. In addition, similar limited changes in the IFN–γ response have been observed upon challenge of vaccinated pigs with wild-type virus (Foss et al., 2002) or booster immunization with MLV vaccine (Meier et al., 2003). Remarkably, the T-cell proliferative response to PRRSV was also not increased by a booster immunization in pigs that had previously been repeatedly exposed to a MLV vaccine, but rather appeared to be suppressed as compared to that elicited by the same vaccine in naïve pigs (Bassaganya-Riera et al., 2004). The mechanism responsible for this unusual effect is currently unknown, but might be related to the persistence of the virus in lymphoid tissues associated with the site of infection (Xiao, et al., 2004). Such sustained presence of virus could adversely affect a subsequent response due to an inherent yet unknown biological property of PRRSV. Clearly, further studies will be required to clarify how the outcome of a PRRSV infection is influenced by the intensity of the IFN–γ response mediated by memory T cells as well as by virus-neutralizing antibodies.

The innate immune response to PRRSV

One characteristic of PRRSV infection that probably contributes to the retarded development of a specific cell-mediated immune response is the apparent lack of an adequate IFN–α response to the viral infection. Usually, virus-infected cells secrete type I IFN and the released cytokine interacts with a subset of naïve T cells to promote their conversion into virus-specific IFN–γ SC (Cella et al., 2000; Cousens et al. 1999; Kadowaki et al., 2000; Biron, 2001; Levy et al., 2003). In contrast, the IFN–α response to exposure to PRRSV is nearly non-existent. Production of IFN–α in the lungs of pigs acutely infected with PRRSV was either almost undetectable, or 159-fold lower than that induced by another pathogen, porcine respiratory coronavirus (PRCV) (Buddaert et al. 1998; van Reeth et al., 1999). Such lack of efficient stimulation of IFN–α production by a pathogen would be expected to have a significant impact on the nature of the host's adaptive immune response, since IFN–α up-regulates IFN–γ gene expression, and thus controls the dominant pathway that promotes the development of adaptive immunity, namely, T cell-mediated IFN–γ responses and peak antiviral immune defenses (Cousens et al. 1997; Levy et al., 2003). In this regard, it has become evident that the link between innate and adaptive immunity in viral infections occurs through the interaction of dendritic cells with type I interferon (Montoya et al., 2002; Though, 2004) and the dendritic-cell controlled polarization of T-cell function (Kapsenberg, 2003). The production of IFN–α by plasmacytoid dendritic cells (pDCs) has an autocrine effect that promotes their functional and phenotypic activations- events necessary for their optimal expression of co-stimulatory molecules and subsequent ability to cause naïve T cells to differentiate into IFN–γ-SC (Cella et al., 2000; Kadowaki et al., 2000; Fitzgerald-Bocarsly et al., 2002; Montoya et al., 2002; Honda et al., 2003). Presumably, PRRSV is a poor inducer of IFN–α production by pDCs since unlike transmissible gastroenteritis virus (Charley et al., 1990; Nowacki et al., 1993) and

type–α CpG oligonucleotides (Guzylack-Piriou et al., 2004) it fails to stimulate the secretion of IFN–α from cultured porcine PBMC (Albina et al., 1998a; Calzada-Nova, et al., 2010). Direct examination of the outcome of the interaction of PRRSV with porcine pDCs will likely reveal important information on the immunobiology of this virus, especially since this virus is susceptible to the antiviral effects of IFN–α (Albina et al., 1998a).

Using mouse models of anti-viral immunity it has been shown that in the absence of IFN–α/β production, the cytokine IL-12 (Orange and Biron 1996) can increase the virus-specific IFN–γ production by T cells (Cousens et al. 1999). Thus, two alternative routes (IL-12- or type I IFN-dependent) can lead to an adaptive Th 1 cell-mediated immune response with potent antiviral effects (Biron, 2001). According to a scenario involving the presence of less than a requisite amount of IFN–α, IL-12 could provide the necessary impetus for the development of an anti-viral IFN–γ response. In this regard, IL-12 mRNA has been detected in porcine macrophages infected with PRRSV (Thanawongnuwech et al., 2001), and transiently in the lungs of PRRSV-infected pigs (Chung and Chae, 2003). However, this pathogen is also apparently a poor stimulator of IL-12 production, since a negligible quantity of IL-12 mRNA or protein was produced by porcine PBMC exposed *in vitro* to PRRSV (Royaee et al., 2004; Calzada-Nova et al., 2010).

Approaches to improve the stimulation of protective immunity to PRRS virus

To compensate for the apparent inadequate innate cytokine stimulation elicited by the infection of pigs with PRRSV, novel adjuvants have been used during immunization. The administration of IL-12 in combination with a live or killed PRRSV vaccine resulted in an increased lymphoproliferative response to this virus (Wee et al., 2001), as well as an enhanced the host IFN–γ response to a modified live PRRSV vaccine (Foss et al., 2002). Similarly, the injection of IFN–α provided exogenously in the form of an expressible cDNA (pINA) was found to exert an adjuvant effect on the vaccine-induced IFN–γ response to PRRS virus (Meier et al., 2004). Remarkably, no significant alteration in the development of the humoral immune response has observed as a result of either of these treatments. Thus, even with such interventions at the initiation of PRRSV immunization, the usual rapid onset of anti-PRRSV antibody production and delayed appearance of VN antibodies (Labarque et al., 2000; Ostrowski et al., 2002; Meier et al., 2003) still occurs. We have observed that the provision of IFN–α cDNA has a more pronounced and sustained effect on the intensity of the cell-mediated immune response (Meier et al., 2004). Likewise, the introduction of a known inducer of IFN–α production in pigs, poly I:C (Derbyshire and Lesnick, 1990), during vaccination was found to temporarily amplify the quantities of PRRSV-specific IFN–γ SC, but is not as efficient as the IFN–α encoding plasmid at enhancing the IFN–γ response to the vaccine. The observation that the inclusion of either IL-12 and IFN–α during immunization increased the intensity of the IFN–γ response to PRRSV validates the proposed role of these two innate cytokines in directing the *in vivo* differentiation of swine Th1 cells, and helps explain the poor virus-specific IFN–γ response that normally develops as a result of the exposure of pigs to PRRSV (Meier et al., 2003; Xiao et al., 2004). It should be noted that the efficacy of a PRRS MLV vaccine is improved by simply producing the vaccine virus in a porcine alveolar macrophage rather than in simian cells (Calzada-Nova et al., 2012). Clearly, regarding the quality and effectiveness of the protective immunity elicited by a PRRS virus biologic there is room for improvement. Consistent with our previously postulated notion of an important role of IFN-α in eliciting protective immunity against PRRSV (Royaee et al., 2004; Meier et al., 2004) in recent studies we found that the PRRS live virus vaccine strain G16X developed in our laboratory, which is capable of eliciting a significant IFN-α response *in vivo*, stimulates the development of significant levels of protective immunity against highly virulent NA type PRRSV isolates that are genetically divergent (heterologous) to the vaccine. In these studies, the novel PRRS live virus vaccine G16X, which was derived from a naturally non-virulent PRRS virus and belongs to NA lineage 5, was capable of eliciting adequate levels of protective immunity against the genetically divergent and high virulence PRRSV isolate NADC20, which as an atypical (ATP) isolate belonging to lineages 8, as well as against a more modern lineage 1 (Canadian-like) isolate with an RFLP 1-22-2 that was isolated from a severe 2011 outbreak of PRRS in a breeding herd located in the American Midwest (Zuckermann et al., unpublished). The ability of this novel vaccine virus to provide adequate levels of protective immunity to heterologous strains is consistent with the growing evidence that the degree of genetic homology of PRRSV ORF5 between the challenge strain and the vaccine is not predictive of the degree of protective immunity elicited (Prieto et al., 2008), but rather, as we have postulated, has more to do with the type of host cell in which the vaccine is prepared (Calzada-Nova et al., 2012), as well the biological properties of the vaccine.

Summary

It is apparent that infection or vaccination with PRRSV elicits in swine an immune response that is insufficient to provide satisfactory protective immunity from the field virus. PRRS virus elicits an immune response that is characterized by an abundance of non-neutralizing antibodies and a paucity of IFN–γ SC. The molecular pathway responsible for generating this type of immunity is unknown at this time, but based on our studies (Meier et al., 2004) it

likely involves the limited induction of IFN–α and IL-12 production and/or inherent structural elements of the virus that promote such a response. We propose that the strong humoral immunity bias of the host response to PRRSV is mostly responsible for the difficulties in the development of a vaccine deemed effective in the field.

References

- Albina, E., Carrat, C., Charley, B., 1998a. Interferon-alpha response to swine arterivirus (PoAV), the porcine reproductive and respiratory syndrome virus. J. Interferon Cytokine Res. 18, 485-490.
- Albina, E., Piriou, L., Hutet, E., Cariolet, R., L'Hospitalier, R.,1998b. Immune responses in pigs infected with porcine reproductive and respiratory syndrome virus (PRRSV). Vet. Immunol. Immunopathol. 61, 49-66.
- Bassaganya-Riera J., Thacker B.J., Yu S., Strait E., Wannemuehler M.J, Thacker E. L., 2004. Impact of immunizations with porcine reproductive and respiratory syndrome virus on lymphoproliferative recall responses of CD8+ T cells. Viral Immunol. 17, 25-37.
- Bautista, E.M., Molitor, T.W., 1997. Cell-mediated immunity to porcine reproductive and respiratory syndrome virus in swine. Viral Immunol. 10, 83-94.
- Bautista E.M., Molitor T.W., 1999. IFN gamma inhibits porcine reproductive and respiratory syndrome virus replication in macrophages. Arch. Virol. 144, 1191-1200.
- Biron, C.A., 2001. Interferons alpha and beta as immune regulators-a new look. Immunity. 14, 661-664.
- Buddaert, W., Van Reeth, K., Pensaert, M. 1998. In vivo and in vitro interferon (IFN) studies with the porcine reproductive and respiratory syndrome virus (PRRSV). Adv. Exp. Med. Biol. 440, 461-467.
- Calzada-Nova G, Schnitzlein W, Husmann R, Zuckermann FA. 2010. Characterization of the cytokine response of pure populations of porcine plasmacytoid dendritic cells to porcine viruses and toll-like receptor agonists. Vet Immunol Immunopathol. 135(1-2):20-33.
- Calzada-Nova G, Schnitzlein W, Husmann R, Zuckermann FA. 2011. North American porcine reproductive and respiratory viruses inhibit type I interferon production by plasmacytoid dendritic cells. J. Virol. doi:10.1128/JVI.01616-10. Vol. 85 (6) 2703-2713.
- Calzada-Nova G, Husmann R , Schnitzlein W, Zuckermann FA. 2012. Effect of the host cell line on the vaccine efficacy of an attenuated porcine reproductive and respiratory syndrome virus. Vet Immunol Immunopathol. Jul 15;148(1-2):116-25. doi: 10.1016/j.vetimm.2012.05.008. Epub 2012 May 11.
- Cella, M., Facchetti, F., Lanzavecchia, A., Colonna, M., 2000. Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent TH1 polarization. Nat. Immunol. 1, 305-310.
- Charley, B., Lavenant, L., 1990. Characterization of blood mononuclear cells producing IFN alpha following induction by coronavirus-infected cells (porcine transmissible gastroenteritis virus). Res. Immunol. 141, 141-151.
- Chinsangaram, J., Moraes, M.P., Koster, M., Grubman, M.J., 2003. Novel viral disease control strategy: adenovirus expressing alpha interferon rapidly protects swine from foot-and-mouth disease. J. Virol. 77, 1621-1625.
- Chung, H.K., Chae, C., 2003. Expression of interleukin-10 and interleukin-12 in piglets experimentally infected with porcine reproductive and respiratory syndrome virus (PRRSV). J. Comp. Pathol. 129, 205-212.
- Collins, J.E., Benfield, D.A., Christianson, W.T., Harris, L., Hennings, J.C., Shaw, D.P., Goyal, S. M., McCullough, S., Morrison, R.B., Joo, H.S., Gorcyca, D., Chladek, D., 1992. Isolation of swine infertility and respiratory syndrome virus (isolate ATCC VR-2332) in North America and experimental reproduction of the disease in gnotobiotic pigs. J. Vet. Diagn. Invest. 4, 117-126.
- Cousens, L.P., Orange, J.S., Su, H.C., Biron, C.A., 1997. Interferon-alpha/beta inhibition of interleukin 12 and interferon-gamma production in vitro and endogenously during viral infection. Proc. Natl. Acad. Sci. 94, 634-639.
- Cousens, L.P., Peterson, R., Hsu, S., Dorner, A., Altman, J.D., Ahmed, R., Biron, C.A., 1999. Two roads diverged: interferon alpha/beta- and interleukin 12-mediated pathways in promoting T cell interferon gamma responses during viral infection. J. Exp. Med. 189, 1315-1328.
- Derbyshire, J.B., Lesnick, C.E., 1990. The effect of interferon induction in newborn piglets on the humoral immune response to oral vaccination with transmissible gastroenteritis virus. Vet. Immunol. Immunopathol. 24, 227-234.

- Fitzgerald-Bocarsly, P., 2002. Natural interferon-a producing cells: the plasmacytoid dendritic cells. Cytokines: receptors and cell signaling, a supplement to Biotechniques:16-29.
- Foss, D.L., Moody, M.D., Murphy, K.P. Jr., Pazmany, C., Zilliox, M.J., Murtaugh, M.P., 1999. In vitro and in vivo bioactivity of single-chain interleukin-12. Scand. J. Immunol. 50, 596-604.
- Foss, D.L., Zilliox, M.J., Meier, W., Zuckermann, F., Murtaugh, M.P., 2002. Adjuvant danger signals increase the immune response to porcine reproductive and respiratory syndrome virus. Viral Immunol. 15, 557-566.
- Goldberg, T.L., Lowe, J.F., Milburn, S.M., Firkins, L.D., 2003. Quasispecies variation of porcine reproductive and respiratory syndrome virus during natural infection. Virology 317, 197-207.
- Gonin, P., Pirzadeh, B., Gagnon, C.A., and Dea, S., 1999. Seroneutralization of porcine reproductive and respiratory syndrome virus correlates with antibody response to the GP5 major envelope glycoprotein. J. Vet. Diagn. Invest. 11, 20-26.
- Guzylack-Piriou, L., Balmelli, C., McCullough, K.C., Summerfield, A., 2004. Type-a CpG oligonucleotides activate exclusively porcine natural interferon-producing cells to secrete interferon-alpha, tumour necrosis factor-alpha and interleukin-12. Immunology. 112, 28-37.
- Halbur, P., 2003. Factors that influence the severity of clinical disease. In; PRRS Compendium. J. Zimmerman and K-J Yoon (Eds.). 2nd Edition. National Pork Board. pp.17-26.
- Honda, K., Sakaguchi, S., Nakajima, C., Watanabe, A., Yanai, H., Matsumoto, M., Ohteki, T., Kaisho, T., Takaoka, A., Akira, S., Seya, T., Taniguchi, T., 2003. Selective contribution of IFN-alpha/beta signaling to the maturation of dendritic cells induced by double-stranded RNA or viral infection. Proc. Natl. Acad. Sci. 100, 10872-10877.
- Kadowaki, N., Antonenko, S., Lau, J.Y., Liu, Y.J., 2000. Natural interferon alpha/beta-producing cells link innate and adaptive immunity. J. Exp. Med. 192, 219-226.
- Kapsenberg, M.L., 2003. Dendritic-cell control of pathogen-driven T-cell polarization. Nat. Rev. Immunol. 3, 984-993.
- Key, K.F., Haqshenas, G., Guenette, D.K., Swenson, S.L., Toth, T.E., Meng, X.J., 2001 Genetic variation and phylogenetic analyses of the ORF5 gene of acute porcine reproductive and respiratory syndrome virus isolates. Vet. Microbiol. 83, 249-263
- Krug, A., Towarowski A., Britsch, S., Rothenfusser, S., Hornung, V., Bals, R., Giese, T., Engelmann, H., Endres, S., Krieg, A.M., Hartmann, G., 2001. Toll-like receptor expression reveals CpG DNA as a unique microbial stimulus for plasmacytoid dendritic cells which synergizes with CD40 ligand to induce high amounts of IL-12. Eur. J. Immunol. 31, 3026-3037.
- Labarque, G.G., Nauwynck, H.J., Van Reeth, K., Pensaert, M.B., 2000. Effect of cellular changes and onset of humoral immunity on the replication of porcine reproductive and respiratory syndrome virus in the lungs of pigs. J. Gen. Virol. 81, 1327-1334.
- Labarque, G., Van Gucht, S., Van Reeth, K., Nauwynck, H., Pensaert, M., 2003. Respiratory tract protection upon challenge of pigs vaccinated with attenuated porcine reproductive and respiratory syndrome virus vaccines. Vet. Microbiol. 95, 187-197.
- Lager, K.M., Mengeling, W.L., Brockmeier, S.L., 1997a. Duration of homologous porcine reproductive and respiratory syndrome virus immunity in pregnant swine. Vet. Microbiol. 58, 127-133.
- Lager, K.M., Mengeling, W.L., Brockmeier, S.L., 1997b. Homologous challenge of porcine reproductive and respiratory syndrome virus immunity in pregnant swine. Vet. Microbiol. 58, 113-25.
- Lager, K.M., Mengeling, W.L., Brockmeier, S.L., 1999. Evaluation of protective immunity in gilts inoculated with the NADC-8 isolate of porcine reproductive and respiratory syndrome virus (PRRSV) and challenge-exposed with an antigenically distinct PRRSV isolate. Am. J. Vet. Res. 608, 1022-1027.
- Levy, D.E., Marie, I., Prakash, A., 2003. Ringing the interferon alarm: differential regulation of gene expression at the interface between innate and adaptive immunity. Curr. Opin. Immunol. 15, 52-58.
- Lowe, J.F., Husmann, R., Firkins, L.D., Zuckermann, F.A., Goldberg, T.L. 2004. Correlation of cellular immunity to Porcine Reproductive and Respiratory Syndrome Virus and clinical disease during outbreaks of PRRS in commercial swine herds. J. Am. Vet. Med. Assoc. 226:1707-1711.
- Lowe, J.E., Husmann, R., Firkins, L.D., Zuckermann, F.A., Goldberg, T.L. 2005. Correlation of cell-mediated immunity against porcine reproductive and respiratory syndrome virus with protection against reproductive failure in sows during outbreaks of porcine reproductive and respiratory syndrome in commercial herds. J Am Vet Med Assoc. 226:1707-11.

- Loewen, K.G., Derbyshire, J.B., 1988. Interferon induction in piglets with polyinosinic:polycytidylic acid complexed with poly-L-lysine and carboxymethylcellulose. Res. Vet. Sci. 44, 132-133.
- Loemba, H. D., Mounir, S., Mardassi, H., Archambault, D., Dea, S., 1996. Kinetics of humoral immune response to the major structural proteins of the porcine reproductive and respiratory syndrome virus. Arch. Virol. 141, 751-761.
- Lopez-Fuertes, L., Domenech, N., Alvarez, B., Ezquerra, A., Dominguez, J., Castro, J. M., Alonso, F., 1999. Analysis of cellular immune response in pigs recovered from porcine respiratory and reproductive syndrome infection. Virus Res. 64, 33-42.
- Meier, W.A., Galeota, J., Osorio, F.A., Husmann, R.J., Schnitzlein, W.M., Zuckermann, F.A., 2003. Gradual development of the interferon-gamma response of swine to porcine reproductive and respiratory syndrome virus infection or vaccination. Virology 309, 18-31.
- Meier, W.A., Husmann, R.J., Schnitzlein, W.M., Osoio, F.A., Lunney, J.K., Zuckermann, F.A. 2004. Cytokines and synthetic double-stranded RNA augment the T helper 1 immune response of swine to porcine reproductive respiratory syndrome virus. Vet. Immunol. Immunopath. 102, 299-314.
- Meng, X.J., 2000. Heterogeneity of porcine reproductive and respiratory syndrome virus: implications for current vaccine efficacy and future vaccine development. Vet. Microbiol. 74, 309-329.
- Mengeling, W.L., Lager, K.M., Vorwald, A.C., Clouser, D.F., 2003a. Comparative safety and efficacy of attenuated single-strain and multi-strain vaccines for porcine reproductive and respiratory syndrome. Vet. Microbiol. 93, 25-38.
- Mengeling, W.L., Lager, K.M., Vorwald, A.C., Koehler, K.J., 2003b. Strain specificity of the immune response of pigs following vaccination with various strains of porcine reproductive and respiratory syndrome virus. Vet. Microbiol. 93, 13-24.
- Montoya, M., Schiavoni, G., Mattei, F., Gresser, I., Belardelli, F., Borrow, P., Tough, D.F., 2002. Type I interferons produced by dendritic cells promote their phenotypic and functional activation. Blood 99, 3263-3271.
- Murtaugh, M.P., Faaberg, K.S., Laber, J., Elam, M., Kapur, V., 1998. Genetic variation in the PRRS virus. Adv. Exp. Med. Biol. 440, 787-794.
- Murtaugh, M.P., Xiao, Z., Zuckermann, F., 2002. Immunological responses of swine to porcine reproductive and respiratory syndrome virus infection. Viral Immunol. 15, 533-547.
- Nelson, E. A., Christopher-Hennings, J., Benfield, D. A., 1994. Serum immune responses to the proteins of porcine reproductive and respiratory syndrome (PRRS) virus. J. Vet. Diagn. Invest. 6, 410-415.
- Nodelijk, G., de Jong, M.C., van Leengoed, L.A., Wensvoort, G., Pol, J.M., Steverink, P.J., Verheijden, J.H., 2001. A quantitative assessment of the effectiveness of PRRSV vaccination in pigs under experimental conditions. Vaccine 19, 3636-3644.
- Nowacki, W., Cederblad, B., Renard, C., La Bonnardiere, C., Charley, B., 1993. Age-related increase of porcine natural interferon alpha producing cell frequency and of interferon yield per cell. Vet. Immunol. Immunopathol. 37, 113-122.
- Orange, J.S., Biron, C.A., 1996. An absolute and restricted requirement for IL-12 in natural killer cell IFN-gamma production and antiviral defense. Studies of natural killer and T cell responses in contrasting viral infections. J. Immunol. 156, 1138-1142.
- Osorio, F.A., Zuckermann, F.A., Wills, R., Christian, S., Galeota, J., Doster, A., 1998. PRRSV: Comparison of commercial vaccines in their ability to induce protection against current PRRSV strains of high virulence. Proc. A. D. Leman Conf. 25,176-182.
- Osorio, F.A., Galeota, J.A., Nelson, E., Brodersen, B., Doster, A., Wills, R., Zuckermann, F., Laegreid, W.W., 2002. Passive transfer of virus-specific antibodies confers protection against reproductive failure induced by a virulent strain of porcine reproductive and respiratory syndrome virus and establishes sterilizing immunity. Virology 302, 9-20.
- Ostrowski, M., Galeota, J.A., Jar, A.M., Platt, K.B., Osorio, F.A., Lopez, O.J., 2002. Identification of neutralizing and nonneutralizing epitopes in the porcine reproductive and respiratory syndrome virus GP5 ectodomain. J. Virol. 76, 4241-4250.
- Royaee, A.R., Husmann, R.J., Dawson H.D., Calzada-Nova, G., Schnitzlein, W.M., Zuckermann, F.A., Lunney, J.K. 2004. Deciphering the involvement of innate immune factors in the development of the host responses to PRRSV vaccination. Vet. Immunol. Immunopathol. 102, 199-216.

- Shi, M., Lam, T.T., Hon, C.C., Hui, R.K., Faaberg, K.S., Wennblom, T., Murtaugh, M.P., Stadejek, T., Leung, F.C. 2010. Molecular epidemiology of PRRSV: a phylogenetic perspective. Virus. Res. 154, 7-17.
- Shi, M., Lemy, P., Singh B., Suchard, M.A., Murtaugh, M.P., Carman, S., D'Allaire, S., Deslisle, B., Lambert, M.E., Gagnon, C.A., Ge, L., Qu, Y., Yoo, D., Homes, E.C., Leung, F.C. 2010. The spread of type 2 Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) in North America: a phylogeographic approach. Virology. 447, 146-154.
- Thacker, B., 2003. Clinical manifestations of PRRS virus. In; PRRS Compendium. J. Zimmerman and K-J Yoon (Eds.). 2nd Edition. National Pork Board. pp.7-16.
- Thanawongnuwech, R., Young, T.F., Thacker, B.J., Thacker, E.L., 2001. Differential production of proinflammatory cytokines: in vitro PRRSV and Mycoplasma hyopneumoniae co-infection model. Vet. Immunol. Immunopathol. 79, 115-127.
- Tough, D.F., 2004. Type I interferon as a link between innate and adaptive immunity through dendritic cell stimulation. Leuk. Lymphoma 45, 257-264.
- van der Linden, I.F., Voermans, J.J., van der Linde-Bril, E.M., Bianchi, A.T., Steverink, P.J., 2003. Virological kinetics and immunological responses to a porcine reproductive and respiratory syndrome virus infection of pigs at different ages. Vaccine 21, 1952-1957.
- Van Reeth, K., Labarque, G., Nauwynck, H., and Pensaert, M., 1999. Differential production of proinflammatory cytokines in the pig lung during different respiratory virus infections: correlations with pathogenicity. Res. Vet. Sci. 67, 47-52.
- Van Rooij, E.M., De Bruin, M.G., De Visser, Y.E., Middel, W.G., Boersma, W.J., Bianchi, A.T., 2004. Vaccine-induced T cell-mediated immunity plays a critical role in early protection against pseudorabies virus (suid herpes virus type 1) infection in pigs. Vet. Immunol. Immunopathol. 99, 113-125.
- van Woensel, P.A., Liefkens, K., Demaret, S., 1998. Effect on viraemia of an American and a European serotype PRRSV vaccine after challenge with European wild-type strains of the virus. Vet. Rec. 142, 510-512.
- Verheije, M.H., Kroese, M.V., van der Linden, I.F., de Boer-Luijtze, E.A., van Rijn, P.A., Pol, J.M., Meulenberg, J.J., Steverink, P.J., 2003. Safety and protective efficacy of porcine reproductive and respiratory syndrome recombinant virus vaccines in young pigs. Vaccine 21, 2556-2563.
- Vezina, S. A., Loemba, H.,Fournier, M., Dea, S., Archambault, D., 1996. Antibody production and blastogenic response in pigs experimentally infected with porcine reproductive and respiratory syndrome virus. Can. J. Vet. Res. 60, 94-99.
- Wee, G.J., Cao, M.J., Liu, W., Kwang, J., 2001. Efficacy of porcine reproductive and respiratory syndrome virus vaccine and porcine interleukin-12. Vet. Therapeutics 2, 112-119.
- Wensvoort, G., Terpstra, C., Pol, J. M. A., 1991. Mystery swine disease in the Netherlands: the isolation of Lelystad virus. Vet. Q. 13, 121-130.
- Xiao, Z., Batista, L., Dee, S., Halbur, P., Murtaugh, M.P., 2004. The level of virus-specific T-cell and macrophage recruitment in porcine reproductive and respiratory syndrome virus infection in pigs is independent of virus load. J. Virol. **78,** 5923-5933.
- Yoon, K.J., Zimmerman, J.J., Swenson, S.L., McGinley, M.J., Eernisse, K.A., Brevik, A., Rhinehart, L.L., Frey, M.L., Hill, H.T., Platt, K.B., 1995. Characterization of the humoral immune response to porcine reproductive and respiratory syndrome (PRRS) virus infection. J. Vet. Diagn. Invest. 7, 305-312.
- Zuckermann, F.A. Husmann, R.J., Schwartz, R., Brandt, J., Mateu de Antonio, E. And Martin, S. 1998. Interleukin-12 enhances the virus-specific interferon gamma response of pigs to an inactivated pseudorabies virus vaccine. Vet. Immunol. Immunopath. 63:57-67.
- Zuckermann, F.A., Martin, S., Husmann, R.J., Brandt, J., 1999. Use of interleukin 12 to enhance the cellular immune response of swine to an inactivated herpesvirus vaccine. Adv. Vet. Med. 41, 447-461.

PRRSV genetic variability and its impact on virulence

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Porcine respiratory and reproductive syndrome virus (PRRSV) is a major swine pathogen responsible for immense economic loss annually. PRRSV first emerged in North America in the late 1980's in near synchrony with its emergence in Europe as the etiological agent of a novel potent swine disease. Not long after, the virus spread to or was detected in most swine rearing countries making it a problem of global relevance. To date, the evolutionary origin of PRRSV is not known although retrospective serologic screening in herds has indicated the virus circulated in pigs for at least several years prior to its official recognition or "emergence". Several hypotheses have been put forth attempting to explain the advent of PRRS however each has its shortcomings.

One hypothesis on the origin of PRRSV postulated a cross-species event in which the parental PRRSV-like virus resided in a rodent species before crossing over to swine and evolving into PRRSV. Had the isolation of such an ancestral strain been possible, it might have provided the first opportunity to compare genetic differences between the parental strain and PRRSV prototype isolates. The degree of difference would have provided clues to how much genetic change was sufficient to enable establishment in a new species as well as emerge in a virulent fashion. Unfortunately, no such ancestral PRRSV-like virus has been detected.

The first inkling of genetic variability and its relation to virulence can be gauged from the initial emergence itself. Clinical presentation of PRRS in both North America and Europe was considered severe with many presenting symptoms shared. Shockingly, however, sequences of prototype isolates from the two continents differed from each other at more than a third of all aligned genomic sites. As a result, the first lesson was the manifestation of PRRS did not require the causative agent to conform to narrow genetic diversity boundaries. Since the initial outbreaks, there has been a gradual build up sequence deposition of select PRRSV genes in various databases in an effort to assess diversity in the field and keep track on its change. Studies on the overall known diversity from these public databases indicated all isolates to date are entirely related to either one of the two prototype isolates (hence the designation of two genotypes). No intermediates have been detected. Nonetheless, there is still substantial genetic diversity within each genotype (more so in type 1 than type 2). With respect to each genotype, time to time there are reports of particularly "virulent" outbreaks. However, one the major hurdle in linking change in genetic diversity to degree of virulence is the lack of a standardized scheme of assessing virulence without which making meaningful comparisons across outbreaks temporally and spatially is problematic. The other major hurdle is most sequencing information available pertains to a very limited region of the PRRSV genome which may not necessarily hold the information to genetic changes linked to altered virulence.

Despite the drawbacks above, there are some examples in which a change in genetic diversity resulted in particularly virulent outbreaks of PRRS. All the examples that follow demonstrate scenarios in which there is clear evidence from PRRSV phylogenetics that virulent outbreak isolates were distinct from either known endemic diversity or diversity for which herds were vaccinated. Global type 2 PRRSV phylogeny indicates PRRSV was first introduced into China as a single event from North America around the mid-90's which gradually led to it becoming endemic. In 2006, "High Fever" PRRS broke out which spread across most of the country affecting up to 2 million pigs. All outbreak isolates from the period were closely related to each other but clearly distinct from the endemic diversity from which they had emerged. The second example is of the "abortion storm" that was reported in 1996. This time phylogenetics indicated isolates belonged to independent outbreaks but all of which were of evolutionary lineages significantly different to those on which the vaccines were designed that had been administered to the herds prior. The third example is from the MN184-related outbreak in Minnesota and other regions in 2001. Once again, isolates from the outbreak all clustered together but not with endemic diversity in the region. This was surprising as there was an active surveillance effort in monitoring the genetic diversity of the region which made it difficult to explain the sudden change in diversity. Later on, it was demonstrated that outbreak isolates were closely related to circulating diversity in Ontario, Canada. A sudden introduction from up north explained the lack of immediate relatedness to local diversity while simultaneously showing how a change in genetic diversity to which herds were normally exposed to triggered a virulent episode of PRRS. In summary, there is clear indication of PRRS being variable in its severity and it is now generally believed this variability is at least partly the result of extensive genetic diversity amongst circulating PRRSV strains in the field. In order to progress towards finely delineating the viral genomic sites associated with changes in pathogenicity, steps need to be taken to standardize virulence evaluation as well as switch to more comprehensive PRRSV sequence surveillance and complete viral genome sequencing.

Managing a pig production system using science-based, systems measurement

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Introduction

In the US pork market, consumers have demonstrated a desire for consistency in quality and safety within a wide variety of product specifications (1). On that basis, pork supply-chain participants have an economic incentive to understand consumer preferences and manage inherent variation within the biological process of pork production within such preferences. Measurement of pork attributes provides the basis for managing production processes within a pork supply-chain, and, therefore, communication and reporting of measures between pork supply-chain participants is increasing within the US industry (2). Integrating such measurements taken at various points along the supply-chain such as at the farm, during transportation, at the harvest plant, and during further processing in conjunction with timely reporting of each respective process outcome can enable managerial optimization of biological factors known to influence variation in both quantity and quality of pork. Scalable, systematic approaches to management of on-farm practices influencing the production of high quality, consistent pork within a pork supply-chain merit consideration. This paper will summarize a management strategy used within The Maschhoffs, LLC pig production system for systematic improvement in consistency of high quality pork within the various markets supplied by the company.

Considerations for measurement in managing a pig production system

"Quality Management" is generally known within business sectors as a process or set of processes that aims to deliver consistent quantities of specific products or services (3). Pig producers stand to benefit from adopting "Quality Management" as a means of ensuring an acceptable level of quality and consistent output from within specific production systems. Successful "Quality Management" relies, in principle, on key measures to provide a basis for continued improvement of the desired outcome. Considerations for determining key measures of a process within a pig production system should include, but are not limited to: biological or financial perspective, leading verses lagging indication, controllable verses uncontrollable variable, and individual or team assessment. A sample of strategic, generally lagging, measures that may be considered for use within pig production to provide for a system-based approach to management are provided in Table 1. Monitoring a combination of such measures in a pig production enterprise enables a systems-based approach to management of interrelated, complex processes; consequently, higher levels of system performance may be achieved.

Pig production systems that aim to improve the quality and consistency of the pork produced must set quantifiable goals and objectives that are coordinated with their respective supply-chain partners, and ultimately reflect the requirements of the end consumer. A well designed measurement scheme will cascade relevant goals and objectives down through the production system, reporting controllable variables to appropriate stakeholders. Managers of various production processes must employ a dynamic set of routine communications aimed at organizing and coordinating limited resources deployed towards achievement of defined goals. Ultimately, specific plans must be developed and executed by both teams and individuals within the production system to close the gap between current levels of performance and those defined within set goals. Specific goals and methods of obtainment are particularly important at the level of the animal caretaker to achieve a sustainable pork supply-chain given the current social climate.

Table 1. Strategic measures within a pig production system to support production of high quality, consistent pork

Measure	Calculation
Quality Carcass Weight Per Per Year	Total carcass weight for which full payment was received from target customer for a year ÷ Average mated female inventory for same time period starting at prior conception
CV in Weight of Carcasses Per Year	Standard deviation in carcass weight for which full value was received from target customer ÷ Average carcass weight of the same carcasses
Sales Premium Per Unit of Carcass Weight	(Total revenue per carcass for which full value was received from target customer – industry average revenue per carcass) ÷ Average carcass weight
Facility Cost Per Unit of Carcass Weight Produced	Total cost of facilities and labor for a defined time period ÷ Total carcass weight produced and subsequently sold resulting from that defined time period
Unit of Feed Used Per Unit of Carcass Weight Produced	Total feed used ÷ Total carcass weight produced and subsequently sold resulting from the feed used

Considerations for science in improving management of pig production system

Processes within scaled-pig production systems are both complex and interrelated and, as such, it is often difficult to determine direct causative relationships among input variables and outcomes relying solely on in-process measurement at the commercial level. Nevertheless, measurement-based strategic and tactical decision making must be timely and informed to ensure that both quality and consistency of output from within a system are maintained or improved. Isolating specific effects of various factors influencing quality and consistency within the production process is essential to informed decision making. Moreover, factors influencing the quantity and quality of the pork produced are becoming increasingly system specific, particularly as the scale of pig production increases and systems increasingly differentiate processes within a supply-chain. Methods used in scientific discovery can be used within a commercial pig production system to determine the influence of specific biological factors on pork quality and consistency. Increasingly, science-based research and development programs are being constructed within pig production systems to fulfill that objective. Design and organization of the research program should be based on the goals and objectives of the production system, available resources and expertise, and standards of expectation set for science within the decision making framework of the system. In general, programs that research broad verses narrow arrays of disciplines considering both short- and long-term goals and objectives within a discipline are most likely to realize both additive and synergistic improvements within the production system. Farm-based factors shown across a variety of research disciplines to influence strategic measures within a US-based, commercial pig production system are summarized in Table 2.

The measured effects of a variety of on-farm factors summarized in Table 2, although individually relatively small in magnitude, suggests significant opportunity exists within commercial pig production to enhance system measures by applying science-based knowledge to alter routine management processes. However, resulting impacts can have both positive and negative consequences on the various participants within a supply-chain and, therefore, comprehensive, quantifiable assessments must be completed with each alteration in process to preserve the output of high quality consistent output.

Table 2. Effect of farm-based factors within research disciplines on strategic measures within a US-based, commercial pig production system

	Calculated percent change in strategic measure per year[a]				
On-farm factor	Carc/ Sow[b]	CV[c]	Sale[d]	F[e]	FCR[f]
Genetics (4,5)					
Dam Line	+1	---	+1	-6	+2
Sire Line	+1	---	---	-1	+1
Health (6,7)					
Immune status	+2	+7	---	-2	+2
Medication program	+1	---	---	-1	---
Housing (8,9)					
Floor area	---	---	+1	-10	---
Group size	---	---	---	---	---
Nutrition (10,11)					
Nutrient source	---	+1	---	---	---
Nutrient level	---	---	+1	---	---

[a] Expected percent change in strategic measure calculated using results from within studies that examined the current on-farm process against alternatives within the discipline.
[b] Quality Carcass Weight Per Sow Per Year
[c] Coefficient of Variation in Weight of Carcasses Per Year
[d] Sales Premium Per Unit of Carcass Weight
[e] Facility Cost Per Unit of Carcass Weight Produced
[f] Unit of Feed Used Per Unit of Carcass Weight Produced

Practicing measurement and science-based research to improve consistency and quality of pork from within a pig production system

In recent times, consumer acceptability of pork bellies and other products generally high in fat that are produced within some US pork supply chains has been reduced. In response to the change in consumer acceptability, some pig processors have begun to measure the iodine value of fat in pork carcasses with the aim of identifying sources of the poorer quality. Prior research suggests pig producers may employ a variety of changes in on-farm processes with varying degrees of success in changing the fat quality throughout the whole of the carcass. In addition, avoiding pork carcasses with unacceptable iodine value can also have a wide range in financial outcomes. (11). Similarly, pork processors have an opportunity to mitigate poorer quality fat through production processes with a range of potential outcomes. In isolation, neither the pig producer nor the pork processor is likely to determine factors for change within their respective processes that may optimize the outcome for the collective supply-chain. However, collaboration among all stakeholders in measuring both the on-farm and in-plant process outcomes as well as research aimed at optimizing measures when considering impacts to both supply-chain participants has served as a basis for sustainable management of the ongoing challenge.

Conclusion

Complexity appears to be increasing among pork consumer preferences and within the numerous pork supply-chains within the US, and presumably throughout the globe. As a result, pig production systems should carefully consider measurement of on-farm processes that ensure success as stakeholders within their specific supply-chain. Consideration should be given to the structure and role of on-farm, systems-based research programs with intent of improving key process measures that result in quantifiable improvement. Finally, increasing information-based communication and coordination among pork supply-chain participants can accelerate the performance of the entire pork supply-chain towards meeting consumer preferences.

References

1. Brewer, M.S. and F.K. McKeith. 1999. J. Food Sci. 64(1):171-174.

2. Kliebenstein, J. B. and J. D. Lawrence. 1995. Am. J. Ag. Econ. 77:1213-1218.
3. Drucker, P.F. 1993. The Practice of Management. New York: Harper Business.
4. Grohmann, N. S. et al., 2014. Midwest ASAS Meetings.
5. Peterson, B.A. et al., 2005. J. Anim. Sci. 83(Suppl. 2):36
6. Peterson, B.A. et al., 2005. J. Anim. Sci. 83(Suppl. 1):175.
7. Wellen, L. et al., 2005. Proceedings of the American Association of Swine Veterinarians Annual Meeting; pp. 19-20.
8. Shull C.M. et al., 2011. J. Anim. Sci. 89(E-Suppl. 2).
9. Wolter, B. F., M. et al., 2001. J. Anim. Sci. 79:1067-1073.
10. Mendoza, O.F. et al., 2010. J. Anim. Sci. 88:3387-3895.
11. Leick, C.M. et al., 2010. J Anim. Sci. 88: 2751-2766.

PRRS, PED PCV2, and SIV: How can we improve our support to the swine industry?

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Introduction

"Insanity: doing the same thing over and over again and expecting different results." Albert Einstein

Globalization, the increased movement of people, swine, supplies, and food pose growing risks to the swine industry. An example of the above are the economic losses and social impact caused by the worldwide spread of porcine epidemic diarrhea (PED), porcine reproductive and respiratory syndrome (PRRS), swine influenza (SIV), and porcine circovirus (PCV2). These losses include, among others, the financial impact caused by increased mortality, decreased reproductive and productive performance, and a significant augmentation in secondary diseases, the purchase of drugs and vaccines, as well as enlarged cost of diagnosis and changes and/or improvements in biosecurity. This dealings have challenged our profession and made us reflect on how we can improve our support to our pork producers and in general, to the swine industry. It is imperative that we ensure that everyone involved in this industry understands how:

a. Disease enters to a farm?
b. A farm develops a plan/ tools to reduce the risk of disease contamination
c. Owners and veterinarian rapidly responds to an important health issue
d. Can promptly diagnose a new disease
e. Can communicate its presence to neighbours, veterinarians and authorities
f. Develop tools/plan to contain the disease from spreading to its neighbourhood/area and country
g. What is an emerging disease?
h. What is food safety?

Travelling around the world and consulting in different countries that before 2013, where and few still remain free of some of the above mentioned diseases, has made me reflect on what would be my "non-negotiables" to avoid continuous chaos in our industry. To my surprise, and in my advice, today, not much has to be invented. I believe that we as an industry have failed in applying the tools that have been provided to us in ensuring a healthier and more productive industry.

So where have we failed as practitioners to apply existing research and technology in order to support our producers?

I. Biosecurity

Preventing the spread between and within pig populations is a critical component of a farm's disease control program. Biosecurity can be termed as: "The set of measures taken to protect herds from the introduction of any new infectious pathogen". Without totally eliminating the risks, a good biosecurity and management program can drastically help the herd to protect its health status. This of course includes external and internal routes of transmission for pathogens, as well as some biosecurity and management measures to stop its transmission. Keeping viruses out of the farm represents a challenging task for modern swine production. Therefore, enforcing a standard application of strong biosecurity protocols to limit vertical, horizontal, mechanical and aerosol transfer of pathogens is imminent. Steps involved comprise creating a biosecurity checklists for farms that include, among others:

a. Risk identification
b. Application of protocols/plans,
c. Training, and continuous education
d. As well as audits and testing of farm personnel

This is easily said, but very difficult to constantly respect and maintain; especially in certain production systems and cultural approaches.

a. Personnel and inanimate objects

To prevent mechanical transmission, disease prevention focuses on incoming materials and people. Personnel and inanimate objects like shoes, clothes, materials and equipment can act as mechanical carriers and transmitters of viruses. It is therefore vital to implement a biosecurity protocol for personnel entry to the farm. More and more new and retrofitted swine facilities include a disinfection room allowing fumigation of incoming materials. Wearing disposable coveralls and plastic boots and applying the "bag-in-a-box" shipping method efficiently prevents pathogen transmission.

b. Pig movement

Strategies to reduce virus circulation are:

a. Adequate gilt acclimation
b. Good colostrum immunity
c. Use of Mc Rebel™ in the farrowing area
d. Wean negative piglets
e. Reduction/elimination of viral challenge to piglets and nursery animals
f. Use of area specific tools and equipment
g. Unidirectional flow in all the farm
h. Strict all in/all out in all areas of the farm
i. Monitoring farm stability when specific and effective test is available
j. Creating a solid population immunity, when an effective vaccine is available
k. Constant risk analysis to find opportunity areas.
l. Continuous personnel training
m. Biosecurity audits

Likewise, older pigs and their secretions can be a source of infection to younger pigs on premises where biosecurity between groups is lacking. Therefore, subpopulation of infected and non-infected pigs play a very important role in the persistence of the infection in the farms and areas.

c. Transport vehicles

Vehicles, as well can carry many pathogens. Most viruses are only moderately resistant to environmental degradation. Researchers have demonstrated that a transport vehicle contaminated with certain viruses can transmit infection to naïve pigs. Effective treatment eliminating viruses from a vehicle has been described as a combination of litter removal, washing, disinfection, and drying, so why have we not implemented this in all farms and abattoirs? Also, one should put up a recovery container for dead pigs at a good distance from the farm, in order to prevent carcass collection trucks from getting too close to the farm. For the same reason, vehicles coming to a farm for picking up live pigs should be empty, clean and disinfected.

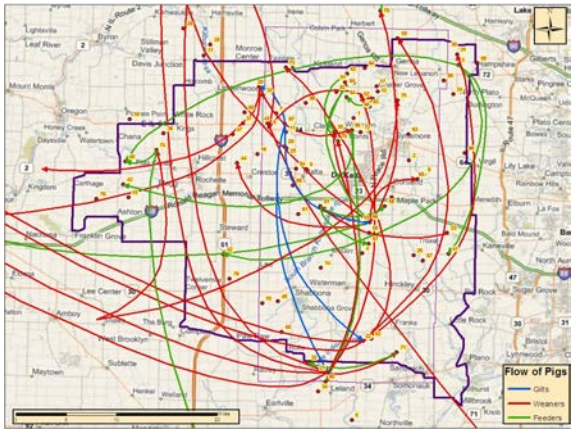


Figure 1. Example of pig movement in a swine production area (Mondaca et al., 2010)

d. Understanding the importance of local proximity and aerosol spread

The possibility of aerosol transmission of several of these viruses has also risen controversy during a number of years, nonetheless, several of them are now recognized to be spread through aerosol. Several filtration systems have been developed to protect swine farms against this mode of transmission, and we should consider them more seriously as an option to protect our farm.

II. Study and practice epidemiology, and implement regional biosecurity and control programs

While veterinarians have developed a variety of strategies to control and eliminate the disease from pig herds, the risk of re-infection remains high even with the best current practices of management and biosecurity. It has been shown over and over that the repeated failures of non-coordinated control and elimination efforts and the ease with which the disease is transmitted from one herd to another strongly suggest that a regional approach is necessary. Therefore, understanding the patterns, causes, and effects of these diseases would certainly reduce their impact. A basic requirement to understand changing local, regional, or global distributions of disease and/or disease risks is the real time access to current information providing alertness of factors potentially associated with disease risk. Developing and/or using more surveillance systems to control disease at a regional level is critical for the swine industry. This information

would allow control in areas of high prevalence and high pig density, while elimination would be feasible in areas of low prevalence and low pig density.

In order for this approach to be successful, we need to changes several paradigms:

a. First and foremost, learn to work together, as a team in order to achieve a common goal…
b. Identify all swine premises in an area
c. Identify pig-related facilities in the area
d. Classify pig sites according to their disease status
e. Design control plans/strategies, and
f. Focus individual projects to combine with existing regional projects

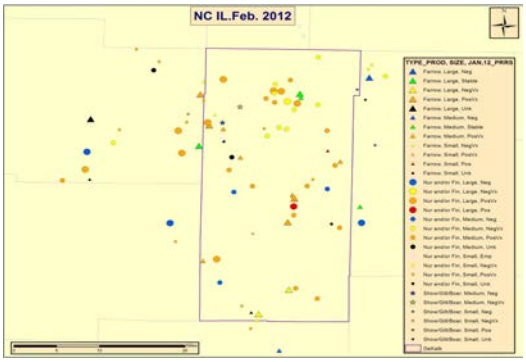


Figure 2. Example of identification of swine sites in a disease surveillance program (Mondaca et al., 2010)

III. Continuous monitoring and diagnostic programs

Continuous disease monitoring, diagnosis and surveillance is necessary to achieve:

a. Early detection of disease outbreaks
b. Ratification of the presence or absence of a specific disease in farm/region
c. Definition of subpopulations
d. Definition of risk factors associated with the spread of disease
e. Planning and setting priorities
f. Evaluation of interventions
g. Resource allocation

Active surveillance has largely been criticized for its cost and labour intensiveness. However, it should be highlighted that it allows for early detection of outbreaks permitting for estimates of spread to farm/areas and permitting the establishment of early and effective control strategies. Surveillance can assist by providing information to make intelligent and more economical decisions rather than waiting until the disease has already spread in the area/region/country.

Essentials components of a monitoring program are:

a. Sample size, always dependent on prevalence, confidence interval and error margin.
b. Type of samples to be collected (i.e. sera, oral fluids, environmental samples, aerosol, etc.)
c. Analysis of all the collected data to gain information of the area under control
d. Establish reporting mechanisms to all the participants of a group
e. Action plans to improve the established working plan and therefore the control of the targeted disease

However the advancement of these programs will not be successful if clarification of the reason(s) for collecting information/different samples types, the use, management and analysis of the data (continuous and the confidentiality) are maintained. Finally, direct benefits such as, health/production improvements and return on investment, and indirect benefits (reduction of lateral infection, improved area knowledge to reduce infection risks, improvement of employees moral, industry perception and enhanced communication among the working group) should occur.

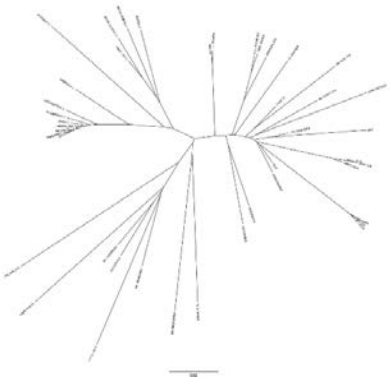
Two final considerations, a good diagnostic laboratory and an excellent diagnostician, with exceptional communication skills, are mandatory to the success of this diagnostic program.

IV. Study and understand molecular biology and most important, apply it to our daily routine

Please read the following statement:

"Today the variation in clinical signs and apparent emergence of new syndromes in these viral diseases is due in part to changes in PRRSV genome. Therefore, the appearance of PRRSV variants with new genetic compositions increases the likelihood of increased variation in the virus and in the diseases it causes, including disease characteristics, modes, and ease of transmission, changes in cell permissiveness, and species specificity, capability of persistence and escape from vaccinal immunity". [11]

Then, please analyze and explain the following dendrogram:



(Murtaugh, 2010)
I think I made my point very clear!

V. Communication and networking

Networks around the swine veterinary community should be as common as Facebook, Twitter or Linkedin, however they are not. These networks should combine practice data with communications amongst swine veterinarians which would help manage swine health issue around the world. Some of these networks already exist (i.e. http://www.swinehealth.ca/CSHIN.php, http://bioportal.ucdavis.edu/). How do they work?

a. They collect data about swine health
b. Data is collected daily and analyzed
c. Information is added by veterinarians and swine experts
d. Information about the diseases and how to control them is produced
e. Information is shared among veterinarians
f. Veterinarians and industry associations use the information to help producers deal with disease
g. Swine veterinarians and specialists hold regular web-based meetings to review and identify new health challenges
h. Participating veterinarian fill a form describing problems they have recently encountered
i. Reports from these meetings are sent to all swine veterinarians, keeping everyone informed about changes to swine health and ways of managing it.
j. The network is also a way to quickly communicate whenever something unusual and important is observed by a veterinarian or laboratory
k. Real time maps of disease surveillance are produced
l. Alerts can be sent on a real time basis
m. All information is kept confidential

In my point of view, PED has shown us that if we do not break status quo and existing paradigms, it will become almost impossible to protect swine herds from emerging disease, help veterinarians solve disease problems, and help the pork industry to maintain current markets and access new markets.

Conclusions

There is no single successful strategy for control of all these viral and emerging diseases. However, we have many answers and options, that for some reason, we have chosen not to use. Recently, experienced has proved that area regional control programs are the answer to future control and possible elimination. Virus control strategies and specific farm and region situations are so variable, it is imperative that experienced practitioners, producers, diagnosticians and

research workers continue to objectively expand their knowledge, communicate and network to better control and eventually eliminate these financially impacting diseases.

References

1. Boutin R (2001). Saint-Hyacinthe; 57-81.
2. Corzo C et al. (2010). *Vir Res*.; 154:185-192.
3. Dee SA et al. (2009). Vet Res 40(4): 39.
4. Dee SA et al. (2004) Can J Vet Res. 68(1); 19-26.
5. Dee SA et al. (2006) Can J Vet Res.70 (3);168-75.
6. Dee SA et al. (2006) Can J Vet Res.70 (1); 28-33.
7. Dee SA et al. (2004) Can J Vet Res 68(3);208-214.
8. Dee S.A et al. (2004). Can J Vet Res 68(2); 128-133.
9. Holtkamp DJ et al. (2013). JSHP. 21(2);72-84.
10. Mondaca E et al. (2014). JSHAP. 22 :(2);84-87.
11. Murtaugh M et al. (2001). JSHP 10(1);15-21.
12. Otake, S et al. (2002). JSHP. 10(2);59-65.
13. Pitkin A et al. (2009). SDEC, U of M.
14. Pitkin A et al. (2009). Vet. Microbiol; 136:1–7.
15. Perez A. (2012). ISVEE: 421
16. Pohl, S. (2007). AD Leman Swine Conf; 86-88.
17. Vincent A. (2008) Adv Virus Res.; 72:127-54.
18. Yeske P. (2012.IPVS, LS011: 63-65,
19. Woo P (2012) J of Virol: 3995-4008.

The emergence of porcine epidemic diarrhea in US swine: Surprises on the road to prevention and control

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Introduction

Porcine epidemic diarrhea (PED), caused by a coronavirus belonging to the genus *Alphacoronavirus*, is a major enteric swine disease of economic significance and has been reported in Europe and Asia since 1971.[1] In the US swine population, PED was first recognized in late April of 2013.[2] Since then, all major swine producing states in the United States have been experiencing epidemics of PED, as well as Canada, Caribbean countries and South America. As of the end of April 2014, PED has been reported in 30 states (http://www.aasv.org/PEDV/SECoV_weekly_report_140430.pdf). The emergence of PED has resulted in a significant production loss in the US swine industry as evident with loss of 4 to 5 million piglets within a year after PEDV was newly introduced.

Clinical presentations

Fecal to oral transmission is the main route of PEDV infection. Clinical manifestations associated with PEDV infection are similar to those with transmissible gastroenteritis virus (TGEV) infection, although these viruses are genetically and antigenically distinct. PEDV-affected pigs become anorectic and lethargic; however, fever is not a clinical response which can be expected in PEDV-infected pigs. Under experimental conditions, body temperature of inoculated pigs was even slightly lower than control pigs, which is probably attributed to the inability to eat and/or rapid loss of nutrients.

The main clinical sign of PED is severe watery diarrhea with occasional vomiting in all ages, leading to dehydration and malnutrition. Under experimental conditions, 4-week-old pigs became diarrheic 3-5 days post inoculation (dpi) when orally inoculated with PEDV (US/Iowa/18984/2013)[3] at the rate of 10^3 plaque-forming units (PFU)/ml and diarrhea subsided by 10 dpi.[4] Neonatal pigs, on the other hand, developed diarrhea within 24 hours post challenge under the same conditions.[5]

The severity and outcome of disease in the field can vary depending upon age as well as other factors such as challenge dose, immunity and other conditions on farms. The mortality rate is often 30-50% in neonates and can reach 100% in piglets born to negative dams. Affected neonates start to die within 2-3 days once diarrhea starts. The casualty can result in 2 up to 4 weeks of production loss. Older pigs, particularly after weaning, generally do not die of PED even if immunologically naïve unless complicated. Yet, weight gain is impacted by the disease. Under experimental conditions, 4-week-old pigs inoculated with PEDV did not gain much weight for 7-10 days after challenge.[4] Such a loss of body weight remained during the 35-day observation period even though average daily gain (ADG) was not statistically different between inoculated and negative control groups after one week. Therefore, wean-to-finish farms should expect 1- to 2-week delay in the time to market after PED outbreak.

Pathology and Pathogenesis

Lesions associated with PEDV infection are also similar to those with TGEV. Grossly, thin-walled intestine filled with watery material is commonly observed in pigs affected by PEDV. Under experimental conditions, this gross observation was well correlated with onset and duration of clinical diarrhea.[4,5] Undigested colostrum or milk can also be found in the stomach of affected piglets although stomach did not show gross and microscopic lesions.

Microscopically, atrophic enteritis is a hallmark lesion throughout the small intestine. Under experimental conditions, significant villous blunting was evident at 3 dpi although PEDV infection of the gut was detected by immunohistochemical staining at 1 dpi when weaned pigs were exposed to 10^3 PFU of PEDV.[4] The significant villous atrophy remained until 7 dpi but not at 14 dpi. In comparison, affected neonatal pigs had PEDV infection in the gut around 12 hours post infection and showed significant villous atrophy at 24 hours post inoculation under the same conditions.[5] Colitis is not a feature with PEDV infection although scattered virus-infected cells can be found in the colon. Furthermore, it appears that organs not associated with the gut are affected by PEDV as no lesions were evident in any of not-gut tissues collected from experimentally infected pigs. The presence of PEDV in these tissues is yet to be determined.

Fecal shedding of PEDV from exposed pigs can starts as early as 24 hours post inoculation. Under experimental conditions, all of the inoculated pigs shed the virus for a week after infection.[4] Although the number of virus shedders declined after 7 dpi, more than 40% of the inoculated pigs still shed the virus in feces at 24 dpi even though they were

no longer diarrheic. This should be taken into consideration for pig movement and herd management since non-clinical pigs can still shed the virus.

Diagnosis and Diagnostics

Observation of acute watery diarrhea in all ages can be a good clinical parameter to initiate diagnostic investigation for PED but not a definitive diagnosis. Such an observation is easier to obtain from farrowing houses when herds are naïve. Growers/finishers may show only loose stool on the floor. As PED is an enteric disease, fecal samples from clinical pigs are considered to be the 'gold standard' for viral testing. Yet, submission of fresh and fixed intestine is strongly recommended so that histological evaluation can be performed to confirm viral enteritis.

PEDV is historically known to be difficult to isolate in cell culture. Experiences with virus isolation attempts on case submissions at Iowa State University Veterinary Diagnostic Laboratory indicate that the success rate would range from 5 to 10% success rate when virus isolation is attempted on Vero cells in the presence of trypsin.[3,8] As a result, nucleic acid-based or antigen-based assays (i.e., PCR, antigen-capturing ELISA) are most commonly used to detect the presence of PEDV in samples. PCR-based assays are much more sensitive than antigen-capturing ELISA. Therefore, antigen-capturing ELISA is not suggested for surveillance purposes. At the same time, caution should be applied when interpreting PCR results with high Ct values until we clearly understand a correlation between PCR results and minimum infectious dose of PEDV.

Recently oral fluid sampling has been recognized as a cost-effective tool for surveillance of numerous pathogens, notably PRRSV and SIV.[6] Although there is no experimental evidence for secretion of PEDV into the oral cavity, PCR testing on paired fecal and oral fluid samples from experimentally infected pigs showed a good correlation in the duration of viral shedding as well as relative quantitation of viral shedding over time.[7] Field observations with use of oral fluid sampling for PEDV have been in agreement with the experimental observations. Thus, oral fluids can be used as a specimen to accurately detect the presence and/or circulation of PEDV in swine herds.

Serology is another tool available for detecting pigs exposed to PEDV. At present IFA and SN tests are available. ELISA tests are under development by numerous researchers and are available on experimental basis. In 4-week-old pigs challenged with PEDV, anti-PEDV antibody (IgG) was detectable by IFA test between 10 and 14 dpi when using 1:40 dilution as cut-off, which was coincided with disappearance of clinical diarrhea among the pigs.[9] IFA titers reached to the peak around 3 weeks post inoculation and then started to decline relatively quickly. The decline seemed to coincide with decrease in the number of virus-shedding pigs. Based on the trend, the IFA antibody level could be below the positive cut-off by 12-15 weeks post infection. An in-house experimental ELISA (whole-virus based) followed the same kinetic pattern but appears to be more sensitive than IFA test.[10] The pigs do developed serum neutralizing (SN) antibody against PEDV after exposure but slowly and to a low level ($\leq$1:16). SN antibodies do not seem to last for a long time after exposure too. Interestingly, previously exposed pigs were clinically protected from subsequent challenge approximately 2 months after the initial infection when pigs were considered seronegative based on IFA test and their SN titers were low. Serum antibody may not have a great predictive value for protective immunity as mucosal immunity is the key for protection against enteric disease. This is an area remained to be further studied to have better understanding protective immune parameters, leading to development of better vaccines or vaccination strategies.

Conclusion

PED emerged in the US unexpectedly, reminding us the nature of transboundary diseases. It is still unknown how the virus was initially introduced to US swine although various factors and mechanisms can be suspected. This incidence as well as recent emergence of other pathogens in US swine emphasizes the urgent need of portal analysis and a better disease surveillance system to develop better preventive strategies against other transboundary diseases including foreign animal disease.

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References

1. Song D and Park B. 2012. Virus Genes 44:167-175.
2. Stevenson GW, et al. 2013. J Vet Diagn Invest 25:649-654.
3. Hoang H, et al. 2013. Genome Announc 1(6):e01049-13.
4. Magstadt D, et al. 2014. Proc Am Assoc Swine Vet, pp 57-58.
5. Arruda P, et al. 2013. Proc Am Assoc Swine Vet, pp 377-378.
6. Ramirez A, et al. 2012. Prev Vet Med 104:292-300.
7. Bower L, et al. 2014. Proc Am Assoc Swine Vet, pp 61-62.
8. Chen Q, et al. 2014. J Clin Microbiol 52:234-243.
9. Hoang H, et al. 2013. Proc Conf Res Worker Ani Dis, Abstract #142.
10. Giménez-Lirola L, et al. 2014. Proc Int Pig Vet Soc Congress (in press).

Influenza virus in swine: Transmissibility within and between populations

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Importance of studying transmission

Infectious diseases continue to have considerable impact on the health and well-being of individual animals and populations and on profitability of individual farms. Most commonly, frequency is measured by evaluating prevalence of infectious agents or evidence of exposure to them. Under certain conditions, incidence could also be calculated. However, for infectious diseases, it is also useful to understand how transmissible they are in a given population. One of the most widely used measures of transmission is the basic reproductive number (R_0), defined as the average number of secondary infections resulting from a typical infectious individual in a completely susceptible population. Different versions of the reproductive number exist, depending on the assumptions used in its calculation and the phase of the epidemic. This measure continues to provide useful insights into different aspects of the epidemiology of infectious diseases and their control. One fundamental insight is the relationship between the R_0 and the herd immunity threshold (Figure 1). The herd immunity threshold sets the target for the proportion of individuals that should be immunized in order to decrease incidence of infection. This has direct implications for long-term control of infections through vaccination, since many of our control strategies are based on regular vaccination of all or a subset of animals. In more general terms, this theoretical relationship provides an insight into the amount of effort that needs to be placed in the control of an infectious disease by applying different control measures [1]. Reproductive numbers could also be used directly in dynamic models of disease transmission within populations to gain better understanding of disease circulation and appreciation of infection control strategies, or to monitor whether control measures are yielding results. Considerable progress has been made in public health and epidemiology of infectious diseases in people since the emergence of SARS, both in terms of extraction of parameters indicative of transmissibility and in the way experts are using them to design effective and sometimes most-efficient infection control strategies. Despite their usefulness for different purposes, their complementary nature to the measures of disease frequency, and sometimes abundance of data, such measures of transmission within swine populations are only beginning to emerge. Influenza in swine deserves to be studied in such a way because of its propensity to spread within and between pig populations and between different species. Influenza virus is often considered to be a virus with a relatively simple natural history, yet it continues to circulate endemically in many swine populations with oscillations that are poorly understood. It is well established that the density of pigs in the area is associated with the likelihood of infection in herds, yet little is known about the exact conditions that facilitate such transmission. It is also considered as a zoonotic infection, but a clearer definition of what type of zoonosis influenza is would be useful for risk assessment and communication purposes. This contribution will therefore focus on a brief review of some interesting recent developments for influenza in swine and other species as it has impact on requirements for data collection and analysis.

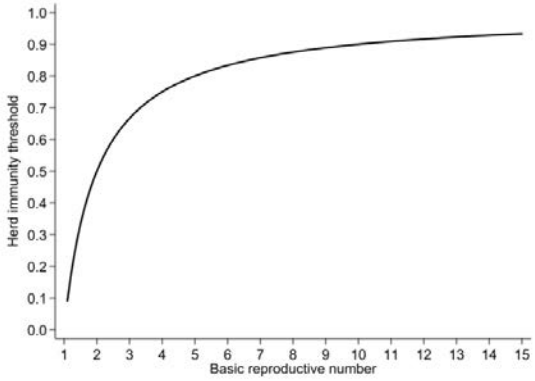

Figure 1. Theoretical relationship between herd immunity threshold and the basic reproductive number. This relationship indicates that infections with higher reproductive numbers require greater herd immunity for their long-term control.

Measures of influenza transmissibility within swine herds and sources of data

Measures of transmissibility are just starting to emerge in the scientific literature for influenza virus in swine populations. Early reports were based on theoretical reasoning, rather than observed data [2]. More recently, estimates of

reproductive numbers were based on real data obtained from experiments and observational studies. These numbers are summarized in Figure 1 [3-5] using fixed effect meta-analysis. It is clear that estimates of reproductive numbers are quite variable. The sources of this variability should be studied further, and this could lead to further understanding of transmission. However, it should also be noted that heterogeneity in reproductive numbers among studies is expected and depends on the contact patterns in populations, *inter alia*. Heterogeneity in reproductive numbers has been observed both for SARS [6] and for influenza in people and is therefore not surprising. It should not prevent us from adapting such approaches in studying influenza and other infectious diseases in swine. It is interesting to note that all of the current estimates of R_0 are obtained using detailed longitudinal measurements of individual animals under field or experimental conditions. While this will likely remain the most accurate method of studying transmissibility, it should be noted that reproductive numbers and other important epidemiological parameters in public health have frequently been obtained from less than perfect data, often just the case numbers in the early phases of the outbreak. For example, the first 205 probable cases of SARS were used to learn about the epidemiology of this disease in 2003[7]. Similarly, case numbers obtained using active or passive surveillance during the 1918 influenza pandemic under different settings have been used to establish the magnitude of R_0[8].

With detailed computerized records being present on many sow and growing pig sites, we should have data that could be used, under certain conditions, to inform us about transmissibility. Examples could include records of abortions in confirmed new incursions of influenza and possibly records of mortality over time due to respiratory signs in cases when influenza virus is confirmed and other pathogens are not involved. Caution must be exercised, since influenza virus in swine rarely cause significant mortality[9].

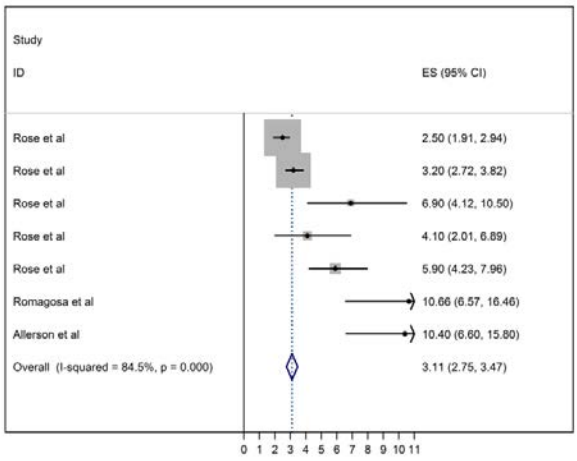

Figure 2. Fixed effect meta-analysis of recent observational studies and transmission experiments reporting estimates of the basic reproductive number. Estimate of the overall R_0 has been provided for illustration purposes, but the value of I^2 suggests large heterogeneity among studies.

Transmission of influenza virus within herds

Role of duration of different periods

Understanding the transmission pattern of influenza in swine populations should be based on a thorough understanding of the natural history of influenza virus infection at the individual animal level. Unlike many other infections in swine populations, influenza has a relatively simple natural history. Under experimental conditions, the period between infection and becoming infectious is 24 hours [9], and the period that a pig sheds the virus in considerable amounts is 5-7 days [9]. Duration of effective immunity over a prolonged period is not well understood[10]. The majority of dynamic models that aim to study influenza in other species assume that influenza virus infection will produce long-term immunity and that immune individuals will remain immune [11]. This gives rise to a susceptible-exposed-infectious-resistant (SEIR) class of dynamic models. This may be a reasonable assumption for studies that aim to evaluate control measures over a relatively short time period in these populations. Such an assumption is probably reasonable for our growing pigs, but perhaps needs to be considered carefully in sow herds. Figure 3 uses a very simple deterministic model, based on simplifying assumptions to illustrate the point that assumption about duration of immunity in sow populations, coupled with an inherently large replacement rate, could have large implications on our understanding of influenza virus circulation. In the case that influenza is assumed to produce a life-long immunity, a single introduction of a virus will result in an outbreak, which will be followed by the fade-out of the virus from this population. In

contrast, if we assume that the average duration of immunity is 1 year, we can expect repeated waves of virus circulation due to infection from the sow population alone, without consideration for the role of circulation in suckling piglets. This perhaps illustrates one additional point: a new clinical outbreak due to infectious disease in a sow herd is frequently ascribed to external breaches in biosecurity. However, at least some of these outbreaks could be due to the natural cycle of infection in an inherently open population and the role that duration of immunity plays.

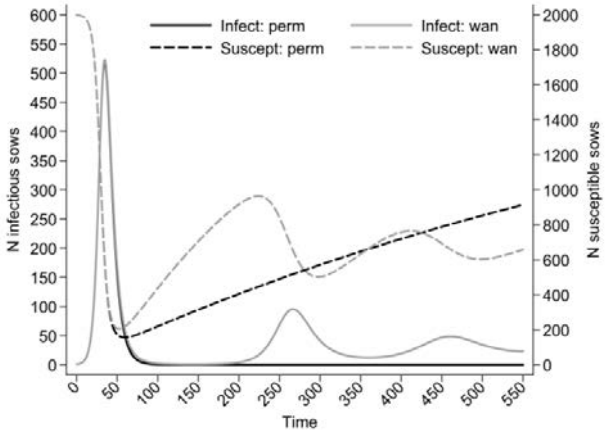

Figure 3. Expected circulation of influenza virus in a sow herd under the assumptions of permanent immunity and waning immunity. Assumptions: (i) sow herd size of 2000 sows, (ii) homogeneous mixing, (iii) R_0=3, (iv) annual replacement rate of 40%, (v) all replacement animals are susceptible, (vi) no contribution of other production classes including suckling piglets (vii) preinfectious period is 1 day, (viii) average infectious period is 6 days. Legend: (i) Infect: perm = number of infectious sows at a point in time under the assumption of permanent immunity, (ii) Infect-wan = number of infectious sows at a point in time under the assumption of waning immunity, (iii) Suscept: perm = number of susceptible sows at a point in time under the assumption of permanent immunity, (iv) Suscept: wan = number of susceptible sows at a point in time under the assumption of waning immunity.

Many influenza control strategies are based on maximizing maternal immunity, which might be present in growing pigs until the late nursery stage. Marked reduction in transmission was reported when piglets were exposed to a virus identical to the influenza virus used to immunize sows[4]. This finding concurs well with the rationale of practitioners who employ this strategy to control influenza virus infection in some herds. In the same study, transmission was slightly reduced when piglets with maternally derived antibodies were exposed to a heterologous virus of the same subtype. At the nucleotide sequence level, the similarity of the hemagglutinin of H1N1 between the virus used for immunization and for challenge was 86%. However, the role of maternal immunity for transmission is also subject to some uncertainty. Authors of a recent observational cohort study reported a high circulation of influenza viruses around weaning time in one of the study farms[12]. In the latter study, piglets had influenza virus positive nasal swabs, despite the presence of a high level of antibodies in their sera. It was therefore postulated that infection could occur in the presence of the colostrum-derived immunity. In another observational study, pigs infected in the presence of maternal antibodies did not seroconvert [5], a finding in agreement with previous experimental work[13]. This could have implications on the circulation of influenza virus in populations of growing pigs, since maternal immunity may persist on average approximately 8 weeks[14], with large variability among individual animals. This time represents a substantial proportion of the productive life of a growing pig. Figure 4 contrasts the expected circulation of influenza virus under two different scenarios in a batch of growing pigs farrowed in a farrowing cohort with 80% of sows immune. The first scenario is based on the assumption of complete protection of maternal immunity against infection in piglets that received maternal antibodies. The second scenario is based on the assumption that piglets will be completely susceptible to infection in the presence of maternal antibodies, and will not develop active immunity after they stop shedding the virus. Although both of these scenarios are unrealistic, they were made deliberately simplistic with the intention to show how maternal immunity could shape the circulation of influenza virus in a closed population. In the former scenario, only the initial proportion of susceptible animals allowed the virus to cause infection in the later phase of the growing period, otherwise circulation would have been stopped completely; whereas in the latter scenario the circulation of the virus starts early with a higher peak. More studies under field conditions would be useful to better understand the circulation of influenza viruses in this early phase of the pig's life.

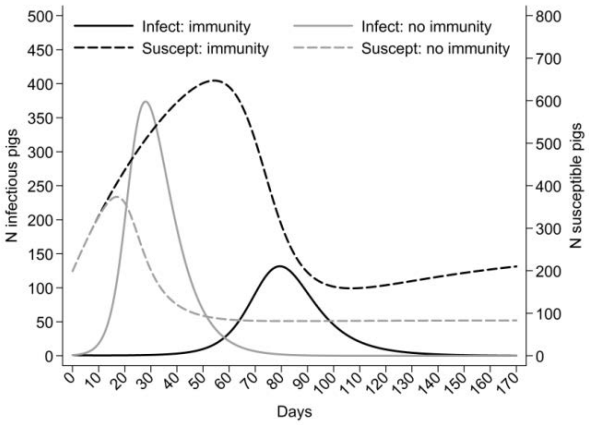

Figure 4. Expected circulation of influenza virus based on a deterministic model in a closed cohort of a growing pig herd under the assumption that maternally derived antibodies offer complete protection, or that maternally derived antibodies (MDAs) do not offer any resistance against infection and do not allow development of active immunity. Assumptions: (i) 1000 pigs in a batch, (ii) homogeneous mixing, (iii) R_0=3, (iv) no contribution of other production classes or batches (v) preinfectious period is 1 day, (vi) average infectious period is 6 days (vii) only 80% of the pigs at time 0 are maternally immune (20% of piglets are farrowed in parity 1 litters and do not have any antibodies), (viii) one infectious pig introduced at time 0. Legend: (i) Infect: immunity = number of infectious growing pigs at a point in time under the assumption that MDAs offer full protection, (ii) Infect: no immunity = number of infectious growing pigs at a point in time under the assumption that MDAs do not offer protection against infection and do not allow development of active immunity, (iii) Suscept: immunity = number of infectious growing pigs at a point in time under the assumption that MDAs offer full protection, (iv) Suscept: no immunity = number of infectious growing pigs at a point in time under assumption that MDAs do not offer protection against infection and do not allow development of active immunity.

Often times, several waves of influenza virus circulation have been reported, both in the scientific literature [5,12,15] and circumstantially, based on clinical impression. The easiest explanation would be to assume that different animals are becoming infected at different times. Although this might be a plausible explanation in some situations, it is puzzling that a recent study identified pigs that with repeated infection with influenza virus after an interval of >4 weeks. The viruses were very similar, and in some cases identical when partial nucleotide sequences of the HA gene were compared.

Role of concurrent circulation of different influenza viruses and different co-infections

Exposure of pigs to different subtypes and variants occurs frequently [16]. In addition, co-circulation of viruses has been documented in the same batch of pigs at different times or simultaneously[5,12]. This has obvious implications on the pattern of influenza circulation in a barn. Co-infections have been documented in the same animals as well, which additionally is a necessary condition for reassortment to occur. In fact, a novel recombinant was detected in a study that involved relatively few herds[5]. This is an interesting addition to a recent study which reported emergence of one recombinant virus per 2-3 years for Eurasian variants measured between 1982 and 2008; before the emergence of pandemic H1N1[17]. In North American herds, multiple recombinants were reported shortly after introduction of pH1N1[18] and their co-circulation in specific well-defined populations should be studied further. Concurrent infections with influenza virus and other respiratory pathogens are also frequent under field conditions. Involvement of influenza virus in porcine respiratory disease complex has been well described. Less is known about the influence that concurrent infections could have on epidemiological parameters of importance for dynamics of influenza at the population level. An interesting perspective comes from a recent comparison between nonimmunocompromised and severely immunocompromised people who were sampled daily for influenza virus until cessation of shedding. The mean duration of shedding in the severely immunocompromised patients was 19 days, as compared to ~6.4 days in the nonimmunocompromised[19].

Role of contacts between different age groups

In a longitudinal study of influenza virus circulation in finisher pigs, Loeffen *et al* reported different patterns of influenza virus circulation between finishing pigs raised in farrow-to-finish herds and in specialized finishing sites[20]. Incidence of influenza was highest at the beginning of the finishing period in farrow-to-finish sites, and at the end of the finishing period for pigs in specialized finishing operations. This high incidence at the beginning of the finisher period

was ascribed to a higher degree of contact between finisher pigs and sows or weaned pigs in operations that house all production classes on the same site and the resulting transmission between them. This finding explains why many farrow-to-finish sites have ongoing circulation of influenza virus. It remains a bit puzzling why some large production systems with much better age separation also have endemic circulation of influenza viruses. At a very general level, the answer is probably in the interaction between: (i) large sow herds where pockets of susceptible animals might allow long-term maintenance of the virus, (ii) mixing of animals from different sow herds with varying degrees of immunity, which might allow maintenance of the virus in a population over longer period of time, and (iii) degree of contact between different age groups during the nursery and finisher phase. It is not uncommon to have more than one nursery or finisher barn on specialized nursery or finisher sites. The reality of some production systems is that there is some overlap between different age groups in different buildings, and this could be a factor contributing to endemic circulation of influenza virus. In order to design more effective infection control strategies for influenza virus circulation, we need to have a better understanding of transmission not only within populations, but also between different production classes. Recent publications also point in this direction, particularly transmission in a sow herd[5,12].

Role of environment and seasonality of influenza

Seasonality of any infectious disease has implications for its transmission and is an important factor when designing control programs. In temperate regions, seasonal influenza in people peaks in the winter months. Contributors to seasonality are still not completely understood although several observational and experimental studies pointed to the role of humidity and temperature in the seasonality[21-24] . Lowen *et al*[22] in a guinea pig model showed that the relationship between transmission probability due to aerosol and relative humidity was not linear. The highest transmission occurred during periods of low relative humidity, decreasing at moderate humidity, then increasing again at 65%, and ceasing at a relative humidity of 80%. The authors concluded that aerosol transmission is most efficient in the experimental model of guinea pigs in conditions of low humidity and low environmental temperature. Interestingly, low temperature also extended the period of viral shedding. Contrary to this, high humidity did not have an impact on the contact spread between experimental animals[25], which led these authors to propose that aerosol transmission plays an important role among humans in temperate regions, whereas contact spread is more important in tropical zones. In contrast, the existence of influenza seasonality in swine populations is not well understood. The general consensus is that a seasonal pattern diminished with the advent of modern swine production systems[10]. One study that explicitly addressed influenza seasonality in pigs was conducted recently in four European countries between 2006 and 2008. No evidence of between-season differences in proportion of influenza-exposure positive pigs or farms could be found on the basis of sampling that was restricted to summer and winter periods[16].

Swine barns are located in different climate regions, and modern production systems have sophisticated systems of environmental control. Microclimate conditions such as temperature and relative (and absolute) humidity could have important implications for spread within different barns and production classes and deserves to be studied more thoroughly.

Transmission between populations located on different sites

Animal density in an area and related indicators have often been considered as a risk factor for prevalent cases of influenza, but little is known about the actual mechanisms of transmission. Some insight may be gained from the recent Australian outbreak of equine influenza, both in terms how the data were recorded and analyzed and conclusions reached. Of importance in this outbreak was the tracing of contacts and accurate assessment of the times when clinical outbreaks occurred. Use of social network analysis and proximity analysis provided some insight into the nature of spread in the early phases of the epidemic [26], whereas time-varying covariates assessed within survival analysis models provided an insightful perspective of the role of environmental factors in the disease spread once movement restriction was in control[27]. Interestingly, the latter study identified the role of time lagged relative humidity and temperature for incident cases of influenza in horse holdings, which was in line with epidemiological and experimental findings of the association between influenza circulation and humidity in human populations. Although it is unrealistic to expect that cases of influenza infections in swine herds will be investigated with the same thoroughness as the cases of a reportable disease, equine influenza, in a completely susceptible population, these reports contain some important lessons for data acquisition and analysis. It is encouraging to see a wide acceptance of the use of premises identification in different North American jurisdictions, either for the purposes of tracing outbreaks or disease control programs. Ultimately it could lead to development of real-time surveillance systems that are based on both spatial and network information. These types of epidemiological models for studying transmission will become a necessity in our highly integrated production systems.

Zoonotic transmission potential of Influenza A virus in pigs

Influenza A virus is a viral pathogen with high relevance for public health due to its zoonotic potential, reflected either in increased transmission events between animals and people, the virulence of some recently emerged variants in human populations, or both. Examples of emerging zoonotic influenza viruses are becoming more frequent in recent times. This includes, the highly pathogenic H5N1 (HP H5N1)[28], and H7N9 influenza virus which emerged recently in China[29]. In North America, swine-origin variant H3N2 virus in the USA caused over 300 human cases in 2012, most of them in people with prolonged exposure to swine during pig shows[30]. Instances of person-to-person transmissions have been postulated in some outbreaks of H3N2 virus. The 2009 pandemic H1N1 virus was linked with swine on the basis of genetic information[31], and between-species transmission has been reported in both directions [32-34]. Prior to emergence of pH1N1, a number of swine-originating reassortant H1N1 viruses caused spillover infections in people[35,36]. Thus, it is of great interest to understand transmissibility of influenza viruses between animals and people, and then subsequently between people. Wolfe et al [37] proposed a useful method to classify zoonoses with respect to this potential. According to this classification, pathogens that transmit from animals to people and then continue to spread efficiently among people have the potential to cause major epidemics in people and are considered to be stage IV zoonoses. Based on the same classification, stage II zoonoses are diseases transmitted between animals and humans, but not between people; whereas a stage III zoonosis will lead to some secondary transmission between people, potentially leading to local outbreaks. This classification was further supplemented by considering the person-to-person transmissibility expressed in terms of the reproductive number in human populations[38]. From this perspective, stage II, III, and IV zoonoses have R=0, <1, and ≥ 1, respectively.

The transmissibility of pathogens in different populations could be assessed using a variety of approaches, including virological studies, transmission experiments, and field studies based on seroprevalence studies and on the basis of data observed during field investigations. Methods for evaluation of self-limited (ie, stuttered) transmission chains, typical for stage III zoonoses, are still under development, but have received heightened interest in recent times; a recent overview summarized developments in this area[39]. De Serres et al[40] initially proposed three approaches to study the transmissibility of communicable diseases close to elimination, a situation that is similar to emergence of stage III zoonoses, via the: (i) proportion of imported cases, (ii) distribution of size of outbreaks, and (iii) distribution of duration of outbreaks. The approaches used were based on an assumed infinite pool of susceptible individuals and are, therefore, particularly limited in well-defined closed populations. Ferguson et al[41] proposed a method based on the number of animal-to-human cases observed, the number of clusters recorded, and the size of the largest outbreak. Further modifications to this methodology were proposed[42]. Using the proposed methodology, it was estimated that the most likely estimate for R_0 for H3N2 swine-origin viruses without the M segment from the 2009 pH1N1 was 0.2, and for H3N2 swine-origin viruses with the M segment was 0.5[42]. This can be used directly in risk assessment. Figure 5 shows the expected size of outbreaks (ie, number of people involved in a single transmission chain) under the assumption that R=0.5. It can be seen that most transmissions will result in infection in a single individual; however there is also a small probability that such transmission could result in a chain that will lead to larger number of individuals before it dies out.

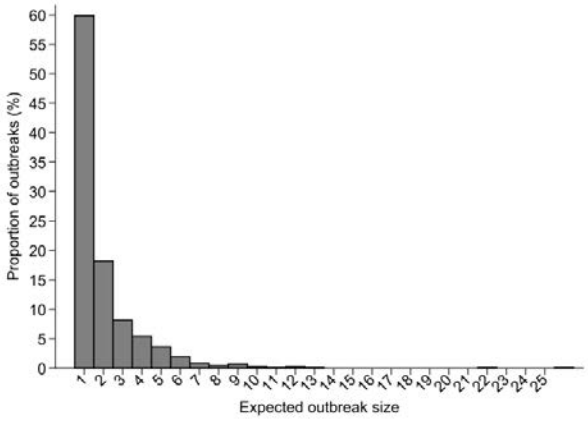

Figure 5. Expected outbreak size in a human population under the assumption that the reproductive number is 0.5, based on 1000 simulations.

Conclusions

Understanding transmission of influenza within and between populations serves to directly inform the infection control strategies at different levels. Recent longitudinal field studies provided interesting insight into circulation of influenza

virus under field conditions. Similar studies are needed under different conditions in populations of growing pigs, and particularly in sows. For such investigations, tools of molecular and infectious disease epidemiology should be used and further developed. In that respect, estimation of parameters that measure transmissibility would be particularly welcomed. For transmission between populations, advantage should be taken of analytical approaches which consider spatial and network characteristics of the populations. For this to occur, however, improved quality of field data is needed. Use of unique identifiers to link data among different data sources will continue to be a necessary requirement and the first step towards that.

References

1. Goldstein E, Paur K, Fraser C, Kenah E, Wallinga J, Lipsitch M. Reproductive numbers, epidemic spread and control in a community of households. *Math Biosci* 2009; **221:** 11-25.
2. Saenz RA, Hethcote HW, Gray GC. Confined animal feeding operations as amplifiers of influenza. *Vector Borne Zoonotic Dis* 2006; **6:** 338-46.
3. Romagosa A, Allerson M, Gramer M, et al. Vaccination of influenza a virus decreases transmission rates in pigs. *Vet Res* 2011; **42:** 120,9716-42-120.
4. Allerson M, Deen J, Detmer SE, et al. The impact of maternally derived immunity on influenza A virus transmission in neonatal pig populations. *Vaccine* 2013; **31:** 500-5.
5. Rose N, Herve S, Eveno E, et al. Dynamics of influenza A virus infections in permanently infected pig farms: evidence of recurrent infections, circulation of several swine influenza viruses and reassortment events. *Vet Res* 2013; **44:** 72,9716-44-72.
6. Bauch CT, Lloyd-Smith JO, Coffee MP, Galvani AP. Dynamically modeling SARS and other newly emerging respiratory illnesses: past, present, and future. *Epidemiology* 2005; **16:** 791-801.
7. Lipsitch M, Cohen T, Cooper B, et al. Transmission dynamics and control of severe acute respiratory syndrome. *Science* 2003; **300:** 1966-70.
8. Vynnycky E, Trindall A, Mangtani P. Estimates of the reproduction numbers of Spanish influenza using morbidity data. *Int J Epidemiol* 2007; **36:** 881-9.
9. Janke BH. Influenza A Virus Infections in Swine: Pathogenesis and Diagnosis. *Vet Pathol* 2013;.
10. Van Reeth K, Brown IH, Olsen CW. Influenza virus. In: Zimmerman J, Karriker L, Ramirez A, Schwartz K, Stevenson G, editors. Diseases of swine.: Wiley-Blackwell; 2012. p. 557-571.
11. Tuite AR, Fisman DN, Kwong JC, Greer AL. Optimal pandemic influenza vaccine allocation strategies for the Canadian population. *PLoS One* 2010; **5:** e10520.
12. Simon-Grife M, Martin-Valls GE, Vilar MJ, et al. Swine influenza virus infection dynamics in two pig farms; results of a longitudinal assessment. *Vet Res* 2012; **43:** 24,9716-43-24.
13. Loeffen WL, Heinen PP, Bianchi AT, Hunneman WA, Verheijden JH. Effect of maternally derived antibodies on the clinical signs and immune response in pigs after primary and secondary infection with an influenza H1N1 virus. *Vet Immunol Immunopathol* 2003; **92:** 23-35.
14. Loeffen WL, Nodelijk G, Heinen PP, van Leengoed LA, Hunneman WA, Verheijden JH. Estimating the incidence of influenza-virus infections in Dutch weaned piglets using blood samples from a cross-sectional study. *Vet Microbiol* 2003; **91:** 295-308.
15. Allerson MW, Davies PR, Gramer MR, Torremorell M. Infection Dynamics of Pandemic 2009 H1N1 Influenza Virus in a Two-Site Swine Herd. *Transbound Emerg Dis* 2013.
16. Kyriakis CS, Rose N, Foni E, et al. Influenza a virus infection dynamics in swine farms in Belgium, France, Italy and Spain, 2006-2008. *Vet Microbiol* 2013; **162:** 543-50.
17. Lycett SJ, Baillie G, Coulter E, et al. Estimating reassortment rates in co-circulating Eurasian swine influenza viruses. *J Gen Virol* 2012; **93:** 2326-36.
18. Ducatez MF, Hause B, Stigger-Rosser E, et al. Multiple reassortment between pandemic (H1N1) 2009 and endemic influenza viruses in pigs, United States. *Emerg Infect Dis* 2011; **17:** 1624-9.
19. Memoli MJ, Athota R, Reed S, et al. The Natural History of Influenza Infection in the Severely Immunocompromised vs Nonimmunocompromised Hosts. *Clin Infect Dis* 2014; **58:** 214-24.
20. Loeffen WL, Hunneman WA, Quak J, Verheijden JH, Stegeman JA. Population dynamics of swine influenza virus in farrow-to-finish and specialised finishing herds in the Netherlands. *Vet Microbiol* 2009; **137:** 45-50.
21. Tamerius JD, Shaman J, Alonso WJ, et al. Environmental predictors of seasonal influenza epidemics across temperate and tropical climates. *PLoS Pathog* 2013; **9:** e1003194.
22. Lowen AC, Mubareka S, Steel J, Palese P. Influenza virus transmission is dependent on relative humidity and temperature. *PLoS Pathog* 2007; **3:** 1470-6.
23. Shaman J, Kohn M. Absolute humidity modulates influenza survival, transmission, and seasonality. *Proc Natl Acad Sci U S A* 2009; **106:** 3243-8.
24. Shaman J, Pitzer V, Viboud C, Lipsitch M, Grenfell B. Absolute Humidity and the Seasonal Onset of Influenza in the Continental US. *PLoS Curr* 2009; **2:** RRN1138.

25. Lowen AC, Steel J, Mubareka S, Palese P. High temperature (30 degrees C) blocks aerosol but not contact transmission of influenza virus. *J Virol* 2008; **82:** 5650-2.
26. Firestone SM, Christley RM, Ward MP, Dhand NK. Adding the spatial dimension to the social network analysis of an epidemic: investigation of the 2007 outbreak of equine influenza in Australia. *Prev Vet Med* 2012; **106:** 123-35.
27. Firestone SM, Cogger N, Ward MP, Toribio JA, Moloney BJ, Dhand NK. The influence of meteorology on the spread of influenza: survival analysis of an equine influenza (A/H3N8) outbreak. *PLoS One* 2012; **7:** e35284.
28. Li KS, Guan Y, Wang J, et al. Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. *Nature* 2004; **430:** 209-13.
29. Lam TT, Wang J, Shen Y, et al. The genesis and source of the H7N9 influenza viruses causing human infections in China. *Nature* 2013.
30. Centers for Disease Control and Prevention (CDC). Influenza A (H3N2) variant virus-related hospitalizations: Ohio, 2012. *MMWR Morb Mortal Wkly Rep* 2012; **61:** 764-7.
31. Smith GJ, Vijaykrishna D, Bahl J, et al. Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. *Nature* 2009; **459:** 1122-5.
32. Howden KJ, Brockhoff EJ, Caya FD, et al. An investigation into human pandemic influenza virus (H1N1) 2009 on an Alberta swine farm. *Can Vet J* 2009; **50:** 1153-61.
33. Pasma T, Joseph T. Pandemic (H1N1) 2009 infection in swine herds, Manitoba, Canada. *Emerg Infect Dis* 2010; **16:** 706-8.
34. Forgie SE, Keenliside J, Wilkinson C, et al. Swine outbreak of pandemic influenza A virus on a Canadian research farm supports human-to-swine transmission. *Clin Infect Dis* 2011; **52:** 10-8.
35. Shinde V, Bridges CB, Uyeki TM, et al. Triple-reassortant swine influenza A (H1) in humans in the United States, 2005-2009. *N Engl J Med* 2009; **360:** 2616-25.
36. Belser JA, Gustin KM, Maines TR, et al. Pathogenesis and transmission of triple-reassortant swine H1N1 influenza viruses isolated before the 2009 H1N1 pandemic. *J Virol* 2011; **85:** 1563-72.
37. Woolhouse ME, Gowtage-Sequeria S. Host range and emerging and reemerging pathogens. *Emerg Infect Dis* 2005; **11:** 1842-7.
38. Lloyd-Smith JO, George D, Pepin KM, et al. Epidemic dynamics at the human-animal interface. *Science* 2009; **326:** 1362-7.
39. Blumberg S, Lloyd-Smith JO. Comparing methods for estimating R0 from the size distribution of subcritical transmission chains. *PLoS Comput Biol* 2013; **5:** 131-45.
40. De Serres G, Gay NJ, Farrington CP. Epidemiology of transmissible diseases after elimination. *Am J Epidemiol* 2000; **151:** 1039,48; discussion 1049-52.
41. Ferguson NM, Fraser C, Donnelly CA, Ghani AC, Anderson RM. Public health. Public health risk from the avian H5N1 influenza epidemic. *Science* 2004; **304:** 968-9.
42. Cauchemez S, Epperson S, Biggerstaff M, Swerdlow D, Finelli L, Ferguson NM. Using routine surveillance data to estimate the epidemic potential of emerging zoonoses: application to the emergence of US swine origin influenza A H3N2v virus. *PLoS Med* 2013; **10:** e1001399.

Nutritionally induced cellular signals that affect skeletal integrity in swine

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Introduction

Insights into skeletal integrity gained from an accidental omission of vitamin D. A recent escalation in lameness and mortality issues in the U.S. swine industry (Madson et al., 2012) were attributed to hypovitaminosis D. An explanation for the abrupt increase in clinical cases of vitamin D deficiency in commercial swine is not apparent. The swine industry has alleviated some of these issues by modifications in vitamin D supplements, but problems still persist (Arnold et al., 2014). Although the number of cases has subsided, the issues spurred interest and questions about bone composition and methods used to accurately assess skeletal integrity. Necropsy reports that describe fractures, callous ribs, and "rubbery" bones may reflect extreme conditions of a nutrient deficiency. More definitive descriptors are needed as guidelines to establish a balance between nutrient inputs required for animal well-being and the environmental issues that often pressure nutrient formulation strategies.

This review will provide a brief overview of basic principles involved in bone composition, the impact of dietary nutrients on bone composition, and a critical assessment of methods used to quantify bone integrity in clinical and research settings as presented earlier (Crenshaw et al., 2013). Additional background will be provided to assess the potential for identification of nutrient-induced cellular signals that may provide diagnostic indicators of physiological perturbations that lead to failures in skeletal integrity in swine. Specific cellular signals involved in vitamin D and bone homeostasis have not been clearly elucidated for pigs, which leads to speculative recommendations (Crenshaw et al., 2014). Our recent research interest in vitamin D and skeletal abnormalities was stimulated by an accidental omission of vitamin D from a premix fed to our research herd. Efforts to explain the symptoms induced has led us to explore new pathways recently discovered. A better understanding of the signaling pathways by which vitamin D alters endochondral ossification will enhance development of treatments to prevent lameness and bone related disorders in pigs.

Bone composition and integrity

Diagnosis of clinical symptoms in nutrient based problems. Before discussing the major nutrient induced signals involved in endochondral ossification, a brief overview of bone composition and methods used to diagnose bone will be summarized from a recent review (Crenshaw et al., 2013). This review provided a foundation for use in diagnosis of nutrient-induced skeletal integrity issues in swine.

Bone tissue composition. The water and fat content of bone varies with age, type of bone, and nutrient inputs. Therefore, the mineral content expressed as a percentage of the dry, fat-free weight is a better descriptor of the extent to which the organic matrix has become saturated with mineral (Crenshaw, 2001). On a dry, fat-free basis, approximately 56% of the entire skeleton is ash. The percent ash varies from 62 to 72% in cortical bone from mature sows (Crenshaw et al., 2013) to 44 to 46% in bones from young pigs that are mostly trabecular such as ribs or vertebrae. Thus, selection of a single bone sample and animal age are critical determinants for comparison if percentage ash is used for diagnostic assessment of mineral adequacy. However, over a range in percent ash, the Ca and P content of bone remains constant. The inorganic ash contains Ca (38 to 40%) and P (17 to 19%) in a 2.1:1 ratio. If Ca and P values deviate from these values, then the analytical methods should be questioned. The amount of ash may change in response to diet, but the Ca and P composition of the ash remains constant.

Bone strength. Bone integrity (soundness) is affected by both the organic and inorganic materials that compose the tissue. Bone tissue is a composite material which requires synthesis of an organic matrix by osteoblast cells that are eventually embedded in an extra-cellular matrix. The matrix is composed primarily of collagen fibrils arranged in helical strands with proteoglycan polymers inter-dispersed within the matrix. With time, hydroxyapaptite-like $[(Ca_3(PO_4)_2)_3 \cdot Ca(OH)_2]$ mineral crystals form within the collagen helical matrix. Systemic hormones and localized growth factors stimulate osteoblast proliferation and differentiation with consequences on the rate and accumulation of the organic matrix. However, the actions do not act directly stimulate mineral crystal formation. Attempts to increase mineralization by over-supplementation of diets will down-regulate homeostatic mechanisms and decrease the efficiency of nutrient use.

The combination of the organic matrix and mineral crystals define the material strength properties of bone. The combination of collagen fibers, which contribute primarily tensile (resistance to stretching) properties, and mineral crystals, which contribute primarily compressive (resistance to compression) properties produces an anisotropic material with properties that cannot be explained by the summation of the individual components. Normal loads imposed on a bone are not singularly a tensile or compressive force. Rather, most forces imposed on live animals

involve a combination of these two forces to produce a bending load. One surface of the bone is compressed while the other surface is under tension (Crenshaw et al., 1981a). Thus, the combination of materials, collagen and mineral crystals, provide a cumulative response to forces that cannot be explained by a single material.

Selection of a single bone for diagnosis of the animal

Which bone to sample? Decisions on selection of a single bone sample to represent the entire animal should be based on how well the bone reflects changes in the entire animal at the age of sample collection. All bones within the skeleton do not grow at the same rate and thus, do not respond the same to nutrient inputs. Use of dual energy x-ray absorpitometry (**DXA**) to measure the entire pig and single bones in response to dietary nutrient inputs illustrates this principle. In young pigs (25 to 30 kg) femur ash provided a better fit to dietary P inputs than fibula ash relative to responses in the entire pig (Crenshaw et al., 2009). The fibula tended to overestimate whole body bone mineral content (**BMC**) at low P intakes, but underestimated BMC at high intakes. As the entire pig, not the femur or fibula, consumed the diet, the bone which reliably predicted the entire pig seemed to be a reasonable choice. However, in a separate study with older pigs (40 to 120 kg) differences among bones (femur, front feet, or hind feet) were not dramatically different in their fit as predictors of whole body BMC (**Table 1**), but predictions based on fibulas over-estimated the whole body BMC. Thus, selection of limb bones from pigs at market weight (120 kg) are not as critical as the bone selected at younger ages (< 30 kg), consistent with earlier conclusions (Crenshaw et al., 1981b).

Table 1. Regression equations for the use of individual standardized [a] bone traits as predictors of whole body bone mineral content of pigs (rPBMC) from 40 to 120 kg

Regression equ|ations	
rPBMC = -0.057 + 1.018*Femur rBMC	R^2 = 0.966
rPBMC = 0.133 + 0.846*Fibula rBMC	R^2 = 0.910
rPBMC = -0.089 + 0.994*Front Foot rBMC	R^2 = 0.906
rPBMC = -0.076 + 0.971*Hind Foot rBMC	R^2 = 0.797
rPBMC = 0.266 + 0.663*Yield Bending Moment	R^2 = 0.620
rPBMC = 0.455 + 0.201*Yield Stress	R^2 = 0.966

a. All values (n=78 total pigs with 6, 24, and 48 each at 40, 80 and 110 kg weight groups respectively) were standardized to the relative values for each trait based on the pig with the largest amount of bone mineral content.

Turn-around time and pitfalls in methods to assess mineral status

Methods to assess bone. Traditional methods used to assess skeletal tissue integrity can be broadly classified into 3 approaches (**Table 2**) which include histology, gravimetric, and mechanical procedures. All 3 procedures, with exception of DXA scans, require a terminal approach and each provide unique, and often different conclusions with regards to nutrient inputs. Each method also has limitations and pitfalls that must be considered in a decision to employ the method or interpret the results for a final diagnosis. Clinical diagnosis requires rapid turn-around to deal with acute issues. Simple, rapid methods, such as visual appraisals, quantifying the incidence of occurrences, and a terminal assessment of the bone ash content are the first-line approaches to deal with acute cases. Long-term, chronic issues can potentially be resolved with additional samples, such as the front foot, collected from marketed animals and submitted for DXA scans to determine mineral content. Use of histology and mechanical tests are more quantitative than measures of bone ash, but require a longer turn-around time for results and are more expensive. Additionally, the measures of bone ash are often only used to diagnosis clinical symptoms already evident in the herd. A method to measure a trait that predicted nutritional adequacy prior to evidence of clinical symptoms would be ideal.

The use of DXA scans of a single bone or foot, a relatively rapid tool for diagnosis, has limitations. First, even given the accuracy of the DXA scans, the results still represent the ash content which is not necessarily a reflection of bone integrity. Second, DXA scans do not identify joint lesions and focal failures which often are predisposing symptoms for lameness.

Table 2. Methods used to assess skeletal tissues in response to nutrient inputs

Method	Test	Prospects	Pitfall
Histology	Terminal	Dynamic	Sample size/time
Gravimetric	Terminal	Standard	Accuracy
DXA	Non-invasive	Whole animal	Ash ≠ Strength
Mechanical	Terminal	Structural Integrity	Irregular shape & Composite material

Inferences for nutritional intervention

Recovery from periods of nutrient deficiencies. A discussion of concepts related to the effects that dietary concentrations of Ca and P have on the accumulation of bone mineral (ash) in pigs is beyond the scope of this review. Deficiencies of these nutrients lead to an under-mineralized bone matrix, deformed limbs (rickets), and spontaneous fractures (mechanical failures). Numerous research papers, reviews and texts have focused on these topics. Guidelines for the amounts of Ca and P supplied and the ratio of Ca to P, especially under conditions of marginal P intake, were relatively well-established until the introduction of phytase supplements as a common feed ingredient. Development of recommendations related to dietary Ca and P supplements in diets that incorporate various phytase products are on-going. Variant feed formulations based on phytase inclusions may contribute to some of the escalated lameness issues, but quality control issues in feed management, which affect phytase stability, are more likely an issue. However, the focus of this paper is to address guidelines for the assessment of bone to establish if animals have been fed diets within an acceptable safe range of nutrient inputs.

The recent escalation of lameness cases associated with vitamin D has highlighted concerns for quality control issues in diet formulations (Arnold et al., 2014). Failures in quality control procedures for feed management may contribute to delivery of diets with un-intentional deficiencies. The inability of growing pigs to recover skeletal mineral content after a brief period of mineral deficiency was recently reported (Aiyangar et al., 2010). Within 4 wk, a 61.6% reduction in whole body BMC was induced in young pigs fed a diet with 70 vs 150% of Ca and P requirements based on NRC, 1998 guidelines. Whole body DXA scans of the same animals at repeated intervals revealed that BMC was not recovered over a subsequent 8 wk period, even if the pigs were fed diets with 150% of Ca and P requirements. However, femurs collected after the 8-wk recovery period had apparently regained equal strength properties as those from pigs fed control diets throughout the entire trial. Recovery of femur strength, but not whole body BMC can be attributed to either preferential partitioning of mineral reserves to load-bearing bones, potentially at the determent of non-load bearing bones, or to a shift in the distribution of mineral reserves within bone to align the limited resources with the direction of applied loads.

These results further illustrate the discrepancy between assessments based on ash and mechanical test properties. Additional disparate results based on ash vs. mechanical properties have been reported in nursery pigs (Rortvedt et al., 2012), growing-finishing pigs (Iwicki et al., 2011) and to an extent by other researchers (Létourneau-Montminy et al., 2011). Guidelines to identify bone strength properties that reflect acceptable ranges have not been defined. Results from mechanical tests of individual bones have been used to describe animal responses to nutrient inputs. The amount of nutrients required to maximize bone strength exceeds the amount required to maximize bone ash (Crenshaw, 1986; NRC, 1998). Thus, the ash content of bone is not directly proportional to the strength properties of bone. Additionally, procedures used to measure bone mechanical traits are not standardized across laboratories. The time required for mechanical test procedures and requirements of specialized equipment often preclude the routine use of these procedures as a clinical diagnostic method. Thus, bone mechanical tests are not recommended for use in clinical assessment of lameness cases, rather such test methods remain as a technique for assessment of hypothesis-driven research projects.

Lessons learned from hump-back pigs. Prior to 2008, we understood that kyphosis was an idiopathic disease which occurred sporadically in swine and produced hump-back pigs with spinal deformities. Although kyphosis was problematic in afflicted herds, the disorder was considered of little consequence to the overall industry. After a flare-up in our research herd in 2008, we have been able to induce kyphosis under controlled conditions by use of diet manipulation. The efforts to characterize the disorder have led to additional insights in bone and connective tissue development that may link lesions of mineralization and osteochondrosis (**OC**).

Although vitamin D has traditionally been indirectly associated with bone development through regulation of Ca homeostasis, a newly discovered hormone has provided additional insights into vitamin D and P homeostasis which involves bone. Traditional pathways for vitamin D regulation of Ca and P homeostasis (**Figure 1A**) have focused on regulation of vitamin D by parathyroid hormone **(PTH)** in response to fluctuations in serum Ca concentrations. The hormones induce changes in target tissues of the kidney, GI tract, and bone. More recently, evidence for a direct role of vitamin D in bone development was provided through identification of the vitamin D receptor **(VDR)** in bone cells and

by new insights in cellular signal pathways that control P homeostasis. The newly discovered phosphaturic hormone, fibroblast growth factor 23 **(FGF23)** has been characterized (Lanske et al., 2014; Martin et al., 2011; and Sitara et al., 2006). FGF23 is primarily produced in the bone osteocyte. FGF23 is responsible for P homeostasis through a pathway that involves feedback regulation between FGF23, vitamin D, and P **(Figure 1B)**.

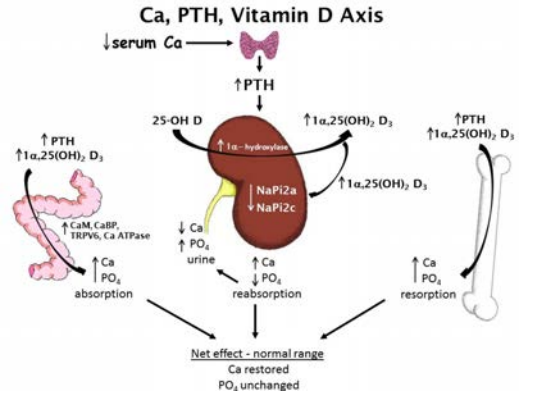


Figure 1 A

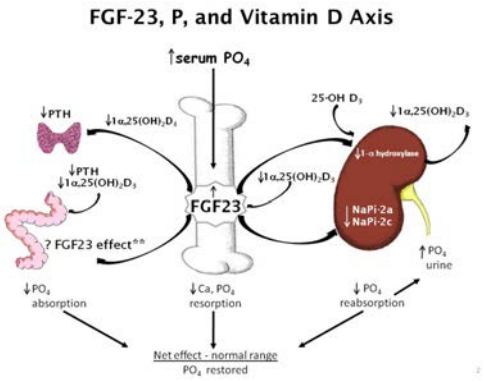


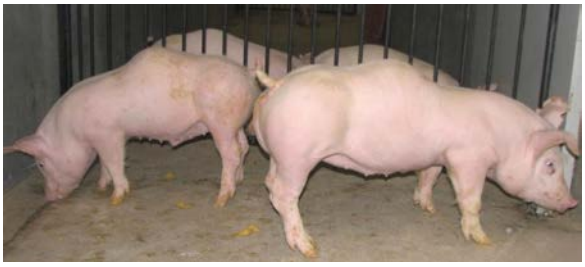
Figure 1 B

Figure 1A and 1B. Comparison of the traditional Ca, PTH, and vitamin D axis to describe Ca homeostasis in response to a reduction in serum Ca with the novel FGF23, P, and vitamin D axis in response to an increase in serum P. (From Crenshaw et al., 2011)

The novel pathway for FGF23, vitamin D, and P homeostasis now offers opportunities to improve P efficiency without compromising skeletal growth and animal well-being. Historically swine diets were fortified with excess Ca and P with minor incentives to improve nutrient efficiency. Constraints on dietary supplemental P are now driven by ingredient costs and environmental concerns. Thus P, not Ca, is typically more limited in swine diets (Crenshaw 2001). New concepts revealed by the FGF23 pathway have challenged the traditional axiom and disclosed signals and feedback inhibition among P, vitamin D metabolites, and PTH with a critical regulatory component attributed to FGF23. A central component that linked FGF23 with P homeostasis and renal function involved identification of bone as the primary tissue for FGF23 synthesis, in essence ascribing an endocrine gland function to bone. By identifying the nutritionally induced responses in the novel pathway for regulation of P homeostasis, applied feeding strategies can be developed to improve efficiency of dietary P use and vitamin D homeostasis in swine.

As mentioned previously, a challenge to explore the alternate pathway for vitamin D-mediated P homeostasis was stimulated, in part, by an outbreak and subsidence of kyphosis (~20% incidence) in pigs produced by the UW Swine Research and Teaching Center (**SRTC**) herd during a 4 month period in 2008 (**Figure 2**). The outbreak and subsidence of kyphosis in the closely monitored research herd occurred with no changes in animal genetics or health status. In a survey of pigs from 3 herds with a high incidence of kyphosis (Nielsen et al., 2005), lesions predominated in the 14th, 15th, and 16th thoracic vertebra. The wedge-shaped vertebra, characteristic of kyphosis, displayed histological lesions characterized as a failure of endochondral ossification. The FGF peptides are implicated in endochondral development (Horton and Degnin, 2009). Gross anatomical skeletal features of FGF23-null mice (Sitara et al., 2006) are reminiscent of skeletal deformities displayed in pigs with kyphosis.

The kyphosis outbreak at SRTC stimulated a series of trials that eventually linked the disorder to an accidental omission of vitamin D_3 from a custom-mixed vitamin premix that was fed to the entire herd for 4 months. The symptoms were initially observed in growing pigs at SRTC fed diets with marginal amounts of Ca and P. However, the outbreak and subsidence of kyphosis in these pigs coincided with the period during which the deficient premix was fed to the gestating sows that produced the pigs with kyphosis.



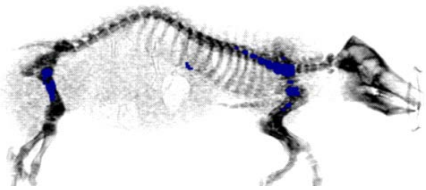


Figure 2. Young pigs (~ 30 kg BW) displaying kyphosis and a DXA scan image of a ~ 20 kg pig to show abnormal spinal column curvature. (From Rortvedt and Crenshaw, 2012).

A controlled study was subsequently conducted to confirm the inferences of a maternal diet effect on vitamin D induced kyphosis in young pigs. The objective was to determine if sows fed a gestation diet without supplemental vitamin D produced pigs that displayed kyphosis. The weaned pigs were fed diets without supplemental vitamin D, and either adequate (120% of requirements, NRC 1998) or marginal (80% of requirements, NRC 1998) amounts of Ca and P. Young pigs fed marginal nursery diets and produced by sows fed a gestation diet with no supplemental vitamin D developed kyphosis (21% incidence) and displayed reduced growth and skeletal mineral content determined from DXA scans by 9 wk of age (Rortvedt and Crenshaw, 2012). At 9 wk, none of the pigs from sows fed diets supplemented with vitamin D (280 IU D_3/kg) displayed evidence of kyphosis. However, by 13 wk, evidence of kyphosis (25% incidence) was detected in pigs fed marginal diets even if the pigs were produced by sows fed diets with supplemental vitamin D. At 13 wk the kyphosis incidence in pigs from sows fed diets with no supplemental vitamin D had increased to 33%. The effects of vitamin D concentrations in maternal diets affected the time required for pigs to display symptoms of kyphosis. The time effect was consistent with an involvement of maternal diets in predisposing pigs to skeletal defects.

Based on earlier research (Aiyangar et al., 2010; Crenshaw, 1986; NRC, 1998), the marginal dietary Ca and P concentrations used to induce kyphosis in the above experiment were not dramatic deficiencies, but may have been exacerbated by lack of supplemental vitamin D. The kyphosis responses were not expected. Either marginal deficiencies of all 3 nutrients exacerbated responses or the life-cycle phases in which the deficiencies were imposed were critical. As discussed in subsequent sections, the vitamin D status of the sows may have contributed to the observed responses.

Maternal carry-over effects. Evidence to support maternal carry-over effects of dietary vitamin D on subsequent pig responses were recently reported (Rortvedt and Crenshaw, 2012; Rortvedt-Amundson and Crenshaw, 2013). Whole body bone mineral density (BMD) was reduced (~20%) in pigs produced by gilts fed 0 or 325 IU D_3/kg and fed diets with no supplemental vitamin D and 120% recommended P requirements. However, BMD was not reduced in pigs fed the same nursery diets if they were produced by gilts fed 1,750 IU D_3/kg during gestation.

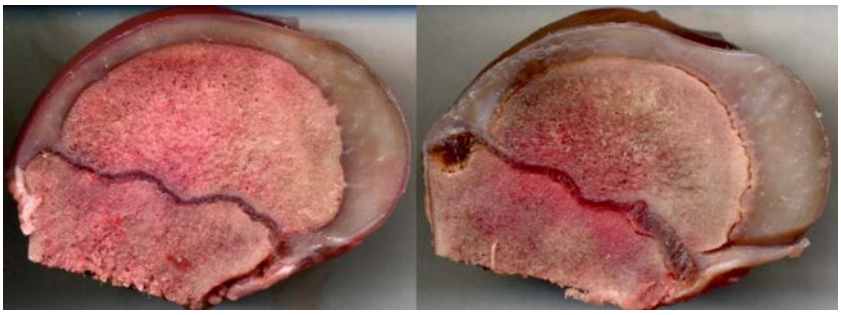
In the same experiment differences due to maternal diet effects in whole body BMD were not detected in pigs at birth and weaning. However, maternal diet effects were detected in mRNA expression of genes involved in vitamin D metabolism in kidney and intestine of the neonatal pigs (Rortvedt-Amundson and Crenshaw, 2014). Maternal diets with excess dietary vitamin D increased mRNA expression of 24,25 hydroxylase (**CYP24A1**) in pigs at weaning, implying an increased ability of the pigs to degrade vitamin D.

The effects due to maternal diets in this experiment and an earlier experiment (Rortvedt et al. 2011) have identified apparent deficiencies that were induced in relatively short time periods even in pigs produced by sows fed the amounts of vitamin D routinely fed to the SRTC breeding herd. Updated estimates of vitamin D requirements (NRC, 2012) imply that the vitamin D supplements in the SRTC diets are not sufficient.

Collectively, these results are consistent with maternal effects of hypovitaminosis D induced bone abnormalities in the fetal pig. The induction of abnormalities are possibly initiated in utero in sows fed diets with marginal or deficient vitamin D concentrations. The abnormalities are detectable at birth and weaning if assessments are based on molecular signals, but the gross abnormalities of bone tissues are not evident until the pigs are nutritionally stressed by marginal Ca and P after weaning. Thus, the measurement of both gross and molecular characteristics in the pig at multiple

developmental stages is necessary to elucidate the critical regulatory signals involved in the initiation of lesions induced in the swine hypovitaminosis D kyphosis model.

Kyphosis - lesions of endochondral ossification. Gross observations of spines from pigs with kyphosis revealed abnormalities of the vertebral growth plate with evidence of retained cartilage, similar to abnormalities reported in pigs with lesions of OC (McCoy et al., 2013; Ytrehus et al., 2007). Other gross abnormalities were not initially observed in live animals, but further analysis of femurs collected from these pigs revealed growth plate abnormalities, which included focal regions of irregular physeal widths (**Figure 3**). These observations support the need to further evaluate changes in molecular signals that are involved in endochondral ossification, especially signals that may be altered by insufficient dietary vitamin D during fetal and early neonatal development.



+D **-D**

Figure 3. Sections of the distal femur to illustrate irregular physeal widths. Sections were collected from 9 wk pig fed +D diets (+D) or diets with no supplemental vitamin D (-D) for 5 wk. (Unreported results).

The femur and vertebrae are both formed by endochondral ossification, a process in which calcified cartilage is coupled with replacement by calcified bone during normal bone formation. An un-coupling of this process leads to an abnormal, wider growth plate, including irregular widths and retained, excess cartilage. The modeling and remodeling of the extracellular matrix **(ECM)** is the rate-limiting step in endochondral ossification (Ortega et al., 2004; Stickens et al., 2004). Critical proteins and signals required for normal endochondral ossification include the family of matrix metalloproteinases **(MMP)**, particularly **MMP9**, a gelatinase, and **MMP13**, a collagenase, and vascular endothelial growth factor **(VEGF)** (Ortega et al., 2004). All 3 signals are directly or indirectly mediated by vitamin D. Evidence to directly link expression of these signals with vitamin D status is not available for pigs.

Lameness research in pigs and other production animals has typically focused on characterizing the later stages of lesions rather than the initiation of lesion development. The swine hypovitaminosis D kyphosis model provides a means to study cellular signals involved in the initiation of growth plate abnormalities as the model provides a method to predictably induce a spontaneous lesion. Characterizing the dynamics of target cells and the cellular signals which are disrupted during abnormal spinal column and femur development of neonatal pigs will potentially lead to specific and effective interventions for prevention of bone abnormalities.

We are continuing to learn from the humpback pig, which has helped us to focus on the pivotal signals that need to be measured. In the past, swine lameness was broadly grouped into either problems associated with mineralization or problems produced by lesions of OC. Experiments to understand the kyphosis incidence in our herd has led to potential signaling pathways that may link kyphosis and OC.

Pivotal signals regulated by nutrient inputs that affect endochondral ossification

Bio-markers for bone. The use of numerous bio-markers to identify bone integrity traits have been attempted, but at best these bio-markers have only proven to be qualitative and of limited value in diagnosis of skeletal lameness. As a result of homeostasis, serum concentrations of nutrients such as Ca and P and even 25-OH vitamin D are of limited value for assessments of skeletal integrity concerns, even though these nutrients are the primary dietary factors that drive skeletal mineral accumulation. A better understanding of the pivotal nutrient-induced cellular signals that affect bone may provide potential use for improvement of methods for rapid diagnosis of swine lameness problems.The pivotal signals that may reflect nutritional and immunological inputs appear to be FGF23, RANKL/OPG, CYP27B1, CYP24A1, VEGF, and MMP.

As discussed previously, FGF23 is involved in vitamin D and P homeostasis. FGF23 is produced by osteoblast and osteocyte cells and is regulated by vitamin D and P (Sitara et al., 2006; Crenshaw et al., 2011; and Lanske et al., 2014). Active vitamin D increases FGF23 production in bone which then acts systemically to up-regulate renal Na-P transporters to increase P reabsorption, thus decreasing circulating P. FGF23 downregulates 1α-hydroxylase and reduces activation of vitamin D (**Figure 1b**). Given the expression of the VDR in chondrocytes, vitamin D potentially has a direct effect on bone tissue production of FGF23. In chondrocyte specific VDR knockout mice, FGF23 release was increased by vitamin D through a chondrocyte-mediated signaling pathway (Masuyama et al., 2006; St-Arnaud, 2008). The signaling factor released from chondrocytes has not yet been elucidated. Our recent data (Rortvedt-Amundson and Crenshaw 2014), revealed an increase in bone FGF23 mRNA expression in nursery pigs fed supplemental D, especially if pigs were fed diets with excess P. The newly identified roles of FGF23 suggest an endocrine role of bone tissue in P homeostasis, beyond that of merely being a storage site for Ca and P (Masuyama et al., 2006; St-Arnaud, 2008).

Excess release of FGF23 may down-regulate activation of vitamin D and induce an apparent vitamin D deficiency, even though vitamin D is supplemented in the diet. This scenario is more likely under conditions of excess dietary P. Swine diets would not typically be expected to contain excess P, but adoption of the use of phytase enzymes to release more P from plant ingredients and the inclusion of ethanol co-products, which contain excess P, has increased the potential for swine diets to contain excess P. Production of excess FGF23 may reduce the efficiency of P use and lead to an over-supplementation of a mineral that contributes to surface water pollution.

To our knowledge, FGF23 gene expression, nor the protein has been successfully measured in the pig until our efforts reported at this meeting (Rortvedt-Amundson and Crenshaw, 2014). The data reported on bone tissue FGF23 mRNA expression via qPCR assays were altered by nutrient inputs, but contained considerable variation. More work is needed to support the role of FGF23 as a pivotal signal in swine. We are more confident in our qPCR results to measure mRNA expression of RANKL, CYP27B1, and CYP24A1 in pig tissues. Measures of VEGF, and MMP have not yet been attempted.

Two main types of cells are involved in bone formation, the osteoblast (**Obl**) and the mature osteocyte. One cell type is involved in bone resorption is the osteoclast (**Ocl**). Bone formation and resorption processes are typically coupled in remodeling. Thus, no net accumulation or loss occurs in adult animals at maintenance, but homeostatic mechanisms can alter the balance. In growing animals, formation exceeds resorption, so bone mineral accumulates. The Ocl are involved in bone resorption. These cells do not have receptors for systemic hormones such as PTH or vitamin D, but are controlled via the Obl lineage of cells. Recent research has focused on the signals and cells that produce the signals to induce Ocl recruitment and activation of resorption at specific locations. Cells of the Obl lineage complete various functions to release signals (RANKL and OPG) in response to systemic hormones.

Support for direct roles of 1,25 OH D in bone formation are based on the presence of the VDR in both chondrocytes and Obl. Most systemic roles of 1,25 D are mediated via mineral homeostasis, however under conditions of rescue diets in VDR-null mice, bone mineralization was restored but growth plate deformities were detected (Li et al, 2002; St-Arnaud, 2008) supporting a direct role for vitamin D in endochondral bone formation. In proliferative and hypertrophic chondrocytes, 1,25 OH D induces the synthesis of an unknown protein that increases FGF23 release and increases the synthesis of RANKL and VEGF. RANKL and VEGF act to stimulate Ocl activation and recruitment to resorb the hypertrophic zone, thus. maintaining a normal chondro-osseous junction and vascular invasion of the calcified hypertrophic zone. This process of endochondral ossification allows bone growth and a normal growth plate width. Disruption of this process results in retention of cartilage in focal regions and development of lesions such as OC.

Links between bone and the immune system. Lameness cases in swine herds are often confounded with herd health status. Our research, to date, has involved pigs from a herd with no major health challenges. Links have been implied between bone lesions and immune function, but direct mechanisms are difficult to establish. Both Ocl and macrophages are derived from the same progenitor hematopoietic stem cells and respond to many of the same signaling pathways. The role for vitamin D in immune response and the extra-renal activation of 25OH to 1,25 OH D as the primary modulator of vitamin D responses are controversial. Treatment with 1,25 OH D has been used to suppress several auto-immune disorders such as rheumatoid arthritis, type 1 diabetes, and multiple sclerosis (Deluca, 2014). Clearly more research is needed to establish the pivotal signals to link bone abnormalities and health challenges.

Conclusions

Clinical cases that involve lameness issues in pigs often lead to questions about the adequacy of nutrient formulations, especially dietary Ca and P, and more recently vitamin D. Attempts to correct the increased incidence of lameness by over-supplementation of nutrients most often does not resolve the problems and may contribute to further complications. Over-supplementation of nutrients, particularly Ca and P, does not necessarily allow pigs to recover from skeletal integrity challenges that may have been imposed by brief periods of deficiency. Accurate diagnosis of nutrient deficiencies require standardized sampling and analysis procedures. Use of DXA technologies offer accurate

and rapid turn-around for specimen analysis of BMC, but simply provide information on the ash content of the skeleton or individual bones.

A better understanding of the cellular signals that control endochondral ossification will lead to methods that are better predictors pf the alterations that induced the lesions. The hypovitaminosis D kyphotic pig model provides an opportunity to characterize the signals involved in the initial stages of lesions in the abnormal growth plates.

References

- Aiyangar, A. K. T. D. Crenshaw, A. G. Au, H. L. Ploeg. 2010. Recovery of bone strength in young pigs from an induced short-term dietary calcium deficit followed by a calcium replete diet. Medical Engineering & Physics 32:1116-1123.
- Arnold, J., D. M. Madson, S. M. Ensley, J. P. Goff, C. Sparks, G.W. Stevenson, T. D. Crenshaw, C. Wang, and R. L. Horst. 2014. Survey of vitamin D concentrations in swine serum across different stages of production and an evaluation of supplemental vitamin D stability in premixes used in swine diets. J. Swine Health Prod. (In review)
- Crenshaw, T. D., E. R. Peo, Jr., A. J. Lewis and B. D. Moser. 1981a. Bone strength as a parameter for assessing mineralization in swine: A critical review of techniques involved. J. Anim. Sci. 53:827-835.
- Crenshaw, T. D., E. R. Peo, Jr., A. J. Lewis, B.D. Moser and D. Olson. 1981b. Influence of age, sex, and calcium and phosphorus levels on the mechanical properties of various bones in swine. J. Anim. Sci. 52:1319-1329.
- Crenshaw, T. D. 1986. Reliability of dietary Ca and P levels and bone mineral content as predictors of bone mechanical properties at various time periods in growing swine. J. Nutr. 116:2155-2170.
- Crenshaw, T. D. 2001. Calcium, Phosphorus, Vitamin D, and Vitamin K in Swine Nutrition. In: Swine Nutrition. A. J. Lewis and L. L. Southern eds. CRC Press. p 187-212.
- Crenshaw, T. D., J. R. Danielson, L. E. Hoffman, and D. K. Schneider. 2009. Femurs are more accurate than fibulas as predictors of whole body bone mineral content in growing pigs. J. Anim Sci 87:(E-Suppl 2) 510.
- Crenshaw, T. D., L. A. Rortvedt, Z. Hassen. 2011. A novel pathway for vitamin D-mediated phosphate homeostasis: Implications for skeletal growth and mineralization. J Anim Sci 89(7):1957-1964.
- Crenshaw,T.D., D.K. Schneider, C.S. Carlson, J. B. Parker, J.P. Sonderman, T.L. Ward, M.E. Wilson. 2013. Mineral concentrations in tissues and lesions of osteochondrosis in bones collected from prolific sows across parities 0 through 7. J. Anim. Sci. 91:1255–1269.
- Crenshaw, T. D., J. L. Reichert, J. R. Booth, D. K. Schneider, and L. A. Rortvedt-Amundson. 2013. Clinical diagnosis of skeletal integrity in swine. Leman Swine Veterinary Conference Proceedings. September 17, 2013. St Paul MN.
- Crenshaw, T. D., L. A. Rortvedt-Amundson, J. A. Cuaron, J. R. Bergstrom, and G. Litta. 2014. TRIENNIAL GROWTH SYMPOSIUM: Vitamin D - Establishing the basics to dispel the hype. J. Anim. Sci. 92:883-886.
- Deluca, H. F. 2014. TRIENNIAL GROWTH SYMPOSIUM: Vitamin D: Bones and beyond. J. Anim. Sci. 92:917-929.
- Horton, W. A., and C. R. Degnin. 2009. FGFs in endochondral skeletal development. Trends in Endocrinology and Metabolism. 20(7): 341-348.
- Iwicki, L. A., J. L. Reichert, J. R. Booth, D. K. Schneider, and T. D. Crenshaw. 2011. Recovery of bone mineralization and strength after a marginal dietary calcium deficiency in growing pigs. J. Anim. Sci. 89 (e-Suppl. 1): 585.
- Lanske, B., M. J. Densmore, and R. G. Erben. 2014. Vitamin D endocrine system and osteocytes. BoneKEy Rpt 3:494. (doi:10.1038/bonekey.2013.228).
- Létourneau-Montminy, M.P., Lovatto, P.A. and Pomar, C. 2011. Effect of phosphorus and calcium depletion-repletion periods on the digestive and metabolic utilization of dietary phosphorus and calcium in growing pigs. J Anim. Sci. Vol. 89, E-Suppl. 3, 180.
- Lin, R., N. Amizuka, T. Sasaki, M. M. Aarts, H. Ozawa, D. Goltzman, J. E. Henderson and J. H. White. 2002. 1α,25-dihydroxyvitamin D-3 promotes vascularization of the chondroosseous junction by stimulating expression of vascular endothelial growth factor and matrix metalloproteinase 9. J Bone Miner Res. 17(9):1604-1612.
- Madson, D. M., S. M. Ensley, P. C. Gauger, K. J. Schwartz, G. W. Stevenson, V. L. Cooper, B. H. Janke, E. R. Burrough, J.P. Goff, and R. L. Horst. 2012. Rickets: case series and diagnostic review of hypovitaminosis D in swine. J. Vet. Diag. Invest. 24:1137-1144.
- Martin, A., S. Liu, V. David, H. Li, A. Karydis, J. Q. Feng, L. D. Quarles,. 2011. Bone proteins PHEX and DMP1 regulate fibroblastic growth factor FGF23 expression in osteocytes through a common pathway involving FGF receptor (FGFR) signaling. FASEB. 25:2551-2562.
- Masuyama, R., I. Stockmans, S. Torrekens, R. Van Looveren, C. Maes, P. Carmeliet, R. Bouillon and G. Carmeliet. 2006. Vitamin D receptor in chondrocytes promotes osteoclastogenesis and regulates FGF23 production in osteoblasts. J Clin Invest. 116(12):3150-3159.
- McCoy, A. M., F. Toth, N. I. Dolvik, S. Ekman, J. Ellermann, K. Olstad, B. Ytrehus and C. S. Carlson. 2013. Articular osteochondrosis: a comparison of naturally-occurring human and animal disease. Osteoarth Cartil.

21:1638-1647.
- Nielsen, L. W. D., P. Hogedal, J. Arnbjerg, and H. E. Jensen. 2005. Juvenile kyphosis in pigs - A spontaneous model of Scheuermann's kyphosis. APMIS. 113(10): 702-707.
- NRC. 1998. Nutrient requirements of swine. 10th revised edition. National Academy Press. Washington,DC.
- NRC. 2012. Nutrient requirements of swine. 11th revised edition. National Academy Press. Washington,DC.
- Ortega, N., D. J. Behonick and Z. Werb. 2004. Matrix remodeling during endochondral ossification. Trends Cell Biol. 14(2):86-93.
- Rortvedt, L. A., Z. Hassen, T. D. Crenshaw. 2011. Growth, DXA skeletal traits, and spinal curvature are compromised within four weeks in pigs fed diets with no supplemental vitamin D. J. Anim. Sci. 90(e-Suppl. 2):1.
- Rortvedt, L.A. and T.D. Crenshaw. 2012. Expression of kyphosis in young pigs is altered by vitamin D supplementation of maternal diets. J Anim Sci. 90:4905-4915.
- Rortvedt, L. A., D. K. Schneider, and T. D. Crenshaw. 2012. Bone ash and strength traits of young pigs fed diets with no supplemental vitamin D were compromised within a four-week trial. J. Anim. Sci. 90 (e-Suppl. 3):564.
- Rortvedt-Amundson, L. A. and T. D. Crenshaw. 2013. Pig bone trait responses to maternal vitamin D intake depend on nursery diet vitamin D and P concentrations. J Anim Sci. 91 (e-Suppl. 2):110.
- Rortvedt-Amundson, L. A. and T. D. Crenshaw. 2014. Maternal and nursery dietary vitamin D concentrations altered tissue mRNA expression. IPVS Congress *(accepted)*.
- Sitara, D., M. S. Razzaque, R. St-Arnaud, W. Huang, T. Taguchi, R. G. Erben, B. Lanske. 2006. Genetic ablation of vitamin D activation pathway reverses biochemical and skeletal anomalies in Fgf-23-null animals. Am J Pathol. 169:2161-2170.
- St-Arnaud, R. 2008. The direct role of vitamin D on bone homeostasis. Arch Biochem Biophys. 473(2):225-230.
- Stickens, D., D. J. Behonick, N. Ortega, B. Heyer, B. Hartenstein, Y. Yu, A. J. Fosang, M. Schorpp-Kistner, P. Angel and Z. Werb. 2004. Altered endochondral bone development in matrix metalloproteinase 13-deficient mice. Development. 131(23):5883-5895.
- Ytrehus B., Carlson C. S., Ekman S. 2007. Etiology and pathogenesis of osteochondrosis. Vet Pathol. 44:429-448.

Porcine T lymphocytes and their contribution to an effective immune response after vaccination

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T cells play a central role in the immune response after infection and vaccination. Two main groups of T cells can be discriminated: T cells with antigen-specific T-cell receptors (TcR) consisting of α and β chains which represent the "classical" T cells, and T cells with TcR γδ chains with less characterized immunological functions. In swine, TcR γδ T cells are a prominent and heterogeneous T-cell subpopulation that is characterized by IFN-γ and IL-17 production. $CD2^+$γδ T cells are mainly responsible for IFN-γ production, whereas IL-17 producers belong in their majority to the $CD2^-$ subset. Beside these functions after polyclonal activation the specific ligands recognized by the respective γδ TcR and the corresponding antigen-specific activation are currently unknown.

Within the TcR αβ T cells two main subsets are characterized: major histocompatibility class I (MHC-I) restricted $CD8^+$ T cells with mainly cytolytic effector functions and MHC-II restricted $CD4^+$ T cells. These $CD4^+$ T cells are involved in the fine tuning of immune responses of the innate as well as the adaptive immune system. After activation via MHC-II presented peptides, $CD4^+$ T cells differentiate into various effector cell populations to accomplish their diverse functions. As a consequence, the differentiated $CD4^+$ population represents a functionally heterogeneous population containing a panel of cell subsets with different effector functions: T_H1, T_H2, T_H17, and regulatory T cells (T_{regs}). In swine, knowledge about these functionally characterized subsets is still rudimental and a range of open questions exists. T_{regs}, identified by the expression of the transcription factor Foxp3, play a crucial role in the down-regulation and modulation of immune responses and in swine the majority of them are defined by a co-expression of CD4 and CD25. Especially $CD4^+$ T cells with high CD25 expression show cell-cell contact dependent regulatory activity. CD25 low positive $CD4^+$ cells seem to be potent producers of IL-10. For an efficacious vaccination these cells might have a negative impact and a stimulation of this subset has to be avoided. More important for a protective immune response are T_H1, T_H2 and T_H17 cells. In contrast to the naïve $CD4^+$ T-cell subpopulation, porcine differentiated $CD4^+$ T cells show the peculiarity of a CD8α expression. The $CD4^+CD8α^+$ T cell subpopulation contains activated as well as memory T-helper cells. $CD4^+CD8α^+$ T cells showing a production of IL-17 can be characterized as porcine T_H17 cells with the capacity for stimulation of neutrophils and are therefore involved in the immune response against bacterial antigens. IFN-γ and TNF-α producing T cells are also included in the heterogeneous $CD4^+CD8α^+$ subpopulation and represent porcine T_H1 cells. CD27 expression discriminates $CD4^+CD8α^+$ T cells into $CD27^+$ and $CD27^-$ cells with characteristics of central and effector memory T-helper cells, respectively.

The generation of $CD4^+$ memory T cells is a hallmark of efficacious vaccines. Consequently, these vaccine antigen-specific memory T cells should be detectable in functional assays. ELISpot analyses can be used to detect the frequency of vaccine-antigen specific IFN-γ, TNF-α or IL-17 producing cells and thereby enable a quantitative analysis of memory cells induced by vaccination. More detailed analyses can be performed by flow cytometry (FCM). FCM enables the identification of the phenotype of antigen-specific cytokine producing cells. Thereby, the involvement of $CD8^+$ cytolytic T cells or the detailed phenotype of the $CD4^+$ T cell population in regard to central or effector memory cells can be identified. Furthermore, FCM provides the possibility to detect multi-functional T cells producing two and more cytokines on the single cell level. The presence of multi-functional T cells may represent a correlate of a successful immune-stimulation. Whether these T cells will furthermore represent correlates of protection has to be elucidated for each vaccine and for each infectious disease.

Genetic basis of host response to PRRS virus infection in pigs

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Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) is the most costly viral disease in pigs around the world. Estimated annual costs in the US alone are $664 million (Holtkamp et al. 2013). Although much emphasis has been placed on development of preventative measures to control the spread and impact of PRRS, including vaccination and regional eradication efforts, PRRS continues to be a major problem in the industry (Darwich et al. 2010, Huang and Meng, 2010, Chand et al. 2012).

Over the past decades, genetic selection has been very effective at developing and improving lines of pigs that produce high-quality lean pork in an efficient manner. This has been accomplished by systematic selection on estimates of the 'breeding value' of potential parent stock based on extensive phenotype recording for growth rate, backfat, meat quality, and reproduction. In principle, these same selection methods can be applied to identify and select pigs that are more resistant to diseases, provided resistance to disease of a pig is at least partially determined by the genetics of the pig (i.e. is heritable) and phenotypes related to disease resistance or susceptibility can be collected on breeding stock.

Although early research showed breed differences in the effects of PRRS virus (PRRSV) infection (Halbur et al. 1998; Petry et al. 2005, 2007; Vincent et al. 2006, Doeschl-Wilson et al. 2009), collection of phenotypic data on the effects of PRRSV infection in breeding stock is problematic because of the need to maintain high health status in breeding herds. However, if genes or genetic markers linked to genes that are associated with resistance or susceptibility of pigs to the PRRSV infection can be identified, this can provide opportunities to select pigs based on marker-assisted selection or genomic selection. Opportunities to investigate the role of host genetics on disease resistance have expanded greatly in the past decade through the rapid developments that have taken place in genomics and in genomics technology. These developments now allow animals to be genotyped for thousands of genetic markers (single nucleotide polymorphisms or SNPs) across the genome, allowing SNPs that are associated with traits of interest to be identified by genome-wide association studies (Goddard and Hayes, 2009).

The purpose of this presentation is to describe ongoing efforts to investigate opportunities to use the genetics of the pig as another tool in the fight against PRRS. Using novel deep genotype and deep phenotype approaches that combine state of the art genomics with state of the art virology, the ultimate goal is to find genomic markers and other biomarkers that can be employed in the development of breeding programs to lessen the impact of PRRSV on the commercial pig industry. Although genetic selection will not offer a single 'magic bullet' solution, especially given the complexity and variability of the PRRS virus, host genetics can be an additional and complementary approach to fight the impact of PRRSV on pork production.

Nursery pig model for host response to PRRSV

Initial studies under the PRRS Host Genomics Consortium

To capitalize on advances in genomics technology to study the role of host genetics in PRRSV infection and develop tools to select pigs for improved resistance or reduced susceptibility, the PRRS Host Genetics Consortium (PHGC) was initiated in 2008 (Lunney et al. 2011; Rowland et al. 2012). The PHGC involves experimental challenge of groups of 200 commercial nursery pigs at experimental facilities at Kansas State University with a specific strain of the PRRSV. Piglets are followed for 42 days following infection, with frequent blood sampling and weighing, along with collection of tissue for DNA for genotyping. All pigs are genotyped with the Porcine SNP60 BeadChip (Illumina), which includes >60,000 single nucleotide polymorphisms (SNPs) across the genome. Serum samples are evaluated to quantify PRRSV viremia using PCR.

Analysis of the first 8 PHGC trials, including over 1,500 pigs infected with PRRSV strain NVSL-97-7895, found that viral load, quantified as area under the curve of logarithm (base 10) viremia from 0 to 21 days post infection (dpi), had substantial heritability (h^2=0.44) (Boddicker et al. 2012, 2013, 2014). Viremia after 21 days was characterized by ~1/3 of pigs showing viremia rebound, which likely reflects immune escape of the PRRSV. Genetics of the host was found to have little impact on whether or not a particular pig showed rebound, as the heritability of rebound was close to zero. Growth following infection was also shown to be moderately heritable (h^2=0.29) (Boddicker et al. 2012, 2013, 2014). Growth rate and viral load were negatively correlated but correlations were not strong: the phenotypic correlation was -0.29 and the estimate of the genetic correlation between growth rate and viral load was -0.46 (Boddicker et al. 2014). These results demonstrated that host response to experimental infection with the PRRSV has a sizeable genetic component.

To identify genetic markers and regions of the genome that are associated with viral load or growth rate following PRRSV infection, genotypes obtained by genotyping all pigs with the 60k SNP panel were evaluated in genome-wide association studies. Results identified a region on swine chromosome 4 (SSC4) of about ½ Mb that was associated with both viral load and growth rate following infection. This SSC4 region explained approximately 15% of the genetic variance for viral load and 11% of the genetic variance for growth rate, indicating the presence of a major gene associated with host response to PRRSV infection. The ½ Mb region was found to be in high linkage disequilibrium across all breeds and lines investigated, which means that it is a region with very little recombination. One SNP in the region, WUR10000125, was found to capture the full effects of the region on viral load and growth rate and this SNP (abbreviated WUR) was used in subsequent analyses. The WUR SNP was found to have significant effects in all trials, with individuals with genotype AB having 5% lower viral load and 14% higher weight gain than pigs with the AA genotype at this SNP. Although the frequency of BB pigs was low, the BB genotype had similar effects as the AB genotype, suggesting the B allele to be dominant over the A allele. The B allele was found to be present in all breeds and lines that contributed to the PHGC trials but at a low frequency (2 to 40%) (Boddicker et al. 2014).

Several other regions of the genome also showed associations with viral load and growth rate following infection (Boddicker et al. 2014). However, the effects of these regions were smaller than those identified for the SSC4 region. One region on SSC1 was found to be associated with mortality in one trial that showed signs of co-infection with other pathogens (Boddicker et al. 2014).

Experimental infection studies with a second PRRSV strain

A second generation of PHGC trials was conducted that involved experimental infection of commercial nursery pigs with another strain of the PRRSV. For these trials, pigs were infected with the more recent, KS-2006-72109 PRRSV strain, which is 89% identical to the NVSL 97 strain at the viral GP5 peptide sequence level. A total of five trials were completed with this virus. Results were very similar to those observed in the initial trials with the NVSL 97 strain, with similar estimates of heritabilities and correlations. The effect of the SSC4 region was also significant for this strain, indicating that the effects of this region may be present across multiple strains of the PRRSV. Estimates of the effect were, however, smaller than observed for the NVSL 97 strain, about 80% of the effect for viral load and about 36% of the effect on weight gain, which may reflect the differences in pathogenicity between these two virus strains (Hess et al. 2014).

Further analysis of the SSC4 region

The ½ Mb SSC4 region includes multiple candidate genes that have been shown to be associated with innate immune response, including members of the GBP family, CCBL2, GTF2B, and PKN2 (Boddicker et al. 2012). Because of the high linkage disequilibrium in the SSC4 region, fine mapping the causative mutation for the SSC4 effect by genetic mapping approaches is problematic. Instead, a functional genomics approach was used, involving the evaluation and analysis of the expression of genes in the region. For this purpose, blood samples at 5 time points from 8 pairs of littermates from one of the PHGC trials, with one piglet being AA and one piglet being AB for the WUR SNP, were subjected to RNA-seq analyses. Results identified one candidate gene in the SSC4 region that showed significantly higher expression in AB versus AA piglets across multiple time points during the trial (Eisley et al., 2013). In addition, allele-specific expression was identified for this gene in AB piglets, with the B allele being expressed more frequently than the A allele across multiple time points. Subsequent analyses of the sequence data identified a splice site variant in one of the introns of this gene, which results in premature stop codon in transcripts produced by the A allele, which are expected to result in production of an incomplete protein (Fritz-Waters et al., 2014). Further work to confirm this as the causative mutation for the SSC4 effect are underway.

Antibody response

Both total (viral N protein-specific IgG based on ELISA, expressed as sample-to-positive (SP) ratio) and neutralizing antibody (Ab) response to the PRRSV were evaluated on blood samples collected at the end of each trial (42 dpi) for all pigs in multiple trials (Trible et al., 2013). Total Ab was shown to be heritable (h^2=13%) but neutralizing Ab response was not. Genome-wide association studies identified the major histocompatibility complex (MHC) on SSC7 as having a major effect on total Ab, explaining 30% of the genetic variance (Hess et al., 2013). The WUR SNP of SSC4 was not found to be associated with antibody response, suggesting that its mode of action may not be through adaptive antibody-mediated immune responses.

Additional gene expression and immune response analyses

To further investigate the dynamics of host response to PRRRV infection and immunological and genetic pathways that are involved in this response, additional gene and protein expression analyses are underway. Initial work using microarrays showed a number of genes that were differentially expressed (Arceo et al., 2013). More detailed microarray and RNAseq analyses are continuing and focus on gene expression changes in blood over time and between pigs that show different responses in terms of viremia and weight gain (Choi et al., 2014). Serum cytokine and chemokines evaluations have highlighted the importance of early interferon-α levels. All PRRSV infected pigs have high 4 dpi serum interferon-α levels; however, pigs with higher viral loads continue to express interferon-α, whereas those with lower viral loads quickly return to pre-infection levels (Choi et al., 2013).

Co-infection trials

In practice, PRRS is often found to increase incidence of secondary infections, resulting in e.g. porcine respiratory disease complex (PRDC) and porcine circovirus associated disease (PCVAD). To investigate the genetic basis of host response to co-infection, several experimental trials in which nursery pigs are infected with both PRRSV and PCV are currently underway. To evaluate host response to PRRS vaccination, half of the pigs in these trials are first vaccinated with a modified live PRRSV.

Reproductive PRRS infection model

It is estimated that 45% of the total costs associated with PRRSV are attributed to reproductive disease (Holtkamp, 2013), yet proportionately little research has focused on reproductive PRRS compared to disease affecting pigs post-weaning. To investigate potential phenotypic and genotypic predictors of reproductive PRRS severity, a large scale experimental infection model was developed and conducted at the University of Saskatchewan (Harding et al., 2012). In this study, 114 pregnant gilts were experimentally infected with a type II PRRSV strain at 85 days of gestation, along with 19 sham-inoculated controls. Gilts were bled on day 0, 2, 6, and 19 post inoculation to evaluate viral load, changes in leukocyte subset counts, and cytokine responses. Gilts were euthanized at 21 days post inoculation (gestation day 106) to evaluate litter outcome and collect tissues from gilts and fetuses. Forty percent of fetuses were autolyzed or decomposed at termination. Genotyping using the porcine SNP60 BeadChip has been completed for all gilts, sires and over 850 viable and dead fetuses. Analysis of phenotypic and genotypic data is ongoing to elucidate mechanisms of fetal death and the phenotypic and genotypic traits associated with PRRS severity (Harding et al., 2013).

Field studies

While experimental infection trials provide great opportunities to investigate the genetic basis of host response under controlled conditions, these finding must be validated in the field. In addition, field studies allow additional effects to be observed and investigated. To this end, multiple field trials have been initiated.

Reproduction PRRS Outbreak Herd

Serum samples were collected on 641 pregnant sows in a sow herd that broke with PRRS. All sows were genotyped using the Porcine SNP60 BeadChip (Serao et al. 2013). The date of the outbreak was identified based on rolling averages of farrowing traits. The date of blood sampling was approximately 7 weeks after the outbreak. Blood samples were evaluated for PRRS viral N protein Ab by ELISA, expressed as SP ratio. Antibody response was found to be highly heritable (h^2=45%) and had strong genetic correlations with multiple reproductive traits after the outbreak, ranging from -0.72 for number of mummified piglets to +0.73 for number born alive. This suggests that antibody response following PRRSV infection (or vaccination) could be used as an indicator trait to select for reduced susceptibility to reproductive PRRS. A genome-wide association study identified two regions on SSC7 that explained large proportions of the genetic variance in SP ratio, including one region in the MHC, which explained 25% of the genetic variance. This region was similar to the region identified in the experimental challenge studies of nursery pigs.

Gilt acclimation study

To study the genetic basis of host response to PRRS and other diseases on the sow herd in a field setting, groups of 10 to 47 naïve commercial F1 replacement gilts were introduced in “health-challenged” herds. Gilts were directly introduced into the cooperating herds, using standard passive acclimation protocols, i.e. no direct challenge for any disease. Data on a total of 923 F1 gilts from 13 genetic sources that were introduced into 18 herds were used in a preliminary analysis. A total of 15 farms vaccinated gilts against PRRSV upon arrival, using a modified live virus vaccine. Individual weights and blood samples were collected on the day of introduction and after the acclimation period (~40 days). Blood samples were also collected at first parity weaning. Blood samples were analyzed for PRRS

by ELISA and expressed as SP ratio. Most gilts were negative on day 0. All gilts were genotyped using the Porcine SNP60 BeadChip.

All traits had low heritability, except PRRS antibody SP ratio after acclimation, which was heritable (h^2 = 0.44). Genome-wide association studies identified multiple regions on SSC7 associated with antibody SP ratio, including the MHC region. Effects of the SSC4 WUR SNP on antibody SP ratio and growth were not significant. Collection of additional health and reproduction data through third parity is currently underway.

Field studies in grow-finish pigs

Multiple field studies are currently underway to evaluate host response to disease in growing pigs in the field. Using the gilt acclimation concept, these studies involve introducing 200 clean nursery pigs into 'health-challenged' finishing barns. Pigs are repeatedly weighed and bled and followed all the way to market.

Conclusions

Host response of nursery pigs to PRRSV infection was found to have a sizeable genetic component under controlled experimental challenge studies. In particular, a major gene for host response to PRRS was identified on SSC4, with a strong candidate gene that is involved in innate immune response. The WUR SNP in this region could be used to select for pigs that are less affected by PRRSV infection in terms of growth rate. However, these effects need to be validated under more complex disease scenarios and in the field. Several such studies are currently under way. Studies on the genetic basis of host response to reproductive PRRS are also underway. While this comprehensive suite of studies is unlikely to identify pigs that are completely resistant to PRRSV, they are starting to unravel the genetic basis of host response to PRRS, leading to the ability to select pigs that are less susceptible to PRRSV infection and to the effects of PRRSV infection on performance. In addition, insight into host response to PRRSV infection can lead to new avenues for development of more effective vaccines and therapeutics.

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References

- Arceo M, Ernst CW, Lunney JK, Choi I, Raney NE, Huang T, Tuggle CK, Rowland RRR, Steibel JP. 2013. Characterizing differential individual response to Porcine Reproductive and Respiratory Syndrome Virus infection through statistical and functional analysis of gene expression. Frontiers in Livestock Genomics. 3:321.
- Boddicker N, Waide EH, Rowland, RR, Lunney JK, Garrick DJ, Reecy JM, and Dekkers JCM. 2012. Evidence for a major QTL associated with host response to PPRS virus challenge. J. Anim.Sci, 90:1733-1746
- Boddicker, NJ, A Bjorquist, RRR Rowland, JK Lunney, JM Reecy and JCM Dekkers. 2014. Genome-wide association and genomic prediction for host response to Porcine Reproductive and Respiratory Syndrome infection. Genetics Selection Evolution. 46:18.
- Boddicker N, Garrick, DJ, Rowland, RR, Lunney JK, Reecy JM, and Dekkers JCM. 2013. Validation and further characterization of a major quantitative trait locus associated with host response to experimental infection with porcine reproductive and respiratory syndrome virus. Animal Genetics. DOI: 10.1111/age.12079
- Chand RJ, Trible BR, Rowland RR. 2012. Pathogenesis of Porcine Reproductive and Respiratory Syndrome Virus. Curr. Opin. Virol. 2:256-63.
- Choi I, Bao H, Kommadath A, Sun X, Meng Y, , Guan LL, G.S. Plastow GS, Rowland RRR, Abrams SM, Lunney JK, Stothard P. 2014. Whole Blood Transcriptome Analyses of Swine Responses to PRRSV Infection. 2014 PAG. Abstract P603
- Choi I, Souza CJH, Araujo KPC, Abrams SM, . Kerrigan M, Lunney JK, Rowland RRR, Lunney JK. 2013. Comparative serum immune responses of pigs after a challenge with porcine reproductive and respiratory syndrome

virus (PRRSV). 2013 North American PRRS Symp. Abstract 29.

- Darwich, L., I. Diaz, and E. Mateu, 2010. Certainties, doubts and hypotheses in porcine reproductive and respiratory syndrome virus immunobiology. Virus Res. 154:123-132
- Doeschl-Wilson, A. B., Kyriazakis, I., Vincent, A., Rothschild, M. F., Thacker, E., and Galina-Pantoja, L. (2009). Clinical and pathological responses of pigs from two genetically diverse commercial lines to porcine reproductive and respiratory syndrome virus infection. J. Anim. Sci. 87, 1638–1647
- Eisley, C., E. Fritz-Waters, I. Choi, J.E. Koltes, N. Boddicker, J. Reecy, J.K. Lunney, S. Carpenter, C.K. Tuggle, P. Liu, J.C.M. Dekkers. 2013. Analysis of gene expression in a region associated with host response to porcine reproductive and respiratory syndrome virus challenge. 2013 PRRS Symposium, Dec. 2013.
- Fritz-Waters, E., J. Koltes, I. Choi, N. Serao, M. Schroyen, J.K. Lunney, J.C.M. Dekkers, C.K. Tuggle, S. Carpenter, J.M. Reecy, 2014. Identification of a Putative Quantitative Trait Nucleotide (QTN) for Host Response to Porcine Respiratory and Reproductive Syndrome (PRRS) Virus Infection. P599, Plant and Animal Genome XXII.
- Goddard, M.E. and Hayes, B.J. 2009. Mapping genes for complex traits in domestic animals and their use in breeding programmes. Nat. Rev. Genet. 10: 381–391.
- Halbur, P. G., M. F. Rothschild, B. J. Thacker, X.-J. Meng, P. S. Paul, and J. D. Bruna. 1998. Differences in susceptibility of Duroc, Hampshire, and Meishan pigs to infection with a high virulence strain (VR2385) of porcine reproductive and respiratory syndrome virus (PRRSV). J. Anim. Breed. Genet. 115:181–189.
- Harding, J., Ashley, C., Ladinig, A., Clarke, K., Ye, Z., Chang, T., Detmer, S., Novakovic, P., Wilson, D., Walker, S., Napper, S., Wilkinson, J., Yates, R., McKenna, N., Lunney, J., Dekkers, J., Rowland, R., Kemp, R., Plastow, G. 2012. Phenotypic variability in response to the PRRS virus in the reproductive model: a potential opportunity. Proc. 2012 Int. PRRS Symposium Pg 24.
- Harding, J., Ladinig, A., Ashley, C., Lunney, J., Plastow, G. 2013. Predictors of fetal survival following PRRSV challenge. 2013 North American PRRS Symposium. Pg 23.
- Hess, A., N. Boddicker, R.R.R. Rowland, J.K. Lunney and J.C.M. Dekkers. 2014. Validation of the Effects of a SNP on SSC4 Associated with Viral Load and Weight Gain in Piglets Experimentally Infected with PRRS virus. Midwest ASAS meetings
- Hess, A., B. Trible, N. Boddicker, R. Rowland, J. Lunney, S. Carpenter, J. C. M. Dekkers. Factors associated with neutralizing antibody response in piglets after experimental infection with Porcine Reproductive and Respiratory Syndrome Virus. Midwest ASAS Meetings, Abstract, March 2013.
- Holtkamp, D.J., J.B. Kliebenstein, E.J. Neumann, J.J. Zimmerman, H. Rotto, T.K. Yoder, C. Wang, P. Yeske, C. Mowrer, and C. Haley. 2013. Assessment of the economic impact of porcine reproductive and respiratory syndrome virus on United States pork producers. J. Swine Health Prod. 21:72-84.
- Huang YW, Meng XJ. 2010. Novel strategies and approaches to develop the next generation of vaccines against porcine reproductive and respiratory syndrome virus (PRRSV). Virus Res. 154:141-149.
- Lunney JK, Steibel JP, Reecy JM, Fritz E, Rothschild MF, Kerrigan M, Trible B, Rowland RRR: Probing genetic control of swine responses to PRRSV infection: Current progress of the PRRS Host Genetics Consortium. BMC Proc 2011, 5:S30.
- Petry, D. B., Holl, J. W., Weber, J. S., Doster, A. R., Osorio, F. A., and Johnson, R. K. (2005). Biological responses to porcine respiratory and reproductive syndrome virus in pigs of two genetic populations. J. Anim. Sci. 83, 1494–1502.
- Petry, D. B., Lunney, J., Boyd, P., Kuhar, D., Blankenship, E., and Johnson, R. K. (2007). Differential immunity in pigs with high and low responses to porcine reproductive and respiratory syndrome virus infection. J. Anim. Sci. 85, 2075–2092.
- Rowland R, Lunney JK, Dekkers JCM. 2012. Control of porcine reproductive and respiratory syndrome (PRRS) through genetic improvements in disease resistance and tolerance. Frontiers in Livestock Genomics. http://www.frontiersin.org/Livestock_Genomics/10.3389/fgene.2012.00260.
- Serão, N.V.L., O. Matika, R.A. Kemp, J. Harding, S.C. Bishop, G.S. Plastow, J.C.M. Dekkers (2013) Genetic analysis of reproductive traits and antibody response in a commercial sow herd before and after a PRRS outbreak. 2013 NA PRRS Symposium Proceedings, p 66.
- Vincent, A. L., Thacker, B. J., Halbur, P. G., Rothschild, M. F., and Thacker, E. L. (2006). An investigation of susceptibility to porcine reproductive and respiratory syndrome virus between two genetically diverse commercial lines of pigs. J. Anim. Sci. 84, 49–57. - See more at: http://journal.frontiersin.org/Journal/10.3389/fgene.2012.00260/full#sthash.G6aVXk3w.dpuf
- Trible, B.R., Popescu, L.N., Wang, Y., Kerrigan, M.A., Rowland, R.R.R. Characterizing the antibody response following experimental PRRSV infection in a large population of pigs. November 29-30, 2012. International PRRS symposium, Kansas City MO.

O.001

Genetic diversity of *M. hyorhinis* field isolates from the United States

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Introduction

Since late 2008 *Mycoplasma hyorhinis* associated disease has been one of the main concerns of the U.S pork industry.[1] It appears that differences in virulence of the infecting *M. hyorhinis* strain, the host immune response, and concomitant infections play a role on disease manifestation.[2] While detection of the bacterium in polyserositis and arthritis cases can be easily achieved through bacterial isolation and PCR, there are currently no genotyping tools available for the characterization of *M. hyorhinis* isolates circulating in swine populations. The objective of this study was to develop a multi-locus sequence typing (MLST) protocol for the characterization of *M. hyorhinis* field isolates.

Materials and Methods

Thirty-eight *M. hyorhinis* isolates obtained in 2010-2011 from diseased pigs in the United States, in addition to one ATCC strain were utilized in this study. These isolates originated from 11 states, 18 systems, three pig stages and seven different lesion sites within the pigs. Isolates were cultured for 7-14 days followed by DNA extraction. The classical MLST scheme was modified to target not only housekeeping genes (slowly accumulate nucleotide changes) but also hyper-variable genes (accumulate changes over a short period of time). This modified scheme would potentially allow for an increased discriminatory power, making this assay more useful for outbreak investigations.[3] After selection of the target genes, primers were designed and PCR amplification was carried out. PCR products were bidirectionally sequenced by standard Sanger sequencing. Sequences were aligned utilizing ClustalW and trimmed to equal sizes. Phylogenetic analysis mas carried out using MEGA 5.2.1 and Bionumerics V7.1.

Results

More than 25 genes where evaluated as potential gene targets with varying degrees of success. The final MLST protocol included the following genes: *ung, pdhB, mtlD, p3, p95.* Variation at the nucleotide level within each gene ranged from 0.5-20%. The number of alleles per gene varied from 3-11, giving rise to 27 sequence types (STs) within the 39 isolates. The dendrogram revealed genetic variation among the examined isolates, with the greatest similarities belonging from isolates of the same owner/system. Two major lineages were observed: A and B, where lineage B had the majority of the isolates examined and all of the ones from Minnesota. The location of isolation within the pig did not correlate with the dendrogram, however, in lineage A the majority of isolates where cultured from pleura. Isolates belonging to the same system clustered together, and in some instances isolates from different geographic location, but same system where identical. In contrast, three isolates from different systems, states and lesion type had a 100% sequence similarity. Both of these cases suggest a common source of pigs (Figure 1).

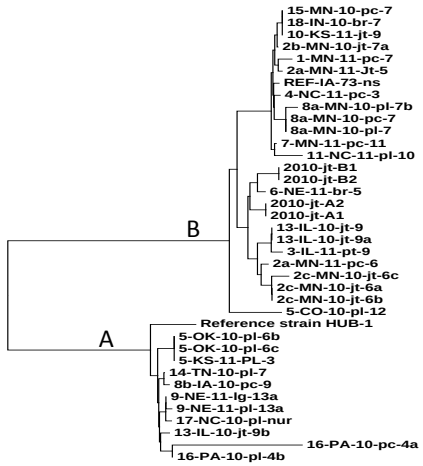

Figure 1. Inferred relationships between 39 *M. hyorhinis* isolates using concatenated sequences for all 5 genes. Identification of each isolate is based on a key (owner number-state-year-lesion-age)

Conclusions and Discussion

This tool will allow to further study the epidemiology and dynamics of infection for this pathogen. Furthermore, this tool will be extremely useful for veterinarians and producers to understand disease outbreaks, to select isolates for vaccine production, and to perform epidemiological studies on the potential origin of a specific isolate. Therefore, the U.S. swine industry will be better positioned to control a pathogen that is responsible for an important part of the mortality observed in the nursery.

Acknowledgments

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References

1. Leuwerke B. (2009) Proc of the Allen Leman Swine Conference. Saint Paul, MN. 36:89-90
2. Gois M. & Kuksa F. 1974. *Zentralbl Veterinarmed B.* 21(5):352-61.
3. Cooper J. 2004. Trends Microb 12(8) 373-377

O.002

M. hyopneumoniae variability within a swine production system

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Introduction

Enzootic pneumonia, caused by *Mycoplasma hyopneumoniae,* is of great economic concern in the swine industry worldwide.[1] In spite of improvements in vaccination strategies and antimicrobial treatment, control continues to be challenging. Genetic variability for *M. hyopneumoniae* has been reported.[2] Historically, we have seen great variation in clinical signs and treatment response within *M. hyopneumoniae*-endemic systems. It was hypothesized that *M. hyopneumoniae* genetic variability exists within the production system. The objective of this study was to characterize the diversity of the *M. hyopneumoniae* variants within a production system.

Materials and Methods

Three nursery-to-finish sites within a PRRS negative system with endemic mycoplasmal infection were chosen for sample collection. The nursery pigs originated from 8 sow farms which received all breeding stock from one multiplier; semen was received from one in-system boar stud. Eight rooms were sourced from a single sow farm; all other rooms comingled pigs from 2 to 4 sources. Tracheal aspirates and bronchial swabs were obtained from 130 four to five month old pigs within 22 total rooms. Bronchial swabs were collected from on-site mortalities, regardless of cause of death. Tracheal aspirates were collected from pigs exhibiting a dry cough via oropharyngeal tube placed into the trachea with negative pressure applied. Samples were quantified at the UMN VDL using a species specific RT-PCR, and then typed using a modified MLVA typing assay.[2] The MLVA typing is based on the number of VNTRs of two loci (P97 and P146) of *M. hyopneumoniae*, as described by Dos Santos et al in these proceedings. MLVA results were further processed using Bionumerics®, and a dendrogram was created to understand the relationships of *M. hyopneumoniae* within the production system.

Results

Ninety five samples were analyzed. Four unique *M. hyopneumoniae* variants were identified (9-15, 11-21, 9-21 and 7-15) [Figure 1]; variant designations indicate number of repeats at each of the two loci, namely P97 and P146. Variant 9-15 was most prevalent, being present in 61 pigs in 79% of rooms testing positive to *M. hyopneumoniae* by standard RT-PCR, and was identified on all 3 sites. Variant 11-21 was present in 26 pigs in 37% of the rooms on Sites 1 and 2; and variant 7-15 was present in 7 pigs in 2 rooms (11%). Variant 7-15 accounted for all the isolates identified in 1 room, as well as one isolate in another room also within Site1. A single isolate of variant 9-21 was identified in one room on Site 2; all other samples from that room were variant 11-21.

Conclusion and Discussion

The four variants identified within this study suggest limited variation; this may be due to the small number of sites sampled and samples collected. No pattern could be constructed tying variant to sow source. Due to the similarity in MLVA loci repeats as seen in Figure1, it is possible that variant 7-15 is an on-site mutation of variant 9-15. We can similarly hypothesize that the single variant 9-21 sample may have resulted from in-situ recombination of variants 9-15 and 11-21. Analysis of clinical signs and morbidity/mortality would be necessary to determine if increased or decreased disease is associated with the observed variants. This is the first time that genetic variation has been identified and reported using MLVA in a US production system; the observed genetic variation is similar to what has been reported in European farms.[2]

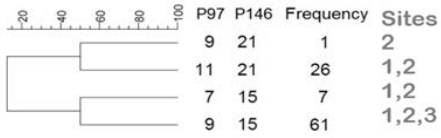

Figure 1. Frequency and relationships of variants 9-21, 11-21, 7-15, and 9-15 in all three study sites.

References

1. Maes D., et. al. 2008. Vet Microbiol, 126 (4):297-309.
2. Vranckx K., et. al. 2011. J Clin Microbiol, 49 (5): 2020-2023.

O.003

Compartmental mathematical model of the within-herd transmission of *M. hyopneumoniae* in a pig herd

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Introduction

Mycoplasma hyopneumoniae is the causative agent of porcine enzootic pneumonia (EP), which affects the industrialized pig production worldwide. In endemic situations, the within-herd transmission is maintained by vertical route from sows to their offspring or by horizontal route mainly between pigs in the same compartment. The objectives of the present study were to develop a mathematical model to estimate the within herd transmission of *M. hyopneumoniae* and to assess the impact of different risk factors.

Materials and Methods

A time-discrete, stochastic compartmental model was developed considering five compartments: "susceptible", "exposed", "acute infectious", "chronic infectious" and "recovered" (Figure 1). Transition parameters were used from the literature [1-5], from own datasets of former studies, and – if no other sources were available - from an expert poll. The model was coded in R software and was run for a 500 sow herd operating in a batch-farrowing system. For each scenario, 1,000 iterations were used to obtain a converged outcome. In the final model, the impact of five different risk factors was evaluated: "duration of suckling period" (Suc), "gilt acclimatization" (Acc), "vaccination against *M. hyopneumoniae*" (Vac), "contact between fattening pigs of different age" (Con) and "presence of co-infections" (Inf). A disease severity index, defined as the proportion of infectious pig-days ($I_a + I_c$), was used to compare the impact of different combinations of risk factors.

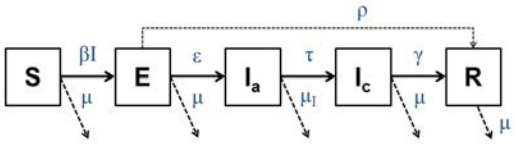

Figure 1. Conceptual design of the compartmental mathematical model considering the transmission of *M. hyopneumoniae* within pig herds

Results

Overall 18 different combinations of risk factors were used. The 'disease severity index' was lowest when gilts were in contact with living pigs during their acclimatization, piglets suckled for 21 days and were vaccinated against *M. hyopneumoniae*, and fattening pigs did not have any contact with other age groups during (re-)stocking of compartments and did not suffer from co-infections. Under these conditions, pigs became acutely or chronically infectious for 0.3% of their lifetime, on average. The disease severity index was highest when gilts did not have any contact with living animals during their acclimatization, suckling pigs got weaned unvaccinated after 28 days, fattening pigs had contact with pigs of other age and were suffering from co-infections. Under these conditions, pigs became acutely or chronically infectious for 26.1% of their lifetime. The impact of the particular risk factors depends on the corresponding age of the pigs (Figure 2)

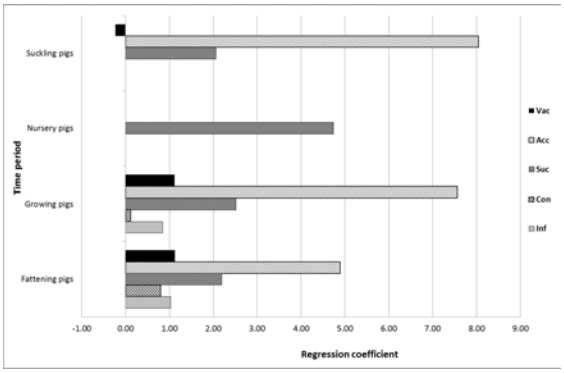

Figure 2. Regression coefficients describing the impact of different risk factors on the transmission of an infection in a compartmental mathematical model of the within-herd transmission of *M. hyopneumoniae*

Conclusions and Discussion

The outcome of the model informs the process of setting up prevention programmes and strategies of controlling EP. The identified intervention measures facilitate the reduction of clinical disease and, thus, can be utilized for a considerable increase of the animals' health and welfare as well as a considerable reduction of the use of antimicrobial drugs.

Acknowledgments

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References

1. Sibila M. et al. (2007) Vet Microbiol. 122:97-107.
2. Gatzka E.M. et al. (2011) Alfter-Imp. Germany
3. Villarreal I. et al. (2011) Vet J. 188:48-52.
4. Sorensen V. et al. (1997) Vet Microbiol. 54:23-34.
5. Kobisch M. et al. (1993) Vet Res 24:67-77.

O.004

Risk factors for *M. hyopneumoniae* piglet colonization during the lactation period

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Introduction

Sow-to-piglet transmission is considered one of the most important aspects of the epidemiology of *Mycoplasma hyopneumoniae*. The degree of piglet colonization at weaning age has been suggested as a predictor of clinical disease at market age[1]. However, sow-to-piglet colonization widely varies among farms and weaning groups, and even within the same farm[2-3]. Therefore, the objective of this study was to identify risk factors associated with *M. hyopneumoniae* piglet colonization during the lactation period.

Materials and Methods

Three sow farms, belonging to three different productions systems (A, B, C) were visited and samples were collected in at least 5 weaning groups at each farm. The following samples were collected at every sampling event: 54 dam nasal swabs, paired piglet nasal swabs and sera. Sampled dams were randomly selected, while stratified by parity. One piglet per dam was randomly selected for sampling. A series of pig specific data were collected from each sampled group, along with climatic data collected on-site during the lactation period. Swabs were analyzed by real-time PCR (Life Technologies) at the UMN-VDL. Sera were tested by ELISA (IDEXX) for antibody detection. A generalized linear mixed model was fitted to test for the association of 9 factors on the risk of piglet PCR positivity at weaning age.

Results

All PCR results obtained from Farm A and C were negative. Positive PCR results were obtained in piglets and/or dams in 5/7 farm visits in Farm B. *M. hyopneumoniae* prevalence at weaning ranged 0-56%, with negative groups detected in between sampling periods with positive results. Dam PCR positive status and piglet weaning age were significantly associated (p<0.001) with piglet *M. hyopneumoniae* colonization at weaning age. A relationship between dam PCR status and piglet age was observed, and is presented in Fig. 1.

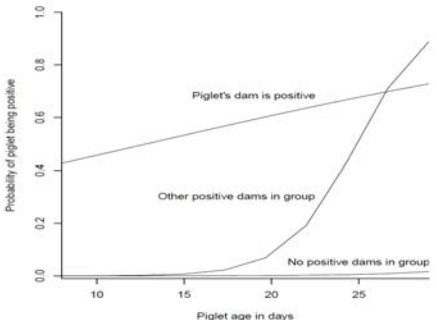

Figure 1. Piglet probability of *M. hyopneumoniae* colonization as a result of dam PCR status and piglet age.

Conclusions and Discussion

Identifying risk factors for colonization at weaning age is of crucial importance to better design control strategies for *M. hyopneumoniae* infections. Under the conditions of this study, shedding of the dam during lactation significantly influenced the probability of piglet colonization at weaning, and this probability increased with piglet age in groups where at least one dam was positive. Our results highlight the influence of the sow in the sow to piglet colonization process, as previously reported[2-3]. Our results contribute to a more comprehensive understanding of the epidemiology of *M. hyopneumoniae* infections.

Acknowledgments

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References

1. Fano et al. 2007. Can J of Vet Res. 71:195-200
2. Villarreal et al. 2010. Veterinari Medicina. 55(7) 318-24
   Nathues et al. 2013. Acta Vet Scandinavica. 55:30

O.005

Determining the ideal sample type/location from wean age pigs to determine sow herd stability and utilizing this information to make inter- and intra-farm comparisons of *M. hyopneumoniae* stability status of six sow herds

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Introduction

Vertical transmission of *Mycoplasma hyopneumoniae* (M. hyo) and epidemiology of M.hyo are not well understood. Previous work has indicated that M. hyo colonization at weaning can negatively impact downstream health.[1] Nasal swabs have traditionally been the sampling location chosen to detect M. hyo in newly weaned pigs. The objective of this study was to assess four sampling types/locations to determine the most sensitive one to detect M. hyo in due-to-wean pigs from six sow herd populations with confirmed clinical Mycoplasma disease downstream.

Materials and Methods

Six sow herds in the Midwest of the U.S.A. were selected based on confirmed clinical cases of M.hyo downstream. Three sampling points for each sow herd were completed, 1-7 weeks apart from one another. Thirty, due-to-wean, poor doing pigs, were humanely euthanized by CO2 at each sampling period (n_{total}=539). Flocked swabs, nasal (NS), oral-pharyngeal (OP), and bronchial swabs (BS) were collected from each pig. Lung homogenate (LH) was made at Iowa State University (Ames, IA, USA). All samples (n=2,154) were tested for M. hyo by qPCR (HMC, Ames, IA). The number of positive samples by sample type was analyzed first using Pearson's chi-square then Pair-wise comparisons made using Fisher's Exact Test. Average cycle time (CT) values by sample type were compared using one-way ANOVA. Means were compared using Tukey-Kramer HSD. Differences were considered significant at P<0.05.

Results

Positive samples were found in 5/6 herds, 11/18 sampling periods and 78/539 pigs. Table 1 shows percent positive and mean Ct for each sample type along with distribution of sample type amongst only the positive pigs. Bronchial sampling (BS) resulted in significantly (P<0.05) more positive samples compared to NS and LH samples. OP swabs were intermediate to BS and LH samples while LH samples were intermediate to OP and NS. Ct levels for BS were significantly (P<0.05) lower than OP, NS and LH.

Table 1. Percent positives and mean Ct value

	Sample Type			
	NS	OP	BS	LH
# pos pigs*	34/539	56/538	75/539	43/538
% of all	6.31[a]	10.41[bc]	13.91[c]	7.99[ab]
Mean Ct	32.17[a]	30.96[a]	28.27[b]	31.22[a]
% of pos pigs[¥].	43.59	71.79	96.15	55.13
# pos sampling periods	9/18	10/18	11/18	9/18
# herds pos**	5/6	5/6	5/6	5/6

[abc] Means differ (p≤0.05);. *≥1 sample positive/pig; [¥] Total of 78 positive pigs**≥ 1/3 sampling period positive/farm

Conclusions and Discussion

Utilizing the most sensitive sampling location is an important step to accurately assess sow herd stability for M. hyo. In this study, all four methods identified 5 of the 6 herds as M. hyo positive and unstable (M. hyo circulation in the farrowing crate). However, BS and/or OP detected the greatest number of positive pigs. Due to this, BS and OP appear to be the most desirable locations to sample this age of pig to determine sow herd stability for M. hyo. Bronchial swabs also detected the greatest amount of M hyo. The Ct difference between 30.96 (OP) and 28.27(BS) is approximately 1log level less; OP only 10% of BS levels. This may explain the ability of BS to detect M. hyo more often than other sample types. Additionally, if sequencing is to be performed, BS is likely to have the most successful sequencing due to the lower Ct levels. Further work into understanding the circulation of M. hyo in the farrowing house is needed and underway.

References

1. Fano, E., CJVR, 2007;71:195–200.

O.006

Variable *M. hyopneumoniae* exposure at the barn level during a field investigation

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Introduction

Developing a meaningful understanding of the dynamics of respiratory disease in finishing pigs can be a complex undertaking. Consideration should be given to a number of potential factors including respiratory pathogens (swine influenza virus – SIV, *Mycoplasma hyopneumoniae* – Mhp, porcine respiratory and reproductive syndrome virus – PRRSv, porcine circovirus type 2 – PCV2 and various other bacteria), exposure to other diseases (often enteric), source of exposure (vertical transmission from sow herd, exposure to older infected pigs or lateral introduction from an outside source), timing of exposure, relationship to exposure with other pathogens, routine procedures (pig movements, stocking rates), environment (air quality and temperature), diet and water availability and quality, and general husbandry. In the investigation reported here, our objectives were to define the pattern of exposure to respiratory pathogens, especially Mhp, at this particular finishing site and to develop a standardized protocol for evaluating other finishing sites in this production system.

Materials and Methods

The investigation was done in a finishing site that received 10 week-old pigs from one sow herd source and nursery site. The pigs were vaccinated with Circumvent® PCV M (Merck Animal Health, Summit, NJ) prior to entering the finishing site. The site contained 20 barns; 12 barns at one location and 8 barns at another location 1 km apart. The two locations were managed as one site with regard to caretakers, equipment, traffic, etc. One barn was filled each week and barns were managed all-in, all-out. Two surveys were conducted. A cross-sectional survey was done at the beginning. Pigs at 10, 14, 18 and 22 weeks of age (WOA) were sampled. For the longitudinal survey, 25 pigs in the 10 WOA barn were ear tagged and sampled again at 14, 18 and 23 weeks of age. At each sampling, blood and a deep oral swab were obtained from each pig. All laboratory testing was done at the Iowa State University Veterinary Diagnostic Laboratory. PCR was performed on the swabs for SIV and Mhp and on serum for PRRSv (North American – NA and European – EU). The Tween 20 Mhp ELISA was performed on serum. Three slaughter checks were performed on pigs that were the last to be sold from their barn; two at 1 and 2 months after the initial sampling, which represented the 22 and 18 WOA pigs in the cross-sectional survey. The third slaughter check was done on pigs from the longitudinal survey barn.

Results

The findings of the initial cross-sectional survey are presented in Table 1. The pigs were PCR positive for SIV and NA-PRRSv at 10 WOA, EU-PRRSv at 18 WOA and Mhp at 18 WOA. Mhp serology exposure demonstrated vaccine induced antibodies at 10 WOA, declining titers at 14 WOA and then increasing titers at 18 WOA, which indicates exposure before 18 WOA. Slaughter checks found a relatively low level of pneumonia; 1.00% average lung lesions for the 22 WOA group (n=55) and 2.06% on the 18 WOA group (n=53).
The findings of the longitudinal survey are presented in Table 2. The pattern of PCR results for SIV, NA-PRRSv and EU-PRRSv were the same as the cross-sectional survey. However, Mhp PCRs were negative and Mhp serology indicated that the pigs were not exposed to Mhp. The slaughter check found little if any pneumonia. Of 46 lungs evaluated, 42 were negative, 2 had 1% pneumonia lesions and 2 had 2% lesions.

Table 1. Cross-sectional survey

Age	PCR - Pools of 5				Mhp Elisa	
	Oral Swabs		Serum - PRRS			
(wks)	SIV	Mhp	NA	EU	Pos.	ODV
10	4/4	0/4	4/4	0/4	17/20	0.575
14	0/4	0/4	0/4	2/4	11/20	0.306
18	0/4	3/4	0/4	0/4	14/20	0.604
22	0/4	0/4	0/4	0/4	19/19	0.901

Table 2. Longitudinal survey

Age	PCR - Pools of 5				Mhp Elisa	
	Oral Swabs		Serum - PRRS			
(wks)	SIV	Mhp	NA	EU	Pos.	ODV
10	4/4	0/4	4/4	0/4	17/20	0.575
14	0/4	0/4	1/4	3/4	4/20	0.171
18	0/4	0/4	0/4	1/4	0/19	0.031
23	0/4	0/4	0/4	0/4	0/23	0.107

Conclusions and Discussion

The Mhp findings from the longitudinal survey were unexpected and demonstrate that the pattern of respiratory pathogen exposure may not always be consistent. This finding has implications for conducting field studies that rely on natural exposure; some method is needed to confirm exposure and timing. Fortunately in the case of Mhp, serology can be used as an indicator of exposure, especially in previously vaccinated animals.

O.007

Assessment of prevalence and severity alongside diagnostic survey to understand impact of therapy to control *M. hyosynoviae* instigated lesions in an endemic herd

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Introduction

Mycoplasma hyosynoviae is one of several agents capable of instigating arthritis in growing swine. Noted as inflammation of the intra-articular tissue of one of more joints accompanied by an increased volume of intra-articular fluid along with other descriptors such as serous, fibrinous, purulent, macrophagic, lymphoplasmacytic, etc[1]. An increase in *M. hyosynoviae* instigated diagnosis has been noted by Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) in the Midwestern United States. From 2003 to 2010 *Mycoplasma* species accounted for 17% of cases at ISU-VDL while in 2010 Mycoplasma species accounted for up to 37% of cases[1]. While the majority of pigs afflicted with infectious arthritis may make it to commercial markets, the concern lies in the welfare of afflicted pigs as well as potential impact on profitability[1]. If a swine operation is involved in the business of raising and developing replacement females, the concern is threefold: 1) Reduction in percent sold from the gilt herd 2) Potential contagiousness of the disease to the sow farm 3) Effect of the infectious arthritis on the females' longevity in the sow herd. In 2013, Probst Miller, Ramirez, and Knoernschild completed a study of an endemically infected herd and the impact of medication on the prevalence and severity of *M. hyosynoviae* instigated lameness. Investigators have over 15 months of monthly quantitative assessments of prevalence and severity across time alongside a diagnostic monitoring plan and intervention. The herd is also positive for *M. hyorhinis* and serology and PCR (rope tests and tonsilar tests) have been done to measure prevalence and severity of both *M. hyosynoviae* and *M. hyorhinis* in this herd pre and post therapeutic intervention.

Materials and Methods

Investigators monitored the prevalence rate and severity of illness by sampling 4 gilts from each pen in 1200 head barns. Four pens per barn were sampled per month over a 3 month period. Sample gilts were tagged and their level of lameness was given a severity ranking of *A (acute), B (subacute), C (chronic),* or *E (euthanize or cull)*. "*A*" pigs showed aesthetic swelling but would likely sell as sound due full mobility. "*B*" pigs also showed swelling on limbs but it was more advanced. These hogs if sound might sell and if not fully weight bearing on all limbs would not sell as selects. "*C*" pigs would for sure end up a market hog vs. a select due to level of swelling and clinical lameness. "*E*" pigs were severely lame hogs that would either go on the cull truck or be euthanized. To further assess presence and intensity of *M. hyosynoviae* and *M. hyorhinis* in the population, the following sampling methodology was completed:

1. Every month for 3 months rope sampling was done in the selected pens.
2. Every month for 3 months serum sampling and tonsilar swabbing was done on the tagged gilts.
3. Fifteen months of prevalence and severity assessments of subpopulations in barns were done per month before, during and after diagnostic tests.

Results

Rope test pcr showed that pathogen prevalence followed rates similar to subjective quantitative assessments of clinical signs. Concurrently, tonsilar swabs of individual animals demonstrated that antibiotic treatment over time reduced the PCR detection of *M. hyosynoviae* in the herd. Our quantitative assessment of clinical signs showed that we were not able to reduce clinical prevalence; but importantly, we were able to reduce severity of symptoms with antibiotic therapy and subsequently increase select gilts sold from 50% to 73.5%.

Conclusions and Discussion

M. hyosynoviae is hard to diagnose and prove correlation with lameness in certain circumstances. In the case of this study, submission of live animals for testing yielded positive results. Coordinated use of novel ELISA tests, rope testing, and tonsilar PCR showed promise as future diagnostic tools for the industry as they allow to track pathogen presence and impact of treatment. Regular quantitative assessment of prevalence and severity of symptoms is essential to understanding impact of intervention.

Acknowledgments

Iowa State for diagnostics; Zoetis for diagnostic financial support

References

1. Gomes Neto JC, Gauger PC, Strait EL, et al. Prod.2012;20(2):82–86.

O.008

Relationship between pig performance and maximum *M. hyopneumoniae*-like lung lesions at slaughterhouse

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Introduction

Mycoplasma hyopneumoniae (M.hyo) is the primary pathogen of swine enzootic pneumonia (SEP), a chronic respiratory disease and one of the most widespread and costly diseases in intensive pig production systems (1,2). Nowadays, there is a lot of information on comparison of methods to evaluate the prevalence and extent of pneumonia lesions in lungs and its correlation as a reliable screening tests (5,6), moreover, another study (4) carried out by Pallares et al., in the south east of Spain, showed as well a strong relationship of macroscopic evaluation of the lungs with histopathological and inmunocytochemical diagnosis ($p<0.001$). The main objective of this study was to establish the impact of M.hyo like maximum lung lesions due to *Mycoplasma hyopneumoniae* on pig performance and its economic impact on the production system.

Materials and Methods

A total of 48.778 pigs were included on this study, all of them Non-Vaccinated, coming from the north-east of Spain, all farms were tested positive to M.hyo. The period of the study was from April 24th of 2012 to August 2nd of 2013, lungs of fattened pigs were examined in the slaughterhouse and the severity of the lung lesions was evaluated using the 0 to 5 method as described previously (3), and all of them were scored by the same person, a highly trained veterinary to avoid differences. The experimental unit on this study is the fattening site. The production parameters evaluated on this study were: ADG (Average Daily Gain), EFCR (Economical Feed Conversion Ratio), % Mortality and medication cost in €. Data were statistically analyzed using the JMP® statistical software version 9.0.3 (SAS Institute Inc., Cary, NC, USA).

Results

The prevalence of maximum lung lesions (lungs with scores of 3, 4 or 5) varies, depending on the batch, from 0% to 65.7%, with an overall prevalence of 15.33%. For each experimental unit, an average of all the production parameters was calculated, as shown in table 1. Batches were classified in 4 different interval groups by quartiles: 0-5%, 5-12.5%, 12.5-25% and >25% of maximum lung lesions score. A different study (7) has shown as well the effect of maximum lung lesions due to M.hyo on ADG, in this particular study between the highest prevalence and the lowest, the difference was 38 grams on ADG, even though the lowest prevalence group was statistically different with all of them. In terms of EFCR between the groups from 0 to 5% and the >25% the difference was 70 grams. On the other two parameters (% of mortality and medication cost), statistically significant differences have been shown between the groups from 0 to 5% of maximum lung lesions score and the rest of them, ranges from 0.78 – 1.21% on mortality and 0.52 – 0.79 € on medication cost; Bak (8) on his study estimated the cost of M.hyo on 2.7€ per pig on infected herds.

Table 1. Pig performance, according to the maximum lung lesions.

% Maximum lung lesions	ADG	Economical FCR	% Mortaliy	MED
0 - 5	686 a	2.50 a	2.17 a	1.82 a
5 – 12.5	663 b	2.52 ab	2.95 b	2.34 b
12.5 - 25	665 b	2.55 b	3.02 b	2.39 b
> 25	648 c	2.57 b	3.38 b	2.61 b

[1]Levels NOT connected by the same letter are significantly different.

Conclusions and Discussion

In this study the ADG, FCR and mortality have been the three parameters more affected by the M.hyo like maximum lung lesions, although statistical differences have been found in all the production parameters evaluated. These results reaffirm the importance of controlling maximum lung lesions as low as possible, with different management strategies in order to improve the efficiency.

Acknowledgments

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

References

1. Madec F. et al. 1992. Prod. Anim. 5: 149-161.
2. Straw B.E. et al. 1989. J. Am. Vet. Med. Assoc. 195
3. Bollo J. et al. Proc. 20th IPVS Congress, vol 1. 104.
4. Pallares F. et al. 2000.Vet. Res. 3: 573-582.
5. Davies PR. et al. 1995. Am J Vet Res, 56: 709-714.
6. Morrison R.B. et al. 1985. Can Vet J, 26: 381-384.
7. Ubeda J.L. et al. 2008 IPVS Congress, vol 2. 205.
8. Bak P. et al. 2008 IPVS Congress, Oral presentation.

O.009

Prevalence of *M. hyopneumoniae* and *M. hyorhinis* in broncho-alveolar lavage fluid (BALF) samples in live animals with and without respiratory problems

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Introduction

The objective of this study was to assess the prevalence of *M. hyopneumoniae(M. hyopneumoniae)* and *Mycoplasma hyorhinis* (*M. hyorhinis*) in broncho-alveolar lavage fluid (BALF) samples from live animals, with and without respiratory problems coming from different European farms.

Materials and Methods

At 10 weeks of age, BALF samples from 5 animals showing respiratory problems (sick animals) and from 10 healthy age-matched pigs were taken. Those healthy animals were sampled again at 20 weeks of age. This procedure was carried in 7 different European farms. BALF samples were processed by means of a *M. hyorhinis* real time quantitative PCR (QPCR) method (1) and a *M. hyopneumoniae* nPCR test (2). When this latter technique offered positive results, a *M. hyopneumoniae* QPCR test was applied (2). QPCR results were expressed as mean log10 (min-max) copies/ml of BALF.

Results

Percentage of *M. hyopneumoniae* (nPCR) and *M. hyorhinis* (QPCR) detection in BALF samples is detailed in Table 1. Overall, *M. hyopneumoniae* detection was lower at 10 weeks of age (27/105, 26%) than at 20 weeks of age (30/58, 52%). There were only 8 out of 58 (14%) animals that were positive to *M. hyopneumoniae* nPCR at both sampling points. Detection of *M. hyopneumoniae* was higher in those animals showing respiratory problems (11/35, 31.4%) than in healthy animals (16/70, 22.8%) (p>0.05). Mean bacterial load of QPCR positive animals showing respiratory problems (5.93 [max=8.60-min=3.89]) was not significantly different from that of healthy ones (6.09 [max=7.98-min=4.24]).

On the contrary, *M. hyorhinis* detection was slightly higher at 10 weeks of age (65/105, 62%) than at 20 weeks of age (30/58, 52%). There were 23 out of 58 (40%) animals that were positive to *M. hyorhinis* QPCR at both sampling points. Detection of *M. hyorhinis* was higher in healthy animals (46/70, 65%) than those animals showing respiratory problems (19/35, 54%) (p>005). Mean bacterial load of QPCR positive healthy animals (5.91 [max=8.53-min=4.37]) was not significantly different from animals showing respiratory problems (6.19 [max=8.37-min=4.41]).

Ten (9%) and eight (14%) pigs were positive to both pathogens at 10 and 20 weeks of age, respectively. Only two animals were positive at both sampling points to both pathogens.

Table 1 Number (percentage) of *M. hyopneumoniae* (nPCR) and *M. hyorhinis* (QPCR) positive BALF samples taken from live pigs at 10 and 20 weeks of age.

Farm	*M. hyopneumoniae*		*M. hyorhinis*	
	10 weeks (n=15)	20 weeks (n=10)	10 weeks (n=15)	20 weeks (n=10)
1	4 (27)	3 (33)	3 (20)	3 (33)
2	0 (0)	2 (20)	13 (87)	9 (90)
3	0 (0)	0 (0)	11 (73)	2 (25)
4	4 (27)	6 (67)	13 (87)	6 (67)
5	6 (40)	3 (50)	10 (67)	1 (17)
6	11 (73)	7 (78)	1 (7)	3 (37)
7	2 (13)	3 (37)	14 (87)	6 (75)

Conclusions and Discussion

M. hyopneumoniae detection in BALF samples was higher in older than younger pigs, while *M. hyorhinis* detection was similar at both age groups. On the other hand, whereas *M. hyopneumoniae* detection was numerically higher in animals showing respiratory problems than in healthy ones, *M. hyorhinis* detection was apparently (in this study) not linked to presence of respiratory problems.

Acknowledgments

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References

1. Clavijo M.J., personal communication
2. Sibila et al., 2012. J Comp Pathol. 147(2-3):285-95

O.010

M. hyorhinis infection in early cases of *M. pneumonia* and evaluation of diagnosis assays

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Introduction

The mycoplasmal pneumonia caused by bacteria of the genus Mycoplasma is a contagious pulmonary disease of swine characterized by a dry non-productive cough, reduced growth rate and poor feed conversion efficiency (1). Due to the controversial role of *Mycoplasma hyorhinis* in this disease, confirmation of the presence of this bacterium and the identification of its role in respiratory disease remains a major challenge. The objectives of this study were to compare different techniques, especially fluorescent *in situ* hybridization (FISH), for diagnosis of respiratory mycoplasmosis in naturally infected pigs, and to evaluate the presence of *M. hyorhinis* in early cases of enzootic pneumonia.

Materials and Methods

Ninety *M. hyopneumoniae* and/or *M. hyorhinis* infected lung tissue samples based on the mosaic of diagnosis (clinical signs, gross and microscopic lesions and PCR results) were used and divided into three groups: group 1, positive for *M. hyopneumoniae* by PCR; group 2, positive for *M. hyorhinis* by PCR; and group 3, positive for both pathogens by PCR. All group samples were evaluated by age of the animal, histopathological changes and presence or absence of viral infection (Flu or PCV2) by IHC. FISH for *M. hyopneumoniae* and *M. hyorhinis* and IHC for *M. hyopneumoniae* were compared using the mosaic of diagnosis as gold standard and sensibility and specificity were determined. For the statistical analysis the following test were used to compare groups: T test for age, Kruskall-Wallys for histopathological findings and Chi-Square for positivity by IHC for viral pathogens.

Results

The average age of animals in group 2 (PCR positive for *M. hyorhinis*) was 57.32 days and 116.31 days (P <0.01) for group 1 (PCR positive for *M. hyopneumoniae*). Microscopic changes were statistically (P <0.01) more intense in group 1 than in group 2. The sensitivity and specificity of FISH for *M. hyopneumoniae* (Fig. 1) was 75 and 100%, respectively, and 40 to 73.3% using immunohistochemistry. The sensitivity and specificity of FISH for *M. hyorhinis* (Fig. 2) was 76.7 to 100%, respectively. The presence of viral antigens (Flu or PCV2) was detected in 53.3% of the samples in group 2 (*M. hyorhinis*) and 13.3% of the samples in group 1 (*M. hyopneumoniae*).

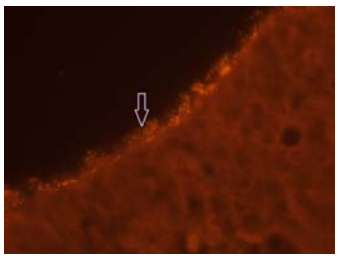

Figure 1. Swine lung sample. *M. hyopneumoniae*, fluorescent labeling attached to epithelial cell in the bronchial surface (arrow). FISH (Cy3).

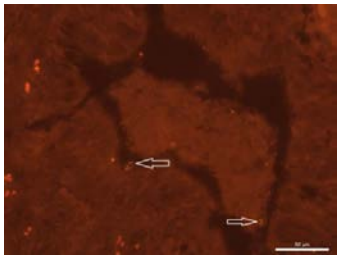

Figure 2. Swine lung sample. *M. hyorhinis* fluorescent labeling (arrows) in epithelial cells and lumen exsudate of bronchiole. FISH (Cy3).

Conclusions and Discussion

These findings suggest that colonization and development of lesions caused by *M. hyorhinis* happen earlier than those caused by *M. hyopneumoniae*. The results also demonstrated that FISH can be a useful tool for diagnosing mycoplasmosis. Similar to observed in other studies (2, 3), the IHC for viral pathogens suggest that coinfection have an important contribution to manifestation of diseases caused by *M. hyorhinis*.

Acknowledgments

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References

1. Thacker and Minnion. 2012. Diseases of Swine.
2. Kawashima et al. 2007. J Vet Diagn Invest.
3. Kixmoller et al. 2008. Vaccine.

O.011

Molecular typing of *M. hyopneumoniae* in clinical samples using MLVA

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Introduction

Mycoplasma hyopneumoniae (*M. hyopneumoniae*) is the cause of enzootic pneumonia in pigs, one of the most important diseases that generate losses in swine production worldwide[1]. The heterogeneity of *M. hyopneumoniae* strains that circulate in swine populations has been reported in different studies[2,3]. A typing approach to investigate outbreaks and to understand the pathogenesis and epidemiology of the bacterium, and to evaluate interventions to control the disease is highly needed. Therefore, the aim of this study was to standardize an assay for the differentiation of *M. hyopneumoniae* strains and to investigate the genetic variability of *M. hyopneumoniae* circulating in the U.S. based on the number of tandem repeats in two important adhesins, P97 and P146.

Materials and Methods

A total of 209 clinical samples submitted to the UMN-VDL during a 6 month period were used in this study. A Multilocus Variable Number Tandem Repeat Analysis (MLVA) was standardized by modifying a previously established protocol[4]. The assay targets the number of repeats in 2 adhesin proteins (P97 and P146) of *M. hyopneumoniae*. Fluorescently labeled primers were combined in a touchdown PCR reaction that was developed to amplify the target loci. Ten microliters of each sample were diluted 1:32 and submitted for capillary electrophoresis. Samples that failed in the capillary electrophoresis analysis were sequenced by standard Sanger sequencing, and loci repeats were manually counted for inclusion in the analysis. MLVA patterns were analyzed in Bionumerics version 7.0.

Results

The two loci were clearly identified on electropherograms according to their size ranges and dyes, and converted to categorical values based on the number of repeats. The Simpson's diversity index for the assay was D=0.908 for P97, D=0.929 for P146 and D=0.979 when both loci were combined. Analysis of the combination of 2 loci revealed 87 MLVA types for all samples in the study (Fig.1). MLVA types were named according to the number of repeats in each locus, P97- P146, respectively. The most frequent was the MLVA type 9-26 (7.2%) followed by type 15-25 (6.2%); 15-21 (5.2%); 11-15 (4.7%) and 14-21(4.3%). In the state of Minnesota 54 MLVA types were observed (n=107 clinical samples). No clustering was observed on the basis of the state where the farm was located.

Conclusions and Discussion

In this study, the MLVA assay was stardardized in our laboratory and a high number of MLVA types were found, suggesting that multiple *M. hyopneumoniae* variants are circulating in U.S. herds and at the state level. Further analysis of samples collected longitudinally from diverse geographic locations and disease types are necessary to investigate if a nonrandom distribution of genotypes is present among highly pathogenic strains.

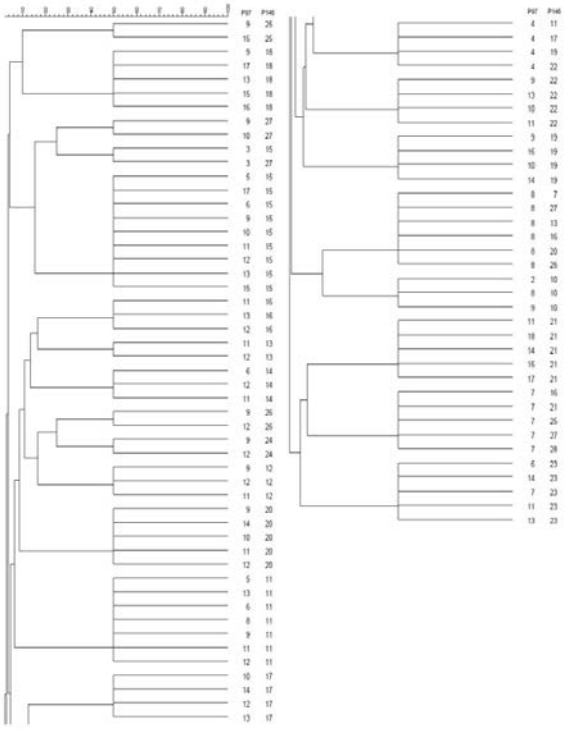

Figure 1. Dendrogram of MLVA types in US revealed 87 MLVA types obtained from 209 clinical samples constructed using Unweighted Pair Group Method with Arithmetic Mean - UPGMA

Acknowledgments

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References

1. Thacker, EL., Minion, FC. 2012. In: Diseases of Swine, 779-797.
2. Mayor, D *et al.*, 2007.Vet. Res., 38, 391–398.
3. Vicca, J *et al*., 2003. Vet. Microbiol., 97:177–190.

Vranckx, K. J *et al.*, 2011. J. Clin. Microbiol., 49, 2020-2023.

O.012

Investigation of the spread of pandemic H1N1 influenza virus in the German pig population – a clinical, serological and virological study

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Introduction

The emergence of pandemic H1N1 influenza A(H1N1)pdm09 virus in the human population in 2009 and its worldwide spread urged increased surveillance activities of swine influenza because this virus consists entirely of segments of influenza A viruses (FLUAV) circulating in swine populations. Receptors in the swine respiratory tract enable the replication of FLUAVs of porcine, human and avian origin. Thus, genetic reassortment may occur after simultaneous infection of pigs with different viruses. This can result in the emergence of new reassortant viruses. A(H1N1)pdm09 virus is circulating in the human population and frequently transmitted from humans to pigs. Human vaccines are adapted to the epidemiological situation each year. Current human vaccines contain two FLUAV subtypes (A(H1N1)pdm09 and H3N2) and one influenza B subtype. For pigs, there are vaccines based on H1N1, H1N2, and H3N2 commercially available in Europe. Yet, cross-reaction of these vaccines is insufficient with the hemagglutinin of A(H1N1)pdm09 virus. Therefore, the objectives of the present study were (i) to investigate the prevalence of A(H1N1)pdm09 in the German pig population and (ii) to assess the need for a vaccine against A(H1N1)pdm09 virus-caused influenza in pigs.

Materials and Methods

Nasal swabs from sick pigs were collected and analyzed using a FLUAV sreening RT-PCR. Influenza A positive samples were subsequently typed using an established RT-PCR typing method after isolation of the viruses. Blood samples from sows and piglets were collected and analyzed using the hemagglutination inhibition (HI) test.
Fifty-three A(H1N1)pdm09 virus-positive pig herds consisting of sows and breeding groups were selected for clinical evaluation using a standardized technical protocol.

Results

From 2011 to 2013, detection of A(H1N1)pdm09 virus and derivative reassortants increased from 2.4 to 5.5% (nasal swabs of pigs from 1592 farms were investigated). Seroprevalences against A(H1N1)pdm09 virus were between 9,5 and 11% of the investigated pig herds (1953 in total).
Of the 53 pig herds selected for clinical evaluation 17 were positive for A(H1N1)pdm09 viruses by RT-PCR and 36 by HI. The clinical signs observed most frequently in these farms were reproductive disorder (40/53), coughing in sows (34/53), fever in farrowing sows (31/53), and abortions (26/53). These were followed by anorexia (20/53) and coughing during rearing (20/53). An increased mortality was recorded in 6/53 farms. Piglet performance was poor in 5/53 farms.

Conclusions and Discussion

The results demonstrate that A(H1N1)pdm09 virus and its derivative reassortants are circulating in the German pig population. The clinical signs comprise influenza-like symptoms like respiratory illness but also a protracted form associated with multiple clinical symptoms. A(H1N1)pdm09 virus and its derivative reassortants are prevalent in the swine population and associated with disease. The disease is more complex than known for influenza which may be due to the virulence of this virus but also due to its interaction with other agents. This new virus needs prevention and control which is limited because the commercially available swine influenza vaccines do not cross-react sufficiently. Consequently there is a need for a vaccine consisting of panH1Nx virus (1).

Acknowledgment

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Reference

1. Dürrwald, R. and Schlegel, M. (2013). Efficient protection of a swine pandemic H1N1 influenza virus vaccine against the newly emerged H1N2 pandemic virus reassortant. 5th European Symposium of Porcine Health Management, 22nd-24th May 2013,Edinburgh, UK

O.013

Full genome sequence analysis of swine influenza lineages circulating in Mexico from 2010 to 2013

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Introduction

Influenza A viruses (IAV) circulating in domestic swine represent an important economic burden to the livestock industry as well as a major human health concern. Pigs can act as an intermediate host for the generation of new IAV strains that can efficiently replicate and spread in the human population, as demonstrated by the recent swine-origin 2009 H1N1 pandemic.

IAVs are characterized by a tremendous genetic and antigenic diversity. Increased worldwide surveillance in relevant host species is needed to better understand the diversity and dynamics of circulating IAVs, to assess the risk of appearance of new strains and to better implement prevention and control measures.

Swine production is an important industry in Mexico. In spite of the large domestic swine population there is little information about the epidemiology of AIVs currently circulating in Mexico and its relationship with the swine IAV lineages circulating in USA and Canada [1-4].

We describe the isolation, full-genome sequencing and phylogenetic analysis of more than 50 IAVs obtained from pig farms in different Mexican States between 2010 and 2013. Our results show that, similarly to the USA, the epidemiology of swine influenza in Mexico is very complex, with multiple clades of H1N1, H1N2 and H3N2 subtypes co-circulating every year.

Materials and Methods

Nasal swab samples were collected from pigs during swine influenza outbreaks in farms from 6 different Mexican States between 2010 and 2013. Virus was isolated in cell culture or chicken embryonated eggs and viral RNA was used as template in a RT-PCR reaction with influenza specific universal primers. The purified cDNA was sheared to a fragment size of 250 bp and amplicon sequence libraries were prepared using Illumina's gDNA Seq protocol that includes end repair, "A" tail addition, adaptor ligation, fragment size-selection and LM-PCR amplification with barcoded primers. Libraries were multiplexed (24 samples per lane), and sequenced on an Illumina HiSeq 2500.

Single-end 100nt reads were filtered to remove low-quality sequences and adapters, and assembled using the Inchworm component of Trinity and curated to yield full-length sequences.

The complete coding region from each viral segment were aligned, along with published sequences representative of the major swine IAV lineages described in the USA and with previously published swine IAV sequences from Mexico. Phylogenetic trees were obtained by the Maximun-Likelihood method with 1000 bootstrap repetitions using the program GARLI Version 0.96b8.

Results

Our results show that, similar to what was described for swine influenza in the USA and Canada, three subtypes (H1N1, H1N2 and H3N2) and several lineages of swine influenza virus are currently circulating in Mexico. All three subtypes could be found in the same year and location, highlighting the high diversity of swine influenza in Mexico

Conclusions and Discussion

Our results greatly increase the available information on swine influenza diversity and circulation in recent years and have important implications for control strategies, including vaccine development, and assessment.

Acknowledgments

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References

1. Anderson, T.K., et al., *Population dynamics of cocirculating swine influenza A viruses in the United States from 2009 to 2012.* Influenza Other Respir Viruses, 2013. 7 Suppl 4: p. 42-51.
2. Lorusso, A., et al., *Contemporary epidemiology of North American lineage triple reassortant influenza A viruses in pigs.* Curr Top Microbiol Immunol, 2013. 370: p. 113-32.
3. Lorusso, A., et al., *Genetic and antigenic characterization of H1 influenza viruses from United States swine from 2008.* J Gen Virol, 2011. 92(Pt 4): p. 919-30.
4. Olsen, C.W., et al., *Triple reassortant H3N2 influenza A viruses, Canada, 2005.* Emerg Infect Dis, 2006. 12(7): p. 1132-5.

O.014

Antigenic characterization of swine influenza viruses isolated in Spain 2009-2011

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Introduction

The assignation of a swine influenza virus (SIV) isolate to a given subtype or lineage is not enough to predict if two isolates will cross react in the haemagglutination inhibition assay (HI) (1, 2). As a matter of fact, it has been shown that isolates sharing the same type of hemagglutinin may have limited or null cross-reactivity (2). The objective of the present study was to analyze the antigenic relatedness in HI of recent field SIV isolates from Spanish farms.

Materials and Methods

Fourteen Spanish SIV isolates (2009-2011) belonging to three SIV subtypes (6 H1N1, including one pandemic H1N1, 4 H1N2 and 4 H3N2) were used as antigens in HI against three groups of sera: a) Mono-specific anti-sera obtained by repeated immunization (4 doses, at 42, 56, 70 and 84 days of age) of naïve pigs with the 14 isolates inactivated with BEI. In this case, sera were tested in triplicate against the homologous strain and then sera were diluted with PBS to 1:160 for H1N1 and to 1:320 for the other subtypes in order to normalize the analysis. b) Sera of pigs immunized using the commercial vaccines available in Spain Gripork® (GP; HIPRA) and Gripovac 3® (G3; Merial). In this case, four groups of immunization (20 pigs each) were included: two doses of GP (group A), one dose of GP plus a second immunization with G3 (group B), one dose of G3 plus a second immunization with GP (group C), two doses of G3 (group D). Unvaccinated pigs were kept as controls (group E). Sera were pooled by groups prior to be analyzed by HI. c) 100 field sera previously tested against commercial H1 and H3 SIV isolates (GD Deventer, The Netherlands) In the analysis of field sera titers obtained with one isolate were compared (1 to 1) with the results obtained with the same sera and the different isolates of the same subtype by means of a correlation analysis and Kappa value with 4 categories (negative, low, moderate, high). In all cases, testing was done at least in duplicate and the threshold for HI was set at 1:40.

Results

One H1N1 isolate failed to produce hemagglutination and was not used as HI antigen.

a) Cross reactivity using mono-specific antisera: Analysis of the cross reactivity in HI using normalised sera showed very low cross-reactivity (≤1:40) between H1N1 isolates. In contrast, cross-reactivity within H1N2 or H3N2 isolates was higher (1:80-1:320), particularly for H3N2.

b) Testing of sera from vaccinated pigs: Clear differences were observed when the different isolates were used as antigens against the pools of sera obtained from vaccinated pigs. For H1N1 isolates, pool A only reacted positively with one isolate at 1:40. Pools B, C and D reacted with three H1N1 isolates at titers 1:80-1:160. None of the pools reacted at significant titers with the pandemic H1N1. For H1N2, pool B, reacted against three isolates (1:40 – 1:160), and C and D reacted only against two isolates (1:80-1:160) while pool A did not recognize H1N2 as expected from the composition of the product. For H3N2, all pools were positive but titers >1:80 were only observed with B and D sera. Sera from E pigs were negative against all isolates.

c) Analysis of field sera: Correlation of results obtained with H1N1 isolates was in general lower (average correlation coefficient = 0.69) than that of H1N2 and H3N2 (average correlation coefficients = 0.79 and 0.84, respectively). Similarly, kappa values were higher for the comparison of HI results within H3N2 or H1N2 compared to H1N1.

Conclusions and Discussion

Spanish H1N1 SIV isolates seem to have a higher antigenic diversity based on HI results than H1N2 and H3N2 isolates. Although HI is still one of the reference techniques for detection of subtype-specific antibodies, the present results emphasize the strong influence on the results that the selection of the antigen may have. The examination of pooled sera obtained from pigs subjected to different protocols of immunization indicated that reactivity against H1N1 and H1N2 was limited and that slight differences in reactivity H3N2 can result in. This suggests the need for a continuous update of available vaccines against SIV. This can be done by means of surveillance of circulating SIV and by developing systems for faster marketing of the new vaccines.

Acknowledgments

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References

1. Van Reeth *et al.*, 2001. Vaccine. 19: 4479-4486.
2. Van Reeth *et al.*, 2004. Virus Res. 103:115-124.

O.015

Surveillance programs in Denmark has revealed the circulation of novel reassortant influenza A viruses in swine

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Introduction

Swine influenza is a respiratory disease caused by multiple subtypes of influenza A virus. Swine influenza virus (SIV) is enzootic in swine populations in Europe, Asia, North and South America. The influenza A virus genome consist of eight distinct gene segments and SIV subtypes are defined by the combination of the gene segments hemagglutinin (HA) and neuraminidase (NA).
In most European countries, the avian-like (av)H1N1, the 2009 pandemic variant (H1N1pdm09), H1N2 and H3N2 subtypes have constituted the dominating SIV subtypes during recent years. In Denmark, the H1N2 subtype is a reassortant between avH1N1 and H3N2 which is different from the dominating European H1N2 subtype (*1*). The prevalence of the H1N1pdm09 virus in swine has increased since 2009 in some countries including Denmark. Here we present the results of the national passive surveillance program on influenza in swine performed from 2009-13.

Materials and Methods

Clinical samples submitted for diagnostic purposes from Danish pigs with respiratory diseases since 2009 were tested for SIV and included in the survey. Between 280 and 527 submissions were tested per year. Routinely, samples were initially tested by pan-influenza A real-time reverse transcriptase PCR assays (rRT-PCR) targeting the M or the NP gene (*1*). All positive samples were then tested by an in-house real-time PCR assay specific for the H1N1pdm09 virus HA gene. Since 2011, selected SIV positive samples were subtyped by partial sequence analysis of the HA and NA genes and a subset of those were full genome sequenced by next generation sequencing. The sequences were analysed and compared to virus sequences present in the GISAID database using standard bioinformatics tools (*1, 2*).

Result

The percentage of submissions that tested positive for SIV increase over the years reaching 47% in 2013. The subtype was determined by phylogenetic analyses of the partial HA and NA gene sequences which showed that the HA belonged to two different H1 lineages - the H1 avian-like SIV and the H1 pandemic (H1pdm09). The NA genes belonged to two different N1 lineages - the N1 avian-like SIV and N1 pandemic (N1pdm09). The majority of samples harboring an N2 gene belonged to the H3N2 SIV lineage, however, a few samples harbored an N2 gene most closely related to human H3N2 viruses from the mid-1990s (*2*). When combining the HA and NA phylogenetic data the results revealed that the two most common subtypes in Danish swine were H1N1 and H1N2 with avian like HA genes. The third most common subtype was the pandemic H1N1pdm09 virus. A single H3N2 different from the vH3N2 virus *(5)* was found in 2013. The results further revealed the presence of new reassortant SIV subtypes in the European swine population comprising either a new combination of known circulating SIV genes or the presence of an N2 gene not previously detected in pigs (*2*). In total, new reassortant SIV subtypes comprised 10 % of the characterized SIV viruses.

Conclusions and Discussion

The results of the passive surveillance program in Denmark revealed that almost 10 % of the circulating viruses were reassorted viruses which were different from the known circulating viruses in one or more gene segments. There is a significant movement of living pigs within Europe which makes it likely that new reassortants will spread. Indeed, reassortants identical to the viruses found in Denmark have recently been detected in Germany (*3*). This further emphasize that European networks on exchange of SIV data like the successful ESNIP3 project should be supported also prospectively.
Detailed knowledge on the genetic and antigenic characteristics of circulating influenza A viruses in swine is of veterinary importance in respect to choice of vaccines. Thus, the use of vaccines with heterologous HA vaccine antigens may elicit insufficient protection or even potentiate disease following natural infection (*4*). In addition, these findings is also important in a human health perspective because new re-assortments may generate new viruses with increased zoonotic potential as seen for the H3N2v virus in the US (*5*).

Acknowledgments

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References

1. R. Trebbien *et al., Virol. J.* **10**, 290 (2013).
2. S. O. Breum, C. K. Hjulsager, R. Trebbien, L. E. Larsen, *Genome Announc.* **1**, (2013).
3. T. C. Harder *et al., J. Virol.* **87**, 10460 (2013).
4. S. Khurana *et al., Sci. Transl. Med.* **5**, 200ra114 (2013).
5. C. L. Loving *et al., J. Virol.* **87**, 9895 (2013).

O.016

Genetic diversity of influenza A viruses in Western Canadian swine

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Introduction

Influenza A viruses (IAVs) cause year-round, acute respiratory disease in swine of any age. In a group of immune competent pigs, most influenza infections are mild and recovery usually occurs within 7-10 days after infection. However, the rapid turnover of the population in a swine herd creates a situation where young, susceptible animals are continually introduced to the population and influenza viruses can persist and change. The genetic and antigenic history of the viruses from a herd can provide insights into the evolution of the virus within the herd and aid in a more scientific approach to control of endemic flu in a herd. Since very little is known about the flu viruses circulating in Western Canadian swine, the main objective of this study was to determine which genetic variants were circulating in this region.

Material and Methods

Samples of lung tissue and nasal swabs from pigs in BC, AB, SK and MB were sent to either Prairie Diagnostic Services, Inc. (PDS) or the University of Minnesota, Veterinary Diagnostic Lab (UMVDL). Most of these samples were collected through passive surveillance of clinically ill pigs, but a small portion was collected as part of a surveillance study. Samples were screened for IAV by Matrix PCR tests, and positive samples were subjected to further analysis where subtyping, virus isolation and sequencing of the hemagglutinin gene (HA) were attempted. Results were reported back to the submitting veterinarian with sequence analysis and compiled for phylogenetic analysis. Evolutionary history was inferred by the Neighbor-Joining method and evolutionary distances computed by the Maximum Composite Likelihood method using Mega 5 software and clustal W alignment.[1,2,3,4]

Results

In 2013, 31 viruses were subjected to HA gene sequencing (4-AB, 9-SK, 20-MB) and the full genome was sequenced for 26 viruses from western Canada collected prior to 2012 (3-AB, 5-SK, 18-MB). Among these viruses were two swH1-alpha viruses,[5] which were common in MB prior to 2009 and virtually disappeared after 2009. Additionally, there were 3 strains of H3N2 virus that were divergent from H3N2 viruses circulating in Eastern Canada and the United States. These viruses were also not similar to each other and were found branching out of different regional clusters (Fig 1).

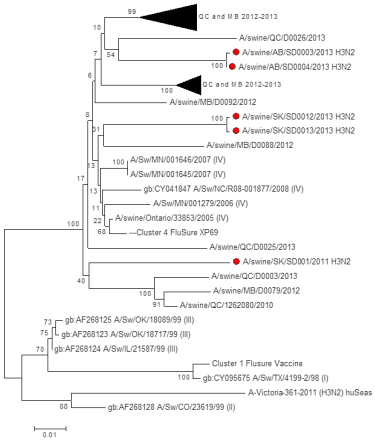

Figure 1. Phylogenetic tree of Canadian and reference H3 viruses.

Discussion

In an ideal world, vaccination would provide complete protection every time, but influenza viruses are expert at evading the humoral immune response by changing their surface proteins. Even slight changes in the three-dimensional protein shape of hemagglutinin can result in less binding or even no binding at all. This antigen-antibody mismatch can mean partial protection or little to no protection, depending on how cross-reactive the antibodies are to other viruses. We must also take into account that flu viruses can spread in the air between barns[6] and that ill workers can introduce human viruses to pigs.[7] This is why a scientific approach to vaccine selection includes genetic and antigenic evaluations of the current strain of virus in the herd, as well as other viruses isolated from the herd or even the region.

References

1. Saitou N. and Nei M. (1987) Molecular Biology and Evolution 4:406-425.
2. Felsenstein J. (1985) Evolution 39:783-791.
3. Tamura K., Nei M., and Kumar S. (2004) Proceedings of the National Academy of Sciences (USA) 101:11030-11035.
4. Tamura K., et al. (2011) Molecular Biology and Evolution 28: 2731-2739.
5. Lorusso A, et al. (2013) Curr Top Microbiol Immunol 370:113-132.
6. Corzo CA, et al. (2013) PLoS One 8:e71444.
7. Nelson MI, et al. (2012) J Gen Virol 93:2195-2203.

O.017

Prolonged detection of swine influenza virus in porcine oral fluids after experimental infection

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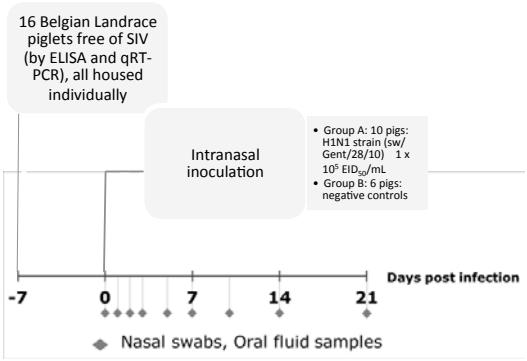
Introduction

The lack of seasonality of swine influenza virus (SIV) infection in combination with the role of swine as "mixing vessels" and the ongoing infections of humans with pH1N1 reassortant H3N2 influenza virus at county fairs, stress the importance of SIV surveillance in order to proactively address the pandemic potential of swine influenza. To date, active surveillance of SIV worldwide is barely done because of the short detection period in nasal swab samples (1). Therefore, more sensitive and reliable diagnostic methods to monitor circulating virus strains are requisite.

Materials and Methods



qRT-PCR was performed using an M gene-targeted RT-PCR procedure. Briefly, all samples were
Extracted: MagMAX Pathogen RNA/DNA kit (Life Technologies)
Amplified: Vetmax Gold SIV detection kit (Life Technologies).
Run on a LightCycler 480 Real-time PCR system (Roche).
Virus isolation was performed using embryonated chicken eggs (ECE) and Madin-Darby canine kidney (MDCK) cells according to procedures previously described (OIE Terrestrial Manual 2008).

Results

SIV was detected with qRT-PCR for a longer time period and with a higher rate of detection in oral fluid samples. Especially noteworthy was the possibility to detect swine influenza virus at 21 dpi in 25% of the oral fluid samples, while all nasal swab samples were already negative at 7 dpi.

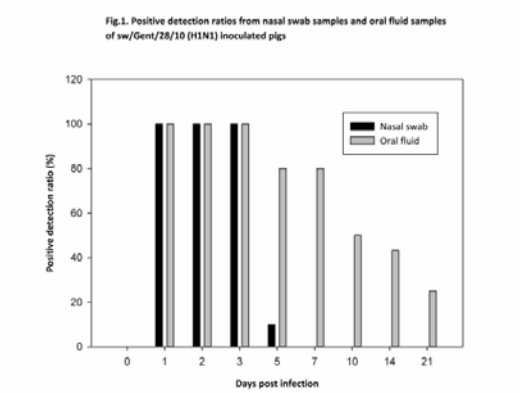


Figure 1. Positive detection ratios from nasal swab samples and oral fluid samples of sw/Gent/28/10 (H1N1) inoculated pigs

Although comparable amounts of SIV were shed in oral fluid and nasal swab samples, when ECE and MDCK-cells were compared for their ability to detect SIV in oral fluid and nasal swab specimens, evidence for VI from oral fluid was rare.

Conclusions and Discussion

Our data indicate that porcine oral fluid samples collected with ropes hold potential for diagnostic purposes and active surveillance seen the possibility to detect SIV RNA for a longer period than in nasal swabs. However, further research is advisory to evaluate the influence of virus subtype and inoculation dose on detectability. The difficulty to isolate SIV from these oral fluid samples could however pose a drawback and has to be studied more intensively.

Acknowledgments

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References

1. Janke et al., 2013.Curr Top Microbiol 370:69-83

O.018

Effect of the electrostatic particle ionization technology on decreasing influenza virus in aerosols from experimentally infected pigs

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Introduction

Influenza A virus (IAV) is considered a significant pathogen for the swine industry because of its effect on pig health and the risk it represents to public health. IAV can be transmitted via aerosols but the role of aerosols in the overall IAV transmission is not fully understood (1,2). It remains unknown which particle size IAV is associated with. This is important in order to evaluate transmission risks to people and also transmission within and between pig units. The electrostatic particle ionization system (EPI) is an air cleaning technology able to reduce airborne agents because of its ability to clump and settle airborne particles potentially improving air quality decreasing the risk of disseminating pathogens. Therefore, the objectives of this study were twofold: a) to evaluate the particle size ranges that IAV is associated with when aerosolized by pigs, and b) the impact of the EPI system in removing IAV from aerosols generated by infected pigs.

Materials and Methods

The EPI system, consisting of a line of stainless steel corona points attached to a stainless steel cable (30KV), was installed at 1.3m height along the length of a 35 m^3 BSL-2 isolation unit at the University of Minnesota. For the purpose of the study 10 pigs were intranasally and intratracheally infected with an H1N1 IAV ($4.4x10^6$ TCID50/ml). Aerosols were sampled for 30 min using a cyclonic air collector as previously described (2) and 1 hour using an Andersen Cascade Impactor able to collect particles as a function of particle size (8 stages that measure particles from 9 to 0.4microns). Additionally, an optical particle counter and an ion meter were used to analyze total particles and verification of ion concentration during the sampling periods. Air samples were collected with the EPI system 'off' and 'on' for 1 hour each time. Oral fluids, clinical signs, coughing and lethargy score were collected at each replicate a.m./p.m. Samples were analyzed by quantitative IAV RT-PCR. Difference in the quantity of virus, removal efficiency and total particle counts by size were calculated during the study with the system 'off' and 'on'.

Results

The qPCR analysis of the Andersen Impactor stages demonstrated that IAV was associated with the largest particle size ranges of 5.8 to 10 microns. After the air was treated with the EPI system there was a significant reduction in the quantity of IA in all size stages (with the exception of stage 2). The most pronounced reduction (up to 2.57 logs) was observed in particles from 9 to 10 microns. Overall the removal efficiency was positive throughout all days in the study (Figure 1).

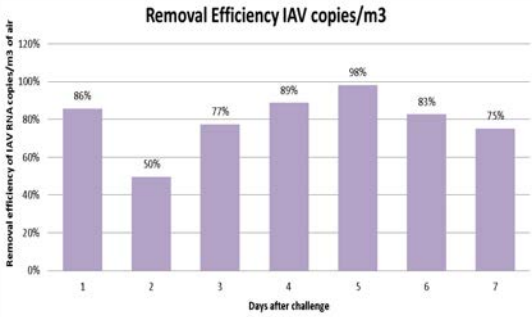

Figure 1. Summary of removal efficiencies (initial concentration of aerosolized virus with system off minus final concentration with system on divided by initial concentration) of IAV particles from infectious aerosol.

Conclusions and Discussion

Results from our study indicate that IAV can be associated to particle size aerosols of various size ranges. Furthermore, IAV aerosols were reduced by the EPI system. Thus air sanitations technologies could play an important role at reducing the load of viral particles from the air; however the significant of this reduction is still unknown. Further studies are needed to test the infectivity of these particles. Decreasing the infectious virus load of IAV in the air of positive pig farms should decrease the likelihood of dissemination of airborne pathogens to neighboring pig sites.

Acknowledgments

This project has been founded by NPB and UMN Signature Program

References

1. Fernstrom and Goldblatt (2012) Journal of Pathogens doi.org/10.1155/2013/493960
2. Corzo C. et al. (2013) PLoS ONE 8(8): e71444.doi:10.1371/journal.pone.0071444

O.019

Influenza A virus endemic circulation in neonatal and nursery pigs in a United States commercial swine farm

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Introduction

Inactivated IAV vaccines used in breeding females provide passive immunity to neonatal piglets through colostrum. However, maternal derived antibody (MDA) may reduce clinical disease but not infection of susceptible piglets (3). Endemic IAV circulation in breeding herds may be due to neonatal swine with inadequate levels and/or types of passive immunity or due to the introduction of a novel IAV (1). The purpose of this study was to diagnose and characterize endemic IAV in neonatal and nursery swine from a US breeding farm using inactivated influenza vaccines in sows.

Materials and Methods

Four breeding farms from the same production system located in the Midwest US using IAV vaccination in the breeding herd were selected. Dams were previously vaccinated with a commercial influenza vaccine and were converted to an autogenous product one week prior to sample collection. Samples included 4,320 nasal swabs (NS) from 12-17 day old piglets within the same litter collected every-other-week between March-May and July-August 2013 for a total of eight collections. Oral fluid (OF) samples were collected from the same group of piglets after transport to the nursery at four weeks of age. Neonatal NS and OF were evaluated for the presence of IAV by a screening and subtyping PCR (VetMAXTM-Gold SIV, Applied Biosystems). Screening IAV PCR was conducted on 1,440 pooled NS with Ct values $\leq$ 38.0 considered positive. Subtyping IAV PCR assays were conducted on individual NS from all positive pools with Ct values $\leq$ 35.0 positive and 35.0 $<$ Ct $\leq$ 38.0 considered suspect. Oral fluids were tested by the same screening and subtyping IAV PCRs. Hemagglutinin, neuraminidase and matrix nucleotide sequences were determined from virus isolates derived from NS.

Results

IAV was detected by PCR in 2.2% of the pooled NS (32/1,440). Subtyping PCR detected 1.8% (76/4,320) of individual NS positive for IAV. The majority of positive NS were detected early during the sample period (March-April). Overall, 56 H1N2, 3 H3N2 and 12 H1/H3 mixed infections were identified. Five samples were positive for only the N2 subtype without a subtypable HA. The largest number of H1N2, H3N2 and mixed infections were detected at week 1 and 2 in farm 2 corresponding with clinical influenza illness. Virus was not detected in NS from farm 2 during the final 6 collections. In addition, H1N2 was the only subtype detected in farms 1, 3 or 4 except for a suspect H3 detected at farm 1 during week 7 (July 22-26). Farm 2 was the only location that demonstrated mixed H1N2/H3N2 IAV infection. Fourteen H1N2 IAV NS isolates derived from Farm 2 were sequenced. Phylogenetically, all isolates belonged to the δ-1 cluster with a 2002 lineage N2 and pandemic Matrix gene.
In the downstream nurseries, 31.0% (49/158) of OF were IAV PCR positive. Farm 2 demonstrated the highest number of positive OF samples, 51.0% (25/49). Farms 1, 3 and 4 demonstrated approximately 15.9%, 20.0% and 30.0% IAV PCR positive OF samples, respectively. Overall, 28.6% of the positive OF samples were H1N2 (14/49), 6.1% H3N2 (3/49), and 18.4% (9/49) subtyped N2 only. Approximately 18.4% (9/49) of the positive OF were considered suspect H1N2 and H3N2 in the subtyping PCR assay and 28.6% (14/49) were untypeable.

Conclusion and Discussion

Despite the use of inactivated vaccines in dams to prevent IAV-associated respiratory disease and infection, a low level of endemic circulation of IAV persisted in nursing pigs. The low IAV prevalence in neonates was in contrast to the much higher prevalence in the down-stream nurseries, consistent with other reports with similar findings (2,4). Sequencing and virus isolation remain in progress at this time. Overall, this study will help guide the management, control and biosecurity measures that may affect the level of influenza circulation on breeding farms and downstream nurseries and provide guidance for using IAV vaccines in swine.

Acknowledgements

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References

1. Allerson M et al. 2013. Vaccine 31:500-505.
2. Allerson MW et al. 2013. Transbound Emerg Dis doi:10.1111/tbed.12053.
3. Kitikoon P et al. 2013. J Gen Virol 94**:**1236-1241
4. Torremorell M et al. 2012. Transbound Emerg Dis doi:10.1111/j.1865-1682.01300.

O.020

Retrospective serological survey of influenza viruses in backyard pigs from Mexico City

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Introduction

The swine influenza virus (SIV) is one of many viral agents implicated in respiratory disorders in pigs.[1] It is present in endemic form in swine farms worldwide in intensive as well as backyard production systems.[2] The aim of the present study was to perform a retrospective analysis of the 2000–2009 period to determine the seroprevalence against pH1N1, hH1N1, swH1N1, and swH3N2 viruses in backyard pigs from Mexico City.

Materials and Methods

Sera obtained from 2094 backyard pigs in Mexico City were used for this study. The following influenza viruses strains were used as antigens: seasonal human influenza (hH1N1) A/Mexico/INER1/2000 (H1N1), pandemic influenza (pH1N1) A/Mexico/LaGloria-3/2009 (H1N1), classical swine influenza (swH1N1) A/swine/New Jersey/11/76 (H1N1) and triple reassortant (swH3N2) A/swine/Minnesota/9088-2/98. We used the standard procedure established by the OIE,[3] for the hemagglutination inhibition assay with the following modifications: we standardized to eight hemagglutinating units (HAU) and serum titers were considered positive when they at a level equal or higher than 1:80. For the statistical analysis we used linear regression to analyze the trend in reactive serum titers across the years.

Results

The swH1N1 virus showed the highest seroprevalence (74%), followed by the swH3N2 (24.2%), pH1N1 (17.8%), and hH1N1 (1.3%) viruses. When antibody titers were simultaneously detected for three subtypes, the ones presenting the highest frequency were pH1N1, swH1N1, and swH3N2 (n = 114). From these subtypes, swH1N1 was present in more occasions (40) followed by swH3N2 (32), and lastly by pH1N1 (12). Regression analysis of the log2 transformed data showed that antibody titers for pH1N1 (b = -149), swH1N1 (b = -0.174) and swH3N2 (b = -0.254) viruses tended to decrease across the years ($P < 0.0001$), whereas antibody titers for hH1N1 (b = 0.008) virus maintained a low and constant seroprevalence, with no trend over time ($P = 0.324$).

Conclusions and Discussion

Our analysis of antibody titers from the 2094 sera tested revealed distinct patterns among the four different influenza viruses. For instance, antibody titers of pH1N1, swH1N1, and swH3N2 tended to decline over time. Antibody titers against pH1N1 were identified in the first years, but they diminished across the years studied. Antibody titers against subtype hH1N1 never exceeded the antibody titers of the other analyzed subtypes. The presence of titers against the pH1N1[4] virus and porcine influenza virus[5] is similar to that reported by other authors. The origin of the hemagglutinin of pH1N1 virus is from classic swine H1N1 virus, that is, genetically similar to this protein, but it is antigenically distinct.[6] In this study, a specific recognition is given for each of the subtypes analyzed. Our results suggest that a virus with similar antigenic properties to that of the 2009 pandemic pH1N1 virus was circulating in the Mexican swine population in 2000.

Acknowledgments

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References

1. Brown IH. The epidemiology and evolution of influenza viruses in pigs. Vet Microbiol 2000; 74:29–46.
2. Torremorell M et al., Transmission of influenza A virus in pigs. Transbound Emerg Dis 2012; 59:1–17.
3. Manual of Diagnostic Test and Vaccines for Terrestrial Animals 2009. Chapter 2.8.8 Swine influenza.
4. Trevennec K et al., Transmission of pandemic influenza H1N1 (2009) in Vietnamese swine in 2009–2010. Influenza Other Respi Viruses 2012; 6:348–357.
5. Avalos GP et al., Influenza Porcina en Mexico. 1ra ed. Espana: Editorial Academica Espanola. 2011.
6. Smith GJD et al., Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. Nature 2009; 459:1122–1125.

O.021

Elimination of two consecutive swine influenza subtypes in a large breed to wean herd

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Introduction

Following success in eliminating SIV from a newly stocked sow farm using autogenous vaccine and Isowean gilts it was decided to attempt elimination in an existing sow herd using internal replacements.[1] Through routine oral fluid surveillance for PRRSv and SIV a 5,600 sow herd in Central Nebraska was identified. This herd was using an influenza control program of two doses of FluSure XP to all incoming gilts with boosters prefarrow. An H3N2 subtype had been isolated and sequenced from nasal swabs and oral fluids. This sequence was compared to commercial influenza vaccines and found to not be similar (< 95%). The decision was made to attempt the elimination with an autogenous SIV vaccine using the replicon technology.[2] Following published research by Corzo, whole herd vaccination was implemented.[3] The protocol included vaccination of all of the internal gilt replacements from weaning through the oldest gilts prior to breeding.

Materials and Methods

The sow farm site and Gilt Developer Unit (GDU) are fully filtered along with the livestock trailer that moves replacement gilts from the GDU to sow farm. All adult swine in the sow herd were vaccinated in September and October 2012, three weeks apart. The entire growing population in the Gilt Developer Unit and nursery received the same H3 antigen only autogenous vaccine in the same time period. Weaned gilt replacements were given booster doses every four weeks until they moved into the finisher for sixteen weeks.

Results

Oral fluid samples from weaned pigs were positive for the H3N2 subtype on October 10th and 17th, 2012. Immediately following the second whole herd vaccination a new subtype H1 Delta appeared in weaned gilts at the GDU. The original H3N2 was eliminated and has never been identified again. The new H1 Delta became the only subtype identified subsequently. A second autogenous vaccine was developed and the same procedure was repeated in January and February 2013. The last PCR positive sample was identified in the youngest weaned gilts at the GDU on February 18, 2013. The farm remained negative on weekly oral fluid sampling until November 25, 2013 when a different H3N2 (93.6%) subtype was identified in two teaser boars.

Conclusions and Discussion

The use of whole herd vaccination was an effective method to eliminate circulating SIV in a sow herd. Following elimination of the first subtype a second (H1 Delta) became apparent. Personal communication with Dr. Corzo indicated a similar situation had occurred in his reseaerch project. The second subtype was eliminated in the same manner as the first. Communication with Dr. Culhane took place on how best to handle the sow herd regarding infuenza boosters.[4] Our decision was to continue to monitor the sow herd and weaned pigs for SIV circulation with oral fluids on a weekly basis. On November 25, 2013 PCR positive samples were identified in two teaser boars. There were no clinical signs in the herd but as the weeks progressed more PCR positive samples were identifed in weaned pigs. The new isolate was sequenced and was identified as a H3N2 Cluster IVA. It was not similar to the previous H3N2 subtype, 93.5%. The two whole herd vaccination procedure will be repeated as previously done. There are no clinical signs of SIV in the sow herd, but weaned pigs are hard to start, becoming infected with the new subtype shortly after weaning. Following the next round of whole herd vaccinations our plan is to continue with the weeky monitoring and at the point of PCR positive samples to monitor and sequence the isolate. Based on the outcome of sequencing a decision would be made to either use the previous sequences in the autogenous vaccine or to prepare a new product with the new sequence for whole herd vaccinations. The owners understand that a new subtype can enter the herd at any time most likely from employee infections. There are no influenza focused employee protocols like daily temperature monitoring prior to entry to the farm. The overall health and performance of the sow herd has improved from the point of the last whole herd vaccination. Customers receiving the weaned pigs have reported better nursery performance.

Acknowledgments

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References

1. Thompson R et al. 2009. Leman Conference p59.
2. Harris Biologics, Ames, IA
3. Corzo C et al. 2012. Journal of Swine Health and Production p283.
4. Culhane M. 2013. Personal Communication

O.022

Vaccination of six-day old vs. twenty seven-day old piglets against PCV2 in the presence of neutralizing antibodies

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Introduction

Porcine circovirus 2 (PCV2) infection is associated with several syndromes in pigs that are devastating to the pig industry. Commercial vaccines against PCV2 are available since 2006 and they have been very helpful for reducing clinical morbidity and mortality associated with PCV2 infection to under 3%, an outstanding accomplishment given that the infection can affect up to 80% of pigs. Most commercial vaccines are labelled for use in piglets three weeks of age or older, however pork producers may prefer to vaccinate piglets in the first week of life to decrease handling and to achieve immunity at an earlier age. A previous study showed that piglets vaccinated against PCV2 at three to five days of age developed a similar humoral immune response as piglets vaccinated at twenty-one days of age. However, sows were naïve to PCV2 to eliminate the possibility of maternal antibody interference with the adaptive immune response (1). The aim of the present study was to assess whether and how PCV2 vaccination of newborn piglets would be impacted by the maturation of the porcine immune system and the presence of maternal PCV2-neutralizing antibodies.

Materials and Methods

Fifty-seven colostrum-fed piglets of PCV2 vaccinated sows from a PCV2 positive pig barn were randomly placed into five treatment groups: early (6-7d: E-VAC) and late PCV2 vaccinated (27-28d: L-VAC), early (6-7d: E-KLH) and late KLH immunized (27-28d: L-KLH), and control group (no treatment: N-VAC). A single dose of Circumvent® PCV vaccine (Intervet Inc., Merck Animal Health) was used in this study. At 36-37 days of age virus challenge (10E5 TCID50 i.n.) was administered to E-VAC, L-VAC, and N-VAC treatment groups. All pigs were serum PCV2 PCR negative prior to viral challenge. A KLH antibody ELISA was performed on the E-KLH and L-KLH serum samples. Saliva and individual serum samples were tested for the presence of PCV2 DNA via qPCR. Serum samples were assayed for neutralizing antibodies against PCV2 (2). Wilcoxon and Kruskal-Wallis tests were applied to data sets with non-normal distributions, and normal distributions of data were analysed using an ANOVA with a post hoc Tukey-Kramer test (JMP ®, version 10.0, SAS Institute Inv., Cary, NC, 1989-2007).

Results

i. To assess the maturity of the immune system we vaccinated with KLH. Humoral immune response against KLH was delayed by one week and remained weaker in young vaccinees compared to older vaccinees. ii. High titres of PCV2 neutralizing antibodies were found in all sera initially, and declined until 4 (E-VAC), 5 (L-VAC), and 6 (N-VAC) weeks of age, respectively, before immediately rising again with statistical differences between vaccinees and non-vaccinees. iii) qPCR on saliva demonstrated N-VAC animals had higher virus loads than both L-VAC and E-VAC piglets four weeks post challenge ($p<0.05$). L-VAC and E-VAC groups were not statistically different ($p=0.24$). iv) Serum qPCR for E-VAC, L-VAC, and N-VAC treatment groups did not show any significant differences post-challenge.

Conclusions and Discussion

Our KLH data indicate that the humoral immune response is delayed and weaker in young piglets in comparison to older ones only during the very early phase of life. With regard to the effects of PCV2 vaccination in reducing salivary viral load, we observed significant differences between non-vaccinated and vaccinated animals but not between early and late PCV2 vaccinees (all vaccinees showed reduced viral shedding). Virus load in sera appeared to be similar in all PCV2 challenged piglets, albeit vaccinees showed a trend of lower PCV2 genome copies in sera than non-vaccinees. PCV2 neutralizing antibodies titres increased earlier in all vaccinees than in non-vaccinees, indicating an immunological booster effect in vaccinees due to infection with PCV2, which was not observed in non-vaccinees.

Overall, this study demonstrates that vaccination with a single dose of Circumvent® PCV vaccine at six to seven days of age provides the same benefit as vaccination at twenty-seven to twenty-eight days of age. Neither the presence of maternal antibodies, nor the partial immaturity of the immune system, appear to outweigh the benefits of early PCV2 vaccination.

Acknowledgments

This work was performed as part of the DVM curriculum engaging all DVM students of the class 2015, their mentors and teachers, as well as support personnel. All animal experiments were conducted based on approved animal use permits. Funding was provided through the Faculty of Veterinary Medicine and the Alberta Livestock and Meat Agency (ALMA).

References

1. O'Neill K.C. et al 2011. Clin and Vacc Immunology 18(11):1865-1871.
2. Eschbaumer M., et al 2013. J Virol Methods 2014 198C:56-63.

O.023

Vaccine efficacy of current commercial vaccines against novel PCV2b variant strains

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Introduction

In the last years, increased numbers of PCV2 vaccine failure cases have been reported in the U.S. (1). These outbreaks commonly occurred in the mid-to-late finisher period. Further molecular epidemiology investigations revealed the presence of a variant PCV2b strain designated as mPCV2b in many of these cases (1). The mPCV2b strain identified in the U.S. in 2012 was very similar to isolates initially described in China in 2010 (2). Indications are that this mPCV2b strain is increasing in prevalence in Asia (3) and now also in the U.S. based on our investigations. The objective of this study was to determine the efficacy of current commercial PCV2 vaccines to protect caesarian-derived-colostrum-deprived (CDCD) pigs and conventional pigs against challenge with mPCV2b.

Materials and Methods

Initially, 26 CDCD pigs were randomly assigned to one of four groups including pigs vaccinated with a commercial PCV2a-based vaccine and challenged (PCV2a-VAC; n=7); pigs vaccinated with an experimental mPCV2b-based vaccine and challenged (mPCV2b; n=7); pigs sham-vaccinated with saline and challenged (positive controls; n=7); and pigs sham-vaccinated with saline without challenge (negative controls; n=5). Vaccination was done on day 0 and day 14. Challenge was done on day 28 using a tissue homogenate containing PRRSV and mPCV2b and the experiment was terminated on day 49. In a follow up project, 50 2-week-old conventional pigs were randomized into five treatment groups with 10 pigs in each group. Pigs remained unvaccinated (positive and negative controls) or were vaccinated at 3 (Ingelvac® CircoFLEX™, Boehringer Ingelheim Vetmedica; Fostera™ PCV, Zoetis Inc; Circumvent® PCV, Merck Inc.) and at 5 weeks of age (Merck group only) with one of three USDA licensed vaccines on the farm of origin. At 11 weeks of age, the pigs were transferred to the research facility and all pigs except the negative controls were challenged with mPCV2b. The experiment was terminated 21 days post challenge.

In both experiments, serum samples were collected weekly and tested for presence of PCV2 DNA by quantitative real-time PCR (4) and for presence of anti-PCV2 antibodies by ELISA (5). Amount of PCV2 antigen in tissues was determined by immunohistochemistry (IHC) (6).

Results

Among the challenged CDCD pigs, 47.6% (10/21) developed severe clinical disease and either died or had to be humanely euthanized between 11 to 20 days after challenge. PCV2 viremia was almost completely absent in the vaccinated groups regardless of vaccine type except for two PCV2a-vaccinated pigs which had detectable PCV2 DNA levels on individual days after challenge. Microscopic lesions typical of PCV2 infection were limited to the positive control group which developed mild-to-severe lesions associated with PCV2 antigen. Clinical disease was not observed in the conventional pigs. PCV2 viremia was significantly reduced in all vaccinated pigs compared to non-vaccinated groups. Microscopic lesions consistent with PCVAD were only observed in the positive control group.

Conclusions and Discussion

Emergence of novel PCV2b variants and association of these isolates with cases of PCVAD has raised concerns over efficacy of current vaccines. Under the conditions of the two studies we conducted, commercial PCV2 vaccines utilized in the U.S. appeared to be effective against mPCV2b challenge; however, further molecular characterization of samples from pigs in the conventional pig study is still in progress. There are several reasons for vaccine failure other than lack of cross protection between the vaccine strain and the field strain. PCV2 vaccine failures could be explained by vaccination compliance issues (no vaccine given, vaccine stored and handled improperly, vaccine used beyond expiration date, wrong dose, etc…) or failure of the pigs to respond appropriately to the vaccine due to other nutritional or health issues.

Acknowledgments

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References

1. Xiao CT et al. 2012. J Virol 86:12569.
2. Guo et al. 2010. Virol J 7:273.
3. Wei et al. 2012. Infect Genet Evol 17:87-92.
4. Opriessnig et al 2003 Vet Pathol 40:521-529.
5. Nawagitgul et al. 2002. Clin Diagn Lab Imunol 9:33-40.
6. Sorden et al. 1999 J Vet Diagn Invest 11:528-530.

O.024

Impact of two different vaccination programs against PCV2 in piglets born from long-term vaccinated sows on performance parameters up to slaughter under Brazilian field conditions

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Introduction

CIRCOVAC® is a PCV2 vaccine registered both in sows and piglets. Vaccination in both sows and piglets has been shown to be an effective strategy to control the damages of clinical (1) and subclinical (2) PCVDs in pigs. Interference of maternally-derived immunity and piglet active immunization is then under question. The present abstract refers to the impact on pig performance (part 1/2) of two different ages of PCV2 vaccination in piglets born from long term vaccinated sows.

Materials and Methods

In a 2000-sow farrow-to-finish operation located in the São Paulo province, Brazil, sow had been vaccinated according to the manufacturer recommendations with CIRCOVAC for more than 4 years. A total of 675 3-week-old piglets were allocated by whole litters randomized on dam parity to 3 experimental groups (G0, G1, G2). Groups G1 and G2 were vaccinated once, IM, 0.5 mL at 3 weeks of age (WoA) and 6.5 WoA respectively; G0 was kept unvaccinated. Each group was housed in 5 pens of 45 pigs. Individual bodyweights were recorded at weaning (3WoA), turn-to-fattening (65 DoA) and before slaughter (at 146 DoA for G0 and G2; at 150 DoA for G1). A standardized bodyweight was calculated at 146 DoA in G1 based on individual fattening average daily weight gain (ADWG). Bodyweight and growth data was analysed by first fitting a heterogeneous variance linear mixed model including group and sex effects as fixed effects. The pen was included as a random effect for the post-weaning and slaughter weights. The Tukey adjustment was used for pairwise comparisons. In addition, models adding the bodyweight at weaning as covariate were fitted to account for weaning weight heterogeneity (SAS® 9.3, Cary, NC, USA). Mortality was analysed using Fisher's Exact test. In addition, blood samples were collected and assayed by PCV2 ELISA for PCV2 antibody titration and by PCR for virus DNA evidencing.

Results

High anti-PCV2 titres were observed at weaning. The serological and virological results demonstrated active PCV2 circulation during the fattening period only in the unvaccinated pigs. The two vaccinated groups reached significantly higher slaughter bodyweights (146 DoA) as compared to the unvaccinated group (G0 vs G1: p=0.0011, G0 vs G2 p=0.0003) .There was no difference between the vaccinated groups (p=0.7576) (Table 1). Identical results were obtained in the models including weaning bodyweight as covariate (data not shown). Survival rate was very high whatever the group with no difference between groups (p>0.05), thus confirming again the subclinical PCVD.

Table 1. Growth and mortality performances

Group	G0 No vaccine	G1 3 WoA	G2 6.5 WoA
BW Weaning	6.4±1.4[a]	6.1±0.8[b]	6.7±0.9[c]
BW end P-W	26.5±3.7 [a]	25.4±2.2 [a]	27.7±2.0 [a]
BW Slaughter[1]	107.5±0.8[a]	111.1±1.8 [b]	111.7±1.2 [b]
ADWG P-W	457±59	439±40	476±36
ADWG Fattening	999±38	1058±14	1037±20
ADWG W-S[1]	808[a]±8	840[b]±11	839[b]±10
Mortality P-W	0%[a]	0.4%[a]	0.4%[a]
Mortality Fattening	2.7%[a]	0.9%[a]	2.2%[a]
Mortality W-S	2.7%[a]	1.3%[a]	2.7%[a]

BW:bodyweight; P-W: Post-Weaning; W-S: Wean-to-Slaugther; [1]: standardized weight at 146 DoA; [a,b]: different superscripts mean a statistical difference in LS-means, p<0.05.

Cconclusions and Discussion

Pigs have been allocated to experimental groups at weaning at different average weights due to the choice of randomization on dam parity and subsequent farm constraints. However, not only the differences have been taken in account in the statistical analysis but the lighter group (G1), in which piglets were vaccinated at 3 WoA appeared to perform as well as the G2 group and significantly better than the control group (G0). These findings added to the serological and virological results confirm a similar good control of PCV2 in the two vaccinated groups. They also can be related to a previous trial run in farms where sows were not vaccinated and where pig growth performance were not negatively impacted by high PCV2 MDA titres at the age of vaccination with the same vaccine (3).

As a conclusion, in the condition of this trial, piglet PCV2 vaccination efficacy was found to be the same, irrespectively if piglets were vaccinated with CIRCOVAC at weaning (3WoA) or at (6.5WoA).

References

1. Pejsak Z. *et al.* 2010. Comp. Immunol. Microbiol. Infect. Dis. 33(6):e1-5.
2. Vila T. *et al.*2014. Proc. 45th Annual meeting AASV, Dallas, TX, USA (accepted).
3. Fraile L. *et al.*2012. Vaccine, 30: 1986-1992.

O.025

Correlation between PCV2 delayed type hypersensitivity test and PCV2-specific IFN-γ responses in CIRCOVAC® vaccinated piglets

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Introduction

The delayed type hypersensitivity (DTH) test has been used for decades as an in vivo assay to measure cell-mediated immunity. Particularly, tuberculin skin test has demonstrated a clinically significant correlation in humans (1). Preliminary trials have shown that DTH test based on the use of CIRCOVAC antigen suspension in CIRCOVAC vaccinated piglets produced promising results for assessing compliance of vaccination (2). The aim of the present study was to asses if the strength of DTH responses were correlated with systemic cell-mediated immunity responses against PCV2 as measured in the IFN-γ ELISPOT.

Materials and Methods

Nineteen 3-week old piglets were selected and distributed in two groups: A (n=9) and B (n=10). Piglets in group A were vaccinated IM with 0.5 mL of reconstituted CIRCOVAC –an inactivated whole PCV2 virus-. B piglets were vaccinated IM with 1ml of a subunit PCV2 vaccine expressed in a baculovirus vector. Four weeks later groups A and B (7 weeks of age), and an additional group C (n=5) of 3 week old unvaccinated pigs (control) were inoculated intradermally in the lower abdomen area with 0.1 mL of the antigen suspension of CIRCOVAC (DTH test).

The DTH reaction was evaluated 18 h after the antigen inoculation considering the development of erythema as the main parameter. Oedema and induration were additional parameters. The diameter (D) of the erythematous reaction was measured with a digital caliper. The affected area was calculated and expressed in mm^2.

In parallel, heparinized blood samples were collected for obtaining peripheral blood mononuclear cells (PBMC). PCV2-specific IFN-γ-secreting cells (IFN-γ-SC) were measured by ELISPOT as described (3) using a pool of 45 15-mer overlapping PCV2 ORF2 peptides (5µg/mL per peptide) as stimulus. As a control, three irrelevant pools of peptides were also included.

Average DTH reaction sizes (erythematous area) and average numbers of PCV2-specific IFN-γ-SC were compared using non-parametric tests. Correlation between DTH test and PCV2-specific responses in ELISPOT were calculated using Pearson's test.

Results

After intradermal inoculation, all pigs except one in group B developed some degree of erythema. Only one pig showed induration and none suffered oedema. The erythematous area was significantly higher in group A (CIRCOVAC) as compared to the other groups (Table 1).

The difference between vaccinated groups for IFN-γ ELISPOT was not significant between vaccinated groups (Table 1).

Significant correlation between DTH test and IFN-γ ELISPOT was only observed in group A:
DTH test = 15.335467 ELISPOT + 51.064127.
$R^2 = 0.49$; $p<0.05$.

Table 1: Mean ± standard deviation of ORF 2 PCV2-specific IFN-γ-SC / $5x10^5$ PBMC and DTH test in PCV2-vaccinated (A and B) and un-vaccinated pigs (C). a>b ($p<0.05$).

GROUP	IFN-γ ELISPOT	DTH test
A	32.4 ± 26.2^a	548 ± 236^a
B	15.4 ± 13.8^a	90 ± 60^b
C	1.1 ± 1.7^b	162 ± 105^b

Conclusions and Discussion

Intradermal injection of the antigen caused some degree of erythema even in unvaccinated pigs. This is common for intradermal tests (e.g. turbeculin test) and requires the delimitation of one or more cut-offs depending of the circumstances (age, previous exposure status, maternal immunity, etc.). In any case, the present study revealed that the intensity of the skin reaction was specific for the vaccine used in group A fulfilling thus initial requirement of a compliance test. Correlation of the DTH test with the ELISPOT was somehow low suggesting that IFN-γ responses only have a partial participation in the reaction observed in the skin test. This is a preliminary assay that should be completed in the future.

References

1. Dietert RR *et al.* 2013. Methods Mol Biol 598, 185-94.
2. Callén A. *et al.* 2013. 45th Annual AASV meeting (*in press).*
3. Fort M. *et al.* 2009. Vet Imm Immunopathol 129, 101-107

O.026

Duration of immunity against PCV2 with Circumvent® PCV M

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Introduction

Porcine circovirus type 2 (PCV2) and enzootic pneumonia caused by *Mycoplasma hyopneumoniae (M. hyo.)* are infectious diseases of swine that continue to challenge the pork industry. Vaccination against both of these diseases has been shown to mitigate the negative effects of infection.[1] Circumvent® PCV M provides producers with a convenient tool for both PCV2 and *M. hyo.* protection.[2] The following studies demonstrate a duration of immunity (DOI) of at least 21 weeks for the PCV2 fraction of Circumvent® PCV M.

Materials and Methods

Fifty, 3-week-old commercial pigs were randomly assigned to one of two treatment groups, Circumvent® PCV M vaccinated (VACC) or placebo control (CONT). The vaccinated group was given 2 mL of Circumvent® PCV M intramuscularly (IM). Pigs in each placebo control group were similarly injected at 3 weeks-of-age with 2 mL IM. All pigs received a booster dose three weeks later with the same treatment. Pigs from the two treatment groups were commingled during the study.

Pigs were intra-nasally challenged with 6 mL of a mixture of virulent PCV2 and PRRSV, 3 mL/nare at 27 weeks-of-age. Pigs were observed daily throughout the study for general appearance and signs of clinical disease. PCV2 viremia and shedding were evaluated by PCR on blood samples, nasal swabs and fecal swabs that were collected weekly. Pigs were euthanized at 34 days post-challenge. Samples of tonsil, mesenteric lymph node and bronchial lymph node were collected. Tissues were placed in 10% neutral buffered formalin and shipped to the Iowa State University Veterinary Diagnostic Laboratory for histopathological examination, and testing for PCV2 antigen by IHC.

Results

One vaccinated and 3 placebo pigs were removed prior to completion of the study for non-vaccine related reasons. The table below provides a summary of the viremia, shedding, IHC and histopathology results. All placebo control animals were positive for viremia, and fecal and nasal shedding after challenge, whereas none of the vaccinated animals were positive for viremia, and only 14 of 24 and 5 of 24 vaccinated animals were positive for fecal and nasal shedding, respectively. The peak of viremia and fecal and nasal shedding was reached by 21 days post challenge for both groups. By the end of the challenge period, all of the vaccinated animals were negative for viremia, and fecal and nasal shedding while all of the placebo animals were still viremic, 21 of 22 (95%) were positive for fecal shedding, and 17 of 22 (77%) were positive for nasal shedding.

Tonsil, mesenteric lymph node and bronchial lymph node samples were evaluated for the presence of PCV2 by IHC. All vaccinates were negative for PCV2 in the lymphoid tissues except for 4 pigs with scores of 1 (sparse staining) for the tonsil, mesenteric or bronchial lymph node. In the placebo group, 4 pigs had scores of 2 or 3 (moderate or extensive staining), and 9 additional pigs had scores of 1 for the tonsil, mesenteric or bronchial lymph nodes. The tissues were also examined microscopically for signs of lymphoid depletion and inflammation. The vaccinated group had only 2 pigs with scores of 1, 2 or 3 for lymphoid depletion and inflammation in the mesenteric and bronchial lymph nodes, while the placebo group had 9 pigs with scores of 1, 2 or 3.

Parameter	VACC	CONT
Viremia	0/24	22/22
Nasal Shedding	5/24	22/22
Fecal Shedding	14/24	22/22
Tissue Infection	4/24	13/22
Lymphoid Depletion	2/24	9/22

Conclusions and Discussion

This study demonstrates that the combined, ready-to-use product Circumvent® PCV M is an effective tool for long-term (at least 5 months) protection against both shedding and disease caused by PCV2.

Acknowledgments

Thank you to the Merck animal services department for their help with these studies.

References

1. Thacker, B, W Wilson, C Francisco, R Schlueter. Circumvent® PCV vaccine: Performance evaluation and serological studies update. 2008. Proc. of the 39th Annual Meeting of AASV, San Diego, California, pp. 153-156.
2. Lehe, K, M Allen, F Roerink, B Thacker. Circumvent® PCV M: A new tool for combination PCV2 and Mycoplasma hyopneumoniae vaccination. 2011. Proc. 42nd Annual Meeting of AASV, Phoenix, Arizona, pp. 145-148.

O.027

Trends in applied PRRSV diagnostics

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Introduction

PRRSV diagnostic tools, methodologies, and applications continue to evolve. The Iowa State University Veterinary Diagnostic Laboratory's (ISU VDL) proximity and service to veterinarians and pork producers throughout the United States provides a reasonable view into the changing landscape of PRRSV diagnostic tools and how they are being applied in North America. The objective of this update is to share some of the more significant trends in PRRSV diagnostics observed over the past 5 fiscal years at the ISU VDL.

Conclusions and Discussion

The number of PRRSV PCR diagnostic assays conducted on an annual basis has increased by more than 300 percent from 2009 to 2013 (Figure 1), while PRRSV antibody testing volumes have remained relatively unchanged. PRRSV testing is increasingly being used as a preventative medicine and/or programmatic monitoring tool to determine the PRRSV status of weaned pigs, gilt or boar replacements, and boar studs. The percentage of diagnostic specimens submitted for PRRSV testing determined to be PRRSV positive has decreased from 27 to 16 percent (PCR) and 37 to 18 percent (ELISA) over this 5 year period.

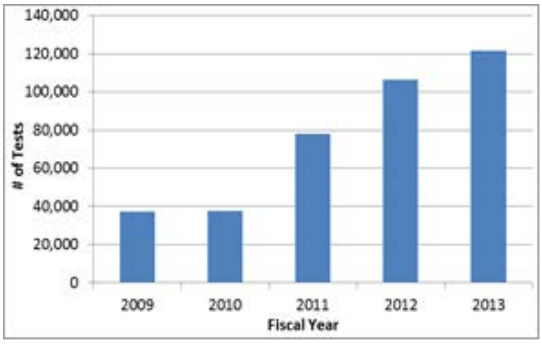

Figure 1. Total number of PRRSv PCR tests conducted at ISU VDL in 2009-2013.

Veterinary diagnostic laboratories have increased their use of commercially manufactured PRRS PCR reagents in lieu of in-house or lab-specific assays. These transitions have largely been made to enhance consistency and convenience at the laboratories. The advent and subsequent wide-spread adoption of oral fluids as a diagnostic specimen has profoundly changed PRRSV diagnostic applications in North America. Oral fluids are being used for both antigen and antibody detection purposes as well as in genetic sequencing applications to diagnose, monitor, and further characterize a growing number of swine pathogens. The number of swine oral fluid diagnostics assays conducted at the ISU VDL has grown from less than 5,000 in 2010, to more than 70,000 in 2013. Increased interest in PRRSV area regional control efforts and an associated increased use of PRRSV ORF5 genetic sequencing data as a tool for monitoring the movement and diversity of PRRSV across regions and production systems over time has been another notable trend in recent years. Significant improvements have and continue to be made to the diagnostic assays, laboratory procedures, and the quality of the diagnostic systems that are putting these tools into practice at veterinary diagnostic laboratories. As one such example, all of the core PRRSV diagnostic assays and methods currently being used at the ISU VDL are either new or have been significantly modified since November 2010. Collaborative efforts are being made to develop streamlined systems that link participating veterinary diagnostic laboratory submissions, corresponding test results, and an interpreted health status of farm sites to spatiotemporal disease management tools for use in area-regional, veterinary clinic, or production system specific PRRSV monitoring and control initiatives. One differential diagnostic technology gap that remains is the inability to use antibody based testing to differentiate pigs vaccinated with modified-live PRRSV vaccine from those infected with wild-type PRRSV. Molecular techniques are presently used for DIVA diagnostic purposes.

Acknowledgments

Iowa State University Veterinary Diagnostic Laboratory Faculty, Staff, and Clientele

References

1. Iowa State University Veterinary Diagnostic Laboratory Laboratory Information Management System (ISU LIMS).

O.028

Bioinformatics for improved pathogen detection: Maintenance of the *virotype*® PRRSV RT-PCR reagents for improved accuracy

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Abstract
Accurate pathogen detection is essential in many fields, ranging from infectious diseases diagnostics in humans and animal medicine to pathogen screening for biosecurity. However, the development of tools for specific pathogen detection can be very complex due to the existence of many pathogenic strains, with varying mutations, alongside the ever-present threat of new emerging strains. Typical examples include the Influenza A virus, infecting multiple hosts including humans, poultry, pigs, and horses, and Porcine Respiratory and Reproductive Syndrome virus (PRRSV), which can have catastrophic economic consequences for the swine industry. Both Influenza A and PRRSV have high mutation rates and regional strain variations exist.
virotype PRRSV NA/EU real-time PCR reagents are designed to detect North American and European PRRSV strains in a multiplex format with an internal positive control. The *virotype* PRRSV NA/EU reagents are derived from the *virotype* PRRSV Kit validated at the Friedrich-Loeffler-Institut for the German market..

Bioinformatics in Assay Design
For assay design, strain sequences for a given pathogen published in the NCBI[1] database are used as template for the pathogen-specific primers and probe design. The specific melting temperature of each assay combination is calculated in silico. The pathogen-specific primer–probe combination is then tested for the optimal temperature for amplification and detection real-time PCR.

Bioinformatics in Assay Maintenance
Bioinformatics are also used for surveillance of QIAGEN assays. Through the routine use of pathogen genomic characterization, the bioinformatics team can access the success rates of current assay oligonucleotide design, and when necessary identify critical sequences that may require assay is adaptation.
In 2013, diligent bioinformatics alerted critical sequence changes, which might impact the accuracy of the *virotype* PRRSV NA/EU Reagent. After notification from the bioinformatics team that the reagents were missing strains of the Midwestern region of US (Iowa), in silico PCR was applied to compare *virotype* PRRSV primer and probe design with PRRSV strains in the database. This analysis allowed for design modification for assay oligonucleotides, which was implemented to maintain accurate detection of the regional PRRSV strains, and maintain detection accuracy of other known strains.

Validation
The analytical sensitivity of the modified *virotype* PRRSV NA/EU oligonucleotides was performed in a translational research lab at Iowa State University (Trujillo). Utilizing purified RNA, from select PRRSV strains, we evaluated several modified oligonucleotides for the *virotype* PRRSV NA/EU Reagent alongside another commercially available PRRSV detection reagent.
Results show that by utilization of bioinformatics data to aid in assay evaluation and redesign, the modified *virotype* PRRSV NA/EU Reagent could accurately detect the regional strain and conventional strains. Furthermore, the modified *virotype* PRRSV NA/EU Reagent demonstrated improved sensitivity of detection as compared to the other commercially available PRRSV detection reagents for the regional isolate (strain Iowa 21).

Conclusion
Academic collaboration and attainment of sequence information for atypical virus isolates coupled with diligent deployment of bioinformatics aided in assessment and successful redesign of oligonucleotides utilized in the QIAGEN *virotype* PRRSV EU/NA Reagent. Furthermore, these partnerships and technology help QIAGEN to perform thorough assay maintenance adaptation to ensure detection of emergent virus genotypes. Diligent deployment of bioinformatic analysis on a regular bases (months), or in response to a reported outbreak, with new sequences continually being added to the internal database through collaboration will insure assay performance. Furthermore, relevant sequences in public databases, such as NCBI, are key players in this effort.
Based on this improved assay performance, the improved *virotype* PRRSV EU/NA Reagent set will be commercially available after March 2014. We encourage commercial users to collaborate with US for the continued assessment and improvement of this assay, as high mutation pathogens continue to evolve and challenge the diagnostic/surveillance methodology.

Acknowledgments
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References
1. National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov)

O.029

The required number of ropes for detection of PRRSV and antibodies in oral fluids: A field validation under Dutch field circumstances

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Introduction

PCR and antibody ELISA tests for (among others) PRRSv were optimised and validated for use in oral fluids (OF) at the GD laboratory.[1]. For practical use in the field however, epidemiological validation of the relative sensitivity (rel Se) and relative specificity(rel Sp) of the use of the OF-sampling method on herd level is necessary. Such a validation provides essential information about the minimal number of ropes necessary for detection of PRRSv by ELISA or PCR in a herd after an outbreak, or in situations with low prevalences.

Materials and Methods

Out of 101 OF samples and 1088 serum samples collected on 37 farms (which were selected by the practitioner based on a known history of PRRS) a selection of in total 42 OF samples was made based on a maximum interval of 48 hours between sampling and arrival at the lab (table 1).

Table 1.

Test	Farms	Pens (OF samples)	Piglets/ pen	Piglets	sampled Pens/farm
ELISA	10	22	10	231	1-3
PCR	14	42	11	481	1-5

For the serum samples the PRRS IDEXX X3 ELISA and the PRRSV real-time PCR were used. For OF the optimized test protocols were applied [1].
The pen prevalence (pp, the number of pens with one or more positive piglets) and the within-pen prevalence (wpp, the number of positive piglets per pen), were determined using the ELISA and PCR results from the individual serum samples, and compared with the OF test results. For the statistical analysis to calculate the rel Se and rel Sp of the OF method, Stata12 and Winepiscope2 was used.
The required number of ropes in lower or higher prevalences (low prevalent: wpp ≥10% and <50%, high prevalent: wpp ≥50%) were calculated for 500 weaned piglets (8-10 weeks of age) in 5 rooms of 10 pens and 10 piglets per pen (Freecalc2).

Results

ELISA: 19 out of 22 pens had at least one positive serum sample; the pp in the 10 farms was 86%. A wpp of ≥50% was found in 15 out of 22 pens (high prevalence), 13 OF samples in these pens were ELISA positive. The rel Se of the OF method was 68% (CI 95%: 48-89%). But when the wpp was low (<50% and ≥10%) (4 pens), no antibodies were detected in OF.
PCR: 21 out of 42 pens in 14 farms had at least one positive serum PCR; the pp was 50% (CI 95%: 34-66%). In 13 out of 42 pens on three farms with a wpp ≥50% (outbreak) all OF samples tested positive. This implied a rel Se of the OF method of 100%. 15 out of 21 pens with at least one positive PCR sample tested positive in OF. The rel Se was 71% (CI 95%:52-91%).
When the wpp was less than 50% in all pens, the rel Se was 25% (CI 95%:0-50%).
In high prevalence conditions, when wpp is ≥50% and the pp is 80-90 %, 2-3 ropes are needed for detection of PRRSv antibodies and virus.
When the wpp varies from low to high prevalence, 4-7 ropes are needed.
When the wpp is <50%, the OF method cannot detect antibodies or virus. Pooled serum samples in 3 of the 4 low prevalent pens were also negative while 1 individual positive sample was found.

Conclusions and Discussion

The OF method is very useful for diagnosing PRRS in case of an outbreak, with 2-3 ropes providing reliable information for diagnosing PRRS. When the wpp and the pp are decreasing, the number of ropes necessary for both antibody and virus detection increases.
Based on data from this trial, the OF method is not suitable for low prevalence situations. However, these data are lacking statistical power. The dataset contains only 4 pens with a wpp > 0% and < 50% for antibodies and 8 pens with this wpp for virus.
Therefore further research is needed to elucidate the usefulness of the OF method in low prevalent, field situations.

References

1. AJ van Hout et al.: 2012 IPVS abstract SO0173
2. Olsen, C et al. 2013. J. Vet. Diagn. Invest. 25(3):328-35.

O.030

Dynamic pattern of antibodies against PRRS in pig farms under tropical environment

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Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) is a disease that produces devastating effects which has plagued the pig industry in the last three decades[1]. After infection, antigen-specific humoral immunity first appears at about 7-10 days post infection, and this can be detected by conventional serological tests[2]. In Venezuela, serological studies shows a range from 42% to 90% of prevalence[3]. However, we have not data about the movement of PRRS antibodies through reproductive herd or downstream in the pig farms hitherto. For this reason we have investigated the kinetics of PRRSV-specific antibodies in several pig farms under tropical environment by using Elisa test.

Materials and Methods

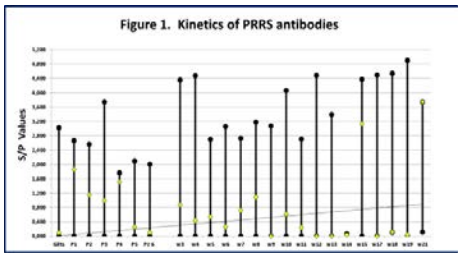
Sera lab data collected from twenty nine pig farms belonging to 6 states were used to this study. Sampling method include those from the breeding herd and downstream pigs. Samples range were from 25 to 83 sera per farm. Sera from breeding herd were grouped according to their conditions, ie, gilts, parity 1 to 6 or greater. In nursery- finish, sera were collected in pig of 3 to 15, 17 to 21 weeks of age. All sera were processed to by using Elisa test HerdChek 2XR PRRS and/or HerdCheck 3X Ab PRRS (Idexx Laboratories Inc, USA).

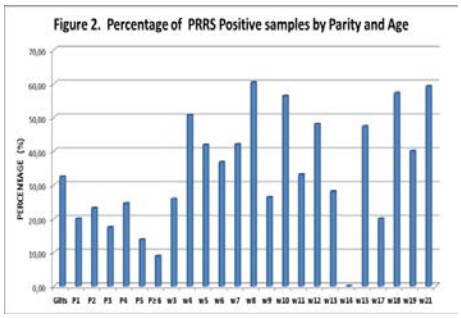
Results

1297 serum samples were analyzed during a two year period. We found PRRS prevalence in all farm breeding herds, we lesser positivity in parity 5 and parity ≥ 6 , 13,7% and 8,9%, respectively (Table 1). In pigs, positive S/P values were detected in every age. S/P values and positivity (ranging 30% to 60%) were high in pigs on 7 to 12 weeks of age, then a new raise of S/P values and positivity in pigs of 15 to 21 weeks of age, indicating a viral recirculation in the finishing period (Fig 1, 2). This viral recirculation can be explained in part because most farms are still continuous flow system (Farrow to Finishing operation).

Table 1. Results of S/P values in the Breeding herd and Pigs

Breeding Herd	Mean	Std Dev	Number	Pigs	Mean	Std Dev	Number
Gilts	0,5251	0,8005	74	*Week 3*	0,5499	0,8722	62
Parity 1	0,2786	0,5596	65	*Week 4*	0,8017	1,0128	75
Parity 2	0,2863	0,5286	65	*Week 5*	0,5061	0,6716	67
Parity 3	0,2706	0,6423	69	*Week 6*	0,5741	0,8861	101
Parity 4	0,2823	0,4659	53	*Week 7*	0,5454	0,6626	50
Parity 5	0,1881	0,3845	58	*Week 8*	1,1790	1,1326	68
Parity ≥ 6	0,1420	0,3695	46	*Week 9*	0,5688	0,9439	57
				Week 10	1,1175	1,1841	64
				Week 11	0,7387	0,9042	15
				Week 12	1,2024	1,3837	75
				Week 13	0,5662	1,0724	25
				Week 14	0,0258	0,0215	10
				Week 15	1,3188	1,4878	72
				Week 17	0,5455	1,1866	25
				Week 18	1,3240	1,4343	42
				Week 19	0,7637	1,3308	15
				Week 21	1,3127	1,2147	44





Conclusions

Under the conditions of this study, there appear to be still high PRRS positivity in the farms studied, mainly in the breeding herd. We also could see two moments of viral circulation. One between 7 and 12 weeks, and the other one between 15 to 21 weeks of age but much more active than the first. In Conclusion, PRRS still remains a problematic disease in our country, despite the prevailing tropical conditions in the regions where pigs are raised.

References

1. Hess, A et al. 2012. Proc. International PRRS Symposium/NSIF Conference. Chicago, Illinois, USA. Abstract # 70. p88.
2. Murtaugh, MP. 2004. Proc. AASV. Des Moines, Iowa, USA. p359-368.
3. Diaz, CT et al. 1998. Proc.15th IPVS Congress 15:313.

O.031

PRRSV surveillance using pre-weaning oral fluid samples detects circulation of wild-type PRRSV

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Introduction

The need for efficient, effective surveillance is particularly true for porcine reproductive and respiratory syndrome virus (PRRSV), which remains one of the most costly diseases of swine worldwide, imposing significant losses on North American (1), European (3) and Asian producers (2). Based on our cumulative global experience, it would seem that achieving control of PRRSV will require that animal health specialists develop the capacity to easily, efficiently, and continuously surveil herds for PRRSV.

The purpose of the present study was to evaluate a method to detect PRRSV circulation in the breeding herd and growing pig populations based on collection and testing of oral fluid samples prior to weaning.

Materials and Methods

Four PRRSV vaccinated commercial swine herds (~12,500/farm) participated in the study. All four herds were endemically infected with PRRSV and all sows had been vaccinated ≥ 2 times with PRRSV modified-live virus vaccines (Ingelvac MLV®, Boehringer Ingelheim Vetmedica, Inc., St. Joseph. MO). Oral fluid samples were collected from 600 litters 24 hours prior to weaning and serum samples from their dams post-weaning. Samples were completely randomized and tested for PRRSV (RT-qPCR and sequencing) and PRRSV antibodies using commercial ELISA Kits (PRRS X3 Ab Test and PRRS Oral Fluids Ab Test, IDEXX Laboratories, Inc., Westbrook, ME) and antibody isotype-specific assays (IgM, IgA, and IgG). In addition, PRRSV ORF5 sequencing was attempted on RT-qPCR-positive samples.

Results

All serum samples (n = 600) were negative and 9 oral fluid samples (n = 600) were positive by PRRSV RT-qPCR. These results are matched from both screening (ISU-VDL) and confirmatory (Tetracore) testing in 2 diagnostic labs. Open reading frame 5 (ORF5) sequencing of 2 of the 9 positive oral fluid samples identified wild-type viruses as the source of the infection.

A comparison of antibody responses in RT-qPCR positive vs. negative oral fluid samples showed significantly higher IgG S/P ratios in RT-qPCR-positive oral fluid samples (mean S/P 3.46 vs. 2.36; $p = 0.02$). Likewise, sow serum samples from RT-qPCR-positive litter oral fluid samples showed significantly higher serum IgG (mean S/P 1.73 vs. 0.98; $p < 0.001$) and Commercial Kit (mean S/P 1.97 vs. 0.98; $p < 0.001$) S/P ratios.

Conclusions and Discussion

The data from the present study suggest that PRRSV herd endemnicity is maintained, at least in part, by a cycle that involves subclinical PRRSV infection in suckling piglets, with transmission to the population of growing pigs occurring as pigs are moved and mixed post-weaning. This study showed that pre-weaning litter oral fluid samples could provide an efficient and sensitive approach to surveil for PRRSV in infected, vaccinated, or presumed-negative pig breeding herds.

Acknowledgments

This project was funded in part by the PRRS CAP USDA NIFA Award 2008-55620-19132. PRRS X3 Ab Test ELISA Kits were provided by IDEXX Laboratories, Inc., Westbrook ME. Testing at Tetracore, Inc., Rockville, MD was provided at no charge.

References

1. Holtkamp et al. 2013. J. Swine. Health. Prod. 21, 72-84.
2. Tian et al. 2007. PLoS One. 2, e526.
3. Velasova et al. 2012. BMC Vet Res. 8, 184.

O.032

Oral fluid samples collected at the abattoir can expedite PRRSV surveillance

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Introduction

The control and/or elimination of PRRSV is a key goal for pork producers because of its impact on animal health and productivity (1). A roadblock to the implementation of effective control programs has been need for herd PRRSV data both at the planning stage and thereafter to monitor progress. Historically, these data were based on testing individual pig serum samples, but recent studies have shown that pen-based oral fluid samples are a more sensitive yet less expensive surveillance specimen (2). The objective of this study was to evaluate surveillance based on testing oral fluid samples collected at an abattoir. Cooperative farms were located in a region where PRRS vaccination was not performed. This made it possible to establish herd status by testing oral fluid specimens for PRRSV antibody.

Materials and Methods

The study was conducted by a cooperative (196 farms) with its own slaughter house and diagnostic laboratory. The abattoir was equipped with 22 pens (40 pig capacity). Pigs from farms were unloaded into pens and pigs from different farms were not mixed, i.e., source farms were easily identified. Over a period of 4 weeks, oral fluids were collected from each pen of ≥15 pigs by suspending a cotton rope in the pen for ~30 minutes. Thereafter, the oral fluid sample was tested using a commercial PRRSV oral fluid antibody ELISA (IDEXX Laboratories, Inc., Westbrook, ME, USA). Samples with a S/P ≥ 0.4 were considered positive.

Results

Oral fluids from 92 farms were collected and tested in a period of 4 weeks (Figure 1). Among the 92 samples (farms) tested, 18 were positive for PRRSV antibody (mean S/P = 3.63). Among the 18 positive samples, 12 had S/P ratios >1.0. Among the 84 negative samples, the mean S/P was 0.195. Each farm was readily identified and immediately notified of the testing results.

Conclusions and Discussion

This approach provided an efficient and cost-effect method to determine farm PRRSV status. Oral fluids were easily collected from pigs at the abattoir despite the stress of travel, mixing, and relocation to new surroundings. At the abattoir, the collection of oral fluids neither disturbed the normal work flow nor required extra personnel.

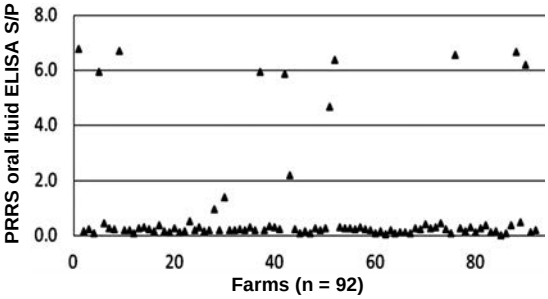

Figure 1. PRRS oral fluid ELISA S/Ps by farm (n = 92)

In the case of PRRSV infection, both PRRSV nucleic acid and antibody are present in oral fluids, but shedding of RNA is highly transient, whereas antibody is detectable for months. In the present example and in other circumstances in which antibody is indicative of herd infection or immune status, antibody ELISAs are usually the test of choice.

ELISA technology is simple, robust, cost-effective, amenable to high-throughput and highly uniform across laboratories - unlike many other diagnostic technologies. Thus, the PRRS oral fluid ELISA was found to be both highly repeatable within laboratories and highly reproducible across laboratories (3).

In this study, the PRRS oral fluid antibody ELISA provided clear discrimination between positive and negative farms, i.e., the mean S/P ratio of positive farms was 30 times higher than negative farms. Implementation of this aproach in conjuction with other measures, e.g., biosecurity, will allow negative farms maintain negativity and positive farms to take steps to control and eventually eliminate PRRSV.

Acknowledgments

Aqualab SAS, Bogota, Colombia

References

1. Neumann et al. 2005. J Am Vet Med Assoc 227:385-392.
2. Olsen et al. 2013. J Vet Diagn Invest 25:328-335.
3. Kittawornrat et al. 2012. J Vet Diagn Invest 24:1057-1063

O.033

Validation of a behavioral test for assessing pain in piglets following castration

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Introduction

Castration is a common surgical procedure performed on male piglets, primarily to prevent the development of boar taint. Castration is a painful procedure, but is largely performed without anesthesia. Ethical questions surrounding this practice have led to the need to develop effective pain control strategies. This work is hindered by a shortage of tools for assessing post-operative pain in piglets. Studies have assessed castration pain using behavior observations (tail wagging, rump scratching, dog sitting). However, this method is time consuming and it is difficult to identify clear indicators of pain (1,2,3,4) The objective of this study was to validate the use of a novel behavioural test for the assessment of pain in piglets following castration using a handling chute.

Materials and Methods

The study was performed using 68 male Yorkshire X Landrace piglets from 13 litters between 4 and 6 days of age. A portable chute specially designed for evaluation of post-operative pain in piglets was used in each trial. The chute measured 177cm in length x 18cm wide x 33cm high, and created a corridor for piglets to walk down with an exit that allowed them to return to their farrowing pen. Adjustable hurdles were placed at two points along the chute, forcing the piglets to step over them to exit the chute. All piglets were trained to navigate the chute at 4 days of age in 4 successive runs, 24 hours prior to castration and testing. Hurdles 10cm in height were used for all tests.

Piglets were assigned to one of four treatments: sham-castration with a saline injection (SS, n=17), castration with a saline injection (CS, n=17), castration with a half dose (0.2mg/kg^{1}) of Metacam® (CH, n=17), castration with a full dose (0.4mg/kg^{1}) of Metacam® (CF, n=17). All injections were administered one hour prior to treatment (castration or sham castration). The time taken to navigate the chute (NT) was recorded at 10 minutes before treatment (PT), immediately after treatment (0 min) and again at 15, 30, 60, 240, 480 and 1440 minutes after treatment. Behaviour of piglets in the farrowing pen was recorded continuously from 30 seconds until 1 hour after treatment, and the posture and activity of study piglets was recorded by instantaneous scan sampling at five minute intervals. Data were analyzed using a mixed model (Proc Mixed, SAS 9.3) with a repeated measures design and fixed effects of treatment, run time, interaction of treatment and run time, piglet weight and pretreatment navigation time, with litter as the random intercept. Behavioral differences were evaluated using generalized estimating equations (Proc Genmod), with results indicating the proportion of observations in which piglets performed each behavior.

Results

At 0 min after treatment SS piglets had a significantly shorter NT than CS piglets (p <0.05), with CH and CF being intermediate. At 15 minutes after treatment, medicated (CH and CF) and SS piglets all had significantly shorter navigation times compared to CS piglets (p <0.01), and at 30 minutes CF piglets showed a tendency for reduced NT compared to CS piglets (p =0.07). No differences in NT were found in subsequent runs. Piglet behavior in the farrowing pen following treatment showed that CF piglets did more nosing and rooting behaviors than CS piglets (p< 0.05). CF piglets also spent less time lying ventrally compared to CS piglets (p< 0.05), with no differences between other treatments. CH piglets spent more time nursing and less time sleeping than SS piglets (p< 0.05), with all other treatments being intermediate.

Conclusions and Discussion

Results of this work indicate that the handling chute was effective at clearly distinguishing different levels of pain at 15 minutes following castration, with some evidence of this response at 0 and 30 minutes. The Metacam® treatments showed a clear benefit at 15 minutes, with piglets' responses being similar to those of sham castrated piglets. The lack of difference between the two drug dosages suggests that a lower dosage may be effective in piglets and merits further study.

Acknowledgments

Project funding was provided by the WCVM Undergraduate Research Fund and the National Pork Board. Strategic program funding was provided by Sask Pork, Manitoba Pork Council, Alberta Pork and the Saskatchewan Agricultural Development Fund.

References

1. Hay M et al. 2003. Appl. Anim. Behav. Sci. 82:201-218.
2. Hansson M et al. 2011. Acta Agr. Scand. 53: 34.
3. Kluivers-Poodt M et al. 2013. Animal 7:7: 1158-1162.
4. Llamas-Moya S et al. 2008. Appl. Anim. Behav. Sci. 111: 133-145.

O.034

Evaluation of the anaesthetic depth during piglet castration under isoflurane anaesthesia - a field trial

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Introduction

To date, surgical castration without anaesthesia is the most common practice in swine production in Europe, although it is evidenced that castration is accompanied by pain and stress (1). In the near future, castration without anaesthesia will be prohibited by welfare legislation in some European countries. Then isoflurane anaesthesia will be one of the options for piglet castration. It permits a safe and rapid anaesthetic induction and maintenance, as well as brief and smooth recovery (2). In combination with an analgesic, isoflurane anaesthesia provides considerable stress and pain reduction (4). Therefore, the objective of this study was to evaluate the practicality and effectiveness of automated isoflurane anaesthesia on commercial swine farms.

Materials and Methods

The study took place in three commercial swine farms in Germany (farm A and B: 200 sows; farm C: 540 sows). A total of 1166 male piglets (age 3 to 6 days) was evaluated for the assessment of the anaesthetic depth. All piglets were given a NSAID treatment directly before castration. Castration and anaesthesia were performed by the farm manager in presence of a veterinarian. All farmers used the PIGNAP Pro® (Agrosystems GmbH, Switzerland) automated anaesthetic device (5Vol.% isoflurane in 30% oxygen; flow rate: 2L / min). The intensity of vocalization and defensive movements of the piglets was rated by using a score system (3). Also the presence or absence of the palpebral and the flexor reflex were noted. Every second piglet was weighed and the oxygen saturation and pulse frequency (OXYPLUS VET, KTMEC INC. medical systems, South Korea) were measured during treatment. For the evaluation of the anaesthetic depth, the weighted piglets were classified into weight groups.

Results

Overall, only 77% of 1166 observed piglets (895) showed a sufficient anaesthetic depth. The rate was 75% in case of farm A, 69% for farm B and 82% for farm C. As shown in Figures 1, the probability for a sufficient depth of anaesthesia decreases with increasing age and weight. The measurements of pulse frequency and oxygen saturation showed an average oxygen saturation of 98% and an average heart rate of 270/min during anaesthesia.

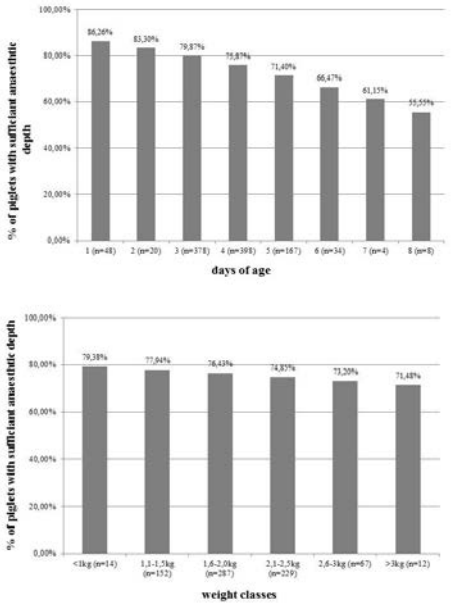

Figure 1. Depth of narcosis in dependency of age and weight

Conclusion and Discussion

Only 77% sufficiently anaesthetised piglets is not an adequate result for the commercial application of this technology. Heavier piglets receive too little anaesthetic agents, in contrast lighter animals too much. This problem could be solved by installing a scale into the anaesthetic device to perform a weight-dependent initiation of anaesthesia to increase the rate of sufficiently anesthetized piglets.

References

1. Hay et al. 2003. Appl Anim Behav Sci 82, 201-218.
2. Hodgson et al. 2006. Vet Anesth Analg 33, 207-213.
3. Hoppe 2011. Univ. Vet. Med. Hannover, Germany, Thesis.
4. Schulz et al. 2007. Dtsch Tierärztl Wschr 120, 177-182.

O.035

Comparison of rectal thermometry to an implantable temperature transponder system in grower pigs

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Introduction

To monitor swine herd health status and for early disease detection, a practical system to determine body core temperatures of large numbers of animals is desirable. Rectal thermometry, which is considered to be gold standard, is time consuming and may influence body temperatures in swine due to manipulation. Several studies confirm the applicability of microchip-systems to assess temperature in laboratory animals[1,2]. It is hypothesized that temperature measured by subcutaneously implanted microchips correlates to rectal temperature and could therefore be used as an indicator for herd health status in swine. In this study, body temperature measured by an implantable temperature transponder was compared to rectal thermometry in grower pigs.

Materials and Methods

Eighteen grower pigs were housed in a barn on straw bedding. Barn temperature varied between 19.0 and 22.0 °C. In each pig two microchips (IPTT-300 transponder system, read out by DAS 7007 reading device of Bio Medic Data Systems (BMDS) (Plexx, The Netherlands)) were inserted at two different positions using the pre-loaded needle assembly. The first microchip was implanted at the left ear base vertically in the subcutaneous tissue. The second microchip was implanted subcutaneously in the *fossa ischiorectalis*. Body temperatures (rectal and subcutaneous temperature) were recorded on three consecutive days both in the morning and in the afternoon (8 am, 4 pm). To generate more data, the experiment was done twice (6th and 12th week of life). For data evaluation IBM SPSS statistics (IBM Corporation, New York, U.S) was used.

Results

Temperature measured in the subcutaneous tissue of the *fossa ischiorectalis* showed a poor, but significant correlation to rectal thermometry (r^2=0.442, p≤0.001). Mean rectal and subcutaneous temperature of the *fossa ischiorectalis* differed significantly (p≤0.001) with a mean difference of 1.58 °C.

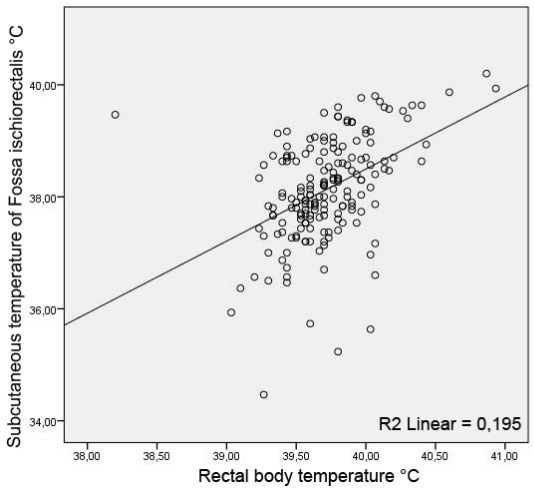

Figure 1. Correlation between rectal and subcutaneous temperature of the *fossa ischiorectalis*

There is also a significant, but poor correlation between mean temperature measured in the subcutaneous tissue of the ear base and mean rectal temperature (r^2=0.222, p=0.002). Mean difference between mean rectal and mean subcutaneous temperature of the ear base was 0.97 °C (p≤0.001); calculated coefficient of determination (R2) was 0.049.

Discussion

According to our data, it is not possible to conclude from subcutaneously measured temperature (ear base and/or *fossa ischiorectalis*) to rectal temperature in individual pigs. Microchips with temperature-sensing capacity are an appropriate tool used in laboratory animals[1,2]. However, the results of the present study indicate, that the application of implantable temperature transponders in research in swine as well as in pork production is highly restricted.

References

1. CILIA, J. et al., 1998, J Pharmacol Toxicol Meth. 1998; 40:21–26.
2. KORT, W.J. et al., 1998; Lab Anim 32: 260 –269.

O.036

Evaluation of lameness prevalence in a large Canadian sow herd and an intervention to reduce prevalence

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Introduction

Lameness is a major welfare concern that affects sow performance and herd profitability, and has been identified as one of the main reasons for culling (1). Hoof lesions represent damage to the structure of the hoof, and have been observed in over 80% of surveyed sows (2). Despite the negative economic consequences of lameness, little is known about its prevalence within commercial sow herds, or how to treat lameness when it arises. The purpose of this study was: 1) to assess the prevalence of lameness and hoof lesions in a commercial sow herd; and 2) to determine the effectiveness of a comprehensive treatment strategy at reducing lameness.

Materials and Methods

The locomotion of sows (n = 3,541, mixed parities) was observed as animals were moved from breeding to gestation stalls at 4 weeks gestation. Sows were assigned locomotion scores on a scale of 0-3 (0=sound, 3=severely lame), based on the Zinpro FeetFirst© scale, and hooves scored for lesions (0-3 scale, Zinpro FeetFirst©). To evaluate a treatment strategy for lameness, 200 sows of mixed parity with a lameness score of 1-3 were assigned to either a control (C) or treatment (T) group (balanced for parity) at four weeks gestation. Sows in the C group were monitored throughout gestation but received no treatment. Sows in the T group were given a rubber stall mat at 4 weeks gestation, received anti-inflammatory (NSAID) injections at 4 and 8 weeks gestation, and a corrective hoof trim at 8 weeks. All sows received a detailed hoof evaluation at 4 weeks gestation, at 8 weeks gestation while restrained in the Zinpro FeetFirst© chute, and again at 13 weeks. Gait scores were again evaluated at 8 and 13 weeks gestation. Back fat and body weight were recorded at 4 and 12 weeks of gestation, and sow productivity was recorded after farrowing.

Statistical analysis was performed in SAS 9.2 (Cary, NC, USA). For the lameness treatment study, the total hoof lesion score (sum of all hoof lesions) was calculated for each hoof, for each of the weeks (4, 8 and 13), as well as changes in the gait score between assessments. Proc Glimmix was used to determine differences in total hoof lesion scores and changes in gait scores over time. A Fisher's exact test was used to determine the association between treatment groups and the prevalence of lameness.

Results

The survey included 59% of the herd, with parities ranging from 0-10 (mean 3.9). Of surveyed sows, 54% had a score of 1 (abnormal gait), 6% had a score of 2 (clear lameness in a particular leg), and 0.23% had a score of 3 (severely lame). Hoof lesions were present in 94% of sows surveyed. The most common hoof lesions were heel overgrowth on the hind feet, and overgrown dew claws on both fore and hind feet.

Among sows selected for the intervention study (n = 198), 70% had an initial gait score of 1, 29% a gait score of 2, and 2% a gait score of 3. At week 4 of gestation there was no significant difference in lameness or hoof lesions between treatments. Between weeks 8 and 13 of gestation a significant reduction in average gait score was found in T sows (p <0.01), and by week 13 a greater number of T sows had a lower gait score (p <0.001). Productivity at farrowing did not differ between C and T sows.

Conclusions and Discussion

Results from the survey found that over 60% of sows had lameness or abnormal gait, and almost all animals had hoof lesions. This raises concerns regarding the well-being of sows and productivity of the herd. The high prevalence of hoof lesions requires more investigation to determine the cause, as this is likely to be herd specific. Results of the intervention strategy show that a comprehensive treatment can have a positive effect, as it successfully reduced the severity and prevalence of lameness over one gestation. A next step will be to refine the treatment to identify the contribution of each component towards lameness reduction. It will also be important to determine effects on sow longevity within the herd, and the cost benefit implications.

Acknowledgments

Funding was provided by Agriculture and Agri-Food Canada's Canadian Agricultural Adaptation Program, the Saskatchewan Agricultural Development Fund and provincial pork councils: Sask Pork, Alberta Pork, and the Manitoba Pork Council.

References

1. Pluym L 2011. Vet Med Czech 3:101-109
2. Anil S S 2007. J Swine Health Prod 15(2):78-83

O.037

Umbilical hernia and differential diagnoses in slaughter pigs

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Introduction

Umbilical hernia is a common developmental defect in pigs (1). Although, several differential diagnoses to umbilical hernia exist, all visible bulges in the umbilical region of pigs are registered as umbilical hernias when slaughtered (2). The prevalence of differential diagnoses to umbilical hernia has not been investigated (3, 4, 5). In a previous unpublished study of 36 pigs registered with umbilical hernia at slaughter, only 55.6% actually had a true umbilical hernia. The object of this study was to investigate the prevalence of umbilical hernia and differential diagnosis in pigs registered as having an umbilical hernia at slaughter.

Materials and Methods

The ventral abdominal wall of pigs registered as having an umbilical hernia was sampled at slaughter, Danish Crown, Ringsted, Denmark. The samples were subjected to a pathoanatomical investigation at Department of Veterinary Disease Biology, University of Copenhagen. For gross examination, all samples were cross sectioned. When paddle-formed proliferations (PFP) were present in the abdominal cavity, tissue was collected for histology and stained with hematoxylin and eosin.

Results

In total, 162 samples were included in the study (Table 1). As it appears from Table 1, eight pathological conditions were diagnosed (umbilical hernia, cysts, paddle formed proliferation, subcutaneous fibrosis, abscess, preputial diverticulitis, peritonitis and patent urachus). Only 17.3% of the samples were umbilical hernia without other pathological manifestations. The most frequent diagnosis was multiple cyst containing hemorrhagic and serous fluid. In 25.9% of the samples, paddle-formed proliferations of vascularized collagen tissue lined by mesothelial cells are present on the internal wall of the abdominal cavity.

Conclusion and Discussion

Failure to differentiate between true umbilical hernia and the variety of differential diagnoses most likely leads to incorrect treatment of animals with impact on animal welfare, use of antibiotic treatment and farm economy.
The presence of paddle-formed proliferations located on the internal ventral wall of the abdominal cavity seems not previously to have been reported.

Table 1. Number of samples and prevalence of lesions registered as umbilical hernia

DIAGNOSIS	NUMBER OF SAMPLES	PREVALENCE
Hernia	28	17.3 %
Hernia and cysts	2	1.2 %
Hernia and PFP[1]	12	7.4 %
Hernia and fibrosis	7	4.3 %
Hernia and abscess	1	0.6 %
Hernia, cyst and PFP[1]	2	1.2 %
Hernia, cyst and fibrosis	1	0.6 %
Hernia, PFP[1] and fibrosis	5	3.1 %
Cyst	46	28.4 %
Cyst and PFP[1]	4	2.5 %
Cyst and fibrosis	6	3.7 %
Cyst and abscess	2	1.2 %
Cyst, PFP[1] and fibrosis	2	1.2 %
PFP[1]	5	3.1 %
PFP[1] and fibrosis	11	6.8 %
PFP[1] and abscess	1	0.6 %
Diverticulitis	2	1.2 %
Fibrosis	9	5.6 %
Abscess	5	3.1 %
Peritonitis	1	0.6 %
Patent urachus and abscess	1	0.6 %
Undiagnosed	9	5.6 %

[1]Paddle-formed proliferations.

References

1. Edwards MJ et al. 1999. Diseases of Swine 8th edition, Iowa State University Press, 704-705.
2. Oberthon JS 2013. Personal message. Ministry of Food , Agriculture and Fisheries, Danish Veterinary and Food Administration.
3. Searcy-Bernal R et al. 1994 JAVMA 204: 1660-1664.
4. Vestergaard K et al. 2010. Pig Research Center, Denmark No 885.
5. Foelger AF 1951. Almindelig patologi for veterinærstuderende og dyrlæger. Den Kgl. Veterinær- og Landbohøjskole, 176-177.

O.038

Comparison of two anesthetic techniques (Azaperone-Propofol and Azaperone-Metomidate) on the castration of sows under field conditions

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Introduction

In veterinary practice, general anesthesia or chemical restraint in swine is required for diagnostic and surgical procedures including hernia repair, cesarean, physical and radiographic examination. Several anesthetic agents have been used in pigs but there are different opinions about its use (3,4). Pigs are difficult to anesthetize animals under practical conditions. For this reason the drugs are administered intramuscularly to produce a degree of sedation are preferred so that the subsequent handling and venipuncture easier to carry (1.2). Pig anesthesia under field conditions requires the use of anesthetics and techniques that are practical and economical but do not require sophisticated or expensive equipment (5).

Material and Methods

10 hybrid sows were used in growth with an average of 37 kg. Two groups (A and B), each group consisted of five sows were randomly formed. Each bristle group A was tranquilized with azaperone at a dose of 0.4 mg / kg body weight by intravenous route followed by application of a dose of propofol to 0.83 mg / kg body weight intravenously. Sows in group B were tranquilized with azaperone a dose of 0.4 mg / kg body weight intravenously followed by application of Metomidate at a dose of 2.5 mg / kg body weight intravenously. Both groups underwent ovariectomy on the left flank. The results of this study were subjected to a completely randomized design with 5 replicates per treatment. The averages of each variable were compared by the Duncan test.

Results

The averages for the induction time, recovery time, heart rate, respiratory rate and body temperature of anesthetized sows azaperone-propofol combination (AP) and azaperone-Metomidate (AM) are seen in Table 1.

Table 1. Averages induction time, recovery time, heart rate, respiratory rate and body temperature of anesthetized sows azaperone-propofol combination and azaperone-Metomidate

Type Anest	T. Ind.. min	T. Rec. min	F.C (L/min)	F. R. (R/min)	T. C. °C
A-P	3.2[a]	51[a]	72[a]	15[a]	38.1[a]
A-M	2.5[a]	78[b]	82[b]	14.1[a]	37.8[a]

(a,b) superscription indicate statistically significant differences ($p < 0.05$).

Conclusions and Discussion

In the present experiment an induction time of 3.2 minutes for the combination (AP) and a time of 2.5 minutes for the combination (AM) was found, no significant difference was observed ($p < 0.05$). The recovery time was greater for the combination than for AM AP combination ($p < 0.05$). From the results obtained and on the conditions under which this study was conducted it is concluded that the anesthetic combination AP can be used to perform minor surgeries such as castrations in growing pigs not involving deep sedation in pigs**.**

References

1. Anderson, L. I. (1977). Anesthesia of swine. N.Z. V. J. 25:319-321
2. Arellano, T. J. (1990). Alternativas con propofol. Rev.Anest. Mex. 2(4): 149-152
3. Bresse, B. A., Constance, E., Dodman, H.N. (1984). Xilacine-Ketamine-Oximorphone: An inyectable anesthetic combination in swime. JAVMA. 184 (2): 182-183.
4. Noorsdy, L. J. (1994). Food Animal Surgery. 3rd ed. VLS Books. U.S.A.
5. Thurmon, C. J., Benson, G. J. (1979). Anesthesia of Swine Under Field Conditions. JAVMA. 174 (6): 594-596

O.039

Welfare (productivity) consequences of the Danish "Yellow Card" debate

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Introduction

During 2010, the "Yellow Card" programme was introduced in Denmark (1). The authorities wished to reduce the use of antibiotics (AB) in the pig production by subjecting herds with high AB usage to fines and regulations. Even before the program was instigated, the public debate made many farmers reduce the AB consumption and the overall usage dropped by ~25% in the following year. Hence, the aim of this project was to determine if this decrease had any effects on animal welfare (measured as daily weight gain and mortality) in affected grower herds (7-30 kg).

Materials and Methods

Herds with an AB consumption of >3.5 kg active compound in the year before June 2010 (1), a reduction in AB consumption of >10% the following year and >500 registered pen places for growers were randomly selected from the national database, Vetstat (2). Organic and outdoor herds, herds that had suffered severe disease outbreaks, had performed eradication programs or made any other major changes during the study period were excluded. AB consumption was calculated as gram active compound AB/pen places and as average number of daily doses given per 100 animals per day (ADD/100 animals/day) (3). Data on number of animals produced, daily weight gain (DGW) and mortality were collected for the entire study period. To test for difference between years before and after June 2010 paired t-tests were performed (significance level 95%) (SAS Enterprise Guide 4.3).

Results

53 grower herds were included in the study for mortality. 87% of these were included in the study on DWG (46/53). The 53 herds had 2922 grower pen places in average (600-11000). AB consumption decreased with approximately 50% in the year following June 2010 regardless of calculation method (Table 1). Overall DWG before and after June 2010 was 449 grams/day (std.dev 64) and 444 grams/day (std.dev 66) respectively (P=0.15). Overall mortality increased from 2.4% (std.dev 1.1) to 3.0% (std.dev 1.5) (P<0.001). Herds with an AB consumption ≥25 ADD/100 animals/day in the year before June 2010 had a significantly higher increase in mortality (62.4%) compared to herds with a AB consumption <25 ADD/100 animals/day (26.6%) (P=0.04) in the same period.

Table 1. Antibiotic (AB) consumption in the participating 53 grower herds

		Use of antibiotics		
		Average	Std.dev	P-value
Gram AB/pen place	Period 1[1]	13.2	7.9	<0.001
	Period 2[1]	6.3	3.4	
ADD/100 animals/day	Period 1[1]	19.6	12	<0.001
	Period 2[1]	9.6	4.8	

[1] Period 1: 1st of June 2009 to 31st of May 2010; Period 2: 1st of June 2010 to 31st of May 2011.

Conclusions and Discussion

The decrease in average DWG of 11 grams/day and the significant 25% increase in mortality suggest that a reduction in AB consumption may affect animal welfare, especially in herds with a high previous AB consumption. The increase in mortality was also significant in a model, which took the longitudinal nature of the data into account (PROC Mixed, SAS Enterprise 4.3). A similar study performed in finisher herds also found a significant increase in the prevalence of abscesses (52%; from 2.9% to 4.4%; P<0.001) and osteomyelitis (67%; from 0.3% to 0.5%; P<0.001) following AB reduction (4). It may therefore be prudent to consider the relevant biological context when implementing restrictive legislation on AB consumption, particularly in herds with vulnerable animals such as recently weaned pigs. To discern if the decrease in DWG is significant more data is needed, as the standard deviation in DWG was larger than expected in the original study design.

Acknowledgments

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References

1. Andreasen M et al. 2012. J Agri Sci Techn A2: 412-416.
2. Stege H et al. 2003. Prev Vet Med 57(3):105-115
3. Jensen V F et al. 2004. Prev Vet Med 64(2): 201-215.
4. Dupont el al. Not yet published data.

O.040

Effects of mixed and uniform parity groupings on feeding behaviour, welfare and productivity of sows in ESF housing

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Introduction

Electronic sow feeder (ESF) systems provide controlled, automated individual feeding for group housed sows. However, within ESF systems, low-ranking sows have been found to receive more aggression and injuries, and have poorer productivity compared to high-ranking sows (1). Low-ranking sows gain entry to the ESF later in the feeding cycle, and are more frequently displaced from entering the feeder (2). As ESF systems are a popular choice for managing large sow herds in groups, information on how to manage low-ranking sows is required, and will benefit welfare and production.

This study compared grouping strategies for sows in ESF systems, looking at mixed parity versus uniform groups of low, medium and high parity sows, to determine their effects on sow feeding behaviour, injury and productivity.

Materials and Methods

Six replicates were performed with approximately 240 sows per replicate. Following confirmation of pregnancy, four groups of 60 sows were formed in each replicate based on parity (low, medium, high parity, and one mixed parity group). Sows were mixed into gestation groups in designated mixing pens where a number of measures were taken to reduce aggression. Five to seven days later, sows were moved to their final gestation pens. Prior to group formation, sows were weighed, body condition scored (BCS) and skin lesions and lameness were assessed. Ultrasonic backfat (BF) measures were collected on a sub-sample of 20% sows. Automated ESF feeding records were collated for individual sows throughout gestation. Scoring for lameness and skin lesions was repeated five days after the initial mixing, and seven days after the final mixing in the gestation pen. Productivity measures, BF and BCS were also recorded at farrowing.

Mixed model analyses (Proc Mixed) were performed in SAS comparing lameness, injury scores, BCS, change in BF depth and productivity between the groups. Average daily feeding duration (FD) and average ESF entry order (EO) of sows in uniform and mixed parity groups, was compared over early, mid and late gestation. Correlations examined relationships between ESF entry order, sow weight and parity.

Results

Average daily FD varied between 15 - 20 mins, with the longest duration being in the first two weeks in the low parity treatment group.

Sows in mixed parity groups had a significantly greater increase in lameness score from pre-mixing to days three and seven after mixing than the uniform groups ($p < 0.01$). Changes in BF over gestation showed significant interactions between treatment and parity score, indicating that sows of parity score 1 and 2 in uniform groups fared better than in mixed parity but lost BF when housed in mixed groups. This was especially true for sows of parity score 1, which lost an average of 4.12 mm BF in the mixed groups. The total average lesion scores increased from premixing to five days after mixing in all treatments, with the uniform high parity group having a significantly lower change in lesion score compared to all other treatments, and the uniform low parity group had the greatest change, being significantly higher than the mixed and uniform high groups. A significant, but weak, correlation was found between BF depth before entering farrowing and the average daily FD, showing that sows with longer feeding times had greater BF levels ($R=0.15$; $P<0.05$). Few differences were found in sow productivity between groups.

Conclusions and Discussion

The preliminary results from this study indicate that housing sows in uniform groups in ESF systems may be a positive management strategy. Sows in uniform groups had a reduced severity of lameness as a result of mixing. Changes in BF over gestation indicate that the well-being of younger sows in particular may be better in uniform groups. Young (parity 1 and 2) sows in uniform groups were able to increase BF, as opposed to loosing it in mixed groups, suggesting that competition at the feeder may be less challenging in the uniform group. Maintaining uniform groups may help reduce mixing injury, with injuries sustained following mixing being equal to or lower than the mixed parity groups. The low parity uniform group had the highest injury scores, however, this appears to be related to the social ability of younger pigs rather than group composition. Management of gilts and young sows to increase sociability is a further management consideration that should be evaluated.

Acknowledgements

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References

1. O'Connell et al., 2003. Anim. Welfare. 12: 239-249.
2. Strawford et al., 2008. Can. J. Anim. Sci. 88: 9-17.

O.041

Handling of forensic cases concerning bruises in pigs

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Introduction

Bruises in pigs inflicted by humans constitute a significant animal welfare problem (1). A bruise is due to a blunt trauma and defined as extravasated erythrocytes beneath and intact epidermis (2). During the past nine years, cases concerning bruises in Danish pigs have been sent for forensic investigation at University of Copenhagen. Central to the evaluation of these cases is an assessment of the age of bruises. Determining the age of bruises is crucial in apportioning blame in a legal context (1). The objective of this study was to elucidate the approach of handling of forensic cases concerning bruises in pigs through a retrospective period.

Materials and Methods

Forensic cases of bruises in pigs investigated at University of Copenhagen were collected retrospectively. Cases concerning bruises in pigs received from 2005 to 2013 were included. Data consisted of photos of the gross lesions, slides for histology and a written description of each case.

Results

In total, 161 cases of bruises in pigs were included in the study. All forensic investigations were requested by the police with the objective to determine the cause and the age of the lesions. To avoid decomposition, tissue was most often frozen prior to submission. In most cases both skin and muscle tissue was submitted.

Upon receipt, the tissue was thawed and all pieces of tissues were photographed. At gross examination, a written description of the localization, pattern and size of bruises was made. Bruises inflicted by humans were localized on the back of the pigs and had a uniform appearance. In many cases, the pattern of bruises reflected the object by which they were inflicted, e.g. sticks, tools or chains (Fig 1.).

Skin and muscle tissue were sampled for histology and stained with haematoxylin and eosin to determine the nature of the lesions. In the subcutaneous tissue, hemorrhage and cellular infiltrations were most often found along the fibrous septa. Changes in muscle tissue were present in the form of necrotic muscle fibers accompanied by infiltration of neutrophils and macrophages. An estimation of the age of bruises was based on the ratio between neutrophils and macrophages compared to studies of wound healing. Wounds of less than four hours are characterized by no

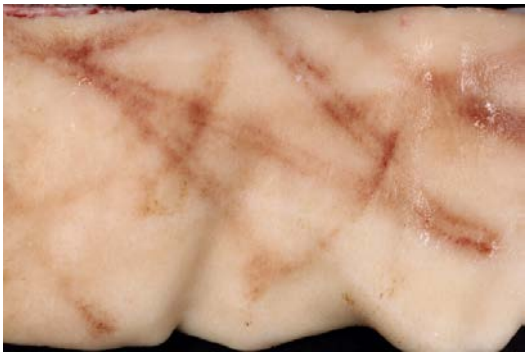

Figure 1. Skin of a pig with bruises with tramline appearance. The uniform pattern of the lesions imply that they were due to blunt trauma inflicted by a human with a stick or a similar object.

or few extravascular neutrophils. Wounds aged between four and 12 hours are dominated by neutrophils and an increasing number of mononuclear cells (2, 3).

Conclusion and Discussion

Cases concerning bruises in pigs should be subject to a gross examination, recording the localization and characteristics of lesions by photographs, and written descriptions. Bruises located on the back of pigs and with a uniform pattern strongly indicate that the bruises were inflicted by humans (Fig 1). Lesions due to bites from other pigs are not uniform in appearance and most often localized on the neck and ears. Moreover, a histopathological evaluation of the affected skin and muscle should be carried out in order to determine the age of the bruises. Based on current knowledge of reaction in bruises (4), it is possible to estimate the age of bruises in pigs to be more or less than four hours. In bruises of more than four hours, an estimation of age can be based on the reaction in wound healing, but there will be some uncertainty.

References

1. Barington K et al. 2013 Vet Rec 173, 21, 526+.
2. Saukko P et al. 2004 Knight's Forensic Pathology 3rd edition, Oxford University Press, 136-173.
3. Raekallio J 1980 Microscopic Diagnosis in Forensic Pathology Eds Perper JA, Thomas Books Illinois, USA, 3-35.
4. Randeberg LL et al. 2007 Lasers Surg Med 39, 403-413.

O.042

Claw lesions in individually and loose housed sows in Greek swine farms

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Introduction

Locomotor problems appear as the second most common sow removal reason (2). Claw lesions are an important underlying cause of locomotor problems in pigs (1). In the European Union all pig herds, from 2013 onwards, should loose house the sows after the fourth week of pregnancy. However, the frequency of claw lesions and lameness among loose housed sows was reported higher than the respective frequency among sows housed in individual stalls (3). Therefore, the objective of this study was the characterization and estimation of the prevalence of claw lesions in three Greek swine herds, in the first 6 months of 2013. One of the herds had fully complied before January 2013 whereas the other two complied after June 2013.

Materials and Methods

Three farrow-to-finish herds with 330 (A), 160 (B) and 800 (C) sows, respectively, were studied. Herds A and B kept pregnant sows in individual stalls whereas in herd C they were loose housed in groups of 8-12. Upon entry of sows to the lactation facilities, their claws were examined for lesions and scored by three, previously trained, farm employees. Scoring followed the Dutch system (4) with some modifications. Specifically, the severity scale run from 1 (no lesion/normal length) to 3 (severe lesion/very long claw) except for the coronary band, which was scored from 1 (no lesions) to 2 (severe lesions) and we scored lateral and medial claws separately. The evaluated anatomical sites were the heel, the sole, the white line, the wall, and the coronary band of the claw, its length (CL) and the length of the dew claws (DCL). The association between lesion score and sow parity, adjusted for herd, was evaluated for significance, for the three anatomical sites most commonly affected, in three generalized ordered logistic models (Stata Statistical Software. College Station, TX). In the analyses, parities were grouped in three categories, namely parities 1 and 2, 3-5, and ≥6.

Results

Four hundred and forty-two sows, of parities 1-10, were scored (127 herd A, 70 herd B, and 245 herd C). At least one lesion was detected in 336/442 (98.6%) of the sows. In herds A and B, the 2 most frequent lesions were CL 117/127 (92%) and DCL 116/127 (91.3%) and in the heel 64/70 (91%) and DCL 56/70 (80%), respectively. In herd C most frequent lesion were in the heel 233/245 (95%) and DCL 223/245 (91%). The herd-adjusted associations between lesion score and parity are in Table 1.

Table 1. Herd-adjusted associations between sow-parity and heel lesion, long claw (CL) and long dew claw (DCL) scores (scaled from 1(no lesion/normal length) to 3 (severe lesion/very long claw).

Claw site	Odds ratio (95% CI)	Odds ratio (95% CI)	
		Lesion Score	
		1vs(2+3)	(1+2)vs3
Heel	1.4(1-1.8)		
CL*		1.6(1.2-2.2)	3(1.8-5)
DCL	3.3(2.4-4.5)		

* the assumption of proportionality of the odds among scores did not hold.

Conclusions and Discussion

Similar to previous studies (5), we recorded very high frequency of claw lesions in all herds. The anatomical sites most frequently affected were the heel area, CL and DCL. The severity of the lesions increased with increasing sow-parity. These results indicate that sow longevity is severely affected by claw health with likely long-term consequences in productivity and welfare.

Acknowledgments

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References

1. Dewey CE et al. 1993. Vet J 34:555-556.
2. Engblom L. et al. 2007. Livestock Sci 106:76-86.
3. Gjein and Larssen 1995. Acta Vet Scand 36:433-442.
4. Hoofs AIJ 2006. http://www.verant-woordeveehouderij.nl.
5. Pluym L. et al 2011. Vet Medicina 56: 101-109

O.043

Pork chop preferences of consumers in four Mexican states

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Introduction

With much of Mexico's pork demand met through international trade, it is as much in the interest of the Mexican as the global pork industry to be aware of the Mexican market preferences. Historically, preference studies on meat have been hindered by practical limitations imposed by short display-lives making it inevitable that different meats have been judged by different consumers. To overcome these limitations a method was developed using digital photographs which allow the systematic assessment of the impact of varying appearance characteristics on consumer choice (1,2,3). The aim of this study was to use these images to identify the most important characteristics of fresh pork which determine choice of consumers from four Mexican states.

Materials and Methods

Consumers (486) were surveyed in Mexico City (204), Merida (120), Guadalajara (102) and Vera Cruz (60). Surveys were conducted using a book of 256 digitally modified photographs specially produced for this purpose so that all consumers see the same pork appearance characteristics. Consumers were chosen at random, asked to select their preferred chops from a series of images and complete a short questionnaire. The response data was divided into three categories for each characteristic; in the first two categories the consumer actually chooses one of the two levels of the given characteristic, whereas in the third category the characteristic is randomly selected (R). The results can be quantified by the definition that if 6 of 8 choices for one consumer are the same for a given characteristic, the choice is a real choice and not random ($P<0.14$). If less than 6 choices are the same, the given characteristic is considered to be randomly selected. This test assumes a binomial distribution of the results ($P=0.5$). For each characteristic, significant differences in the number of choices can be observed using the χ^2 test.

Results

About half of the consumers used colour and/or fat cover in their decision making process for pork, whereas only a third of consumers used marbling and drip suggesting that these latter characteristics are less important in the choice process (Figure 1). Of those who used fat cover, more than 5 times the consumers showed a preference for lean than fatty meat cover. Differences were less marked between consumers who used colour, with only 1.5 times more preferring the dark than the light pork.

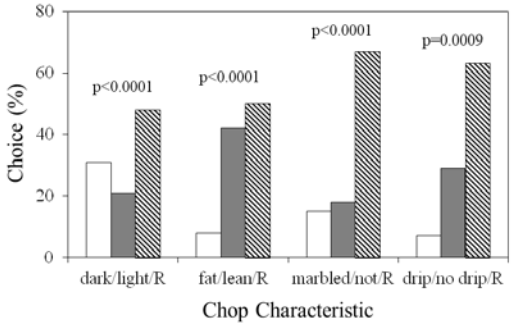

Figure 1. Mexican consumers' pork choice preferences

The trends are similar to those in the 2001 study of 751 consumers from Chihuahua, Mexico undertaken with Dr Alarcon-Rojo (2,3). However, the current data shows 10% less consumers choosing light red pork and 20% less choosing lean meat. These differences may indicate that fewer Mexican consumers use the given criterion at the levels presented or they may indicate regional effects. In the present study, no regional differences were found in preferences of consumers from the four states surveyed, but Chihuahua was not one of these states.

Conclusions and Discussion

Colour and fat cover were more important in consumer choice than marbling and drip. A slight preference for dark than light red meat was observed, whereas lean fat cover was much preferred over fatty. These preferences are favourable for the industry in that variations within the 'normal' range of pork colour allow the industry to meet consumer preferences and fat cover is largely a matter of trimming. Of the consumers who used marbling and drip in their decision, marbling was relatively evenly divided, but more consumers preferred no drip than the presence of drip. Nowadays, drip issues are often countered by the use of an absorbent pad.

Acknowledgments

Fondo Sectorial Sagarpa-CONACYT No. 109127 "Fresh meat quality indicators".

References

1. Ngapo TM et al. 2007. Food Qual Prefer 15:349-359
2. Ngapo TM et al. 2007. Food Qual Prefer 18:26-36.
3. Ngapo TM et al. 2007. Food Qual Prefer 18:139-151.

O.044

Effect of immuno-castration on meat quality characteristics

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Surgical castration is a traditional practice to eliminate boar taint, an unpleasant smell of androstenone and skatole that accumulated in the fatty tissue of male pigs. Due to surgical castration is not a welfare practice for animals thus many countries will ban it as in EU by the year 2018. Immuno-castration is the alternative way to eliminate boar taint by immunizing boars using a synthetic analogue of GnRH coupled to a large carrier protein (Improvac, Zoetis). Several studies have reported the efficiency of immuno-castration on boar taint elimination and also on growth performance improvement. However, the effect of immuno-castration on meat quality attributes have not been widely studied and there have been no consistent findings across the studies. Therefore more extensive investigations are required especially in the local conditions. The present study was conducted in commercial rearing conditions in Thailand to evaluate the effects of immuno-castration on meat quality traits.

Materials and Methods

Thirty crossbred pigs [(Large white X Landrace) X Duroc]: surgically castrated pigs (SC; n = 15) and immuno-castrated pigs using Improvac® vaccine (Zoetis) (IC; n = 15) were used in this study. Pigs were fed a commercial diet ad libitum and were slaughtered within the same week at average weight 120 kg. A pH at 1 h post mortem (pH_1) was taken from Longissimus muscle (LM) at the last rib position during chilling carcass. After overnight chilling, LM was excised from carcass then collected sample from the first rib to the tenth rib. pH at 24 h post mortem (pH_{24}) and meat color was measured at the tenth rib position then muscle sample was subsequently cut into chops for measuring sarcomere length (Cross et al., 1981) and shear force (at 1 d and 5 d ageing). Sample chops for shear force measurement were vacuum packaged and stored at -20 °C until analysis.

All data collected in the study were analyzed using mixed model procedures with pig considered to be a random effect in the testing fixed effect of differences between treatment groups (SC or IC).

Results

Carcass weight was not significantly different between treatments (Table 1). Sarcomere length, pH_1, pH_{24} and color was not different ($P > 0.05$) except IC showed lower a* value than SC ($P = 0.091$). Immuno-castration was no effect on shear force value ($P > 0.05$). However, after 5 day ageing, shear force value of IC was slightly less than SC ($P = 0.166$).

Table 1. Effect of castration method on meat quality traits

Trait	Method[1]		SE	P-value
	SC	IC		
Carcass weight (kg)	93.34	88.58	2.86	0.248
Sarcomere length (µm)	1.04	1.03	0.01	0.113
pH_1	7.13	6.94	0.08	0.102
pH_{24}	5.61	5.59	0.03	0.711
L*	47.96	48.95	1.49	0.642
a*	3.22	2.73	0.19	0.091
b*	9.96	9.79	0.56	0.832
Shear force 1 d (kg)	7.92	7.14	0.45	0.231
Shear force 5 d (kg)	7.53	6.62	0.46	0.166

[1]Method : SC = surgical castration, IC = immuno-castration

Conclusions and Discussion

Overall, immuno-castration was no effect on pH and color except IC had a trend to have lower a* value than SC. Pauly et al (2009) showed no effect of immuno-castration on neither pH nor color. While Gispert et al (2010) reported that IC had higher a* value than SC. In the present study, shear force value of IC was slightly less than SC after 5 d ageing. In agreement with Pauly et al (2009) that explained the tenderization could be caused by the compensatory growth of IC at the end of the finishing period. Kristensen et al (2002) and Bee et al (2006) reported that compensatory growth before slaughter influence the increase of proteolytic potential and tenderization rate.

In conclusion, there was no significant difference in meat quality characteristics between surgical castration and immuno-castration in this study.

Acknowledgments

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References

1. Bee G et al. 2006. J Anim Sci 84 : 191-203.
2. Cross H R et al. 1981. Meat Sci 5:261-266.
3. Gispert et al. 2010. Meat Sci 85 : 664-670.
4. Kristensen L et al. 2002. J Anim Sci 80 : 2862-2871.
5. Pauly C et al. 2009. Animal 3:7 : 1057-1066.

O.045

Loin meat quality of an improved genetic line of Spanish Duroc

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Introduction

Spanish pig production is led to obtain pork which is appreciated by consumers in price and quality. The appreciation of the quality of fresh pork by consumers is related to several aspects such as juiciness, tenderness, the water holding capacity etc. In addition the fat content can highly vary in a same genetic line between individuals therefore it becomes necessary to obtain animals, "in vivo", under an homogeneous inter and intra muscular fat percentage. The objective of this work is to evaluate the pork quality parameters of a genetic TOPIGS Duroc sire line.

Materials and Methods

Information from fresh loins was available from 239 animals (all TOPIGS Duroc gilts). Animals were slaughtered according to EU Council Directive 1009/2009/EC (2009)[1] with a live weight of 103.54±9.6 kg. The analyses were determined in the *Longissimus lumborum* muscle. Meat quality determinations carried out were the following: Water-holding capacity (WHC), expressed as percentage (Grau and Hamm, 1953)[2]; Cooking losses (CL), as the differences in the weight of raw and cooked samples expressed as percentage (Honikel, 1998)[3]; Intramuscular fat (IMF) assessed by AOAC (1990)[4] procedures and expressed as percentage on fresh matter; Shear force (SF) assessed with a Warner Braztler device using a QTS-25 texture analyzer (Brookfield CNS Farnell, Borehamwood, Hertfordshire, England) equipped with a load cell of 25 kg and Texture Pro V. 2.1 software and expressed in Newtons (kg/cm^2).

Results

To evaluate the intramuscular fat content results (Figure1) three different groups were considered: 1 (Low, IMF ≤3); 2 (Medium, IMF >3.1 <6); and 3 (High, IMF >6). The percentage of animals and the average of IMF content in the three groups were: Group 1: 17.57% (2.53%); Group 2: 62.7% (4.58%); Group 3: 19.6% (7.9%).

In Table 1 is showed the Pearson´s correlation coefficients in pork quality from Duroc genetic line. The IMF is positively correlated to the WHC ($p<0.01$) and negatively with the CL ($p>0.05$) and SF ($p<0.01$). It is remarkable the correlation between CL and SF (0.463**).

Conclusions and Discussion

The IMF content in the major of the samples (4.58%) is adequated for fresh and meat product (Reixach et al. 2009[5]; Rincker et al., 2008[6]). It is well known that the fat content adds an extra value to products, especially to the cured ones. Meat quality coefficients evolution are in accordance with the usual trend of these atributtes for pork. High IMF implies a lower SF and higher WHC which involves an increase in juiciness and tenderness. A major homogenity of Duroc genetic sire line as respect the IMF is required in order to obtain an apreciated quality for fresh and processed meat.

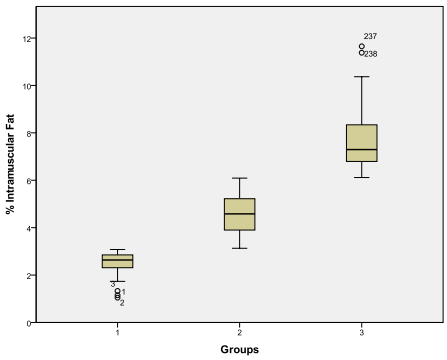

Figure 1. Distribution of the samples in accordance to the intramuscular fat content (%).

Table 1. Pearson´s correlation coefficients in pork quality from Duroc genetic sire line.

	IMF	WHC	CL
IMF			
WHC	0.178**		
CL	-0.126 ns	-0.148*	
SF	-0.197**	-0.132*	0.463**

*, **: Significance levels of $p<0.05$ and 0.01, respectively; ns: not significant

Acknowledgments

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References

1. EU Council Directive 1009/2009/EC (2009).
2. Grau and Ham. 1953. Natuwissenschaften 40, 29-30
3. Honikel KO.1998. Meat Sci. 49, 447-457.
4. AOAC. 1990. Official Methods of Analysis.
5. Reixach et al. 2009. Livestock Sci. 123, 63-69.
6. Rincker et al. 2008. J Anim Sci. 86, 730-737.

Birth weight implications for ovarian development in gilts

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Introduction

The development of the hypothalamic-pituitary-gonadal axis occurs largely during pre-natal life. Within the ovary, processes such as migration, proliferation, degeneration and meiosis of germ cells, and folliculogenesis determine the follicle population at birth and potentially the number of follicles that can be recruited during postnatal life (1). There is evidence of a delay in follicular development in runts, probably in the activation of primordial follicles (1). Hence, this study was designed to test the hypothesis that altered growth in utero, associated with poor early postnatal growth, when ovarian development is still proceeding, may impair fertility in gilts.

Materials and Methods

New-born female pigs (n = 64; TOPIGS genotype), born to 1st parity sows and in litters of 10 to 15 pigs, were identified as falling into two birth weight groups: high (HBW: 1.5 ± 0.2 kg) and low (LBW: 1.0 ± 0.2 kg) littermates. Gilts were slaughtered at a fixed age (80.8 ± 1.2 days): Age group (AG) or at a fixed body weight (35.2 ± 1.4 kg): Weight group (WG), and the ovaries collected. Blood samples were taken at slaughter for leptin (Lep) and IGF-1 analysis. The diameter of the antral follicles present on the ovaries was measured and one ovary was fixed in Bouin's solution for 24 h, embedded in paraffin, and serial sectioned to determine the number of healthy primordial, primary, pre-antral and antral follicles. Data were analyzed as a randomized complete block design, where litter of origin was blocked, and the comparison between means was performed by t-test.

Results

In the AG, HBW gilts had higher body weight (BW) at slaughter compared to their LBW counterparts (P<0.05; Table 1). On the other hand, LBW gilts from the WG were older at slaughter (P<0.05; Table 1). Birth weight did not exert a negative effect on total antral follicle (TAF) number or size, considering the gilts' age or BW at slaughter. Moreover, blood levels of leptin or IGF-1 were not affected by birth weight in both experimental groups (Table 1) and no associations were found between those metabolic hormones and TAF number. The histological evaluation of the ovaries showed that birth weight did not affect the population of the different classes of ovarian follicles in either AG or WG gilts (Table 2).

Conclusions and Discussion

In the present study, ovarian development was evaluated at a fixed age or body weight to avoid any disadvantages of a lower growth rate in the LBW females. Follicle development at this stage has progressed up to the secondary follicle stage, and is close to the onset of tertiary (antral) follicle development (2). Although a delay in follicular development in LBW females has been demonstrated previously (1), our data suggest that, within the age or weight evaluated, follicular dynamics was not impaired by birth weight, despite the differences in growth rate. It was observed a litter of origin effect for TAF, primordial and primary follicle numbers which revealed the importance of the use of littermates when designing experiments of this kind to account for the differences due to family. Although birth weight affects postnatal growth, its effects on ovarian development are still controversial. Further studies are necessary to better understand this issue.

Table 1. Antral follicle (AF) sizes, Lep and IGF-1 levels in LBW and HBW gilts

Parameter	Age			Weight		
	LBW	HBW	s.e.m	LBW	HBW	s.e.m
Age, d	-	-	-	82.9 [a]	77.0 [b]	1.20
BW, kg	34.50[a]	40.10[b]	1.60	-	-	-
OW, g	0.32 [a]	0.39 [a]	0.10	0.41 [a]	0.26 [a]	0.11
AF number	4.00 [a]	5.60 [a]	2.20	62.10 [a]	10.70 [a]	23.20
Foll 1mm	0.00 [a]	0.12 [a]	0.10	0.30 [a]	0.03 [a]	0.21
Foll 2mm	0.50 [a]	0.32 [a]	0.20	1.00 [a]	0.23 [a]	0.60
Foll 3mm	0.46 [a]	1.14 [a]	0.50	0.21 [a]	0.90 [a]	0.35
Lep, ng/mL	2.74 [a]	2.69 [a]	0.08	2.82 [a]	2.76 [a]	0.08
IGF1ng/mL	350.4[a]	341.6 [a]	15.4	293.9 [a]	340.7 [a]	18.6

[a,b] Within a row, means with different supperscripts differ (P<0.05)

Table 2. Histological evaluation of follicle numbers in LBW and HBW gilts

Parameter	Age[§]			Weight[§]		
	LBW	HBW	s.e.m	LBW	HBW	s.e.m
Primordial	27,255	29,090	3,140	15,760	24,962	4,898
Primary	16,770	17,610	2,220	13,630	17,760	3,343
Pre-antral	2,860	2,435	188.0	3,460	3,230	692.0
Antral	13.00	0.00	9.20	21.90	15.60	6.00

[§]Birth weight did not affect follicle numbers in AG or WG (P>0.05)

Acknowledgments

We gratefully acknowledge CNPq, Capes and Fapemig.

References

1. Da Silva-Butkuss P et al. 2003. Reproduction 126: 249-258
2. Dyck GW& Swierstra EE 1983. Can J Anim Sci 63: 81-87

O.047

Sow reproductive performance using Triptorelin Gel and fixed-time AI in commercial swine farms

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Introduction

Triptorelin Gel (OvuGel®) is a gel formulation containing the GnRH analog, triptorelin. A single dose of Triptorelin Gel deposited intravaginally 96 hours after weaning induces ovulation 40 to 48 hours later, thereby facilitating a single fixed-time insemination 24 hours following treatment without regard to estrus. The objective of this study was to further demonstrate the use of Triptorelin Gel under a variety of management conditions in commercial swine farms.

Materials and Methods

The study was conducted at six farms in the United States. Conventional artificial insemination (AI) was used on one farm, while others used post-cervical AI. Breeding eligible sows (BES) were blocked by parity and body condition score at weaning and randomly assigned to either Triptorelin Gel or Contemporary (Control) treatments. Control BES (n=1479) were inseminated following normal farm SOP on the day detected in estrus, and 24 hours later if still in estrus. Triptorelin Gel BES (n=1475) were treated 96 hours post-weaning and inseminated once 22+/-2 hours later. Pregnancy was determined by ultrasonography approximately 30 days after AI. Data were subjected to analysis of variance using the PROC MIX procedure of SAS (version 9.2) to determine main effect of treatment, replicate, and treatment by replicate interactions. Differences between treatment means were tested on least squares means estimates using the T test at $P<0.05$.

Results

Reproductive performance data are presented in Table 1. There was no difference among Triptorelin Gel sows bred once and Control sows bred an average of 1.9 times in weaned sow farrowing rate/weaned sow utilization (no. farrowed/no. weaned, $P=0.41$), born alive ($P=0.66$) and total born ($P=0.60$). This resulted in live pigs per 100 sows of 1100 for Triptorelin Gel sows and 1081 for Control sows. Fewer semen doses were required for the single fixed-time insemination, therefore, total born per semen dose was greater ($P<0.01$) for Triptorelin Gel sows than for Control sows (11.9 vs. 7.5, respectively).

Table 1. Reproductive Performance at Six Farms[1]

	Control	Triptorelin Gel	*P*-Value
Breeding Eligible Sows	1479	1475	.
Inseminated by 7 Days Post-Weaning	1363	1475	.
Number Semen Doses	1.9	1.0	0.0001
Sows Pregnant	1257	1261	.
Pregnancy Rate (no. pregnant/no. weaned)	85.0	85.5	0.68
Sows Farrowed	1224	1239	.
Farrowing Rate (no. farrowed/no. bred)	89.8	84.0	.
Weaned Sow Farrowing Rate (farrowed/weaned)	82.8	84.0	0.41
Total Born	14.2	14.2	0.60
Born Alive	13.1	13.1	0.66
Total Born/Semen Dose	7.5	11.9	0.0001
Live Pigs per 100 Sows	1081	1100	.

[1] The data presented in this table are raw means. Sows farrowed spring and summer of 2013.

Conclusions and Discussion

Because only Control sows which expressed estrus were inseminated, while all Triptorelin Gel-treated sows were inseminated once, comparing conventional farrowing rates (no. farrowed/no. bred) is not appropriate. Thus, we suggest that weaned sow farrowing rate or weaned sow utilization rate (no. farrowed/no. weaned) and live pigs per 100 weaned sows are the most appropriate economic measures to compare farrowing performance. In this study, sows treated with Triptorelin Gel and inseminated once had both a weaned sow farrowing rate and a litter size similar to Control sows inseminated multiple times during estrus. By breeding all weaned sows on a single day, utilization rates of weaned sow inventory increased and improved throughput. Our data show that breeding all weaned sows on a single day allow sows to be induced to farrow with no increase in number of stillborn pigs. Furthermore, 92% of the induced sows farrowed on the same day. This decreased the number of days spent on assisted farrowing and Day 1 pig care. Also, 90% of the piglets were the same age (20 days) at weaning. After Triptorelin Gel implementation in the spring of 2013, average pregnancy rate of weaned sows at three farms increased from 82.3% (7289 pregnant/8857 weaned sows) to 85.8% (7187 pregnant/8377 weaned sows) compared to the same time period in the previous year, indicating improvement in weaned sow utilization. Conventional AI and post-cervical AI produced similar results. These data demonstrate that Triptorelin Gel effectively synchronizes time of ovulation in weaned sows, thereby facilitating a single fixed-time AI without regard to estrus. As a result, the cost of labor and semen is reduced, while a high level of reproductive performance is maintained.

Birth weight effects on reproductive tract development and puberty onset in gilts

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Introduction

In the last decades, selection for improved prolificacy has resulted in higher litter sizes. However, selection for higher litter sizes has increased the proportion of low birth weight (LBW) piglets (3). There is evidence that LBW piglets have poor postnatal development, such as growth performance, muscle accretion and intestine morphology (1). However, there is little information on its effects on the development of the reproductive tract in female pigs. Therefore, the objective of the present study was to investigate some biometrical and hormonal parameters, as well as the onset of puberty in light birth weight gilts.

Materials and Methods

New-born female pigs (n = 38; TOPIGS genotype), born to 4th- 6th parity sows and in litters of 10 to 15 pigs, were identified as falling into two birth weight groups: high (HBW: 1.47 ± 0.25 kg) and low (LBW: 0.95 ± 0.26 kg) littermates. Gilts were weighed regularly throughout the production phases. Daily estrus detection with direct mature boar contact began at about 140 days (d) of age until onset of estrus, which was defined as a standing reflex in the presence of the boar. Gilts were subsequently slaughtered when no standing reflex was shown anymore. Blood samples were taken at 80 d of age for leptin and IGF-1and at slaughter (~180 d) for leptin analysis. After slaughter, the reproductive tracts were recovered and dissected for biometrical measurements. Data were analyzed as a randomized complete block design, where litter of origin was blocked, and the comparison between means was performed by t-test.

Results

HBW gilts had a higher postnatal growth performance compared to their LBW counterparts (P<0.05; Table 1). Notwithstanding the difference in growth performance, age at puberty was similar between groups but body weight at slaughter was higher in HBW gilts (P<0.05; Table 2). Moreover, the biometric data showed that birth weight did not exert a negative effect on the development of the reproductive tract or on blood levels of either leptin or IGF-1 (Table 2). Birth weight was highly correlated with weight at slaughter (r=0.57, P=0.002) and with leptin levels at 80d. Additionally, age at puberty was negatively correlated with growth rate (r=-0.47, P=0.004) and with IGF-1 levels at 80d (r=-0.61, P=0.001), but positively correlated with leptin levels at 80d (r=0.38, P=0.02).

Conclusions and Discussion

Evidence of a better growth performance in HBW animals has been previously demonstrated (1). Additionally, high growth rate gilts attain puberty earlier than low growth rate gilts (4). In this regard, our data suggest that heavier gilts at birth will grow faster and attain puberty earlier. In fact, gilts that attain puberty earlier are the most fertile in the breeding herd. Despite the differences in growth rate, the develpoment of the reproductive tract was not impaired due to birth weight. On the other hand, leptin and IGF-1 levels directly affect puberty attainment, which has also been shown by others (2,5). Although birth weight affects postnatal growth, its effects on the development of the reproductive tract are minor.

Table 1. Mean body weight (wt) of LBW and HBW gilts from birth to 180 days

Parameter (kg)	Treatment			
	LBW	HBW	s.e.m	P
Birth wt	1.0	1.5	0.2	
Weaning wt	6.0	7.3	0.2	<0.05
Wt 63d	26.0	31.0	0.8	<0.05
Wt 180d	102.4	117.6	3.1	<0.05

Table 2. Biometric data of the reproductive tract and hormonal analysis in LBW and HBW gilts

Parameter	Treatment			
	LBW	HBW	s.e.m	P
Age at puberty, d	178.0	172.4	4.1	NS
Body wt, kg	102.4	117.6	3.1	<0.05
Backfat, mm	8.9	9.1	0.4	NS
Mean ovarian wt, g	7.4	8.0	0.3	NS
Ovulation rate	14.5	15.0	0.5	NS
Vaginal length, cm	11.0	12.1	0.6	NS
Uteri horn length, cm	85.8	88.8	2.1	NS
Oviduct length, cm	21.6	23.3	0.7	NS
Leptin at 80d, ng/ml	2.3	2.6	0.2	NS
IGF-1 at 80d, ng/ml	334.6	345.2	12.1	NS
Leptin at slaughter	3.5	3.7	0.2	NS

Acknowledgments

We gratefully acknowledge CNPq, Capes and Fapemig.

References

1. Alvarenga ALN et al. 2013. Rep. Fertil. Dev. 25:387-395.
2. Barb CR et al. 2005. Dom. Anim. End. 29:186-192.
3. Foxcroft GR et al. 2006. J. Anim. Sci. 84:E105-E112
4. Kummer R et al. 2009 Reprod. Dom. Anim. 44:255-259.
5. Patterson JL et al. 2010. J. Anim. Sci. 88:2500-2513.

O.049

25 Hydroxycholesterol regulates progesterone production in porcine luteinized *in vitro* granulosa cells

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Introduction

Progesterone (P4) is a steroid hormone synthesized and secreted in the ovary cells (1,2). Its maiA118n function is to prepare the endometrium for embryo implantation (3,4). Successful implementation, among other factors, depend upon the proper regulation of the synthesis of P4 (5,6), therefore insufficient concentrations of P4 at the time of implantation would increase the chances of embryo mortality (7). *In vitro* studies of P4 production in luteal cells of rats and primates (8), with 25 hydroxycholesterol (25-OH) as analog oxysterol, shows an inhibitory effect which was attributed to decreased mRNA CYP (9). Granulosa cell (CG) culture model for determining the effect of 25-OH in steroidogenesis, currently plays a significant role in numerous hormone regulators studies. Therefore the aim of the research was to determinate the effect of 25-OH in P4 production in porcine luteinized CG *in vitro*.

Materials and Methods

CG were obtained from ovaries of sows pre-pubescent. To luteinized CG, were cultured for 96 hrs at 37 °C, 95 % relative humidity and 5% CO2. The first 48 hrs with MEM medium and fetal bovine serum (FBS) and the second 48 hours free MEM with FBS. Once luteinized, was added 10 ug/ml of 25-OH (Sigma). The control culture was incubated only with medium. The experiment was carried out by performing three independent replicates, and the application of the treatment was performed in triplicate within each repeat . The culture supernatant was collected at 24 and 48 hours to examine the production of P4 . To quantify the levels of P4, *EIA commercial kit -1561 08/07 manufactured by DGR IntrumentsGmbH , Germany was used. Division of DRG International (Inc.Frauenbergstr . 18 , D- 35039 Marburg)* based on the principle of competitive binding . Individual comparisons between means were performed by Tukey test . The level of significance was $P < 0.05$.

Results

The addition to the culture medium with 25-OH was shown to increase the production of P4. When cells were treated with 10 ug/ml of 25-OH increased observed until 3 times the production of P4, both at 24 hours (27.89 ng/ml) and at 48 hours (26.94 ng/ml) compared to untreated cells or control (2.04 ng/ml at 24 and 48 hours 7.68 ng/ml), these levels of P4 production have significant difference ($p < 0.05$). Comparing recorded at 24h and 48h of the treatment group, the difference was not significant ($p > 0.05$).

Conclusions and Discussion

Travert et al (2006) conclude that the rat Leydig cells are able to use 25-OH as a precursor in the synthesis of testosterone. In other studies using immunoassay procedures were able to detect the formation of pregnenolone from 25-OH in cells cultured pig granulosa (12) and in cultured bovine luteal cells (13) . Babischkin et al. (1997) also reported the conversion of 25-OH progesterone syncytiotrophoblastic human placental cells. In conclusion, this study indicated that 25-OH would be acting as a substrate in the biosynthesis of P4 in porcine luteinized GC. We suggest that such action is achieved through its direct pregnenolone same conversion that could be because 25-OH is cleaved from its side chain easily and quickly through the mitochondrial membranes separate the enzymatic process. Moreover we can assume that as the 25-OH acts as precursor for the biosynthesis of P4 may regulate the expression of some genes steroid .

References

1. Niswender y col 1994. Biol Reprod 50: 239-247.
2. Monniaux y col. 1997. J Reprod Fertil Suppl 51, 3–23.
3. Mann y Lamming 2001. Reproduction 121, 175-180.
4. Green y col 2005. Anim Reprod Sci 88, 179-189
5. Joachim y col 2003. Steroids 68, 931-940.
6. Blois y col 2004. J Immunol 172, 5893-5899.
7. Ashworth 1991. Anim Reprod Sci 26, 311-321.
8. Christeson y Devoto 2003. Reprod Biol Endocrinol 1:90
9. Chaffin y col 2000. Mol. Hum. Reprod. 6, 11-18.
10. Björkhem 2002, J Clin Invest 110(6),725-730.
11. Travert y col (2006. Reprod Toxicol 22, 564–570.
12. Veldhuis y Rodgers 1987. J Biol Chem 262, 7658–7664
13. Gu y col 1990. Biochem Bioph Res Co 169, 455–461

Effect of genetic background and birth weight on the financial results of growers and finishers

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Introduction

By order of the Dutch Product Board for Livestock and Meat, at Swine Innovation Centre Sterksel the effects of genetic background and birth weight on the performance and financial results of piglets, growing and finishing pigs were investigated. Genetic background (TPI; TOPIGS Profit Index) was expressed as the genetic index for daily gain, feed intake and lean meat percentage of the growing and finishing pigs.

Materials and Methods

In total 624 weaned piglets (48 pens x 13 piglets) and 576 growing and finishing pigs (48 pens x 12 growing and finishing pigs) (Tempo boar x (Dutch Landrace x Dutch Large White) sow) were used in the experiment. Pigs were followed from birth until delivery to the slaughterhouse. There were four experimental treatments. See table 1.

Table 1.

Treatment	Genetic indexes 1	Birth weight Piglet 2	Number of pens
1	High	High	12
2	High	Low	12
3	Low	High	12
4	Low	Low	12

[1] Sows with a high genetic index for daily gain, feed intake and lean meat percentage of the growing and finishing pigs were inseminated with a boar with a high genetic index; sows with a low genetic index were inseminated with a boar with a low genetic index.
2 High birthweight is higher than 1,340 gram; low birth weight is lower than 1,340 gram.

At weaning piglets were grouped by genetic background, birth weight and gender. Five weeks after weaning, pigs were moved to the unit for growing and finishing pigs. Pigs out of the same weaning pen stayed together. The growing and finishing pigs were fed ad libitum. They received a starter diet for five weeks, a grower diet for four weeks and a finisher diet until delivery to the slaughter house.

Results

Genetic background

Yield per delivered piglet was similar in high and low genetic piglets. The gross margin per delivered growing and finishing pig, however, was € 4.72 higher in high genetic than in low genetic growing and finishing pigs. The difference in gross margin per growing and finishing pig per year is even higher because high genetic growing and finishing pigs were delivered 3.5 days earlier to the slaughter house.

Birth weight

Yield per delivered piglet was € 1.80 higher in high birth weight piglets. The gross margin per delivered growing and finishing pig was numerically, but not significant, € 0.80 higher in high birth weight growing and finishing pigs. The difference in gross margin per growing and finishing pig per year is higher because high birth weight growing and finishing pigs were delivered 5 days earlier to the slaughter house than low birth weight growing and finishing pigs.

Table 2. Financial results (in € per delivered finisher) of finishers per TPI and per category of birth weight

	Low TPI	High TPI	Low Birth weight	High Birth weight
Income	145.47[a]	151.58[b]	147.12[a]	149.92[b]
Cost piglet	37.2[a]	38.00[b]	36.19[a]	39.01[b]
Feed costs	79.36	79.95	80.07	79.24
Mort. costs	0.95	0.95	0.95	0.95
Other costs[1]	4.3	4.3	4.3	4.3
Yield	23.66[a]	28.38[b]	25.61	26.42

[1]costs for health, electricity,water,heating and other
[a,b,c] averages with a different letter within treatment within arrow are different ($p<0,05$)

Conclusions and Discussion

In conclusion, financial results of growing and finishing can be improved by using sows and boars with a high genetic index for daily gain, feed intake and lean meat percentage. The genetic index can be used in the decision whether a sow should be replaced or not. Besides, the farmer can decide to use boars with a high genetic index. Pigs with a high birth weight have a better performance and higher financial results during both the weaning period and the growing and finishing period than low birth weight pigs. Birth weight is highly repeatable, in other words, sows that have heavy piglets will also have heavy piglets in the next litters. Thus, birth weight of the piglets can be used in the decision whether or not to replace a sow. High genetic growing and finishing pigs with a high birth weight are delivered 8.6 days earlier to the slaughterhouse than low genetic pigs with a low birth weight. Therefore, it is financially interesting to keep these pigs in different compartments.

Acknowledgements

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O.051

Detecting levels of autozygosity in two pure lines of pigs using genomic and pedigree data

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Introduction

Intense animal selection and breeding strategies have been used to improve several animal traits within the livestock sector. The fewer animals kept for breeding, the greater is the selection intensity; therefore, faster genetic progress can be accomplished (5). One of the concerns involving the massive use of specific sire lines to improve desirable traits is the loss of genetic diversity and the accumulation of high levels of homozygosity among animals, which could be detrimental to reproduction, conformation, and growth traits (2). The objectives of this study were to estimate and to compare levels of homozygosity using both pedigree and genotype data in two female pure lines of pigs, Landrace (LA) and Large White (LW).

Materials and Methods

The animals used in this study were from the BRF/SA private company (BRF-Brazilian Foods). The complete pedigree records for the LA breed contained information on 84,611 animals and for the LW 50,348 animals under selection. They traced back 12 generations, with an average length of 6.41generations for the LA and 5.55 generations for the LW. The pedigree records for the LA and LW were evaluated using the R-statistical environment with the pedigree package. The inbreeding coefficient (F_x) based on the pedigree of the animals was calculated using Wright's Coefficient (3). Animals were genotyped with Illumina 60K SNP Chip. The genomic inbreeding estimated with runs of homozygosity (ROHs) were calculated with PLINK using a sliding window of 50 SNPs, a minimum ROH of 50 SNPs with a minimum length of 1000 kb. One heterozygous SNP and one missing SNP genotype were allowed within the sliding window (4). Identified ROH were then used to estimate individual genomic inbreeding coefficients (FROH):

$$F_{ROH} = \frac{\Sigma_k \text{ Lenght } (ROH_k)}{L}$$

Where "k" was the number of ROH for each individual in Kb and "L" was the total swine genome length (2,808,525Kb, Sscrofa10.2, Aug 2011).

Results

After sample and SNP quality control, 1168 LA (91 M and 1077 F) and 1094 LW (114 M and 980 F) with 58,911 SNPs remained for the analysis. When all the generations were included in the analysis, the Fx calculated based on the pedigree ranged from 0 to 0.139, with an average of 0.014 in the LA, and from 0 to 0.062 for LW, with an average of 0.021. The average inbreeding of the LA animals using the ROH was 0.094 ranging from 0.012 to 0.184 and for LW was 0.106 ranging from 0.008 to 0.181. None of the homozygous regions were shared among all the animals within or across breeds. A region on SSC14 was identified with the highest number of homozygous regions among LA animals. This region harbors two important genes: *CXCL12* and *TFAM*. The *CXCL12* gene was associated with immunological traits in LA piglets, especially with disease resistance, being very important for the survivability of the animals (4). The *TFAM* gene plays an important role in porcine gametogenesis and embryo preimplantation and development, having broad implications for cell physiology and evolutionary biology (1).

Conclusions and Discussion

For diversity studies, the levels of recombination, inbreeding and segregation can be fairly estimated based on pedigree information. However, such parameters can be accurately calculated using high-density genomic data. The use of pedigree information alone to calculate levels of homozygosity and degree of relationship between animals underestimated the levels of inbreeding in both swine breeds analyzed, because of the limited number of generations that are traced back. Implementation of genomic tools to better estimate the correlation coefficient between animals will improve the accuracy of EBVs, consequently the selection efficiency, among other potential advantages. Although no inbreeding differences between breeds were found using FROH, the identification of conserved homozygous regions among individuals might reveal important findings related to the evolutionary standpoint for the pork industry.

References

1. Antelman et al.: 2008, *J Cell Physiol.* 217(2):529-43.
2. Burrow: 1993, *Animal Breeding Abstracts*. 61 (11).
3. Nomura et al.: 2001, *J. Anim. Sci.* 79:366-370.
4. Scraggs et. al.: 2013, *J Anim Breed Genet.*
5. Wang et al.: 2012, *Mol Biol Rep*. 39(3):2417-27.
6. Weigel: 2001, *J. Dairy Sci.* 84(12):2789-85.

Single serving sows to enhance boar performance and reduce cost of production

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Introduction

Pig production needs to accelerate its genetic improvement to produce pigs with improved food conversion, reduced carbon costs and a carcase quality that reflect local and international customer requirements. The use of the pig in biological products also demands the maximum output per boar.

Current breeding programmes utilise two or even three artificial insemination matings after weaning to achieve an 82+% farrowing rate and producing a total of 13+ pig born (alive and dead).

These production targets were taken as a base line to develop alternative methods of enhancing boar production.

Single serving sows with boars have been recognized as a successful method of breeding for over 30 years. But the technique has never become a routine breeding technique, partly because if the boar is infertile the results are disastrous.

Methods

Semen

Boars semen was pooled (minimum 2) to avoid the likelihood of any one boar being infertile (despite visual examination of the semen). Sperm concentration was a minimum of $2x10^9$ sperm per insemination in a semen dose of 75 ml.

Semen delivery system

Sows were served using a controlled boar exposure breeding stall in groups of 3. Seven minutes were allowed for each group of 3 matings. The sow was mated using a foam tipped catheter and a semen delivery system allowing for a hands free insemination. The sow was allowed to climax and move the semen into her uterus and oviduct under peristaltic contractions.

Mating programme for Single Served Group

Weaned sows

The sows were weaned on day 0

There was no boar exposure (boars not present in the building) until day 4

Sows were heat checked in the morning of day 4. Sows in oestrus were noted but not bred.

Sows were heat checked in the morning of day 5. All sows in oestrus were immediately mated and the quality of the mating recorded (good, average, poor).

Sows were heat checked in the morning of day 6. All sows which recorded a poor mating on day 5 and in oestrus on day 6 were remated (recorded as a double mating – there was less than 1% of matings in this group). All sows which recorded an average or good mating on day 5 were ignored. All sows, which were in oestrus for the first time on day 6, were mated and the mating recorded (good, average, poor).

Sows were heat checked in the morning of day 7. All sows which recorded a poor mating on day 6 and in oestrus on day 7 were remated (recorded as a double mating). All sows which recorded an average or good mating on day 6 were ignored. All sows that were in oestrus for the first time on day 7 were mated and the mating recorded (good, average, poor). They were remated on day 8 (recorded as double mated).

Return sows and gilts Late Sows (in oestrus for the first time after day 7). (double mated).

Sows or gilts that return to oestrus once were mated once in the morning of day 1 and by a boar on day 2.

Gilts (double mated)

Gilts were mated twice by AI in the morning of two consecutive days (AM,AM) and the quality of the mating recorded.

Mating programme for Double Served Group

All sows were mated AM/AM following heat detection on day 4 plus post-weaning. Returns and gilts were double mated AM/AM after their oestus was detected.

Results

Breeding	# bred	# farrow	FR %	Total born/sow
Single	2856	2555	89	13.9
Double	4066	3375	83	13.3

FR= Farrowing rate

There is a statistical difference between the results favouring single service, this is to be expected as the single serving group would be naturally more fertile.

Cost implications

There is an obvious benefit in reducing AI purchases to achieve pregnancy. However, the author prefers that the same genetic cost be spent on utilising boars of higher genetic merit to reduce feed conversion and enhance carcase quality.

The major savings are:

Time to mate the sows

Catheter and equipment costs

Time to collect boars

Need to house and feed extra boars of poor genetic merit.

Conclusions

Single serving appropriate weaned sows by AI can be easily achieved by today's pig industry.

The system effectively reduces the boar power required by a factor of 2 - instantly increasing genetic improvement to focus on food conversion for example.

Reduction in breeding time allows the farm to reconsider its stockmanship priorities towards focusing on farrowing and weaning.

For further details see: Muirhead, Alexander, Carr. (2013) Managing Pig Health 5M Publishing, ISBN 978-0-9555011-5-9

Sperm morphology results from thirty North American boar studs

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Introduction

Boar Studs in North America may or may not do objective morphological analysis on every ejaculate before processing. General recommendations are that acceptable minimums should be 70-80% normal morphology[1-3]. Guidelines have been established for assessing morphology[4]. A 10% decrease in abnormal sperm (from 30% to 20%) has been shown to increase total born by .08 pigs[5]. Fully processed doses were evaluated from 30 boar studs over the course of one year to determine characteristics of doses currently being used in North America.

Materials and Methods

Fully extended semen doses submitted to the Swine Vet Center Andrology Lab (a CIVAL member andrology lab) were evaluated over the course of a year. Doses were determined to be acceptable for distribution by the boar stud and were submitted for end product monitoring for sperm counts, morphology, and bacterial culturing. Prior to the start of data collection, adjustments to the computer settings were made until correlations were determined to be >.9 between human assessment of morphology and the CASA assessment. Morphological abnormalities counted included Distal Midpiece Reflux (DMR), Coiled Tails, Proximal Droplets, and Distal Droplets. Twelve of the studs are performing objective morphological analysis on every ejaculate and using morphology as a screening tool for whether ejaculates are acceptable for processing. In each case a representative sample of sperm (50) are counted after sperm are fixed using either sodium citrate or hypertonic saline. A cutoff of 70% normal is used. The other 18 are only doing subjective analysis on ejaculates at the time of processing.

Results

A total of 4439 doses were evaluated from 30 boar studs. A mean of 532 sperm were evaluated per dose (Std. Deviation=110). Overall 7.3% of the doses were found to have <70% morphology. 31.6% of the doses had 70-80% normal morphology. Studs doing objective screening (n=3181 doses) had 5.3% of submitted doses below 70% actual normal morphology compared to 12.1% (n=1258 doses) of doses from studs performing subjective morphology (P<.0001). There was a similar % of ejaculates (31.2% and 32.5%) of samples falling in the 70.0-79.9% normal morphology for boar stud performing objective and subjective morphology respectively.

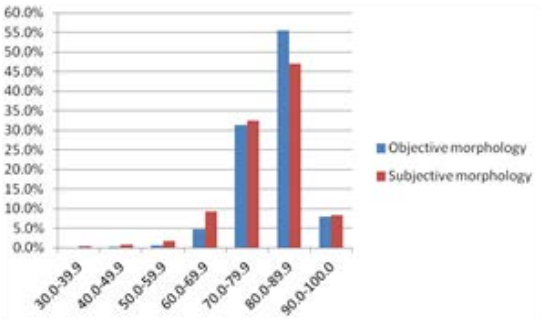

Figure 1. The % normal morphology assessed on 4439 doses of semen from 30 studs over a year, dependent on whether the stud assesses morphology on each ejaculate objectively or subjectively. *P<.0001*

Conclusions and Discussion

The data suggest a clear benefit to performing objective morphology assessment at the boar stud on each ejaculate, even when small sample numbers are counted. For the industry to change from a cutoff of 70% normal morphology to 80% normal morphology would result in a large portion (1/3) of the doses from North American studs being unacceptable for distribution. The mean heritability for sperm cell morphology has been estimated at 0.31[6]. Genetic selection should incorporate semen quality. As the swine industry strives for lower sperm counts to increase the influence of higher index boars, increases in minimum acceptable morphology for ejaculates may be desireable. Incoporating sperm morphology into the genetic selection index would seem necessary to be able to make significant progress.

References

1. Althouse GC et al. 1998. Swine Health Prod 6:128.
2. Chenoweth PJ. 2001. Proc Ther Conf p. 49-55.
3. Flowers WF. 1998. IPVS Proc p. 45-52.
4. Kuster et al. 2003. Therio 61:691-703.
5. Feitsema et al. 2005. Proc 7th ICPR. p. 51-60.
6. See T. 2002. NC State Extension 25:6.

O.054

Comparison of pooled feces and oral fluids for culture of *Salmonella* from swine

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Introduction

Salmonellosis is a prevalent disease in both swine and humans. During 1998-2002, *Salmonella* represented the most commonly reported bacterial cause of foodborne outbreaks in humans. One challenge for developing on-farm control methods for *Salmonella* is cost-effective diagnostic protocols, especially related to sample collection for culturing or performing other laboratory assays. In feedlot cattle, a rope technique has been described to determine *Salmonella* status.[1] In swine operations, using a similar rope technique would enable producers to more easily evaluate the *Salmonella* status of finishing pigs. The objective of this project was to compare culture of oral fluid collected with ropes to culture of pooled fecal samples for determining the *Salmonella* status of finisher pigs.

Materials and Methods

Two cohorts (barns) of swine were selected from one production company for participation. Each cohort was housed at a different finisher site. Barn A had 40 pens that held 20-22 pigs per pen. Barn B had 12 pens; 8 pens that held 100 pigs each and 4 pens that held 25 pigs each. Samples were collected every 2 weeks from 10 to 26 weeks of age For fecal pool samples, 5 X 5g (25g total) fresh feces were collected from the floor and pooled into one sample. For oral fluid collection, cotton ropes (1/2 inch diameter, 50 inches in length) were tied to pen gates and the pigs were allowed to chew on the ropes for 30-60 minutes. Twenty- four fecal pools and 48 rope samples were collected at each time point. Samples were cultured using standard methods. The prevalence of positive culture results were calculated for both pooled fecal and oral fluid (OF) samples for each sampling period. Comparison of prevalence between OF culture and fecal culture was conducted using McNemar's Test. The relative sensitivity of OF culture to pooled fecal samples (gold standard) was calculated for each sampling period, for each cohort and for all sample time points combined.

Results

A summary of pen level results is presented in Table 1. Both cohorts were positive for *Salmonella* during the study period by either method. The proportion of *Salmonella* positive cultures ranged from 0-50% and 0-40% for fecal and OF samples, respectively. For Barn A at the pen level, the relative sensitivity of OF was 10.4% and the relative specificity was 96.7%. For Barn B at the pen level, the relative sensitivity of OF was 50% and relative specificity of 89.7%. For both barns combined, the relative sensitivity of OF was 18.33% and the relative specificity of 95.6%.

Table 1. Pen level culture results –no. of samples

Barns A & B combined		Ropes		
		Pos.	Neg.	Total
Feces	Pos.	11	49	60
	Neg.	10	168	178
	Total	21	217	238

Conclusion and Discussion

Validating the sensitivity and specificity of OF culture for assessing the *Salmonella* status of swine would provide a valuable addition to the long term goal of using oral sampling as a routine diagnostic test for *Salmonella* on swine farms. In our study, in all but two sample periods, OF culture yielded the same results at the barn level (positive/negative) compared to fecal culture. However at the pen level, OF culture had poor sensitivity compared to pooled feces. Oral fluid sampling could be beneficial at the barn level for detecting *Salmonella*, but further development is needed to improve sensitivity to the level achieved by culturing pooled feces.

References

1. Smith, D.R., et al. 2002. Behavior of cattle towards devices to detect food-safety pathogens in feedlot pens. *The Bovine Practitioner* 36:5-9.
2. Prickett,J., et al. 2008. Detection of Porcine reproductive and respiratory syndrome virus infection in porcine oral fluid samples: a longitudinal study under experimental conditions. *J Vet Diagn Invest* 20:156-163.
3. Funk J. Pre-harvest food safety diagnostics for *Salmonella* serovars. Part 1: Microbiological culture. *J Swine Health Prod.* 2003;11 (2):87-90.

Effect of mannoprotein in the pre harvest phase on *Salmonella* sp. seroprevalence and carcass contamination in pigs

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Introduction

The success of *Salmonella* control in pork depends on the intervention in all steps of the production chain. Post harvest measures have been proved to be cost-effective in preventing pork contamination (4). However, in regions where the herds have a very high prevalence, on farm control is also needed (1). Thus, additional interventions in the pre harvest phase is an interesting way to decrease *Sallmonella* herd prevalence in a short period of time (3), and consequently reducing the risk of carcass contamination (2). The mannanprotein prebiotic effects are mainly related to: type I fimbriae agglutination, macrophages activation and promoting specific bacteria growth that avoids *Salmonella* colonization (5). The aim of this study was to validate the mannoprotein effect on *Salmonella* seroprevalence and carcass contamination in a large Brazilian swine agroindustry.

Materials and Methods

The study was carried out in six finishing pig herds located in south of Brazil. Three batches were fed with mannoproteins Actigen® (treatment group). Prebiotic was provided as follow: 35 days (weaning): 1600 g/ton; 36-50 days: 800 g/ton; 51-slaughter: 400 g/ton. Three other batches, without any treatment, composed the control group.

Sampling sizes were calculated to estimate the prevalence in the batch, with a 10% absolute error and 95% of confidence level, considering herd population of 600 animals and carcass population 4000/day.

Blood of 55 animals was randomly collected in two moments: at beginning of the finishing period (first day) and four days before slaughter. Additionally, 60 pigs not belonging to the treatment groups, but slaughtered at the same days were systematically collected at bleeding and defined as the contemporary group. Before chilling, 40 carcasses were sampled using sterile sponge in four points (400cm^2). Blood samples were submitted to ELISA-Typhimurium (5) and carcass samples to bacteriological culture.

The seroprevalence and *Salmonella* isolation frequencies were compared with Wald qui-square test, utilizing PROC LOGISTIC procedure in SAS 9.2 to Windows. Copyright © 2012 SAS Institute Inc.

Results

Seroprevalence was much higher at slaughter age (170 days old) than at the beginning of the finishing period (60 days) in both groups. The treated group presented a significantly lower prevalence of seropositive pigs and carcass contamination when compared with the control group (**Table 1**).

Table 1. Prevalence (CI 95%) of positive samples in control (CG) and treatment (TG) groups

	Groups	
Sampling	CG	TG
Housing	2.4 (0.05-4.7)	3 (0.3-5.6)
Slaughter	50.3^a (42.6-58)	98.7^b (97-100)
Contemporary	72^a (65.5-78.6)	100^b
Carcasses	18^a (8-28)	0^b

Different (a, b) superscripts in the same row indicate statistically significant differences *p* 0.05.

Conclusions and Discussion

Seroconvertion occurred mainly at the finishing phase as previously demonstrated (7, 8). According to the seroprevalence results, Actigen® showed a protective effect against *Salmonella* transmission within the batches, and also reduced carcass surface contamination, reinforcing the hazard of delivering highly prevalent batches on the carcass contamination (2).

Acknowledgments

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References

1. ALBAN, L.; STÄRK, K. D. Preventive Veterinary Medicine, v. 68, p.63-79, 2005.
2. BAPTISTA, F. M. et al. Preventive Veterinary Medicine, v. 95, p. 231-238, 2010.
3. BOYEN, F.; et al. Veterinary Microbiology, v. 130, p. 1-19, 2008.
4. GOLDBACH, S. G.; ALBAN, L. Preventive Veterinary Medicine, v. 77, p. 1-14, 2006
5. MORAN, C. A. 2004. Alltech's Annual Symposium: re-imagining the feed industry, pp. 283-296 (20).
6. KICH, J. D., et al., Journal of Veterinary Diagnnostic Investigation. v. 19, n. 5, p. 510-517, 2007.
7. SCHWARZ, P.; et al. 2006 IPVS Proceedings, (19).
8. SILVA, L. E.; et al.. Arquivo Brasileiro de Veterinaria e Zootecnia, v. 58, n. 4, p. 455-461, 2006.

O.056

Comparative effect of Avilamycin and Carbadox in nursery pigs diets in presence of hemolytic *E. coli*

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Introduction

Carbadox (Mecadox®, Phibro®) is a quinolone antibiotic used in feed for pigs after weaning to control enteropathogens and will have some risk for animals and people (1). Avilamycin (Surmax®/Maxus®, Elanco®) is an Orthosomycin (Oligosaccharide antibiotic) with effect vs Gram + bacteria and have an indirect effect controlling *E. coli* infection changing the coli-bacteria's adhesive proteins. Hereby the bacteria loose the ability to adhere to the intestine wall and also their disease-producing abilities (2). Avilamycin is safe for use in livestock because is poorly absorbed in the gut and has 0 withdrawal day, and used exclusively in food animals.

Materials and Methods

216 cross-breed commercial pigs (PIC) were randomly designated to 36 pens (6 pigs of mixed gender per pen) with the following treatments: T1: Control (no medicated feed), T2: Control + 80 ppm Avilamycin (Surmax®/Maxus®), T3: Control + 55 ppm Carbadox (Mecadox®). The experimental unit was the pen and there were 12 replicates by treatment. Feed and water were administered *ad libitum*. There were two diets (21 – 35 and 36 to 56 days). Diets met or exceeded NRC requirements. All treatments were simultaneous in the same barn, starting around 21 days old (at weaning) and finished at 56 days old. Individual pig weights were recorded at day 0, 7, 14, 21, 28 and 35 of treatment. Feed consumption was recorded daily by pen. Temperature in the barn and rain-fall were recorded daily. Feed samples of each treatment were collected for any further analysis. Rectal swabs from pigs with diarrhea were also collected and sent to the laboratory for analysis. Live weight, average daily gain, average daily feed intake, feed to gain (calculated) and gain to feed (calculated) were collected and reported by pen. Sick pigs were treated and concomitant therapy was recorded. Statistical analysis was done by RCBD (3).

Results

Body weight per treatment is reported in Table 1.

Table 1. Body weight.

Day	0	7	14	21	28	35
T1	6.22	7.00	9.24	12.20	15.84	19.64
T2	6.22	7.31	9.76	12.79	16.26	20.48
T3	6.18	7.18	9.49	12.60	16.09	20.46
P	<0.98	<0.61	<0.37	<0.36	<0.72	<0.37

(a, b) Superscripts indicate statistically significant differences within main effect (p<0.05). Multiple means comparison Tukey-Kramer method (p< 0.05).

Productive results are reported in table 2.

Table 2. Productive results.

Treatment	DFI	ADG	FC
T1	531	383	1.387[a]
T2	520	407	1.278[b]
T3	522	408	1.280[b]
Probability	<0.76	<0.12	<0.0002

(a ,b) Superscripts indicate statistically significant differences within main effect (p<0.05). Multiple means comparison Tukey-Kramer method (p< 0.05).
DFI: Daily feed intake (grams per day).
ADG: Average daily gain (grams per day).
FC: Feed conversion (consumption/gain).

12 rectal swabs were taken from pigs with diarrhea. All pigs came from T1. All samples were positive to abundant alpha-hemolytic *E. coli*.

Conclusions and Discussion

Treatments 2 (Avilamycin, Surmax®/Maxus®) and 3 (Carbadox, Mecadox®) produce the same statistical results and better than T1 (Control).
There are numeric but not statistical differences between T2 and T3, maybe due to sample size, pathogenicity of the bacteria involved, or presure infection due to concomitant infection, management, whether conditions or diet.
This study demonstrated avilamycin is a safe alternative to use to medicate nuresery diets for swine for control of pathogenic *E. coli*.

References

1. Health Council of the Netherlands: Dutch Expert Committee on Occupational Standards (DECOS). Carbadox. The Hague: Health Council of the Netherlands, 1999. Publication no. 1999/06OSH.
2. Friis Ch. 1993. Hyologisk. No. 8, 19-22.
3. NCSS 2007, Number Cruncher Statistical System.

O.057

Oldies making a comeback, sounds familiar?

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Introduction

Oedema disease is a sporadic disease of weaner pigs characterized by nervous signs usually with low morbidity and high mortality rates, caused by *E coli* nearly all of which are alpha-hemolytic. Most of them belong to a very limited number of serotypes (1). The objective of this paper is to first show that many non recognized strains are capable of causing oedema disease, the large prevalence of multi-resistant strains as well as the need of combining ETEC and ED strains in vaccines for them to be effective in the field.

Materials and Methods

During the second half of 2012 after a very wet season and after a huge invasion of rodents in piggeries located in northern New South Wales and south-east Queensland a total of ten outbreaks of oedema disease and post-weaning scours with high morbidity and mortality were investigated.
Four piggeries were located in northern NSW and six in QLD.
Pigs were examined clinically, onset of disease started most commonly 4 to 7 days after weaning and in some cases between 10 to 14 days after weaning, morbidity range from 20 to 60% and mortality range from 60 to 90% being both morbidity and mortality higher in farms with mix infections.
At least 75% of the outbreaks had a mixed infection of post-weaning scours and nervous signs and samples for bacterial culture were collected from duodenum and colon during post-mortem examination. E coli isolates were tested for antibiotic sensitivity at the Biosecurity Queensland Veterinary Laboratories and serotyped by Queensland Health Forensic and Scientific Services

Results

All isolates (100%) were resistant to tetracyclines, 80% were resistant to Trimeth-sulfa and lincospectin, and 70% were resistant to neomycin, amoxicillin and apramycin. All isolates (100%) were sensitive to ceftiofur.

Table 1. Sensitivity test results by state.

	NEO	AMO	APRA	T/S	CEF	TET
NSW 1	+	-	+	-	+	-
NSW 2	-	+	-	-	+	-
NSW 3	+	-	-	-	+	-
NSW 4	-	-	-	-	+	-
QLD 1	+	-	+	-	+	-
QLD 2	-	+	+	-	+	-
QLD 3	+	-	-	+	+	-
QLD 4	-	-	-	+	+	-
QLD 5	-	+	-	-	+	-
QLD 6	+	-	+	-	+	-

Neo=Neomycin, Amo=amoxicillin, Apra=apramycin, t/s=trimethoprim/sulfa, Cef=ceftiofur, tet=tetracyclines

Table 2. Serotyping of *E coli* isolates

NSW	QLD
E coli inactive	E coli ONT:HR
E coli O139 stx2	E coli O8:H28
E coli ONT:H1	E coli O149:H10
E coli O153:HR	E coli O98:HR
E coli O147	E coli O149:H-
E coli ONT:H- stx2	E coli inactive
E coli O149:H10/50	

NT=non typable

Table 3. Morbidity and mortality pre and post-vaccination.

	Pre-vaccination		Post-vaccination	
Farm	Morbidity	Mortality	Morbidity	Mortality
NSW1 S	40	70	5	2
NSW2M	75	90	10	3
NSW4 S	30	60	2	0
QLD1 M	60	85	4	1
QLD4 S	25	65	2	0
QLD5 M	20	90	1	0

As many of the E coli isolates were multi-resistant strains or farms had very few options for treatment isolates were sent to Treidlia Biovet Pty Ltd for vaccine production. A total of four farms require a single isolate for a vaccine to achieve control of morbidity and mortality two in NSW and two in QLD O139 stx2, O149:H10/50, O149:H10 and O149:H- respectively however six farms (two in NSW and four in QLD) required a combination of at least two different strains and consistently involving either an *E coli* inactive or a non-typable *E coli* O.

Conclusions and Discussion

It has been reported that oedema disease syndrome is caused by E coli strains that usually belong to serotypes O139, O138 and O141 (2) however during this investigation only one farm had O139 and the other had a wide range of serotypes many of the non-typable or inactive but that were needed for full vaccine efficacy. Many of the apramycin resistant strains were O149, O147 and non-typable ones which is consistent with findings by Hunter (3).

References

1. Bertschinge, H.U. (1998) Diseases of Swine 8th ed. Iowa State University Press, Ames.
2. Taylor, D.J. (1995) Pig Diseases 6th ed. St Edmundsbury Press, Suffolk.
3. Hunter, J.E.B. et al (1992) *Proceedings of the 12th International Pig Veterinary Society Congress*, The Hague, Netherlands, p.245.

Analysis of twelve *E. coli* virulence genes observed in Australia in 2012 and 2013

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Introduction

Escherichia coli (E. coli) is known to cause disease and morbidity in pigs and remains one of the most important diseases of suckling and post weaning piglets worldwide (1). Real time PCR has previously been reported as a useful tool to identify the *E. coli* virulence genes (VG) for toxins, fimbriae and adhesions (2). In this study we compared annual frequencies of VG observed in porcine diagnostic specimens tested in our laboratory.

Materials and Methods

Specimens received from sick piglets during 2012 and 2013 were cultured on MacConkey and blood agar plates and incubated at 37°C overnight. Colonies identified as *E. coli* were lysed in 100uL nuclease free water by boiling for 10 minutes at 98°C, centrifuged and the supernatant used directly in the PCR. The multiplex real time PCR (2) identified toxin genes: ST1(STa), ST2 (STb), LT1, EAST, Stx2e; and fimbriae/adhesion genes: F4(K88), F5(K99), F6(987P), F18, F41, AIDA and EAE.

Results and Discussion

Of the 314 *E. coli* isolates (197 in 2012, 117 in 2013), 42.7% were negative for all VG for both years and have not been included in further statistical analyses.

Virulence genes were observed in haemolytic *E. coli* (HEC) and also in non-haemolytic *E. coli* (NHEC). The prevalence of VG for HEC dropped from 93.2 to 84.2%, while for NHEC it increased from 35.8 to 44.3% for 2012 and 2013 respectively. Toxin genes were seen with adhesions and without. The HEC were 8.6 times more likely to have toxin genes with adhesions than without, while the NHEC were 3.1 times more likely to have toxin genes without adhesions. In 2012, 7.2% of the HEC had adhesion factors without toxin genes, however, no similar isolate was found in 2013.

The prevalence of each VG is shown in Figure 1. EAST and F4 are the most prevalent toxin and adhesion genes respectively. Fimbriae F41 was not observed at all.

Twenty five distinct pathotypes were observed, but only 11 of those were observed in both years (Figure 2). Six pathotypes were twice as prevalent one year than the other (marked with * Figure 2), however, the statistical significance of this difference cannot be determined because many of the actual number of observances is very low (five or less). The remaining 14 pathotypes were seen in one year only, again at very low numbers.

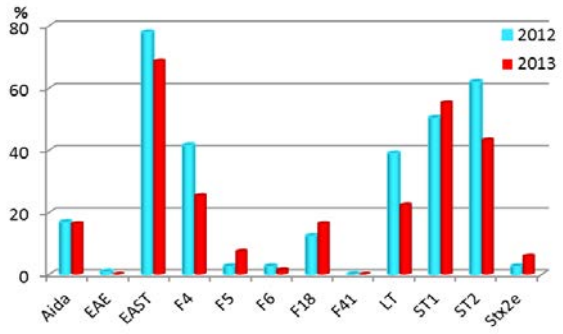

Figure 1. Prevalence of *E. coli* virulence genes.

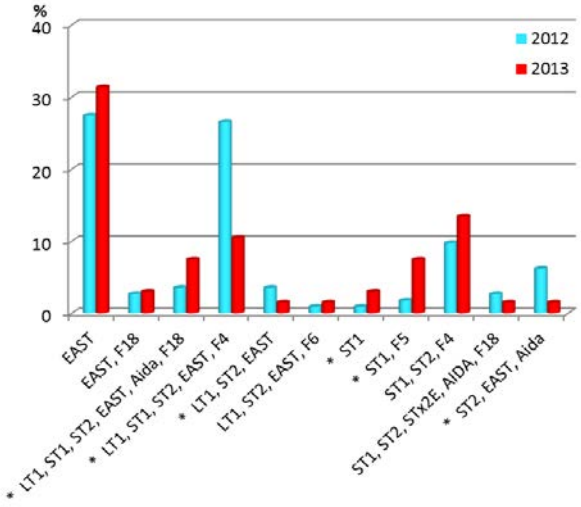

Figure 2. Prevalence of pathotypes observed in both 2012 and 2013.

Conclusions

With 14 of the 25 pathotypes seen in one year but not the other, further testing is necessary to determine if this variation is a 'normal event' and may occur each year, or if it is unique to this period.

The increase in the number of NHEC with VG over the two year period suggests that these isolates must be tested for VG routinely, regardless of the age of the host.

References

1. Ngeleka M. et al. 2003. J Vet Diagn Invest 15:242-252.
2. Limm T. M. et al. 2013. Proceedings 6th Asian Pig Veterinary Society Congress, Vietnam OR45

O.059

Expression of cellulose and curli fimbriae by *E. coli* isolated from piglets with edema disease

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Introduction

Edema Disease (ED) is caused by some serotypes of *Escherichia* (*E.*) *coli*, that colonize the small intestine and produce a Shiga-like toxin. Microscopic lesions are referable to a degenerative angiopathy, affecting the small blood vessels. The lesions can occur in different organs and tissues. Disease is generally only seen 1 to 4 weeks after weaning. The virulence of the strains involved is associated with a plethora of different factors. Among them, the ability of the bacterium to adhere to intestinal epithelial cells and to colonize the digestive tract have been identified as a crucial step in the development of the lesions. In some *Enterobacteriaceae* the capability to colonize hosts and to survive in the environment is linked to biofilm formation. The structures contributing to its formation are different. Among them, flagella, fimbriae, secretory proteins and curli fibers have a complex interaction and play different roles in the development of the biofilm. Certain isolates of *E. coli* express the so called rdar (rough dry and red) morphotype, representing the biofilm formation at liquid-solid interfaces. This morphotype is made up of the expression of cellulose and curli fimbriae. Others morphotypes such as pdar (pink, dry and rough) or saw (smooth and white) are described. The former is associated to the cellulose expression, while the latter is linked to an absence of the expression of both the above mentioned structures (2). This study investigated whether the expression of the biofilm components cellulose and curli fimbriae was found among 65 field strains of *E. coli*, isolated from piglets affected by ED.

Materials and Methods

65 strains of *E. coli* isolated from swine affected by ED were used in this study. The colony morphology was studied on Eosine Methylene Blue agar and on Congo Red agar (CRA) media. On CRA the morphotypes were read as described in other Enterobacteriaceae (2). The ability to express curli fimbriae and to produce cellulose were evaluated as described (1). The plates were incubated at 28±1°C and at 37±1°C for 48-96 hours, aerobically.

Results

At 28°C 6/65 strains (9,2%) showed the rdar morphotype representing a co-expression of cellulose and curli fibers. 59/65 (90,8%) resembled the saw morphotype, characterized by an absent expression of both the structures. At 37°C three morphotypes were found: rdar, sar (smooth and red) and saw. Rdar morphotype was seen in 2/65 strains (3,1%), while sar in 47/65 (72,3%) strains. Among them, 18/47 (38,3%) were cellulose positive strains, while 29/47 (61,7%) strains did not express cellulose. The saw morphotype was recovered in 16/65 (24,6%) strains.

Conclusions and Discussion

Both pathogenic and commensal *E. coli* isolates can produce cellulose, curli fibers, or both. It is noteworthy that the expression of the components is prone to various factors, such as phase variation and environmental conditions (2). In our study, at 28°C the majority of the tested strains expressed the saw morphotype, while at 37°C, the sar was the most recovered type. The rdar morphotype was found in a low percentage of the strains. Indeed, at 28°C only the 9,2% co-expressed the cellulose and curli fibers. Among sar morphotype we have found a varied expression of cellulose behaviour. Indeed, 18/47 (38,3%) expressed the character, while 29/47 (61,7%) did not. Calcofluor binding indicates a variable production of cellulose, but this bond is not specific for cellulose. In this case, some strains did link other proteinaceous compounds. So, another method should be used in order to confirm the involved substance. Moreover, we have found a purple (very red) colour in 38/65 (58,5%) strains at 37°C, and 4/65 (6,1%) at 28°C. This could suggest a greater amount of curli fibers or might be linked with a mutation in the genetic background. In human beings, the majority of isolates from septic episodes have been found to express curli even at 37°C. On the other hand, uropathogenic *E. coli* show expression only at ambient temperature. During ED episodes, *E. coli* isolates expressed curli fibers and cellulose more frequently at 28°C, but curli fibers were expressed in 49/65 (75,4%) strains at 37°C. Invasion of the host cells has been shown to be mediated by curli fimbriae. The obtained data indicate a different expression of both the studied structures. They are involved in the biofilm formation, which may play a role in the virulence of the *E. coli* strains. In any case, other studies must be conducted in order to know if the structures are prone to phase variation and/ or there are different genetic expression in *E. coli* strains from ED.

References

1. Hancock, V et al. 2007 FEMS Microbiol Lett 267: 30-37.
2. Romling, U et al. 2000. Mol Microbiol 36: 10-23.

O.060

Study on phylogenetic groups of *E. coli* strains involved in edema disease

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Introduction

Escherichia (E.) coli can be divided into seven phylo-groups (A, B1, B2, C, D, E and F) on the basis of the genetic substructure typical of this species. In human medicine a correlation between virulence and phylogroup of *E. coli* strains is known. In particular strains causing extra-intestinal infections are more likely to belong to phylogroups B2 or D (7). In veterinary medicine, some works suggest that the commensal E. coli phylogroups are not randomly distributed. They are connected to specie, diet, climate and body mass (1, 6). On the contrary, studies on pathogenic *E. coli* are few. In this work we analyzed 66 E. coli strains isolated from pigs affected by Edema Disease (ED) and belonging to the three most frequently involved serogroups: O138, O139, and O141.

Materials and Methods

Serogrouping was perfomed by a rapid agglutination test (4). PCR. DNA was extracted from pure culture by boiling and analyzed according to the described protocol (2). Table 1 summarized the involved genes and the obtained phylogroup.

Table 1. Quadruplex PCR (2).

Genes involved in the PCR (3)				Phylo-group	Next step
arpA (400bp)	chuA (288bp)	yjaA (211bp)	TspE4.C2 (152bp)		
+	-	-	-	A	
+	-	-	+	B1	
-	+	+	-	B2	
-	+	-	+	B2	
-	+	+	+	B2	
+	-	+	-	A or C	Confirm group C
+	+	-	-	D or E	Confirm group E
+	+	-	+	D or E	Confirm group E
+	+	+	-	E or Clade I	Confirm group E

Moreover, for certain strains others PCR were conducted in order to confirm the C or E groups (2).
Due to a non-specific product group C confirmation PCR was performed without primers for internal control.

Results

Results are summarised in Table 2.

Table 2: Results

		Phylogroup		
		A	D	Tot
Serogroup	O138	1	15	16
	O139	1	29	30
	O141	20	0	20
	Tot	**22 (33%)**	**44 (67%)**	66

Conclusions and discussion

Most of the tested strains (67%) belong to the D phylogroup, unlike a previous publication related to diseased swine (8) reporting a prevalence of group A. 33% of the analyzed strains belong to group A, which is considered less invasive in human medicine. In order to reach a better evaluation of the role of the different groups, studies on their distribution among commensal *E. coli* in Italian swine farms are needed. Indeed, actual data on the distribution of phylogenetic groups among commensal *E. coli* in swine are few and discordant. Reports of prevalence of B1 phylogroup in healthy swine in Thailand (5) and of A phylogroup in France (3) suggest the influence of geographical location. Our results show that, among *E. coli* involved in ED, strains belonging to the same serogroup most frequently show the same phylogenetic group. Other studies must be conducted in order to establish if there is a connection between phylogroup and virulence factors (O and eventually F antigens). This analysis can become a useful tool in evaluating potential pathogenicity of *E. coli* isolates. The applied method was confirmed to be an efficient and low-cost system to identify *E. coli* phylogroups.

References

1. Baldy-Chuzdik K et al. 2010. Vet Microbiol. Sep 18;131(1-2):173-84.
2. Clermont O et al. 2013. Environmental Microbiology Reports 5(1), 58–65
3. Escobar-Paramo P et al. 2006 Enviroment. Microbiol. 8 (11), 1975-1984
4. de Leeuw PW and Guinée PAM 1981. Laboratory Diagnosis in Neonatal Calf and Pig Diarrhoea p 160
5. Lay KK et al. 2012 Foodborne Pathog. Dis. Nov;9(11):992-1001
6. Gordon DM, Cowling A 2003. Microbiology. Dec;149 (Pt 12):3575-86.
7. Johnson JM, Stell AL 2000. J Infect Dis. Jan;181(1):261-72.
8. Wang XM et al. 2010. FEMS Microbiol Lett. May;306(1):15-21.

Experimental reproduction of mucohemorrhagic diarrhea following inoculation with *B. hampsonii* clade 1 (Canadian isolate 30599)

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Introduction

The first Canadian diagnosis of *B. hampsonii* clade 1 was in November 2011 from grow-finish pigs with bloody, mucoid diarrhea. This was a unique event, since all previous *B. hampsonii* cases diagnosed by our laboratory were clade 2. Since its first diagnosis, we have identified *B. hampsonii* clade 1 in the absence of other *Brachyspira* spp. in 19 other cases of diarrhea from 7 farms. The objective of this study was to determine if Canadian *B. hampsonii* clade 1 isolate 30599 is pathogenic to pigs.

Materials and Methods

Eleven five-week-old pigs, testing negative for *B. hyodysenteriae*, *B. pilosicoli*, *B. murdochii* and *B. hampsonii* by culture and/or PCR, were inoculated intra-gastrically with 10^8 genome equivalents of *B. hampsonii* clade 1 (30559) on 3 consecutive days following an overnight fast. Six pigs were similarly sham inoculated and housed in a separate room. All were fed a commercial, non-medicated diet. Feces were collected daily and scored for consistency (0=normal, 1=wet cement, 2=runny, 3=mucoid, 4=bloody). Inoculated pigs were euthanized and necropsied at peak clinical signs or 13 dpi if non-diarrheic. Controls were necropsied on 14 dpi. *Brachyspira* spp. was isolated in feces and terminal colon by culture (BJ and CVS agar). Zones of hemoylsis detected within 96 hours were touch-sampled, routinely processed and the *nox* gene sequenced. The concentration (copies/g) of *B. hampsonii* clade 1 was determined in feces and colon using an in-house Sybr Green RT-PCR. Samples collected at termination were tested for *Lawsonia intracellularis* (Li), salmonella, porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2).

Results

Nine of 11 inoculated pigs developed severe diarrhea (8 bloody, 1 mucoid) between 7 and 10 dpi, and were terminated between 8 and 12 dpi. One of 6 control pigs demonstrated runny diarrhea on 4 dpi. Moderate to severe colitis was observed in 7/11 inoculated pigs, but in no control pig. All pigs tested negative for salmonella, Li, PRRSV, and PCV2. *B. hampsonii* clade 1 was isolated from the colon of 9/11 inoculated pigs but from no controls. *B. intermedia* was isolated from colon of the remaining 2 inoculated and from 3/6 controls. *B. hampsonii* concentration in colon ranged from 10^6 to 10^9 genome equivalents/g. *B. hampsonii* was not identified in feces or colon of control pigs.

All inoculated pigs shed *B. hampsonii* clade 1 in feces. The onset of fecal shedding was 5.4 (±1.6) dpi. In the two inoculated pigs that did not develop diarrhea, shedding was confined to one day and low levels (~10^5 genome equivalents/g). In inoculated pigs that developed bloody or mucoid diarrhea, a higher concentration was detected over multiple days. Peak concentrations ranged from 10^7 to 10^{11} genome equivalents/g. The characteristic microscopic lesion of moderate to severe mucopurulent colitis with necrosis was observed in 9/11 inoculated pigs. Two pigs had mild lesions that were not discernible from the control pigs. These were the same pigs that did not develop diarrhea.

Conclusions and Discussion

This experiment confirmed the pathogenicity of *B. hampsonii* clade 1 using Canadian isolate 30599 in susceptible pigs. Moreover, dietary manipulations such as the feeding of excessive amounts of soybean meal were not required to induce mucohemorrhagic colitis. Although the present experiment was not designed to compare the relative pathogenicity or virulence of clade 1 and clade 2 *B. hampsonii*, the prevalence of mucohemorrhagic diarrhea following challenge in this experiment was similar to our previous experiments assessing the pathogenicity of *B. hampsonii* clade 2 (Canadian isolate 30446)[1]. Burroughs *et al*. have also reproduced bloody diarrhea following experimental *B. hampsonii* challenge[2] using clade 2 and a "strongly hemolytic *B. intermedia* isolate widely believed to be *B. hampsonii* clade 1. These results, along with the frequent isolation or detection of clade 1 and 2 *B. hampsonii* in diagnostic samples in the absence of *B. hyodysenteriae*, demonstrate it's is causal association with the outbreak of mucohemorrhagic diarrhea underway in western Canada since 2009.

References

1. Rubin JE, et al. 2013. *PLoS One*. 8:e57146.
2. Burrough ER, et al. 2012. *J. Vet. Diagn. Invest.* 24:1025-1034.

O.062

Diversity and distribution of "*B. hampsonii*" in U.S. swine herds

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Introduction

Outbreaks of dysentery in swine herds across North America have been reported to cause significant economic losses since the late 2000s. A novel strongly hemolytic species, "*Brachyspira hampsonii*", was isolated from cases of mucohemorrhagic diarrhea, clinically indistinguishable from that caused by *Brachyspira hyodysenteriae*. The novel pathogenic "*B. hampsonii*" was found to consist of two distinct clades (I and II), of which clade I was more frequently isolated from farms in the U.S.[1]. The genotypic diversity and epidemiological significance of both clades remains unknown. The aim of this study was therefore to characterize the strains of "*B. hampsonii*" circulating in U.S. swine herds, and investigate their distribution, relationships and epidemiology using multi-locus sequence typing (MLST).

Materials and Methods

A new MLST scheme was established based on six housekeeping genes, four of which (*est*, *glpK*, *pgm* and *thi*) used primers previously proposed for a MLST scheme for the genus *Brachyspira* sp.[2], and two of which (*adh* and *gdh*) were newly developed. A preliminary sample set of 40 "*B. hampsonii*" isolates (28 clade I and 12 clade II) originating from 25 sites, 13 systems and five major swine rearing states across the U.S. in 2009-2013 were obtained from the University of Minnesota Veterinary Diagnostic Laboratory's culture collection. These isolates were characterized and analyzed by MLST on three levels (intra-site, inter-site/intra-system and inter-system). The PCR conditions consisted of an initial denaturation at 95°C for 15 min, followed by 35 cycles of 94°C for 30 sec, 48°C for 1 min and 72°C for 1 min, and lastly a final extension at 72°C for 10 min. The nucleotide sequences of isolates for each locus were compared to determine the number of alleles. The isolates were then characterized as a genotypic sequence type (ST) based on their combination of alleles, and clonal STs were grouped into clonal complexes (CCs). The concatenated nucleotide sequences were used to evaluate the relatedness of genotypes of both clades in the U.S.

Results

The evaluated "*B. hampsonii*" isolates represented 11 STs (four of clade I and seven of clade II) and three CCs (one of clade I and two of clade II) (Figure 1). No more than one ST was found in each site, and in general, different sites owned by a system shared a common ST. Sites and systems that were positive for "*B. hampsonii*" over several years were found to be infected with the same STs over time. Only one system was found to have isolates of more than one clade, however, within that system, each clade was specific to a site.

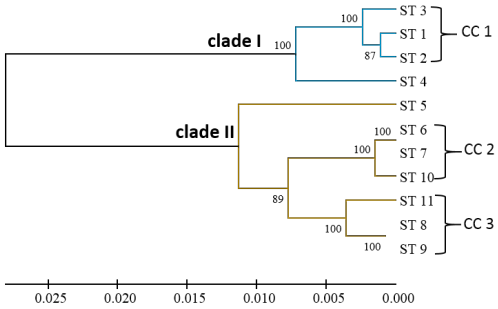

Figure 1. UPGMA dendogram depicting molecular relatedness of 11 "*B. hampsonii*" STs. The length of each concatenated sequence is 4,113 nucleotides, and the total length of the scale represents 25 substitutions per 1000 base pairs of nucleotide sequence.

Conclusions and Discussion

"*B. hampsonii*" genotypes were found to be epidemiologically related to their site and system of origin. The detection of the same genotype in a site temporally, despite histories of apparently successful "*B. hampsonii*" elimination, suggests the possibility of a re-infection from a source within the system. From this preliminary study, "*B. hampsonii*" clade II in the U.S. showed more genotypic diversity than clade I. This is the first study to characterize "*B. hampsonii*" genotypes circulating in swine herds and to elucidate their epidemiology and relatedness.

Acknowledgments

We thank the University of Minnesota Veterinary Diagnostic Laboratory for "*B. hampsonii*" isolates.

References

1. Chander Y et al. 2012. Journal of Veterinary Diagnostic Investigations 24(5):903-910
2. Rasback T et al. 2007. Microbiology 153(12), 4074-4087

Minimal inhibitory concentration of *B. hyodysenteriae* strains isolated from pigs in Brazil

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Introduction

B. hyodysenteriae is the primary agent of swine dysentery, characterized by mucohaemorrhagic diarrhea (11). An increase in the number of clinical cases of this disease has been observed, especially in the United States and Canada since 2007 (2). In Brazil, approximately eighteen new outbreaks of swine dysentery were diagnosed since 2010; a fact of great importance, due to previous history of sporadic isolated cases of low economic impact. Currently, all major swine producing regions of the country have confirmed cases of swine dysentery. One explanation for the increase in the number of cases is a decrease in sensitivity to the antimicrobials commonly used. Thus, the assessment of antimicrobial resistance is essential for the complete understanding of the current outbreak. The aim of this study was to determine the patterns of minimum inhibitory concentration of Brazilian *B. hyodysenteriae* strains isolated from pigs with swine dysentery.

Materials and Methods

Samples: Twenty two strains of *B. hyodysenteriae* were isolated from pigs clinically affected with diarrhea and colitis from states of Brazil. Isolation: All fecal and intestinal smear samples were inoculated using swab smears on plates with selective medium for *Brachyspira* sp. TSA agar (8) and incubated for at least three days at 42°C in jars with anaerobic atmosphere. Multiple passages were performed to obtain pure colonies. PCR: The technique of double amplification for *B. hyodysenteriae* and *B. pilosicoli* (6) was used. Minimum Inhibitory Concentration: Antimicrobial susceptibility testing were performed on each isolate to tiamulin (0.063 to 8 µg/ml), valnemulin (0.031 – 4 µg/ml), doxycycline (0125-16 µg/ml), tylvalosin (0.25 to 32 µg/ml), lincomycin (0.5 to 64 µg/ml) and tylosin (2-128 µg/ml) using the VetMIC ™ Brachy SVA plates (see 2). Each well was inoculated with 0.5 ml of Brain Heart Infusion Broth (BHI), 10% of fetal bovine serum and about 10^6 CFU/ml *Brachyspira* sp. Results were interpreted as the lowest concentration inhibiting visible evidence of growth (increased turbidity of the medium).

Results

All samples were confirmed as *B. hyodysenteriae* by PCR. The proportion of samples with low sensitivity compared to wildtype were 90.9% to tiamulin, 91% to valnemulin, 95% to doxycycline, 95% to tylvalosin, 100% to lincomycin and 95% to tylosin (11). Results of median, mode, MIC 50, MIC 90 for the different antibiotics tested are shown in Table 1.

Table 1. Median, mode, minimum inhibitory concentration value at which 50% and 90% of isolates of *B. hyodysenteriae* sensitive (MIC 50, 90) for each antibiotic tested.

Variable	Median µg/ml	Mode µg/ml	Minimum and Maximum µg/ml	MIC 50	MIC90
Tiamulin	8	8	0,063->8	8	8
Valnemulin	2	4	0,031->4	2	>4
Doxycycline	2	2	1-8	2	8
Tylvalosin	16	32	1-32	16	32
Lincomicina	64	64	2->64	64	>64
Tylosin	>128	>128	4->128	>128	>128

Conclusions and Discussion

Tylosin had high MIC50 and MIC90 results, similar to the literature (1, 3, 4, 5, 6, 7, 9, 12 and 14). The high frequency of resistance found to tylosin is not surprising, as this antimicrobial has been used widely in recent years. Tylvalosin had high MIC results, when compared to literature. For lincomycin, the MIC50 was higher compared to the reported literature; however, MIC 90 was close to what was found by (4) and (1). For doxycycline, the MIC50 values were close to those found by (14), (1), although the MIC90 was higher. Valnemulin results were close or similar to those already reported. MIC50 results to tiamulin were higher than those in the previously studies, however MIC90 were similar to the results found by many authors (1, 3, 4, 5, 6, 7, 9, 12, 14).

The study has confirmed that a high proportion of *B. hyodysenteriae* strains in Brazil are multi-resistant to the few antimicrobial agents that are available to treat and control this disease. This is critical to the health and welfare of pigs in Brazil, as well as the economy of the Brazillian pig industry. Strict biosecurity is urgently required to prevent the spread of resistant isolates. Collaboration and effective planning by the industry is needed to tackle and eradicate this serious emerging disease threat.

Acknowledgment

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References

1. Álvarez, L. et al., 2013. In: *International Conference on Colonic Spirochaetal Infections in Animals and Humans. Surrey*,82,
2. Chander, Y. et al., 2012. *J. VET. Diagn. Invest*. 24, 5, 903–910.
3. Hidalgo, A. et al. 2011. *Antimicrob. Agents. Chemother*. 55, 7, 3330–3337.
4. Hidalgo, A. et al. 2009. *Res. Vet. Sci.* 87, 7–12, 2009.
5. Karlsson M., et al., 2003 , *J. Clin. Microbiol*. 41, 2596–2604.
6. Karlsson M., et al., 2002. *Vet. Microbiol.* 84, 123–133, 2002.
7. Karlsson M., et al., 1999. *FEMS Microbiol. Lett*. 172, 255–260.
8. La, T. et al., 2003. J. *Clin. Microbiol.*, 41, 7, 3372–3375.
9. Mirajkar N. S. & Gebhart C. J. 2013. In: *International Conference on Colonic Spirochaetal Infections in Animals and Humans.* Surrey, 53.
10. Novotná, M. & Skardová, 2002. *Vet. Med. – Czech*, 47, 4, 104–109
11. Pringle, M. et al., 2012 *.Act. Vet. Scan.*, 54, 54, 2012.
12. Rohde, J. et al., 2004. *Vet. Microbiol*. 102, 25 –32.
13. Taylor, D. J. & Alexander, T. J. L. 1971. *Br. Vet. J.*,11,58-61.
14. Zmudzki, J., et al. 2012 *Pol. J. Vet. Sci.* 15, 2,259–265.

O.064

Testing oral fluid samples to diagnose swine dysentery in commercial farms

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Introduction

Oral fluids are becoming commonplace among swine practitioners because of the ease of collection. A further advantage is oral fluids sample the population (the pen) rather than an individual.

The effectiveness of such a tool to detect enteric pathogens by PCR such as *Lawsonia intracellularis* has been proven[1]. Our objective is to assess if oral fluid samples are an adequate farm diagnostic tool for the detection of Brachyspira species. Warneke et al showed that if a rope is soaked into a mix of pure Brachyspira culture or mixed with oral fluids or feces, the bacteria can be cultured from the liquid obtained out of this rope. Then they demonstrated similar results from oral fluids using inoculated pigs. Our goal is to assess if the technique is efficient in a commercial farm.

Materials and Methods

A swine-dysentery (SD) positive finishing farm was selected. The attending veterinarian made the diagnosis based on clinical signs (hemorrhagic diarrhea) and positive cultures for *Brachyspira hyodysenteriae* of fecal samples.

Four cotton ropes were used in four randomly selected pens in each barn on the four barn farm. Sixteen total ropes were placed. The ropes were tied to a pen divider. Only one pen had access to a rope. The ropes were left in place for 15 minutes. At the end of the sampling time, oral fluids were collected into a plastic bag and transferred to a sterile tube. Five rectal swabs were randomly taken in each rope sampled pen to determine SD status. A total of 20 individual fecal swabs were cultured.

The samples were refrigerated and shipped on ice to the Veterinary Diagnostic Laboratory at University of Minnesota to be cultured and tested by Nox-Restriction Fragment Length Patterns[3].

The statistical unit was the pen. Therefore, a single positive fecal swab in a pen was considered a positive pen. To compare both of the techniques, a Chi-square test was used.

Results

The results are presented in Table 1. Chi-square analysis showed no significant difference among the two sample methods (p>0.01).

Table 1. Culture followed by Nox-RFLP results.

Barn	1				2				3				4			
Age	1 week placed				2 weeks placed				3 weeks placed				4 weeks placed			
Clin. St.	Hemorrhagic Diarrhea				Hemorrhagic Diarrhea				Hemorrhagic Diarrhea				Hemorrhagic Diarrhea			
Pen	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
OF	N	N	N	N	H	H	H	H	H	H	H	H	H	H	H	H
F	M	M	H	H	H	H	H	H	H	H	H	H	H	H	H	H

Clin. St.: Clinical Status, OF: Oral fluids, F: Feces, N: Negative, H: Positive for *Brachyspira hyodysenteriae*, M: Positive for *Brachyspira murdochii*

Conclusions and Discussion

Brachyspira hyodysenteriae was isolated from the oral fluids collected from severely clinical pigs. Although *Brachyspira murdochii* and *Brachyspira hyodysenteriae* were isolated from the individual fecal swabs in Barn 1, the oral fluid collection failed to culture any *Brachyspira spp*. these oral fluids samples. These samples were from the youngest pigs (first week of placement in finishing) on the farm. Further evaluation in young finisher pigs as well as subclinical pigs needs to be assessed. Early detection prior to clinical signs will speed treatment decisions. The technique will also allow wider assessment of a population for potential surveillance efforts to control and eliminate this costly pathogen.

Acknowledgments

The authors want to aknowledge the farm owner and attending veterinarian for the assistance in this project.

References

1. LaVigne et al. 2013. 44th AASV proceedings p71.
2. Warneke et al. 201. 36th International Conference on Colonic Spirocheteal Infectionsin Animals and Humans 43.
3. Rhode et al. 2002 J. Cli. Microbiol. 40 (7) 2598-2600

Clinical, pathological and therapeutic assessment of *C. difficile* infections

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Introduction

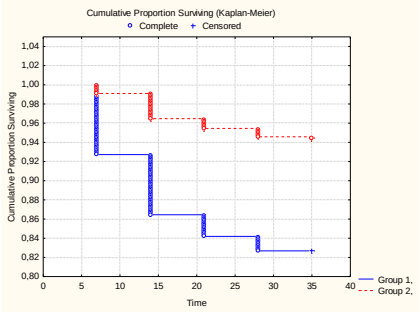
In neonatal piglets *Clostridium difficile* is one of a major cause of death with or without diarrhea (3,4) The disease has been reported in USA, Germany, Canada, Spain and Japan (5) In Argentine, *C difficile* infection was reported in 2009 (2). Prevalence in piglets 1 week-old varies from 26% to 74% (5). Our objectives in the present study were: a) to describe the pattern of field cases of *C. difficile* infection in three herds and b) a comparative therapeutic approach for control of the infection.

Materials and Methods

Farm 1 (F1): Farrow-to-finish multisite of 2,500 sows with an annual PWM of 7.6%. Farm 2 (F2): On site farm of 800 sows with annual PWM of 6.9%, which reported a suddenly 40% of mortality in 2 days-old piglets. Farm 3 (F3): A farrow-to finish herd of 200 sows with PWM of 10.6%. In total 50 necropsies were performed, Samples of small and large intestine were taken for histopathology and colonic swabs for. *C difficile* detection from F2. *C. difficile* was isolated on cefoxitin-fructose agar from stool specimens, that were treated with alcohol for spore selection. The isolates identified as *C. difficile* were cultured anaerobically in brain heart infusion broth for 4-5 days and detection of A/B toxins were perfomed by an automated immunoassay VIDAS® *C. difficile* toxin A&B (bioMérieux). Before definitive diagnoses, BMD® at 250 g/ton/feed was administered to sows before and after farrowing. In F2, a comparative therapeutic approach was performed. Age susceptible piglets with and without clinical signs were randomly selected in two groups: G1 (194 pigs) was orally administered Metrovet® (25 mg/kg/bw) and in G2 (200 pigs) Tylan® at 12 mg/kg/bw was injected. Collected data of piglet mortality during the first 35 days post-treatment were recorded and statistical comparison was made with χ2-test with p= 0.05 as level of significance.

Results

In F1 no clinical signs were observed. In F2, affected pigs showed, dyspnea, weakness, lethargy, hypothermia, abdominal pain, subcutaneous edema and death within hours. PWM dropped from 40% at the beginning to 20% during the 2nd week, and remained for 4 weeks. In F3, hyperemia, abnormal postures and hypothermia was recorder in 10% of litters in < 7 day-old pigs. In F1, mesocolon edema was found in 42% of pigs and 75% in F2 plus dehydration, subcutaneous edema and watery diarrhea in 25%. In F1 and F3 microscopic lesions comprised edema of the colonic mesothelium with perivascular infiltration of neutrophils and hyperplastic and reactive lymphoid nodules. The colonic lumen showed fibrinopurulent exudates with a focal necrosis of surface epithelium with a focal infiltration of neutrophils in la lamina propria and lumen resembled a "volcano" lesion. In F2, only colonic edema, enlarged Peyer patches and hyperplasia of goblet cells were observed. *C. difficile* isolated strains were positive to toxins A and B. In F1 and F3, the use of BMD in sows seems to control the PWM. In F2, reduction of PWM by Tylan® (G2) treatment was statistically significant with respect to Metrovet® (G1) ($p < 0.0010$) (fig.1)



Conclusions and Discussion

Clinical signs associated with high PWM in neonatal pigs resembled those reported (1,2,3,4). Edema of mesocolon, reactive lymphoid aggregates and focal supurative colitis are the pathological key-marks (2,3,4)and were seen in F1 and F3. Subclinical infection in F1 and F3 might be negligent if routine postmortem studies were not performed (2,4). *C. difficile* was isolated and prove to be toxigenic. Prophylactic treatment of sows with BMD® was effective in the reduction of the course of PWM in F1 and F3. In F2, an *in vivo* therapeutic testing of showed that Tylan® was more effective than Metrovet® in the reduction of PWM in coincidence with those reported in the literature (3, 5).

Acknowledgments

This work was partially supported by grants PICT 2010-0961) and Secretaria de Ciencia y Técnica UNLP.

References

1. Arruda H E et al. 2013. Anaerobe 22: 104-110
2. Cappuccio J et al. 2009. Braz J Vet Pathol 2:35-40
3. Songer JG et al. 2006. Anaerobe 12: 1-4
4. Songer J G, Uzal F 2005. J Vet Diagn Invest 17: 528–536
5. Alvarez-Perez et al 2013. Vet Science 95: 358–361

O.066

Infection dynamics of *L. intracellularis* in nurseries

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Introduction

Lawsonia intracellularis (LI) is a common cause of enteritis in pigs[1]. Enteritis in nurseries accounts for the major part of antimicrobials consumed in Danish pig production[2]. In many herds treatment of enteritis is initiated regularly in nursery pig batches. Little is known of whether the underlying infection dynamics of LI follows the same pattern from batch to batch. Differences in infection level between batches of nursery pigs constitutes a problem for herd diagnosis, prognosis and decisions on treatment strategy of LI induced enteritis.

Materials and Methods

A study was performed in three Danish pig herds with a history of LI induced diarrhoea in the nursery unit from October 2011 until April 2013. All herds used all in all out management with cleaning and disinfection before moving a new batch of weaned pigs into a nursery room. Feacal samples were obtained from 15 randomly selected pigs in each room at the beginning of an outbreak of diarrhoea starting a minimum of 14 days after weaning in order to avoid cases of *E. coli* post-weaning diarrhoea. The samples were tested for LI by qPCR[3] at the National Veterinary Institute, Technical University of Denmark. For statistical analysis a non-parametric Kruskal Wallis rank sum test was performed using R software[4].

Table 1. Description of trial herds

	Herd A	Herd B	Herd C
Herd size/pigs	3600	2300	2800
Room size/pigs	531	206	368
Batches studied	15	16	17
Pigs studied	217	241	254
Age at outbreak*[1]	35 [28;42]	24 [14;42]	21 [17;42]
Diarrhoea prev.	37.3 %	38.3 %	38.9 %
Range of LI among pigs*[2]	4.2 [0.0;7.9]	0.0 [0.0;8.1]	3.9 [0.0;8.1]
Range of LI among batch*[2]	4.0 [1.8;5.7]	0.5 [0.0;5.9]	3.5 [0.3;6.5]

*[1] Days after weaning, median [min;max]
*[2] Log10 bacteria / gram faeces median [min;max]

Results

LI qPCR results were obtained from 712 pigs. The LI qPCR distribution of data is shown as medians per herd in Table 1 and as boxplots per batch in Figure 1.
At the outbreak of diarrhea the level of LI shedding was significantly different between the three herds ($p < 2.2e-16$), and also within each of the three herd there was an overall significant difference in LI shedding between batches ($p < 0.05$).

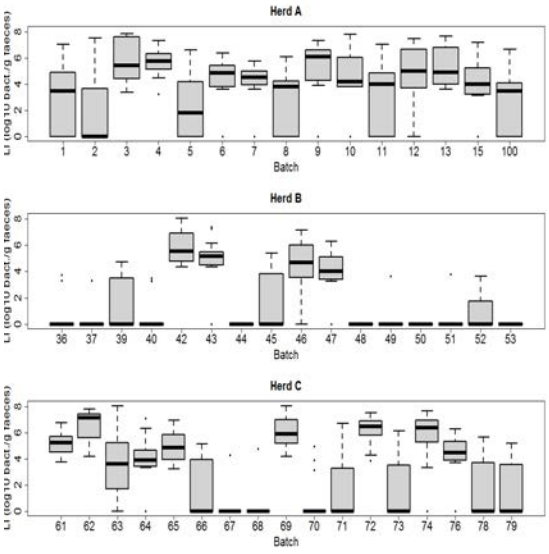

Figure 1. Boxplot of LI (Log10 bacteria/gram faeces) per batch per herd

Conclusions and Discussion

Lawsonia intracellularis shedding in faeces at outbreak of diarrhoea differed significantly between herds but also between batches of nursery pigs within the same herd.
The observed variation should be taken into account in herd diagnostic and prognostic activities based on outbreaks of diarrhoea. Also design and timing of antibiotic treatment programs should reflect that bacterial load of LI may vary between batches.

Acknowledgments

DVM Alex T. Jacobsen and DVM Lola K.T. Leihardt.

References

9. Stege H et al. 2000. Prev. Vet. Med. 46, 279–292.
10. DANMAP 2012. ISSN 1600-2032.
11. Ståhl M et al. 2011. Vet. Micr. 151 (2011) 307-314.
12. R Development Core Team 2011. ISBN 3-900051-07-0, http://www.R-project.org/.

O.067

Monitoring *L. intracellularis* infection in herds with serology and oral fluid (qPCR)

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Introduction

Monitoring *Lawsonia intracellularis* infection in pig herds has relied on serology and DNA based assays using faeces. Serology provides a good estimate of the timing and prevalence of infection, whereas quantitative real time PCR (qPCR) indicates the severity of infection (1,2,3). Oral fluids have recently been used to monitor herd health, either through pathogen or antibody detection. Oral fluid samples collected on cotton ropes represent a group of pigs within a population, rather than individual animals (4). The aim of this study was to compare the ability of qPCR amplification from oral fluid to detect the timing and severity of *L.intracellularis* infection relative to the traditional serology assay.

Materials and Methods

Matching oral fluid and serum samples were collected from multiple age groups on ten commercial wean-to-finish pig herds (every 3 to 4 weeks between 6 and 23 weeks of age). One oral fluid sample was collected from each pen of less than 40 pigs and two oral fluid samples were collected from larger pen sizes. Nucleic acids were extracted from oral fluid with the MagMax 96 Viral RNA isolation kit (Applied Biosystems) and *L.intracellularis* DNA was amplified with a TaqMan real time PCR assay (2). Blood was collected from 10 pigs per pen, representing at least 5% of pigs, and tested for *L.intracellularis*-specific IgG using the Bioscreen Enterisol Ileitis ELISA. Simple linear correlations between both the prevalence and magnitude of *L.intracellularis* DNA in oral fluid and serum antibody for each pen were tested in GenStat 16th edn.

Results

L.intracellularis DNA was detected in oral fluids collected from 58 of the 88 pens (65.9%) and from 97 of the 152 individual oral fluid samples (63.8%), from 7 of the 10 herds. *L.intracellularis* numbers ranged from 2.6 x 10^2 to 3.2 x 10^7 and *L.intracellularis* DNA was detected in pigs ranging between 12.5 weeks and 19 weeks of age.

L.intracellularis infection was detected by serology in all 10 herds, with 76 of 88 pens seropositive (86.4%). The mean seroprevalence increased to more than 80% of finisher pigs in herds 1, 3, 7, 8, 10 and 12, with the mean antibody concentration (expressed as percent inhibition, PI) greater than 55. Seroprevalence was less than 67% and the PI was below 46 in the remaining herds. In six herds, *L.intracellularis* was detected in oral fluid at the same time as antibody was detection in sera. However, in the remaining four herds qPCR detection of *L.intracellularis* was either absent or delayed relative to the presence of *L.intracellularis* antibodies (Table 1).

Table 1. Pig age when *L.intracellularis* infection was first detected in oral fluid (qPCR) or sera (ELISA).

Herd	ELISA detection (weeks)	Oral fluid qPCR detection (weeks)
1	19	19
2	19	19
3	15	Not detected
4	19	19
5	15	15
6	14.5	14.5
7	12.5	12.5
8	10	13
9	16	Not detected
10	14	Not detected

A significant correlation was demonstrated between the number of *L.intracellularis* (log_{10} transformed) detected in oral fluids and the concentration of *L.intracellularis* antibodies in sera ($R = 0.44$, $p < 0.001$), and also between the prevalence of ELISA positive and qPCR positive animals ($R = 0.39$, $p < 0.001$).

Conclusions and Discussion

The results suggest that qPCR detection of *L.intracellularis* in oral fluid is less sensitive than serology with the current sampling protocol. However, the qPCR may prove useful in herd monitoring with more frequent collection of oral fluid, or in herds where the prevalence and *L.intracellularis* titre are higher. It is not clear from this study whether *L.intracellularis* found in the oral fluid of pigs indicates current active infection or ingestion and passage of dead bacteria through the gut.

Acknowledgments

Financial assistance was provided by the Australian Cooperative Research Centre for High Integrity Pork.

References

13. Pedersen K et al. 2012. BMC Vet Res 8:198.
14. Collins A et al. 2014. Vet Microbiol 168:455-458.
15. Collins A 2013. Agriculture 3:536-555.
16. Prickett J and Zimmerman J. 2010. Anim Health Res Rev 11:207-216.

Effects of either previously vaccinated or antibiotic treated Lawsonia intracellularis naturally infected pigs on spreading of an experimental *Salmonella* infection

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Introduction

An infection with *Lawsonia intracellularis (L.i.)* in growing pigs can be controlled either by prophylactic vaccination or antibiotic treatment. Both are common measures. By an antibiotic treatment, however, the intestinal flora (eubiosis/activity/composition) is affected (1, 2). This experimental study aimed to investigate whether there is a difference in a following experimental *Salmonella* infection in pigs vaccinated in contrast to pigs treated previously with antibiotics.

Materials and Methods

In 3 consecutive trials a total of 72 potentially naturally *L.i.* infected pigs were housed in two groups and fed a conventional meal diet ad libitum (low Cu content: 27.2 mg/kg DM). Pigs of group $VAC^{+}AB^{-}$ were vaccinated against *L.i.* as suckling piglet (Enterisol®Ileitis). Group $VAC^{-}AB^{+}$ (PCR *L.i.* pos.) was treated with tylosin (10 mg/kg bw) for 5 days at the start of the trials. Two days after finishing the antibiotic treatment an experimental oral infection with *S.* Derby (dose per pig: $1.04x10^{8}$) was done in two pigs of each of the both groups. These artificially infected pigs ("seeders") were housed individually (2d) until infection was confirmed by culture test from the faeces. After that both pigs were set back into their original group. A four week follow-up investigation started (12 rectum swaps/pig, cultural S. detection in faeces). Statistical analyses (*S.* Derby positive pigs) were performed by Fisher`s exact test, differences were considered significant when $p \leq 0.05$.

Results

Table 1. Number of pigs, gender, body weight at start and end of the trial, daily feed intake, daily body weight gain and feed conversion ratio in the trials

Group	$VAC^{+}AB^{-}$	$VAC^{-}AB^{+}$
Pigs in total (n)	n=36	n=36
n (male/female)	18/18	24/12
body weight start/ end (kg)	23.2±2.38/ 56.2±4.80	23.1±1.85/ 56.5±4.05
Daily feed intake (kg)[1]	1.76	1.80
Daily body weight gain (kg)	0.789±0.09	0.798±0.07
Feed conversion ratio (feed:gain)[1]	2.23	2.25

[1] on group basis during the trials

In pigs treated with tylosin *Salmonella* infection was much more pronounced (significant differences in *S.* Derby positive pigs between the groups (p=2.155E-04) (Table 2).

Table 2. Follow-up *Salmonella* excretion in faeces comparing the two groups

Group	$VAC^{+}AB^{-}$	$VAC^{-}AB^{+}$	$VAC^{+}AB^{-}$	$VAC^{-}AB^{+}$
	artificially infected		contact pigs	
Pigs in total (n)	6 (3x2)	6 (3x2)	30 (3x10)	30 (3x10)
Swabs in total (n)	72 (6x12)	72 (6x12)	360 (30x12)	360 (30x12)
S. Derby positive pigs (n)	6 (100%)	6 (100%)	1 (3.33%)[a]	13 (43.3%)[b]
S. Derby positive swabs (n)	9 (12.5%)	16 (22.2%)	1 (0.28%)	20 (5.55%)

Conclusions and Discussion

The therapy of porcine proliferative enteropathy in growing pigs is often done by a repeated antibiotic therapy. This treatment may affect the gastrointestinal flora which seems to be more prone for *Salmonella* infection shortly after the antibiotic therapy. The risk for a higher *Salmonella* prevalence in practice within the fattening period (in cases of repeated antibiotic therapy) could be slightly higher than shown here after a single antibiotic treatment only.

References

1. Beloeil P A et al. 2007. Vet Res. Nov-Dec; 38(6):835-48.
2. Van der Wolf P J et al. 2001. Vet. Microbiol. 78:205-219.

Fecal detection of *Lawsonia intracellularis*, *Brachyspira hyodisenteriae* and *Brachyspira pilosicoli* by PCR. Cross sectional study in four farms

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Introduction

Diarrhea in growing-finishing pigs is frequently asssociated with Laws*onia intracellularis* (LI), *Brachyspira hyodisenteriae* (BH) and *Brachyspira pilosicoli* (BP) (1). Clinical differentiation among them is difficult and laboratory confirmation is necessary (2). Multiplex PCR is a rapid and sensitive test that can be applied on fecal samples from live animals (1).

The objective of this work was to detect fecal shedding of LI, BP and BH in diarrheic pigs by PCR and correlated it with diarrhea score and percentage of affected pigs in four commercial swine herds.

Materials and Methods

Four farrow to finish swine farms between 500 and 1000 sows participated in this study. Fecal samples were taken in sterile bags from pigs with diarrhea of 8, 11, 14, 17, 20 and 24 weeks old. (n=10 from each age) Diarrhea was categorized (1-4) according to Winkelman (3), and percentage of diarrheic pigs at each sampled age was recorded. Information about the use of antibiotics in each farm was obtained. The DNA was extracted (ZR Fecal DNA MiniPrep, Zymo Research Corp, Irvine, CA, USA) and a multiplex PCR was applied according to La et al. (1). Results were expressed as a percentage of positive samples in each age group and farm.

Results

LI DNA was detected in the four farms. All farms were negative to BH and BP. The relationship between diarrhea score and positive pigs is presented in table 1. Table 2 shows the percentage of positive pigs and the percentage diarrhea registered in each sampled age.

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Table 1. Relationship between diarrhea score and PCR results.

Score	(-) PCR LI	(+) PCR LI (%)	Total
1	99	26 (20,8)	125
2	57	28 (32,9)	85
3	3	0 (0)	3
4	2	9 (81,8)	11

Table 2. Percentage of detection of LI and diarrhea at each sampled age.

	Farm 1		Farm 2		Farm 3		Farm 4	
Age (weeks)	% det	% dia	% det	% dia	% det	% dia	% det	% dia
8	0.0	1.9	0.0	5.7	30.0	2.4	0.0	3.0
11	0.0	5.5	0.0	5.3	0.0	2.5	0.0	3.7
14	72.7	4.2	4.8	3.5	0.0	2.0	44.4	3.0
17	25.0	1.2	0.0	20.8	0.0	0.9	100.0	4.4
20	0.0	3.3	60.0	6.5	20.0	1.9	40.0	1.4
24	45.5	3.6	40.0	5.2	70.0	2.3	77.8	1.8
% total	28.0		18.6		22.6		43.6	

% det: % detection of LI. % dia: % of diarrhea at each sampled age

Conclusions and Discussion

Almost 25% of pigs with diarrhea score 1 were positive to LI. However, on farms 1 and 4 the detection of LI was associated with a high percentage of diarrhea score 2 and 4. The above mention score of diarrhea was associated with LI infection (4). The previously mentioned findings show the variability of clinical presentation of LI. In the four herds, DNA of LI was detected between 14 and 24 weeks of age. Differences in detection rates could be associated with intermittent excretion, biosecurity and hygiene measures or the uses of tiamulin or tylosin in feed (4). The lack of detection of BH and BP may be explained by the low prevalence of these agents. In farm 4, the high level of detection was associated with a clinical outbreak of LI.

The use of PCR allows us to determine the agent associated with diarrhea and could be used to determine the age of risk of clinical outbreak in each farm.

Acknowledgments

This work was supported by PICT 2010-0961 and Secretaria de Ciencia y Técnica, UNLP.

References

1. La T et al. 2006. Lett. Appl. Microbiol;42,284–288.
2. Nathues H et al. 2007. J.Vet. Med 54, 532-538.
3. Winkelman, N. 1997. 28th Annual Meeting AASV. 353-358.
4. McOrist S et al 2012. Diseases of swine 10th.811-820

O.070

Multiplex Luminex assay for detection of antibodies against three major proteins of ASFV

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Introduction

Serology has been been widely used in ASFV control programs in the Iberian Peninsula and Sardinia as a tool for the detection of ASFV carrier animals. Several ASFV structural and non-structural proteins have being identified as candidate antigens for serological tests (1,2). Among these, structural proteins p30, p54, and p72 are the best described, most highly studied, and most widely used in commercial ASFV serum antibody ELISAs.

Although ELISA is the most widely used tool for screening purposes, it is limited to measuring a single biomarker at a time. The development of spectrally-distinguishable fluorescent beads-based technology (Luminex® Corp, Austin, TX) make it possible to detect antibody against different antigen targets in a single reaction using antigen-coupled beads (3). The purpose of this study was to evaluate the serum antibody response against three major ASFV proteins (p30, p72 and p54) in a single assay using a multiplex fluorescent microbead-based immunoassay (FMIA).

Materials and Methods

Serum samples were collected over time from 9 pigs experimentally inoculated with isolate NHV, an attenuated ASFV that produces a chronic form of ASF. Serum samples were collected at day post inoculation (DPI) 0, 6, 12, 15, 19, 26, 33, 40, 47, 54, and 61. Plasmids containing the p30, p72, and p54 genes were overexpressed in *E. coli* and the recombinant His-tagged fusion p30, p72, and p54 polypeptides were purified by nickel-affinity chromatography. The covalent coupling of purified recombinant p30, p72, and p54 polypeptides was performed as previously reported (4). The absence of matrix effects that may affect assay results were investigated, e.g., interactions among antigens, non-immune specific binding, and multiplexing cross-interference. In addition, different assay parameters, e.g., coupling conditions, reagent concentrations, incubation time and buffer composition were optimized.

Results

The serum antibody response to ASFV polypeptides p30, p72, and p54 polypeptides between DPI 0 to 61 is shown in Figure 1. Results are presented as the mean serum S/P ratio. Antibody was detected at 6 DPI against p72 (1/9, 11%) and at 12 DPI for both p30 (9/9, 100%), and p54 (8/9, 89%). All pigs (9/9, 100%) were positive at DPI 12 for p30, at DPI 15 for p54, and at DPI 19 for p72.

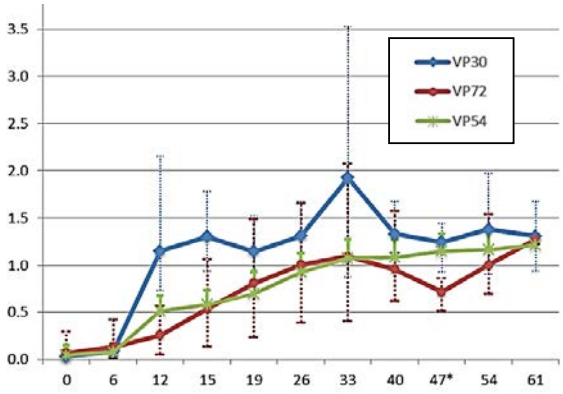

Figure 1. Serum antibody responses (S/P ratio) over time against ASFV p30, p54, and p72 as detected using a multiplex fluorescent microbead-based immunoassay (FMIA)

Conclusions and Discussion

This is the first study describing the use of Luminex technology for specific detection of circulating antibodies to ASFV. This novel approach may be used as first screening tool to explore the utility of reactivity of different antigens in different clinical situations. As an open diagnostic platform, future applications of this assay may include evaluation of other candidate antigens, as well as other sample types such as oral fluid. Preliminary results on experimental samples showed that p30 provided the best diagnostic performance and suggests that p30 would be an appropriate antigen for the development of other assay platforms, e.g., ELISA.

Acknowledgments

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References

1. Gallardo et al., 2009. Clin Vaccine Immunol 16: 1012-1020.
2. Cubillos et al., 2013. Virus Res 173:159-167.
3. Pang et al., 2005. J Immunol Methods 302:1-12.
4. Giménez-Lirola et al., 2014. Clin Vaccine Immunol 21:85.

Efficacy of real-time RT-PCR for quantification of the N gene from different PorPV isolates

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Introduction

Blue-eye disease (BED) in pigs is endemic in Mexico, and was first described in the early 1980s (1). Three genogroups have been associated with the various clinical presentations of disease (2). The diagnosis of PorPV infection has traditionally been determined by hemagglutination inhibition (HI) assays and virus isolation (3). Furthermore, the susceptibility of cell lines to infection is variable and depends on the viral genogroup (4). The viral genome has been detected using a nested RT-PCR method, but this procedure has only been used in research to detect the prototype strain, LPMV (5). It is not known whether this method is capable of detecting other PorPV strains. Because of the low sensitivity and lack of adequate quantification methods, it is essential that faster and more sensitive diagnostic methods for the detection of PorPV strains be developed. The aim of our study was to develop and evaluate the efficacy and sensitivity of a quantitative real-time RT-PCR (qRT-PCR) assay that can detect PorPV strains from the three different PoRV genogroups. We also evaluated the sensitivity and specificity of the qRT-PCR assay following the experimental infection of pigs with PorPV.

Materials and Methods

Nine different PorPV strains obtained from 1980–2003 were used to validate the qRT-PCR assay. Viral stocks were titrated on GMK cells grown in 96-well plates. Cultures were evaluated for detect the presence of cytopathic effect, hemagglutination and indirect immunofluorescence. The Reed and Muench method was used to calculate the titer, expressed as the 50% tissue culture infectious dose ($TCID_{50}$). For statistical purposes titers were transformed into $\log_{10}$ values. Nasal (n = 47) and oral (n = 47) swab samples were obtained from eight 6-week-old growing pigs. These pigs were experimentally infected *via* intranasal inoculation with 4 mL of the PAC-3 strain virus (1×10^6 $TCID_{50}$/ml). The swab samples were collected on different days after infection and during the period of clinical disease. This time frame spanned from two days prior to infection through to 17 days post-infection (dpi). Total RNA from infected culture supernatants and swab samples were extracted using a QIAamp Viral RNA™ Mini Kit (Qiagen, Inc, USA) according to the supplier's specifications. The RNA extracted from the samples was used in the quantification of the N gene of the PorPV. Real-time RT-PCR quantification was carried out following a previously described procedure (6).

Results

The limit of detection for the developed assay was 10^2 copies of synthetic RNA. Viral RNA from PorPV was detectable at $TCID_{50}$ of 0.01. Significant differences between viral RNA quantification and titrated virus from nine PorPV strains were observed (Table 1). For nasal and oral swab samples that were collected from experimentally infected pigs, the qRT-PCR assay was more sensitive (87.1–83.9%, respectively) for the detection of positive samples compared with methods involving isolation of virus.

Table 1. Quantification by qRT-PCR and virus titration for various PorPV strains

PorPV Strain	HA titration $TCID_{50}$ /mL	CPE titration $TCID_{50}$/mL	IIF titration $TCID_{50}$/mL	RT-PCR quantification relative $TCID_{50}$/mL (RNA copies/mL)
PPMV	0	7.73	5.79	9.14 (10.18)
LPMV	2.80	3.80	6.37	10.27 (11.58)
PAC-1	3.30	7.06	6.80	10.06 (11.32)
PAC-2	0	9.06	5.04	9.89 (11.11)
PAC-3	0	7.53	5.96	10.37 (11.70)
PAC-4	5.30	8.30	9.80	10.08 (11.57)
PAC-5	2.80	4.80	8.30	10.23 (11.53)
PAC-6	2.80	4.80	8.27	9.90 (11.12)
PAC-9	0	7.53	9.06	9.47 (11.82)
Mean[a]	1.88*	6.73**	7.26**	9.94*** (11.32)
SEM	0.64	0.60	0.54	0.13

*, **, ***Mean values are significantly different ($P < 0.05$). [a]Values are expressed as Log_{10}. SEM, standard error of the mean; HA, hemagglutination; $TCID_{50}$, 50% tissue culture infectious dose; CPE, cytopathic effect; IIF, indirect immunofluorescence.

Conclusions and Discussion

The implementation of highly sensitive assays that yield results quickly will be of great assistance in the eradication of PoRV from Mexico. We also believe our developed qRT-PCR assay for diagnosis will help reduce the spread of this viral infection to other countries.

Acknowledgments

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References

1. Moreno-Lopez J et al.1986. Arch Virol 91:221-231.
2. Sanchez-Betancourt J et al. 2008. Res Vet Sci 5:359-67.
3. Stephano et al. 1988. Vet Rec 122:6-10.
4. Reina J et al. 2001. J Clin Pathol 54:924-26.
5. Cuevas et al. 2009. Vet Immunol Immunopathol 127:148-152.
6. Rivera-Benitez J et al. 2013. Arch Virol. 158:1849-56.

O.072

ELISA quantification of anti-PEDV IgA antibody in colostrum as a potential tool to monitor PEDV protection in breeding herds

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Introduction

Porcine epidemic diarrhea virus (PEDV) was introduced into North America in 2013 resulting in an urgent need for reliable PEDV diagnostic assays. Recently our group developed an ELISA based on the S1 portion of the PEDV spike gene. The diagnostic sensitivity of the S1-ELISA on serum was determined to be 100% and the diagnostic specificity was 94% based on 239 field samples. Passively-acquired colostral immunity protects suckling pigs for up to 4-14 days of age (1). Studies revealed that high titers of secretory IgA antibodies in milk correlated with protection of suckling pigs against enteric coronoviruses, such as TGEV (2,3) and PEDV (4), while IgG antibodies in milk generally provided poor protection to suckling pigs (2) and presence of neutralizing antibodies in serum does not correlate with protection of newborn piglets (4). The objective of this study was to modify an existing IgG-based PEDV ELISA for detection of IgA in colostrum and milk to monitor PEDV protection and to test that assay on colostrum samples from dams with known PEDV status.

Materials and Methods

A total of 133 colostrum samples from individual sows from 3 different herds were used. Among the samples, 102 PEDV negative colostrum samples were collected from two Iowa farms in 2010 as described previously (5). The TGEV and PRCV status of these farms was unknown. The remaining 31 samples were obtained from an Iowa farm in November 2013 which had been infected with PEDV in May 2013. This farm was known to be PRCV positive and TGEV negative.

For the ELISA development, an existing IgG PEDV ELISA based on the S1 protein was utilized and slightly modified for usage on colostrum. Specifically, all samples were tested for anti-PEDV IgA and IgG and results were compared.

Results

Among the colostrum samples, 90.3% (28/31) of samples from the PEDV positive farm were positive for anti-IgG PEDV antibodies while 100% (31/31) were positive for anti-IgA PEDV antibodies. Considering the PEDV negative farms, 8.9% (9/102) of the samples were positive for anti-IgG PEDV antibodies and all samples were negative for anti-IgA PEDV antibodies suggesting a higher specificity for the IgA-based assay (Fig. 1).

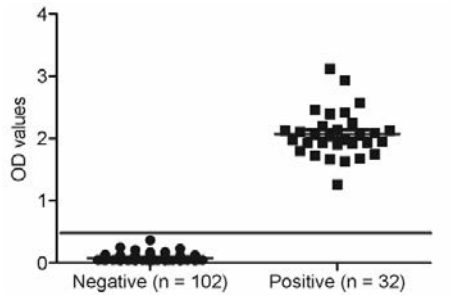

Figure 1. Distribution of IgA PEDV ELISA results on colostrum samples by negative or positive farm classification. Assay cut-off = black line.

Conclusions and Discussion

The presence of serum IgG antibodies in the dam against pathogens such as PEDV that replicate primarily in mucosal surfaces is not always correlated with protection of her piglets. In such cases, testing colostrum or milk for the presence of IgA may be be appropriate. As there is no PEDV vaccine currently available in the USA, pregnant sows have been deliberately exposed to the intestinal contents of infected piglets in PEDV-infected farms, thus artificially stimulating lactogenic immunity in an attempt to reduce the duration and magnitude of PEDV outbreaks. However, because intestinal contents may not have homogenous titers of PEDV, the induction of a solid lactogenic immunity may not be consistent across the population. Therefore, monitoring of IgA titers in colostrum and milk samples is important to assure the piglets receive adequate passive immunity. The S1-ELISA for IgA colostral antibodies developed in the present study provides a sensitive and specific tool for monitoring passive immunity for PEDV. Further studies are warranted to investigate the persistence of IgA secretion into milk during lactogenesis.

References

1. Song and Park 2012 Virus Genes 44:167-168.
2. Bohl et al. 1972 Infect Immun 6:289-301.
3. Saif et al. 1972 Inf Immun 6:600-609.
4. Ha et al. 2010 K J Vet Public Health 34:53-58.
5. O'Neill et al. 2012 Vet Rec 171:425-431.

O.073

Novel diagnostic tools for *E. rhusiopathiae* infection

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Introduction

Swine erysipelas caused by *Erysipelothrix rhusiopathiae* continues to be an important disease of pigs (1). *E. rhusiopathiae* is typically diagnosed postmortem and the gold standard is isolation of the bacteria from tissues of dead pigs. Additional tools for diagnosis of *Erysipelothrix* infection include immunohistochemistry (IHC) techniques, PCR and serology. Further characterization of *Erysipelothrix* isolates is commonly conducted by pulsed field gel electrophoresis (PFGE), serotyping, ribotyping, randomly amplified DNA, and recently the use of complete sequencing of isolates (1). The objective of this study was to evaluate traditional and novel detection tools for *Erysipelothrix* spp. on different sample types (tissues, serum samples, oral fluids) obtained from pigs with known and unknown *Erysipelothrix* status.

Materials and Methods

Samples tested included serum samples, tissues and oral fluids from experimentally infected pigs (serotypes 1a, 19), vaccinated pigs (serotype 2), from non-infected negative control pigs and from pigs from the field with unknown *Erysipelothrix* status. The diagnostic tests utilized are summarized in Table 1.

Table 1. Diagnostic assays utilized and what they detect.

Assay	Sample type			Detects
	Tissues	*Serum samples*	*Oral fluids*	
Direct isolation	+	+	+	Isolate
Enrichment	+	+	+	Isolate
ELISA		+	+	Antibody
FMIA		+	+	Antibody
IHC	+			Antigen
Real-time PCR	+	+	+	DNA

Results

Among 170 cases evaluated by both IHC and direct culture, there was a disagreement on 25 cases (14.7%) with the IHC assay determined to be highly sensitive and specific for detection of *Erysipelothrix* spp. antigen in formalin-fixed, paraffin-embedded tissues (2).

When direct and enrichment cultures were compared side-by-side, it was determined that the enrichment method was markedly more sensitive. Specifically when utilizing CNA agar, direct culture identified 38/466 samples as positive (8.2%) while 360/466 of the samples (77.2%) were positive by the enrichment method (3). When different tissues (spleen, lungs, heart, tonsil, liver, kidney) from pigs experimentally infected with *Erysipelothrix* were tested by real-time PCR, enrichment culture, and conventional PCR, the agreement between real-time PCR and enrichment culture was excellent (4). The same number of positive samples (27/37) was detected by the enrichment broth culture method and by the multiplex real-time PCR (4).

For oral fluid samples, real-time PCR was found to be a sensitive method (Fig. 1) with an overall detection rate of 100% shortly after inoculation, whereas *E. rhusiopathiae* was successfully isolated in only approximately 25% of experimentally infected pigs (4). Anti-*Erysipelothrix* IgM and IgG antibodies in serum and pen-based oral fluids were detected between 4 and 8 days post inoculation (Fig. 1) (4).

Method	1	2	3	4	5	6	7	8	9	10	11	12	13	14	21	28
Culture	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
RT-PCR	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
ELISA	-	-	-	-	M	M	M+	M+	M+	M+	M+	M+	M+	+	+	+
FMIA	-	-	-	-	M	M	M+	M+	M+	M+	M+	M+	M+	M+	+	+

Figure 1. Examples of results obtained at different days post *E. rhusiopathiae* inoculation (1-28) with 4 different assays on pen-based oral fluid samples. M=detection of IgM. FMIA= fluorescent microbead-based immunoassay.

Conclusions and Discussion

Recently developed *E. rhusiopathiae* diagnostic assays have provided veterinarians much better tools for surveillance of pigs for erysipelas. It has become standard procedure in many diagnostic laboratories to utilize either enrichment culture or real-time PCR assays for sucessful demonstration of *Erysipelothrix* spp. in various samples. In antimicrobial treated animals, IHC assays should be considered a good alternative to culture. In addition, oral fluids appear to be an appropriate sample for rapid detection of *E. rhusiopathiae* in pigs but are also suitable for long-term surveillance based on IgM and IgG antibody responses.

Acknowelegements

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References

1. Opriessnig T Wood R 2012 Diseases of Swine 10:750-759.
2. Opriessnig T et al. 2010 J Vet Diagn Invest 22:86-90.
3. Bender et al 2009 J Vet Diagn Invest 21:863-868.
4. Pal et al. 2010 J Appl Microbiol 108:1083-1093.
5. Giménez-Lirola et al. 2013 J Microbiol Methods 92:113-121.

O.074

Influenza A virus (IAV) surveillance using pre-weaning oral fluid samples

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Introduction

IAV is a major respiratory pathogen in contemporary production systems (2). IAV circulates endemically in all age groups, including suckling pigs (1). That is, IAV transmission and infection occurs in piglets despite the presence of maternally-derived antibodies. The purpose of this study was to evaluate the feasibility of IAV surveillance in breeding herds using pre-weaning oral fluid samples from litters of piglets.

Materials and Methods

Four commercial breeding herds vaccinated with autogenous vaccine participated in the study. Gilts were vaccinated at 9, 12, and ~24 weeks of age and again one week post-farrowing. Oral fluid samples were collected from 600 litters 24 hours prior to weaning and serum samples from their dams post-weaning. Litter oral fluid samples were completely randomized and tested for influenza A virus (virus isolation, qRT-PCR, subtyping and sequencing). Sow serum and litter oral fluid specimens were tested for IAV nucleoprotein (NP) antibody using NP blocking ELISAs and NP isotype-specific assays (IgM, IgA, and IgG).

Results

All litter oral fluid specimens (n = 600) were negative by virus isolation. 25 oral fluid samples were positive by qRT-PCR, based on screening and confirmatory testing in 2 diagnostic labs. 18 of the 25 qRT-PCR-positive oral fluid samples were submitted for sequencing hemagglutinin (HA), neuraminidase (NA), and matrix (M) genes (insufficient sample was available for the remaining 7). No HA and NA gene sequences were obtained, but M gene sequencing was successful for all 18 samples. Genetic analysis revealed that the M genes were identical (GenBank accession no. KF487544) and belonged to the triple reassortant swine influenza virus M gene (TRIG M). The M gene sequence obtained was identical to a previous GenBank submission JX444793/A/swine/Ohio/A01203624/2012(H3N2).

The cumulative proportions of IgM- and IgA-positive samples differed significantly by specimen type. The proportion of IgM and IgA-positive samples was significantly higher in sow serum and litter oral fluid, respectively ($p < 0.01$). No significant difference was detected in the proportion of IgG- and blocking ELISA-positive responses on the basis of specimen type.

Conclusions and Discussion

The circulation of IAV in vaccinated sow herds was detected in oral fluid samples collected from litters of pigs prior to weaning. This study supported the use of oral fluid sampling as a means to conduct IAV surveillance in pig populations and demonstrated the inapparent circulation of IAV in piglets. Thus, influenza surveillance can be done easily and effectively using pre-weaned piglet oral fluid samples. In particular, oral fluid offers the potential to conduct surveillance with fewer samples than required for individual pig testing.

Acknowledgments

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References

1. Corzo A et al. 2012. J. Swine. Health. Prod. 20, 283-289.
2. Holtkamp D et al. 2007: Part I. Swine News 30 (3). North Carolina Cooperative Extension Service.

Prevalence of chlamydiosis in the Polish pig population based on the serological and nested PCR survey

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Introduction

Chlamydia infection in pigs have been noted from 1955 but were and still are considered as non-important pathogens of pigs because are often found in association with other pathogens. Chlamydia infections in breeding sows, boars and piglets occur more often than originally thought [3]. *C. abortus*, *C. pecorum*, *C. psittaci* and *C. suis* can infect pigs and should be noted that the mixed Chlamydia infection occur very often[2]. The Chlamydia infections in swine is associated with disorders of the intestines and genital tract, respiratory disease, and conjunctivitis. However, asymptomatic infections have also been detected in many herds. The aim of the studies was undertaken to evaluate the occurrence the Chlamydia infection in pig population in Poland based on the serological and nested PCR survey.

Materials and Methods

The 15,174 samples of swine sera, 400 vaginal swabs and 25 aborted foetuses were collected in 2008-2013. The serological examination were performed using complement fixation test (CFT). Serum was considered as positive when a partial inhibition of haemolysis was observed at the dilution of 1:32. DNA extraction from swabs was performed using the commercial QIAamp DNA mini kit (Qiagen), following the manufacturer's instruction. The vaginal swabs (n=400) and aborted foetuses (n=25) were tested by nested PCR. The PCR reaction amplified the *ompA* DNA sequences specific for *Chlamydia* sp. in the first step and in the second specific for *Chlamydia suis*. The amplification was describe previously. Additionally, the samples of biological material were divided on samples from pigs with and without clinical signs and both groups were compared.

Results

Results of serological studies are presented in table 1. The percentage of positive results in subsequent years was: 0.12; 0.0; 1.38; 0.5; 1.33, 1.71, respectively. The results of nested PCR are presented in table 2 and confirmed presence of two species *C. susis* and *C. abortus*. The analysis of samples from pigs with and without clinical symptoms showed that no correlation to clinical signs was detected (table 3).

Table 1. Serological results

year	number of tested samples	positive results	percentage of positive results
2008	7,444	9	0.12
2009	1,942	0	0
2010	434	6	1.38
2011	2,801	14	0.5
2012	1,500	20	1.33
2013	1,053	18	1.71
totally	1,5174	49	0.32

Table 2. Nested PCR results

kind of samples	number of samples	number of positive results (percentage)	species of Chlamydia
swabs	400	19 (4.75%)	*C. suis*
		2 (0.50%)	*C. abortus*
foetus	26	1 (3.85%)	*C. abortus*
totally	425	22 (5.17%)	*C. abortus/suis*

Table. 3. Comparison the nested PCR results (pigs with and without clinical signs)

kind of samples	number of samples from pigs with clinical symptoms	numbers of samples from pigs with clinical symptoms
swab	200 (10*)	200 (11*)
foetus	13 (1*)	13 (0*)

*number of positive results

Conclusions and Discussion

Obtained results showed that the percentage seropositive results is similar in subsequent years. Comparing our PCR results with other publish data, it can concluded that the results are differentiated in each part of Europe [1,3]. For example in a German study, 2 uteri (n=42) of sows were positive for *C. abortus.* Whereas the studies performed in Switzerland in 2004, showed that percentage of seroprevalence of *Chlamydiace* was 63% but the newest molecular studies (real-time PCR) of fetuses showed that percentage of positive samples for *Chlamydiaceae* is significantly lower (0.35%)[3]. The results showed that *Chlamydia* sp. are presented in the Polish swine population and the *C. suis* is dominant but the *C. abortus* occurs much less and there is no correlation to clinical signs.

References

1. Donati M. et al. 2009 Vet Rec 164, 173-174.
2. Englund S et al. 2012 BMC Vet Res 8:9.
3. Koschwanez M et al. 2012. J Vet Diag Invest doi:10.1177/1040638712452729

Use of bronchoalveolar lavage in swine respiratory diseases diagnosis and physiopathology

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Introduction

The clinical diagnostic of respiratory diseases in pigs is not easy, due to the diversity of the etiologic agents. Previous works illustrated the effectiveness of bronchoalveolar lavage (BAL) in detecting *Mycoplasma hyopneumoniae*, *Pasteurella multocida* and *Haemophilus parasuis* in pigs presenting respiratory symptoms[1, 2].

The two clinical cases described in this study illustrate the use of BAL to identify the germs present in the early stages of the respiratory disease in order to help the practitioner to choose quickly the most efficient treatment and to prevent new outbreaks.

Materials and Methods

BAL fluid was collected in two commercial swine farms with respiratory problems affecting pigs batch after batch. The sampled pigs were ill fatteners (respiratory and depression signs and over 40°C rectal temperature), the clinical signs having begun less than 2 days before in the considered batches. The characteristics of the outbreaks are described in table 1. Samples were transferred within 12 hours to the local diagnostic lab under positive cold for bacteriology and PCR for *Mycoplasma hyopneumoniae* (M.h).

Table 1. Characteristics of the outbreaks

Farm	**1**		**2**	
Animals in the batch	177		205	
Age (d)	130		100	
Vaccination	None		M.h, PRRS	
N rooms	2		2	
Animals per room	89	88	103	102
Pens per room	10*	10*	4	4
Animals per pen	11 to 12	11	23 to 28	25 to 28
Ill animals per room	33	36	47	52
% ill animals	37	41	46	51
% min ill per pen	9	0	16	21
% max ill per pen	82	64	60	70

* with 2 empty pens

Results

On the 168 BAL, isolated germs were *Haemophilus parasuis* (H.p), *Pasteurella multocida* (P.m), *Bordetella bronchiseptica* (B.b), and *Actinobacillus pleuropneumoniae* (App). M.h PCR was also positive in farm 1. The results are summarized in tables 2 and 3. Influenza PCR tested on the lungs of 3 euthanized pigs per farm was negative.

Table 2. Identified germs

Farm	**1**				**2**			
Room	**A**	**B**	**Total**	**%**	**A**	**B**	**Total**	**%**
Ill animals	33	36	69		47	52	99	
No germ*	0	8	8	11.6	13	11	24	24.2
H.p	23	15	38	55.1	25	38	63	63.6
P.m	14	15	29	42.0	16	16	32	32.3
B.b	32	9	41	59.4	5	0	5	5.1
M.h (PCR)	28	12	40	58.0	0	0	0	0
App	0	0	0	0	1	0	1	1.0
Association	33	16	49	71.0	13	13	26	26.3

* including 'polymicrobial' results, with *Streptococcus suis* found 6 times

Table 3. Germ frequencies in single-germ samples

Farm	**1**		**2**	
Germ	**N**	**%**	**N**	**%**
H.p	3	25	38	78
P.m	6	50	7	14
B.b	1	8	3	6
M.h	2	17	0	0
App	0	0	1	2

In farm 1, among the 49 BAL in which multiple germs were found, 34 involved B.b, H.p, M.h, and/or P.m. In farm 2, among the 26 BAL in which multiple germs were found, 24 were a P.m and H.p association.

Conclusions and Discussion

The clinical presentation and the morbidity are of high inter-pen variability. The number of BAL has no equivalent to our knowledge. The 19% rate of samples with none of the searched pathogens is in accordance with previous work[2]. H.p has been isolated in 60% of the samples, much more than the 2% found in a previous publication[2]. The difference of M.h isolation rate between the two farms can be explained by a farm, a vaccination and an age effect. These results were followed by implementation of a M.h vaccination in farm 1 with immediate satisfaction, and a H.p vaccination in farm 2 with partial satisfaction, additionnal investigations being in process.

This work results in two different examples of the lung flora composition. The picture could have been as instructive with less samples per farm. PRRS PCR could have been performed. BAL sampling didn't delay the implementation of a treatment, but helped questionning its rationality and how to prevent new outbreaks.

References

1. Fablet C et al. 2010. Vet. Microbiol. 143,238-245.
2. Pommier P. et al. 2006. Rev. Med. Vet. 157 (3), 153-157.

O.077

Effect of time of second Improvest® dose relative to time of harvest on the growth performance of immunologically-castrated barrows compared to physically-castrated barrows and gilts

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Introduction

Improvest® (*gonadotropin releasing factor analog-diphtheria toxoid conjugate*, Zoetis) is used to immunologically castrate male pigs. It is given in 2 doses, with the 2nd dose, which effectively castrates the animal, given in late finishing (3-10 wks prior to harvest). Immunologically-castrated barrows (IC) have been shown to have improved growth performance compared to physically-castrated barrows (PC) and gilts (G) (1, 2). There has been limited research carried out on the effects of varying the timing of the 2nd Improvest® dose relative to the time of harvest on the growth performance of IC relative to other sexes. Such information is needed to predict the optimum time for giving the 2nd dose. The objective of this study was to evaluate the effect of increasing the time between 2nd Improvest® dose and harvest on the growth performance of IC compared to PC and G.

Materials and Methods

The study was carried out as a RCBD with 12 treatments:

- Trt 1-4: IC given 2nd Improvest® dose at either wk 14, 16, 18, or 20 of age; harvested at wk 24 of age;
- Trt 5 and 6: IC given 2nd dose at wk 20 of age; harvested at either wk 26 or 28 of age;
- Trt 7-9: PC harvested at either wk 24, 26, or 28 of age;
- Trt 10-12: G harvested at either wk 24, 26, or 28 of age.

A total of 288 pigs, housed in groups of 3 (8 groups/treatment), were used. The first Improvest® dose was given to pigs on all IC treatments at the start of study (wk 9 of age). Diets were formulated for requirements of intact males (3); pigs had *ad libitum* access to feed. All feed additions and feed in the feeder at pig weighing were recorded. Data were analyzed using the PROC MIXED procedure of SAS (Cary, NC, USA). Differences between means were considered different at $P \leq 0.05$.

Results

Treatment effects on performance from wk 9 of age are presented in Table 1. Compared to PC, G had lower ADFI but greater G:F and ADG (except for pigs harvested at wk 28). In general, IC grew faster than PC but the difference between these 2 sexes for ADFI and G:F varied with time of harvest. The feed efficiency of IC given the 2nd Improvest® dose at wk 20 and harvested at wk 24 was greater ($P < 0.05$) than for any of the other sex treatments; ADG was also the greatest for this treatment but treatment differences were not always statistically significant ($P > 0.05$).

Table 1. Effects of treatment on growth performance.

Treatment Code[1]	ADG, g	ADFI, kg	G:F, kg:kg
1 – IC 14 24	1023^{ab}	2.93^{a}	0.350^{bcde}
2 – IC 16 24	1015^{ab}	2.97^{a}	0.343^{def}
3 – IC 18 24	1003^{bc}	2.77^{bc}	0.363^{b}
4 – IC 20 24	1048^{a}	2.77^{bc}	0.378^{a}
5 – IC 20 26	1039^{ab}	2.93^{a}	0.355^{bcd}
6 – IC 20 28	975^{cd}	2.93^{a}	0.333^{fg}
7 – PC 24	971^{cd}	2.89^{ab}	0.336^{fg}
8 – PC 26	958^{de}	2.98^{a}	0.323^{gh}
9 – PC 28	921^{ef}	2.96^{a}	0.311^{h}
10 – G 24	908^{f}	2.53^{e}	0.358^{bc}
11 – G 26	901^{f}	2.61^{e}	0.345^{cdef}
12 – G 28	895^{f}	2.63^{cd}	0.340^{ef}
SEM	15.4	0.054	0.0051
P-value	<0.001	<0.001	<0.001

[1]Treatment number, sex, time of second Improvest® dose, and time of harvest, respectively.

Conclusions and Discussion

These results suggest that the greatest advantage of IC over PC occurred when maximizing the time that IC grow as intact males (i.e., allowing no more than 4 wk between the 2nd Improvest® dose and harvest). Also, increasing the time between 2nd Improvest® dose and harvest, either by giving the 2nd dose earlier or harvesting pigs later, reduced, but did not eliminate, the growth performance advantages of IC compared to PC. In line with these results, previous research has shown that IC have greater ADG, ADFI, and G:F than PC after the second dose (1,2) but that these differences decrease with time over this period. However, another study (4) reported no effect of time of 2nd dose on ADG or G:F. Further research to evaluate this concept is warranted.

Acknowledgments

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References

1. Zamaratskaia G et al. 2008. Reprod. Dom. Anim. 43:351-359.
2. Fàbrega E et al. 2010. Livest. Sci. 132:53-59.
3. NRC. 2012. Natl. Acad. Press.
4. Lealiifano A et al. 2011. J. Anim. Sci. 89:2782-2792.

O.078

Prevalence of *Salmonella spp.* in slaughtered pigs and antimicrobial resistance of *S. enterica* subsp. *enterica* serovar 4,[5],12:i:- isolated from slaughtered pigs and pathological samples in Italy.

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Introduction

Salmonella spp. are recognized as major zoonotic pathogens of economic significance in animals and humans. Recently a monophasic variant of *S.* Typhimurium, *Salmonella enterica* subsp. *enterica* serovar 4,[5],12:i:- (*S.* 4,[5],12:i:-) has emerged in pigs and caused outbreaks of salmonellosis in humans in several countries worldwide (1). The main objectives of this study were: 1. to evaluate the prevalence of *Salmonella* spp. and the most frequent serovars in slaughtered pigs; 2. to asses the rate of antibiotic resistance in *S.* 4,[5],12:i:- strains isolated from slaughtered healthy pigs and pathological samples from clinical pigs.

Materials and Methods

From February to November 2013, 1230 ileocolic lymph nodes were randomly collected from heavy pigs (9-10 months old, 160 kg BW) belonging to 41 batches coming from as many herds. Thirty pigs were sampled for each batch and the samples collected were analysed following the ISO 6579:2002/Amd 1 Annex D:2007 in order to evaluate the prevalence of *Salmonella* spp. Suspected *Salmonella* colonies were inoculated on lysine desossicolate agar (XLD) and brillant green agar (BGA). All strains presuntively identified as *Salmonella* were confirmed by slide agglutination and serotyped according to the White-Kauffman –Le-Minor scheme.
Fifty-three strains of *S.* 4,[5],12:i:- isolated during the survey at the slaughterhouse (group I) and 41 strains of *S.* 4,[5],12:i:- (group II) isolated from clinical pigs sent to the laboratory for diagnostic purpose, were tested using a broth microdilution technique (Sensitre® TREK Diagnostic Systems) for their susceptibility to 14 antimicrobials. Isolates were classified as resistant, susceptible or intermediate to antimicrobials in accordance with the breakpoints proposed by the Clinical and Laboratory Standards Institute (CLSI 2008). Intermediate isolates were grouped with susceptible isolates.

Results

Salmonella spp. was isolated from 229 out of 1230 lymph nodes (18.6% CI95% 16.5%-20.9%) and 38 batches out of 41 were positive. A total of 21 different serovars of *Salmonella* were identified.
S. 4,[5],12:i:- was the most prevalent serovar (4.3%) followed by *S.*Rissen (3.2%) and *S.*Derby (2.7%) while *S.*Typhimurium showed a prevalence of 0.74%.
All strains of *S.* 4,[5],12:i:- showed resistance to at least one antimicrobial and 90.4% of them showed multiresistance (resistance to 4 or more antibiotics). The most common pattern of resistance profile was the ASSuT profile (48.9% of strains) followed by the ACSSuT profile (41.5% of strains). The rates of resistance to the antibiotics tested were reported in table 1. The resistance to the antimicrobials did not differ significantly between the two groups of *S.*1,4,[5],12:i:- tested (group I and II) except for CIP (0% group I and 12.2% group II; $p<0.01$) and NAL (22.6% group I and 56.1% group II; $p<0.01$).

Table 1. Rate of resistance to the antibiotics tested of *S.* 4,[5],12:i:- strains tested.

Antibiotic	% of resistant strains
Ampicillin (A)	92.5%
Chloramphenicol (C)	42.5%
Streptomycin (S)	100%
Sulfamethoxazole (Su)	100%
Tetracycline (T)	90.4%
Gentamicin (GEN)	58.5%
Florfenicol (FFN)	40.4%
Kanamycin (KAN)	40.4%
Trimethoprim (TMP)	29.8%
Nalidixic acid (NAL)	37.2%
Colistin (COL)	20.2%
Cefotaxime (FOT)	6.4%
Ceftazidime (TAZ)	11.7%
Ciprofloxacin (CIP)	5.3%

Conclusions and Discussion

The prevalence of *Salmonella* serovars in this study showed an overall decline of serovar Typhimurium. To some extent this reduction has been counteracted by an increase in prevalence of the emerging serovar 4,[5],12:i:-, characterized by isolates with multidrug resistance (ASSuT and ACSSuT profiles) as described in several European countries. In addition the development of resistance to ciprofloxacin and cephalosporins is reason of concern, due to the essential role of these antimicrobials for the treatment of infections in humans.

Acknowledgments

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References

1. Hopkins K.L. et al. 2012. Euro surveill 17 :pii=20275.

O.079

Natural *L. intracellularis* infection in young pigs: Effects on nutrient digestibility (total tract) and growth rate in vaccinated or not vaccinated animals

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Introduction

The infection with *Lawsonia intracellularis (L.i.)* in pigs is often accompanied by diarrhea, as well as by a reduced feed intake and impaired growth (1, 2). The hypothesis of this study was that in herds with signs of porcine proliferative enteropathy due to *L.i.* (mainly the posterior small intestine is affected) the reduced performance is not only the result of the already mentioned lower feed intake but may also be caused by decreased digestibility rates of nutrients.

Materials and Methods

In 3 consecutive trials a total of 27 potentially naturally *L.i.* infected pigs (bw: 19.0 ± 1.50 kg; 9 pigs/group) were fed a conventional diet ad libitum (XP: 176 g, XF: 23.5 g, XL: 33.6 g, ME: 13.8 MJ/kg diet) including 0.5 % Cr_2O_3 as marker. Animals were alloted to 1 of 3 groups: not vaccinated, without clinical findings = $VAC^{-}CF^{-}$; with clinical findings (soft to liquid faeces) = $VAC^{-}CF^{+}$; vaccinated (Enterisol®Ileitis; as suckling piglet) = VAC^{+}. Pigs were housed individually and fed the diet for 10 days, divided in a 5-day adaptation period and a 5-day faeces collection period to determine apparent digestibility of the organic matter (OM), crude protein (XP) and starch. Shedding of L.i. with the faeces was confirmed by qPCR. Statistical analyses were performed by one-way ANOVA (procedure GLM), differences were considered significant when $p \leq 0.05$.

Results

Shedding of *L.i.* was seen in all groups but with the numerically highest numbers of genome equivalents in group $VAC^{-}CF^{+}$. Additionally, the animals in group $VAC^{-}CF^{+}$ had the significantly lowest DM content in the faeces (Table 1). This gives some evidence that the pigs were still clinically affected during the digestibility trial.
As shown in the table, the digestibility rate of OM was in the tendency (*p=0.0537*) and the digestibility rate of XP significantly reduced in the clinically affected group. Regarding the performance parameters, pigs of the group $VAC^{-}CF^{+}$ showed the lowest daily feed intake, daily body weight gain as well as the highest feed conversion ratio.

Table 1: Faeces quality (dry matter content, DM), total tract digestibility rates (organic matter [OM], crude protein [XP], starch), daily feed intake, daily body weight gain and feed conversion ratio in young pigs shedding *Lawsonia intracellularis*

		VAC^{-} CF^{-}	VAC^{-} CF^{+}	VAC^{+}
DM content of faeces (g/kg)[1]		245[a] ±16.8	211[b] ±19.8	236[a] ±18.4
L.i. genome equivalents (lg)		5.83 ±2.35	7.70 ±1.83	6.00 ±2.89
Total tract digestibility rates (%)	OM	86.9 ±1.81	84.8 ±2.19	86.4 ±1.46
	XP	83.9[a] ±2.03	80.7[b] ±2.57	83.0[a] ±1.72
	Starch	99.0 ±0.15	98.8 ±0.26	98.9 ±0.27
Daily feed intake (g)[2]		1207 ±119	1165 ±148	1320 ±142
Daily body weight gain (g)[2]		852[ab] ±100	800[b] ±139	920[a] ±60.0
Feed conversion ratio (feed:gain)[2]		1.42 ±0.08	1.47 ±0.15	1.43 ±0.12

[1] determined during the digestibility trial [2] due to technical problems, only trial 2 and 3 were considered

Conclusions and Discussion

This study showed that even clinically mild cases of PIA lead to impaired digestibility rates of nutrients despite of compensatory hind gut digestion - at least partly. Especially starch that escaped the precaecal digestion and absorption is fermented more or less completely in the hindgut. Stressful field conditions are possibly more serious with regard to the already in this short experimental study observed in the tendency lower body weight gain in affected animals.

References

1. McOrist S et al. 2003. The Pig Journal, 51, 26-35.
2. Jacobson M et al. 2003. Research in Veterinary Science 74, 163-169.

O.080

Effect of prolonged anorexia on development of clinical signs associated with porcine periweaning failure-to-thrive syndrome (PFTS)

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Introduction

Porcine periweaning failure-to-thrive syndrome (PFTS) is a clinical condition, typically identified within 7 days of weaning, where affected piglets become anorexic, lethargic and then progress to debilitation requiring euthanasia (1). The estimated flow-prevalence of PFTS is currently reported to be 4.3% and the most commonly reported clinical signs associated with PFTS are anorexia, repetitive sham chewing, and weight loss (2). However, research conducted to date has not been able to elucidate definitive risk factors, etiologic agent(s), effective treatments, or the pathogenesis, associated with the syndrome (3). It has been hypothesized that the majority of histologic lesions and clinical behaviors currently associated with PFTS are complicated by the prolonged anorexia that PFTS-affected piglets experience. The objectives of this project were to investigate behavioral, biochemical, and histologic changes associated with anorexia in piglets at weaning and to determine their response, once fasted, to the re-introduction of feed.

Materials and Methods

Study 1: Twelve piglets, 21 days of age, were randomized to one of two groups, matched by litter, sex, and housed individually. Group 1 (FED; n=6) was fed a weaned pig diet ad libitum for 8 days. Group 2 (FAST; n=6) was fasted for up to 8 days. Piglets were monitored twice daily and closely examined for evidence of hypothermia, weight loss/gain, general demeanor, posture, infectious disease, and behavioral changes. All piglets had blood samples taken on day 0, 4, and 7 for biochemical analysis. At the completion of the trial (day 8) all piglets were humanely euthanized and comprehensive necropsy examinations performed.

Study 2: A separate group of 21 day old piglets were assigned to one of two groups matched and housed in an identical manner to the piglets in Study 1. Group 1 (FED; n=6) was fed a weaned pig diet. Group 2 (FAST-FED; n=6) was fasted for up to 8 days and then had the weaned pig diet introduced on day 8. Blood samples were taken every 2 days and 1 day after the re-introduction of solid feed. Ad libitum access to water was available to all piglets in both studies.

Results

Study 1: All FAST piglets developed repetitive sham chewing starting as early as 3 days fasted with all piglets demonstrating the behaviour by 5 days fasted. None of the FED piglets developed these behavioral changes. The most predominant biochemical change noted between the groups was an increased level of beta-hydroxybutyrate (BHB) in the FAST piglets evident by 4 days fasted ($P<0.001$, using multilevel linear regression). Two FED piglets developed mild diarrhea by day 5. None of the FAST piglets exhibited any clinical disease. Body condition scoring was an insensitive method of determining which pigs were fasted, even after 7 days of fasting.

Study 2: The FAST-FED piglets developed the same behavioural and biochemical changes as the FAST piglets in Study 1. None of the piglets developed clinical disease. All FAST-FED piglets consumed feed within 5 minutes of re-introduction of feed, following which their BHB levels rapidly normalized from the elevated levels experienced during the fasting period. Histology results are pending.

Conclusions and Discussion

This study demonstrated that serum BHB was elevated in fasted piglets and could be detected by 4 days of anorexia while piglets that ate normally maintained a normal serum BHB. Measurement of BHB could be a potential diagnostic test to help identify PFTS-affected piglets earlier in the development of the syndrome or post-weaning anorexia in general. All of the fasted piglets developed repetitive sham chewing while the piglets that ate did not. This suggests that that the repetitive sham chewing seen in PFTS-affected piglets is associated with prolonged anorexia. Piglets exposed to a fast and then re-introduced to feed ate readily suggesting that the piglets did not develop a food aversion during the prolonged fast. The sham chewing did not prevent them from seeking out the food once re-introduced. The fasted piglets were difficult to identify visually even after 7 days of fasting suggesting that reported cases of PFTS-affected piglets were likely anorexic for extended periods of time and were not identified in the early stages of the syndrome.

Acknowledgments

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References

1. Huang, Y., *et al. J Swine Health Prod*. 2011;19(6):340-344.
2. O'Sullivan *et al. J Swine Health Prod.* 2014;22(1):24–28.
3. Huang, Y., *et al. J Vet Diagn Invest*. 2012;24(1):96-106.

O.081

Monitoring antimicrobial use in the French pig production: The INAPORC panel

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Introduction

Monitoring antimicrobial use in food-producing animals is a key element of a control programme of antimicrobial resistance. The French pig industry has developed a tool, named INAPORC Panel, to measure the quantities of antibiotics used in farms by age group and to specify antimicrobial usage pattern. This communication presents the methodological approach applied and some preliminary results obtained.

Materials and Methods

A panel has been established, consisting of 169 pig farms, randomly selected in the National Swine Database of Identification (BDPORC). These volunteer farms were representative, after adjustment, of the French pig herd population, characterized through activity, localization and productivity. Technical parameters (such as number, weight, and mortality rate of pigs at each age group) were collected from farmers. Inventory of antimicrobials they bought in 2010 were collected from their drug suppliers (veterinarians, medicated feed manufacturers). Finally, during a phone call, farmers described their antimicrobial usage pattern: categories of animals treated, reasons for treatment, dosages and treatment durations implemented. The two indicators recommended by the European Medicines Agency (1) were used to express the results for each category of animals: number of Animal Daily Dose per animal product (ADD/a) and number of Animal Course Dose per animal product (ACD/a). The results of sows were expressed relative to the number of piglets weaned. Calculations were performed using the dosage and treatment duration as defined in the summary of product characteristics.

Results

In 2010, most treatments were administered to piglets in post-weaning units (57 % of ACD/a; 73 % of ADD/a). Important differences between farms were observed: half of the treatments in post-weaning units were attributable to 25 % of farms.

Medicated feed premixes were the most commonly used pharmaceutical form (44 % of ACD/a; 74 % of ADD/a) (Figure 1) which confirms results of a previous study (2). Three antimicrobial classes represented two thirds of total number of ACD/a: polypeptides (33 %), penicillins (22 %) and tetracyclines (13 %). "Critically important antimicrobials" (third- and fourth-generation cephalosporins and fluoroquinolones) represented only a small fraction of total number of ACD/a (5 % and 3 % respectively).

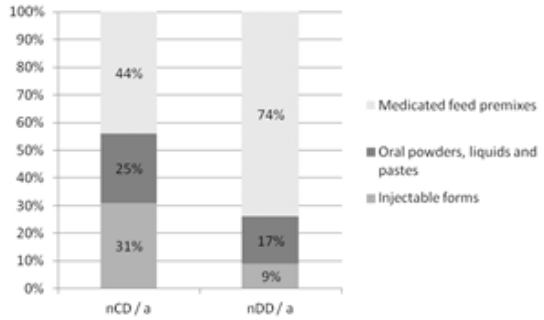

Figure 1. Part of antibiotics use by pharmaceutical form

Conclusions and Discussion

Since 2010, the French pig sector and the veterinarians have voluntarily limited the use of 3rd and 4th generation cephalosporins (3). Consequently, their exposure level decreased by 62 % between 2010 and 2012 (4). More generally, the level of exposure of pigs to all families of antibiotics decreased by more than 18 % between 2010 and 2012 (4).

Finally, this study provides the French pig industry with reliable baseline data which complement available results already published by the French Agency for Veterinary Medicinal Products (ANMV) (4). It adjusts some data downwards and most importantly, it specifies antimicrobial usage pattern by age group. The panel, which is renewed in 2014, should help professionals to strengthen their strategy for an efficient reduction of antimicrobial usage, as recommended by French public authorities in the Ecoantibio 2017 Plan (5).

Acknowledgments

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References

1. EMA/286416, 2012.
2. Chauvin C, 2010. Rapport Anses. 33 pages.
3. Chouët S et al., 2012. Bulletin GTV, 64, 55-56.
4. ANMV, 2013. 69 pages.
5. EcoAntibio 2017 Plan, 2012.

O.082

Investigating the benefits of anti-infective metaphylaxis in finishing pigs

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Introduction

Pigs that die from PRDC in the late finishing period (> 15 weeks post weaning) represent a significant economic loss due to high treatment costs and lost revenue. In US production systems it is common to apply anti-infective metaphylaxis (AM) to control the impact of PRDC. To date, there have been no published studies exploring the potential benefits of these strategies. In this study, the value of using AM in late finishing pigs endemically infected with PRDC but free of PRRSv was examined.

Materials and Methods

A total of 732 pigs (89 -100 days post-weaning) from four AIAO Wean to Market (WTM) sources (148-199 pigs/Lot), were enrolled in the study. At each site pigs were housed together in a single pen, individually identified, and divided in to 2 cohorts based on body weight, sex, and rectal temperature. The cohorts were subjected to either control (C) or AM (Tulathromycin 2.5 mg/kg IM, Zoetis, Florham Park, NJ, USA). Pigs were monitored and re-weighed on day 21. Post treatment weight gain over the 21 day period was used as a measure of well-being and endemic disease. Data was analyzed using ANOVA with co-variation for starting weight and sex.

Results

Across all replicates there was no difference in weight gain between AM and C pigs (17.1 kg vs. 16.7 kg, p=0.34, Table 1). There was an effect of replicate for both starting weight (p<0.001) and weight gain (p<0.001). No interactions between treatment and replicate were detected. There were no differences in lot level weekly mortality between lots prior to (0.118%) or during the study (0.12%) and no pigs enrolled in the study died.

In the lightest weight quartile of the pigs at enrollment, there was an improved weigh gain in the AM pigs (18.5 kg vs. 16.4 kg, p=0.005, Table 1) that was not present in any of the other starting weight quartiles. There was no interaction between treatment and replicate in the lightest weight quartile of the pigs suggesting that the effect is consistent across sites, sources, and time.

Conclusions and Discussion

The results of the present study suggest that the universal application of mass AM under routine commercial health conditions in the US with antibiotics known to be effective against all of the major bacterial components of PRDC cannot be rationalized, either in terms of improvements in animal well being or added economic value. These data are the first that the authors are aware of that have evaluated the impact of AM under conditions controlled for lot level effects. In our experience, lot-to-lot variation in performance is much larger than the perceived impact of AM and therefore understanding the impact of AM within lots is critical to quantifiying its value.

In this study the lightest weight pigs did respond to AM by increasing their growth rate suggesting that there may be an infectious disease component to the slower growth rate of pigs prior to 80-100 days post weaning. It can be inferred that AM used in the lightest pigs at 100 days post weaning improves economic outcomes, minimizes the need for additional anti-infectives, and improves animal well being through a reduction in disease. Under the conditions of this study AM used across the entire group resulted in negative economic outcomes, as the cost of the intervention was born by the group but without subsequent improvement in performance. In addition, this suggests that the mass application of AM in pigs late in the growing period increases the total amount of antibiotics used without an observable improvement in animal well being. Devising strategies that can identify and then target the animals that are most likely to respond to AM would significantly improve animal well-being. These are likely to be animals with disease but no overt clinical signs, and so they may require more sensitive disease detection modalities. These data suggest that growth rate alone at 100 days post weaning may be a useful predictor of which pigs would have improved well being and result in an economic gain for the producer with AM.

Table 1. Weight gain (kg) in pigs over 21 days following AM

Variable	All[1]	Lightest 25% at enrollment[2]
Weight Gain C	16.7	16.4
Weight Gain AM	17.1	18.5
SEM	0.2	0.4
P	0.340	0.005

[1] n=732, start weight=30.5 kg
[2] n=193, start weight=26.1 kg

Acknowledgments

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O.083

Assessment of colostrum management strategies at commercial sow farms using immunocrit ratios

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Introduction

To date, the quantification of immunoglobulins (Igs) to assess colostrum management has been difficult to implement routinely in veterinary practices. Recently, a simple, rapid, and inexpensive method that measures passive transfer from dam to piglet was described (1). Blood samples of 1-day-old piglets can use the "immunocrit" method by testing the sera and calculating an immunocrit ratio (IR). Benchmark immunocrit values and the ability to assess colostrum management with IR in commercial situations have not been validated. The objectives of this study were to 1) evaluate alternative sampling techniques in neonatal pigs, 2) generate a benchmark for a desirable IR value in commercial populations and 3) determine if a correlation exists between immunocrit level and production parameters.

Materials and Methods

Objective 1- A sample collection from 17 piglets at 1-day of age was attempted via the cephalic, medial, and lateral saphenous veins, and tail docking. A paired t-test was performed on the IR of these 17 piglets to determine if a smaller serum sample (30 µL) would have comparable results to the volume (50 µL) previously validated (1). Objective 2- Thirty litters were sampled from each of the 9 sow farms. A small, medium, and large piglet was chosen, based on birth weight (BW), from each of the 30 sows per site. Gender, BW, sow parity, the birth dam's ID, total born alive, AM/PM birth, farrowing date, adjusted wean weight (ADJWW), and mortality were recorded for each piglet. Blood (1 mL) was collected via the cephalic vein of piglets approximately 24 h old by using a 22-ga x ¾-in long needle and a 1-mL syringe. Samples were treated as previously described with an adaption to increase serum centrifugation time from 5 to 10 min (1). Piglets were the experimental unit of concern for this study. Descriptive statistics including: mean (µ), standard deviation (SD), and range values were estimated for IR by farm, and scatter plots of IR were generated. The percentage of piglets with 1 and 2 SD below the mean were calculated to determine consistency in IR and therefore consistency in colostrum management. Objective 3- Correlation analyses between ADJWW and IR, as well as number of pigs weaned and IR, were performed (SAS 9.2, Cary NC). The effect of IR on pre-weaning mortality (PWM) and average daily gain (ADG) was analyzed using a linear mixed-effects model with IR, AM/PM birth, gender, parity, and total born alive as the fixed effects and farm and sow as random effects (SAS 9.2, Cary NC). P values < 0.05 were considered significant. To determine a practical IR sample size, calculated precision (distance from the µ to limit) was estimated based on a 95% confidence limit.

Results

No mortality was associated with sample collection via the cephalic vein. For the immunocrit test, a p-value of 0.3708 resulted when comparing 30 µl (0.113±0.018) vs. 50 µl (0.114 ± 0.0138) samples. A mean IR of 0.098 ± 0.026 was found for this operation. The correlation coefficient between IR and ADJWW was low (R = 0.16, P < 0.001). Parity was the only trait that impacted IR (P < 0.05). In 5/9 sow farms, P1 sows had the lowest IR. In 6/9 (66%) farms, the highest IR values were found in piglets born with the heaviest birth weight and those with heavier weaned weights. A sample size of 30 piglets is considered statistically adequate.

Conclusions and Discussion

Collecting blood via the cephalic vein was determined to be the most efficacious. The paired t-test comparing sera samples determined, should a veterinarian be unable to obtain the full 1 ml of blood, that a serum sample size of 30 µl would produce similar IR results as a 50 µl sample. Target IR in research farm conditions has been reported to be 0.125 (3). The mean IR for this operation is markedly lower than Vallet's research herd's mean. While the overall commercial distribution is considered within normal limits, it suggests that improving management practices in the lower averaging barns within this system could increase mean IR. Parity was the only trait that impacted IR (P < 0.05). Producers with high replacement rates may benefit from the knowledge that the offspring of P1 sows are at an immunological disadvantage. If a producer were to have a high replacement rate and concurrently discover that their measured IR rates were below commercial average, this indicates that these animals should be the caretakers' primary focus in moving forward with colostrum management improvement strategies.

Acknowledgments

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References

1. Vallet JL et al. 2013 Vet J 195: 91-97.

O.084

Timing of primary lesions and secondary repair processes during early onset of osteochondrosis in young pigs

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Introduction

Osteochondrosis (OC) is one of the main causes of leg weakness in breeding sows and finishing pigs. The prevalence of OC in farms may reach as high as 90%[1]. This high prevalence and the considerable strain it imposes on the welfare status of pigs and on farmers economically, make it a pressing issue in today's porcine industry[2]. In order to alleviate this matter and create improved strategies for farmers to deal with OC it is important to get a better understanding of the disease and especially the primary processes. One current theory that explains the pathogenesis is a micro-traumatic event, which causes a sudden interruption of the blood supply of the growth cartilage during endochondral ossification[1]. This results in focal necrotic areas of the growth cartilage, which subsequently develop into osteochondrotic lesions. These lesions may or may not repair during growth, as, due to age-dependent changes in cartilage metabolism, there is a point of no return after which osteochondrotic lesions will not regenerate. Therefore, if the prevalence of OC is to be reduced through any form of intervention, knowledge of the timing of both occurrence of lesions and the repair process is essential. The aim of this study is to gain a understanding of the timing of development and repair processes of OC in the pig and to determine the point of no return.

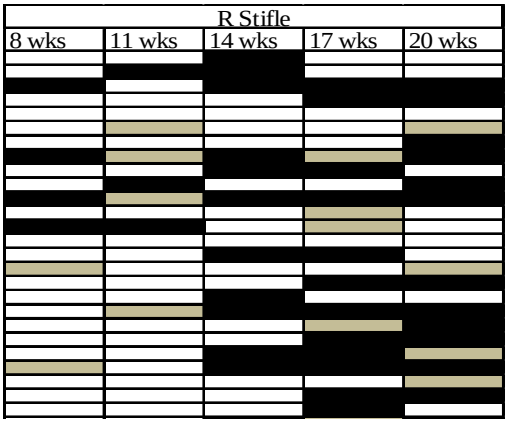
Materials and Methods

In this study, we followed 26 weaned pigs longitudinally for a period of 4 months using a specifically selected set of in-vivo techniques. Some of these, such as X-rays, are already widely used to detect lesions in other species, but haven't been tested exhaustively in the pig yet. Other newer techniques in the field were also used, such as pressure plate technology. These were tested also for their capacity of high-through put screening and non-invasive character. As the goal was to study OC-development, experimental conditions were tailored to increase the risk of OC-development (pigs with high risk of developing OC, ad-libitum feed for high growth rate, fully slatted slippery flooring,). Measurements were taken at five points in time, all separated by an interval of three weeks. At these time points all pigs were examined visually, with the pressure plate, sampled for blood to detect cartilage biomarkers (C2C and CP2) and with X-rays. We focused on known predilection sites occurring in specific joints – the elbow, knee and hock.

Results

Primary interpretation of the results from the X-rays indicate lesions and repair processes did occur in a large proportion of our pigs up to age of 20 weeks. Table 1 displays the X-ray results from the right stifle of 26 animals. Results from other in-vivo techniques are still being processed currently and will be presented in more detail during the congress.

Table 1. X-ray results of the right stifle, white is negative, black is positive and gray the interpretation of the X-ray was inconclusive. Each row represents an animal.



Acknowledgements

Financial support was obtained from The Dutch Technology Foundation (STW), the Institute for Pig Genetics B.V.(IPG) and The Product Board Animal Feed (PDV).

References

1. Nakano T et al. 1987. Can J Animal Science 67, 883-901
2. Jørgensen, B et al. 1998. Livestock Production Science 54, 167-171.

Preweaning mortality in nine Danish herds

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Introduction

Preweaning mortality is the major contributor to pig mortality. In Denmark the average mortality for 2012 in the suckling, nursery grower-finisher period was 13.7%, 2.9%, and 3.6%, respectively. The causes of death in the suckling period are dependent on the age of the piglet. The objective of this study was to examine mortality between herds and different age groups of suckling piglets.

Materials and Methods

In 9 herds with an average preweaning mortality of 17% (12-23%), approximately 1000 live born piglets per herd were ear tagged. All live born piglets from sows in the same farrowing group were included. Pigs that died in the farrowing section were stored in a freezer before shipment to the laboratory for pathological examination. For each piglet ear tag number and day of death was recorded.

Based on the pathological findings the most likely cause of death or euthanasia was established for each piglet. The causes of death where reduced to the following eight categories: not viable, sepsis, hernia, trauma, arthritis, starvation, enteric disease, and miscellaneous. Piglets categorized as not viable were either weak born, immature or had a birth weight below 700 gram. The weak born piglets were piglets with empty stomachs, no tear on hoofs or skin, and no other pathological findings.

The suckling period was divided into three age-groups; day 1-4, day 5-11 and day12 to weaning. For each period, major overall causes of death were identified. The results are shown as an average percentage across all herds and an interval (min-max) that shows the variation between herds. Statistical analysis of the cross tabulations was done by chi-square tests in Proc Freq in SAS.

Results

A total of 1465 piglets from 9 herds were subjected to pathological examination. Within each herd 116-244 piglets were examined. In the age-groups 59% (41-74%), 21% (9-31%), and 20% (8-30%) of the examined piglets died, respectively. The proportion and numbers of each diagnosis in the three age-groups are shown in Figure 1. The differences in causes of death in the three age-groups are statistically significant (P<0.0001).

The causes of preweaning mortality in each herd are shown in Figure 2. The figure shows the relative proportion of the diagnosis in each herd. The differences in causes of death in the 9 herds are statistically significant (P<0.0001).

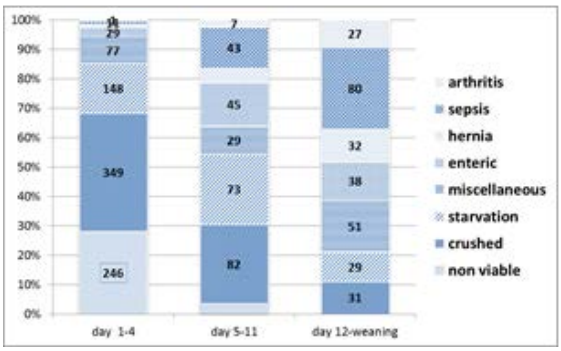

Figure 1.Distribution of causes of death in 3 age-groups in farrowing units across 9 herds

Conclusions and Discussion

The results from this study indicate a considerable variation in the causes of death between herds and between the three time periods. To ensure the right advice for preventing preweaning mortality in the individual herd pathological examination of a larger number of piglets could be a good staring point.

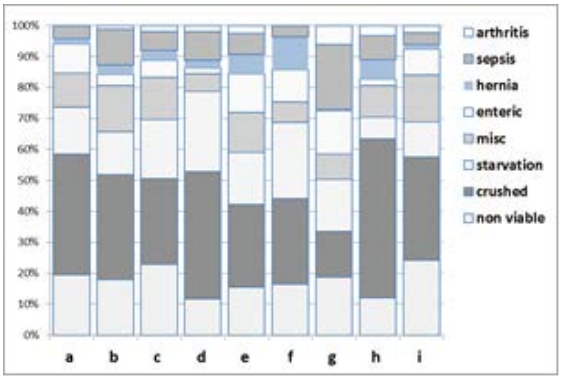

Figure 2. Distribution of causes of death in 9 herds. The numbers of autopsied pigs in herd A-I were: 116, 166,161, 161, 161, 170, 244, 156, and132 piglets

Acknowledgments

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References

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An easy-to-use scoring system for monitoring tail biting lesions in pig herds

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Introduction

Tail biting in pigs is considered as a sign of reduced welfare in pig breeding (production disease), as tail biting in wild living *Suidea* is not reported. Tail biting is also economically important because of reduced performance and carcass quality (1). A better understanding of the problem is necessary to develop appropriate control strategies. In several studies, well defined tools for scoring tail biting lesions are used (2). Scoring lesions is often time-consuming and several often advanced scoring systems are used which are difficult to use in practice. The aim of this study was to investigate tail biting in a pig herd based on an easy-to-use monitoring system.

Materials and Methods

The herd was a commercial gilt producing pig herd and consisted of two pig stables each containing 17 compartments. In each compartment, there was a central hall with six pens on each side, with a total of 96 (first stable) or 108 (second stable) pigs per compartment. This pig herd had a recurrent problem of tail biting. A scoring system was used to monitor tail biting lesions in different successive groups of pigs. Tail lesions were monitored in six compartments (three from each stable). All pigs of these compartments were monitored weekly during four successive weeks, starting when pigs were 10 to 13 weeks (equally distributed between two stables). The scoring system was based on the scoring system described by Zonderland et al. (2), but with some modifications. The lesions were categorized into six groups based on the stage of lesions (table 1.).

Table 1. Categories used in the scoring system

Category	0	1	2	3	4	5
Stage of lesions	No lesions	Small lesions, no blood	Blood, no loss of tissue	Blood, + loss of tissue	Advanced lesions	Old and healed lesions

Zonderland et al. (2) used three parameters with a subdivision in different categories for each of them. We have combined this into one single parameter with six categories. The structure of a compartment was schematically presented underneath each category of lesions. The number of animals for each category was noted.

The scores were noted in a complementary excel sheet, and calculations of the prevalence of each category, total prevalence and the distribution of lesions per compartment were performed. The scoring was conducted on a weekly basis for a four week period.

Results

In total, 557 pigs were monitored four times. The prevalence of tail lesions (score ≥1 and score 1 separately) during the four-week period in the different compartments is presented in Table 2.

Table 2. Percentage of tail lesions at compartment level

	Time (weeks)				
Compartment[1]	1	2	3	4	Mean
A	38[2] (38)[3]	28 (28)	16 (15)	16 (15)	24 (24)
B	60 (59)	56 (54)	31 (25)	32 (24)	45 (41)
C	31 (30)	46 (46)	27 (27)	21 (20)	31 (31)
D	46 (38)	51 (26)	51 (28)	30 (7)	44 (25)
E	35 (28)	25 (18)	30 (17)	24 (12)	28 (19)
F	31 (30)	30 (29)	30 (17)	17 (12)	27 (22)
Mean	40 (37)	39 (34)	31 (22)	23 (15)	33 (27)

[1] compartments A-C were in stable 1, compartments D-F in stable 2 ; [2] total prevalence of lesions (score ≥1); [3] prevalence of score 1-lesions

The overall prevalence of tail biting lesions was 33%. The prevalence of lesions declined with increasing age of the pigs i.e. from 40% at first monitoring to 23% during the last monitoring. The lesions were milder at the beginning (37/40 = 93% score 1) than at the end (15/23 = 65% score 1) with increasing age (lower proportion of score 1 in affected pigs). There was a large variation between different compartments in the prevalence and evolution of lesions, and also between different pens within a compartment (data not shown).

Conclusions and Discussion

This pig herd had a high prevalence of tail biting (33%). The prevalence of lesions declined with increasing age, but more pigs with severe lesions remained at the end of the observation period. Lesions categorized as score 1 were only minor lesions, which could rapidly heal. As there was a large variation between compartments and pens within a compartment, it is important to include a sufficient number of pigs and different compartments and pens. The farm-based monitoring system proved to be very useful to assess in a objective way the prevalence and severity of tail biting lesions in pigs. The system could be very valuable to assess the effect of control measures against tail biting lesions.

References

1. SchrØder-Petersen D L and Simonsen, 2001. Vet J 162:196-210.
2. Zonderland J et al., 2003. RIAH report 18. Praktijkonderzoek, Lelystad, The Netherlands

O.087

Different PRRSV genotype 1 isolates induce apoptosis in macrophages and dendritic cells differently

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Introduction

Different PRRS virus (PRRSV) strains may exhibit different immunobiological properties (1). At present, PRRSV is known to replicate in porcine alveolar macrophages (PAM), an in different types of dendritic cells (DC) such as bone marrow-derived DC (BMDC) and monocyte-derived DC (MoDC) (2). The virus may damage target cells by inducing necrosis in infected cells but may cause also apoptosis of bystander cells. The main objective of the present study was to assess the potential of three different PRRSV-1 strains for inducing apoptosis in different susceptible cells.

Materials and Methods

Cells were obtained from 3-week-old PRRSV-negative piglets. BMDC and MoDC were generated as reported before (3). Three genotype 1 strains were used: 3249, 3262 and 3267 (1,4). Cultures of PAM, BMDC and MoDC were infected with the PRRSV strains (0.1 MOI, 37°C) and examined for apoptosis induction at 19, 30 and 60 h of incubation. For this purpose, cells were fixed overnight with 3% paraformaldehyde (-20°C) and then were stained with anti-caspase-3-FITC and anti-PRRSV-Alexa610-PE (N-protein) antibodies. Readings were done in a fluorescence microscope. Replication of the virus was assessed by qRT-PCR.

Results

Infection of the different cell culture types firstly revealed a different cell-type susceptibility to the infection. While PAM and MoDC showed clear labeling for N protein at 19 h, for BMDC, staining of infected cells was not clearly visible until late times. According to qRT-PCR results, strain 3262 replicated with less intensity than the other two strains. Regarding the development of apoptosis, all the examined strains induced apoptosis mostly in bystander PAM already at 19 h of incubation although at a different extent. Strain 3267 caused strong apoptosis in bystander cells while with strain 3262 only some caspase-3 positive cells were seen. This was correlated with the intensity of replication of the virus, as revealed by the anti-N protein labeling (Fig. 1). In MoDC, strain 3267 was again the one causing the strongest apoptosis. Replication of the virus was less intense in MoDC than in PAM. For BMDC, significant apoptosis was not seen until 60 h.

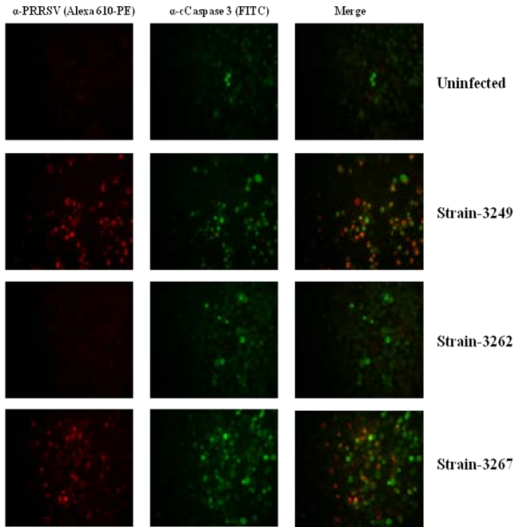

Fig.1. Staining for N-protein of PRRSV and caspase-3 in cultures of PAM infected with PRRSV-1 strains 3249, 3262 and 3267 (0.1 MOI, at 19 h).

Conclusions and Discussion

Different PRRSV-1 strains may cause different degrees of apoptosis in bystander cells. The susceptibility of PAM and different types of DC to PRRSV-1 infection seems to be different.

Acknowledgments

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References

1. Gimeno et al. 2011. Vet Res 42:9.
2. Silva-Campa et al. 2010. Virology396: 264-271.
3. Carrasco et al. 2001. Immunology104: 175-184.
4. Darwich et al. 2011. Vet Microbiol150: 49-62.

Efficacy study of the electrostatic particle ionization technology on PRRSV and influenza artificial aerosols

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Introduction

Influenza and PRRS virus are two of the most important swine pathogens transmitted by aerosols (1,2). Influenza A virus (IAV) is also considered an important zoonotic agent and an important public health concern for human and specifically for swine workers (3). The electrostatic particle ionization system (EPI) is a technology able to reduce airborne agents because of its ability to clump and settle airborne particles and improve air quality potentially decreasing the risk of disseminating pathogens. Therefore, the objective of this study was to evaluate the removal efficiency of the EPI system located at different levels from the ground on the quantity of PRRSV and IAV in artificially generated aerosols.

Materials and Methods

The EPI system, consisting of a line of stainless steel corona points attached to a stainless steel cable (30KV), was installed at 3 different levels (1, 2 and 3 meters) along the length of a 35 m^3 isolation unit at the University of Minnesota. The room was filtered, mechanically ventilated and fitted with environmental controls. Aerosols were generated using a Collison nebulizer located at the inlet level (3m distance from the floor). Artificial aerosols were sampled using an Andersen Cascade Impactor able to collect particles as a function of particle size (8 stages that measure particles from 9 to 0.4microns). Additionally, an optical particle counter and an ion meter were used to analyze total particles and verification of ion concentration during the sampling periods. Air samples were collected with the EPI system 'off' and 'on' for 30 minutes each time. Three replicates were performed with the EPI lines connected at 1, 2 and 3 meters from the air sampler level which was placed on the floor. Samples were analyzed by quantitative PRRS and IAV RT-PCRs. Temperature and relative humidity data were collected during the entire study. Difference in the quantity of virus, removal efficiency and total particle counts by size were calculated for both viruses during the study with the system 'off' and 'on'.

Results

The qPCR analysis of the Andersen Impactor stages for both PRRS and IAV demonstrated a significant removal efficiency of viral particles from the air when the EPI system was on.!The most pronounced reductions (up to 3.5 logs for PRRSV) were observed at 3m in the particle size range of 5 to 10 microns. Overall the removal efficiency increased as the distance of the EPI line increased to the air sampler located at ground level (Figure 1).

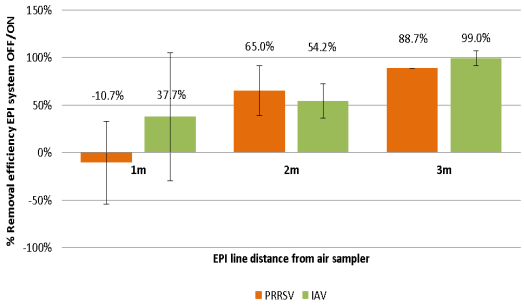

Figure 1. Summary of removal efficiencies (initial concentration of aerosolized virus with system off minus final concentration with system on divided by initial concentration) and standard deviation of PRRSV and IAV particles at 3 different distances of the EPI lines.

Conclusions and Discussion

Our results indicated that particle size and distance to the source of ions (or air collection device) influenced the efficiency of the EPI system when aerosols were artificially generated. The distance of the EPI lines to the source of aerosols is a key aspect to take into account when considering this air treatment. However, further studies are needed to demonstrate the effectiveness of the EPI system at reducing viral particles generated by infected pigs under field conditions. Decreasing the infectious virus load of PRRSV and IAV in the air of positive pig farms should decrease the likelihood of dissemination of airborne pathogens to neighboring pig sites.

Acknowledgments

This project has been founded by NPB and UMN Signature Program

References

1. Otake S. et al. (2010) Vet Microbiol 145(3–4): 198–208
2. Corzo C. et al. (2013) PLoS ONE 8(8): e71444.doi:10.1371/journal.pone.0071444
3. Shu B. et al. (2012) Virology 422, 151-60

O.089

Towards the identification of host molecular pathways associated with PRRSV vaccine efficacy using genomics

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Introduction

The initial interaction of porcine reproductive and respiratory virus (PRRSV) with the immune system is of critical importance for immunological and clinical outcomes. PRRSV infection is associated with inefficient or aberrant immune responses: weak and delayed neutralizing antibody responses and erratic IFN-γ producing T-cells specific response. The lack of vaccine correlates capable of predicting protection has largely hampered the development of new efficacious PRRS vaccines. The objective of this study was to determine if systems biology (1) could be used to identify molecular pathways associated with "good" and "bad" PRRSV vaccine responders in terms of their intensity of IFN- γ secreting cell (SC) responses.

Materials and Methods

Landrace x Large White 4-week old piglets were vaccinated (n=32) intramuscularly with a genotype-1 commercial vaccine (2ml, diluted according to manufacturer instructions), and control pigs (n=10) were inoculated with sterile saline solution. Heparinized blood samples were collected at 6 weeks post-vaccination for obtaining PBMCs. Vaccine-specific IFN-γ-SC were measured by ELISPOT as described (2). In parallel, for each animal PBMCs were cultured in triplicate with MEM supplemented with 10% fetal calf serum, PHA! (10µg/mL) and the vaccine (moi=0.1). ELISPOT results were expressed as number of PRRSV-specific IFNγ-secreting cells / 500.000 PBMC (Media ± SD). Total RNA was extracted from PBMCs using standard methods (3). RNA quality was assessed and samples were run on Affymetrix Snowball microarray platform (4). Regulated transcripts with false discovery rate (FDR) <0.2 were identified using Partek genomic suite and gene lists were further analyzed using Ingenuity pathway analysis (IPA).

Results

Piglets were classified in 2 vaccine groups: "good responders" (n=3) harboring high IFN-γ-SC values (54.3±8.1) and "bad responders" (n=3) with low values (22.3±4.5). Data from non-vaccinated control pigs (n=3) were used for normalizing values in the genomic analysis. In the "bad responder" group, a greater number of transcripts were down-regulated in comparison to the "good responders" (FDR<0.2, *P<0.001*) (Fig.1). The IPA results indicated that IFN-γ was one of the most important inhibited upstream regulator with 6 ($P<4.9\ 10^{-9}$) and 28 ($P<3.3\ 10^{-20}$) IFN-γ regulated molecular pathways in "good" and "bad" responder pigs, respectively (Fig. 2). Differential regulation of transcripts encoding acute phase proteins and pro-inflammatory cytokines were also identified.

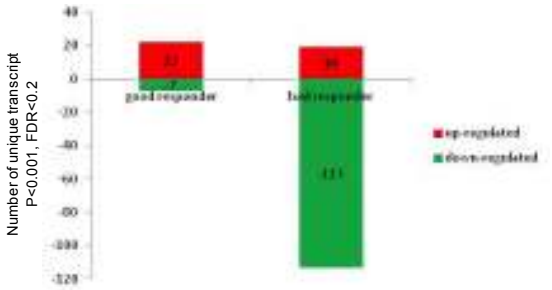

Fig1. Number of unique non-redundant regulated transcripts with a FDR<0.2 (P-value <0.001). Transcriptional response were measured from PBMCs isolated from "Good responder" (n=3) and "Bad responder" (n=3) pig groups and normalised to PBMCs isolated from the same non-vaccinated control pigs (n=3).

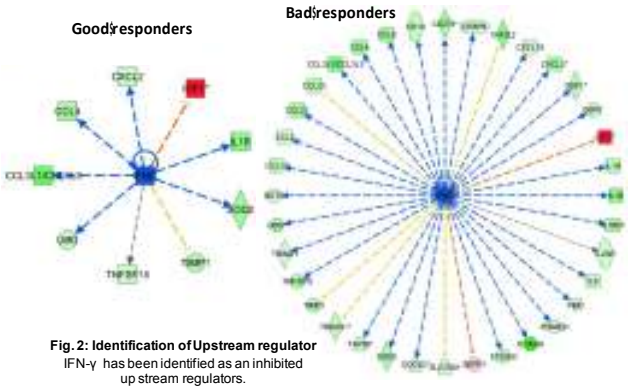

Fig. 2: Identification of Upstream regulator IFN-γ has been identified as an inhibited up stream regulators.

Conclusions and Discussion

Pigs with worse cellular immunity after vaccination harboured greater number of inhibited IFN-γ-regulated transcripts. The present data justify additional research on PRRSV immunology using these systems biology to enhance our understanding of PRRS vaccination eficacy

Acknowledgments

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References

1. Querec(TD(et(al.(2009.(Nat(Immunol,(10:116925(
2. Díaz(I(et(al.(2012.!Vet(Res,(43:30
3. Ait-Ali T et al. 2011. Immunogenetics,!63:437-48
4. Freeman TC et al. 2012. BMC biology, 10:90

Load closed homogenized model in PRRV elimination programs in four Danish farms

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Introduction

In Denmark there is an increasing motivation to eliminate PRRSV from positive sow, nursery and finisher sites to optimize production results, and to increase value of traded pigs. Load, close and Homogenize L-C-H has been proven to be a successful model to control and eliminate PRRSV from farms in North America (1). This study evaluates PRRSV elimination from 4 different farms in Denmark, using the same basic L-C-H model for the sow site, for each farm a detailed elimination plan was implemented for each nursery site.

Materials and Methods

The L-C-H programs were performed in 2 acutely and 2 chronically affected sow herds with either on or offsite nursery (Table 1).

Table 1. Case descriptions

Case herds	NM	HM1	HM2	RV
No sows	1384	500	300	900
Production	F-30kg 2 site	F-30 kg 2 site	F-30 kg 1 site	1site gilt prod.
Gilt acc.	Off-site	Off-site	Off-site	On-site
PRRS clinical expression	Acute Type 2 Weak repro signs	Chronic Type 2 Clinical signs nursery	Chronic Type 2 Clinical signs nursery	Acute Type 2 Repro Weak/ still born

The L-C-H procedure was started on all farms between the periods of April 2013 to July 2013. Together with the L-C-H procedures, strict McREBEL rules were applied on all farms and sites.

Table 2. General L-C-H timeline

Week 0	1st Breeding herd mass vaccination with Ingelvac® PRRS MLV + gilts loaded for 200 days. Herd Closure	Piglet Vx with Ingelvac PRRS MLV around weaning age
Week 4	2nd Breeding herd mass vaccination with Ingelvac PRRS MLV	
Week 9	Monthly test of weaned pigs. blood samples per age group (PRRS PCR & ELISA)	
Week 12/16	Stop of Piglet vaccination (in those farms with piglet vaccination, NM, HM1, HM2)	
Week 29	Naïve SPF sentinels placed in breeding unit. Only farm NM and RV	!!

Results

5 weeks after the second mass vaccination, pigs were PRRSV PCR negative (at BIVRC, Hannover) in all the case herds. In farm RV, 25 blood samples per week were collected from pigs one week post weaning and also at 9-10wks of age getting negative results tested by PCR and ELISA (IDEXX3X). The first newborn gilts after second mass vaccination were placed as sentinels in the breeding unit 29 weeks after second mass vaccination. In farm NM sentinels were purchased SPF PRRSV naive gilts. No sentinels became positive (ELISA) at any time in both farm NM and RV. In farm NM, HM1 and HM2, 5 pigs at each age group in the nursery were tested by PCR and ELISA (IDEXX X3) monthly. In farm HM2 pigs from 7 weeks of age were found PCR positive (vaccine strain) once, 8 weeks after second mass vaccination. This batch was removed, and no PCR positive results were found. The first 2 batches in farm NM were tested by oral fluid, OF. PCR positive batch at end of nursery in left over batches were found at 2 occasions 24 and 29 weeks after second mass vaccination, (vaccine strain).

Table 3. PCR batch results in the nursery at different time points after 2nd breeding herd mass vaccination

Farm\weeks post 2. vacc	4 W	8W	12W	16W	18W
NM	-Neg OF	Neg OF	1 of 8 Pos	1of 8 Pos	1 of 8 Pos
HM1	All Neg	1/8 pos	All neg	All Neg	Al Neg
HM2	All Neg	1/8 pos	All Neg	All Neg	All neg
RV	Neg	Neg	Neg	Neg	Neg

Conclusions and Discussion

Testing results showing PCR negative weanead piglets 5 weeks after 2nd mass vaccination and antibody negative sentinels on farm NM and RV showed strong evidence of lack of circulation of PRRSv in sow sites. Farm HM decided to go for a gilt acclimatization and vaccination program for some additional month, to avoid infection from nearby positive finisher herds. These results from 4 different production systems highly suggest, that the L-C-H model is an efficient method for potential PRRSV elimination programs in acutely and chronically infected farms. Intensive and frequent testing is essential following the L-C-H program, to identify residual PRRSv in single batches and remove those as soon as possible.

References

1. Linhares D et al. AASV Annual Meeting. 2013:57

O.091

Characterization of PRRS risks related to small non-commercial pig sites in a PRRS area-regional control project in East Central Colorado

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Introduction

The transmission of PRRS virus occurs via multiple mechanisms. The term "regional-spread" describes PRRS outbreaks where virus appears to move between farms within a large area, but all the mechanisms and potential sources of new virus are not understood. The risk of small populations of non-commercial pigs infecting large commercial farms has not been quantified. Most non-commercial pigs in this region are exhibition pigs, which are commonly purchased at public sales featuring pigs from producers across multiple states. The objective of this project was to attempt to quantify the risk small non-commercial pig sites may represent to regional-spread of PRRS to larger commercial operations.

Materials and Methods

This project was conducted from 2010-2013 in East Central Colorado. This region participated in a PRRS-Area Regional Control program, initiated by commercial herds in Yuma, Kit Carson, and Cheyenne counties. The project began with the Colorado State University Extension agents contacting youth livestock show members (4H/FFA) within the region. Educational meetings were conducted to introduce youth to this project and the opportunity to enroll. Veterinarians contacted youth enrolled in the project and visited each site in May. Sites were mapped, demographics collected, pigs were vaccinated for PCV2 and M.hyo at no charge to the youth, and serum samples were taken from individual pigs. At county fairs in July, Swiffer® cloths were used to collect fluids from the snout and mouth of individual pigs, which is a non-invasive sampling method developed especially for this project[1]. Serum samples were tested for PRRS ELISA and PCR, while snout wipes were tested for PRRS PCR, all at the Health Management Center (HMC), Ames, IA.

Results

There were 25 pig sites housing exhibition pigs sampled in 2010, 22 sites in 2011, 28 sites in 2012, and 17 sites in 2013. The May (pre-show) 2010 sampling had 1 pig PRRS PCR positive from the 83 pigs sampled, 2011 had 5 pigs positive on 2 sites of the 75 pigs sampled, 2012 had 2 pigs positive, both from the same site from the 103 pigs sampled, and 2013 had zero pigs positive from the 62 sampled (Table 1). The 5 PRRS PCR positive pigs in 2011 contained 3 distinct PRRS sequences. Seven of the eight PRRS PCR positive pigs originated from public sales, which featured pigs from various producers across multiple states. In July, no pigs at any of the fairs were found to be PCR positive via the snout wipes.

Table 1. PRRS serology from May (Pre-show)

Testing	2010	2011	2012	2013
Sites Sampled	25	22	28	17
Sites PCR+	1	2	1	0
Sites Elisa+	1	2	1	0
Pigs Sampled	83	75	103	62
Pigs PCR+	1	5	2	0
Pigs Elisa+	2	9	6	0

Conclusions and Discussion

This study is a step to better understand the mechanisms of regional-spread of PRRS. Small non-commercial pig sites are a potential PRRS risk to the large commercial operations in the region, as PRRS virus was found within this show pig population. Due to the frequent close proximity to commercial sites, the more obvious risk mechanisms would be potential indirect spread by fomites, and potential infection via aerosol. The risk level of aerosol transmission is likely low however, due to the small size of these sites and lesser quantity of PRRS virus potentially aerosolized from these sites. This project was also valuable in educating and building relationships with youth exhibitors. The percent of exhibitor sites enrolling increased from an estimated 33% in 2010, to an estimated 75% in 2013. Show pig exhibitors learned the value of being PRRS negative and now commonly ask breeders their PRRS status, and communicate a preference for PRRS negative pigs. Also, communication between exhibitors and commercial pig producers has improved via this project.

Acknowledgments

Dave Amundson, Midwest Farms, Burlington, CO
Mick Livingston, CSU/Kit Carson County Extension
Jess Waddell, Sutton, NE

References

1. Dobesh K et al. 2012. AASV

O.092

Genetic and geographic variation of PRRSV among the sites of a single company

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Introduction

There are several hypotheses regarding spread and transmission patterns of porcine reproductive and respiratory syndrome virus (PRRSV): these can be broadly categorized based on the presence or absence of human intervention. Analyzing the relationship between genomic and geographic distance among PRRSV isolates can provide insight into routes of virus spread and thereby serve as an important tool in disease control. A positive correlation between genomic and geographic distance would support the theory of distance-dependent transmission via wind and wildlife vectors, while a lack of positive correlation would suggest further investigation into long-distance transmission of PRRSV (i.e., transport vehicles and semen routing). This study analyzes the relationship between genetic distance of PRRSV isolates and geographic distance among the farms in a single swine management and consulting company.

Materials and Methods

ORF5 sequences of 603 nucleotides were obtained from diagnostic reports. A matrix was generated using BioPortal® to compare all pairs of nucleotide sequences. Physical addresses for each site were obtained from a company database and converted to latitude and longitude coordinates using Google Maps. Geographic distances between sites were calculated using PASSaGE2 and a matrix was generated comparing all pairs of coordinates. The Mantel test was used to compare the two resulting matrices using XLStat® with a confidence interval of 95% and significance level at P = 0.05.

Results

Twenty-five isolates from 25 sites were analyzed using XLStat®. The matrix of genomic distances (25 x 25) was compared to the matrix of geographic distances (25 x 25) applying the Mantel test. The null hypothesis was defined as a lack of correlation between the matrices; the alternative hypothesis established the existence of correlation. The statistics from the partial Mantel test for the correlation between geographic distance and nucleotide similarity was r(AB) = 0.082. Statistical analysis of 10,000 permutations indicated a non-significant relationship (P = 0.147).

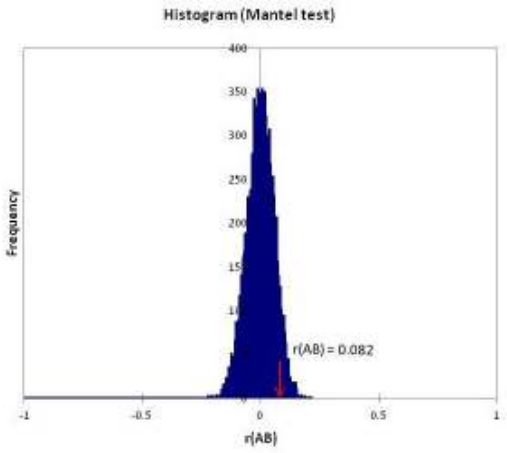

Figure 1. Mantel statistical values for the null hypothesis, defined as a lack of correlation between PRRSV isolates' genomic similarity and geographic distance between respective isolates' sites of origin. The red arrow shows the position of the statistical value (r(AB) = 0.082, in the zone of non-rejection of the null hypothesis; P = 0.147) calculated from the original, non-permutated data in the frequency distribution curve generated from 10,000 random permutations of the data.

Conclusions and Discussion

Results of the bivariate Mantel test prompted failure to reject the null hypothesis, as the computed P-value was greater than the significance value alpha = 0.05. This suggests that there is no direct correlation between geographic and genomic distances among the sequences and corresponding sites analyzed. The hypothesis that PRRSV is transmitted via distance-dependent routes must be rejected and prompts further investigation into alternative, non-distance-dependent methods of virus spread such as transmission of PRRSV via transport vehicles. This is an initial exploration of this novel methodology of analysis. Veterinarians and swine producers can utilize the results of this type of study as an aid to identifying gaps in biosecurity and in prioritizing the allocation of resources to improve biosecurity measures against PRRSV transmission.

O.093

PRRSV outbreak in a pig unit by infected semen

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Introduction

In April 2013, the main boar stud of the island of Ireland was diagnosed with PRRSv (Porcine Reproductive and Respiratory virus) infection. This stud was supplying semen to around 70% of producers.

One 100 SPF (Specific Pathogen Free) sow unit had PRRSv clinical signs of early farrowings, stillborns, agalactia, lack of appetite in sows, newborn piglet scour with poor antibiotic response and cachexia in piglets and weaners.

Materials and Methods

Clinical investigation consisted of a farm visit in order to assess the clinical signs. Further follow up visits were carried out during the year. Thirty blood samples were collected at different stages of production and analysed for PRRS seroconversion (ELISA IDEXX) in AFBI, Stormont (Belfast). Any positive PRRS serum (ELISA) was sequenced by PRRS-RT-PCR in IVD, Hannover (Germany). Five unused semen bags dated up to the 4th of April were collected and tested for E.U. PRRS nucleic acid (PCR) in AFBI, Stormont (Belfast). Any positive samples were tested by Real-time Multiplex RT-PCR kit detection of E.U., North American (N.A.) and Highly Pathogenic (H.P.) PRRS viruses in IVD, Hannover.

Results

Table 1 shows the production parameters before and after PRRSv infection.

Twenty three of the 30 blood samples were positive to PRRSv (ELISA). Sows inseminated before March 2013 were negative to PRRSv (ELISA) whereas the ones inseminated from March 2013 were positive to PRRSv (ELISA). Six of the 23 PRRSv positive (ELISA) serum samples collected were positive (RT-PCR) to PRRSv E.U. nucleic acid indicating viremia in the sows. Finishing pigs were still negative to PRRSv (ELISA) two weeks after the closure of the stud farm.

Three of 5 semen bags collected were positive (RT-PCR) to PRRSv E.U. nucleic acid. These bags were dated the month of March. Nucleotide sequence identification of orf7 gen in the semen and serum supplied matched 97% with the PRRSv E.U. reference Lelystad.

Conclusions and Discussion

The positive results in the semen bags indicate that infection at the stud farm was present for a period of time before it was closed down. Furthermore, nucleotide sequence matching proved that the herd became infected by semen. An attenuated-live PRRSv vaccine was introduced to the breeding herd immediately after diagnosis. Clinical signs disappeared within 3 months and production parameters recovered slightly.

Table 1. Productive parameters before and after PRRS infection.

	A	B	Difference
Conception Rate (%)	95.0	93.6	-1.4
Live Born/Litter	15.1	13.5	-1.6
Dead Born/Litter	0.9	1.2	+0.3
Weaned/Litter	11.5	9.9	-1.6
Pre-Weaning Mortality (%)	20.1	28.0	+7.9
Non-Productive Days	10.1	14.7	+4.6

A= 6 months prior to the outbreak (1 Oct 12 – 31 Mar 13)
B= 6 months after the outbreak (1 Apr 13 – 30 Sep 13)

PRRSv is enlisted as a Notifiable Disease in Northern Ireland (1). The prevalence of infection in 2011 and 2013 was 36.6% and 43.9% of the Northern Irish pig producers, respectively (2).

Semen has been described as a route of infection experimentally (3). There are not many studies and/or confirmed reports of PRRS infection by contaminated semen in the field. This is now a real concern due to the widespread use of artificial insemination in pig production. Better biosecurity controls are required to be implemented in stud farms in order to avoid the spread of PRRSv to naïve herds, as has occurred in Ireland or Switzerland (4).

References

1. The Diseases of Animals (Northern Ireland) Order 1981. Schedule 1, Part III.
2. Borobia J 2011 & 2013. Unpublished data.
3. Christopher-Hennings J et al 1995. Journ Vet Diagn Invest 7:456-464.
4. BVET (2012) http://www.bvet.admin.ch/aktuell/01617/04492/index.html?lang=it&msg-id=46913

O.094

Relationship of microscopic lesions and viral load in fetal implantation sites for type 2 PRRSV infected pregnant gilts

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) continues to be the most costly diseases facing the North American swine industry resulting in reproductive losses estimated to be over $500 million annually[1]. However, after nearly 25 years of PRRS research, the pathogenesis of fetal death during infection is still poorly understood. In most of the cases of abortions associated with late gestation infections of PRRSv, microscopic lesions in the internal organs of the fetuses are rare and inconsistent[2]. This suggests that fetal death might not be a consequence of PRRS virus replication in the internal organs, but that fetal implantation sites may play an important role in the pathogenesis of fetal death[3]. The objective of this study is to determine whether PRRSv RNA levels in endometrium correlate with microscopic lesions present in the fetal implantation sites of pregnant gilts infected with type 2 PRRSv.

Material and Methods

A total of 113 PRRSv-naïve high-health pregnant gilts were intramuscularly and intranasally inoculated with PRRSv (10^5 $TCID_{50}$ total dose) and 19 negative control gilts were sham inoculated on gestation day 85±1. At 21days post inoculation, dams and their litters were humanely euthanized for necropsy examination. The adjacent uterus and placenta was collected for all fetuses and samples were fixed in 10% buffered formalin for microscopic examination. An in-house quantitative PCR for the inoculated PRRSv was performed on frozen endometrial tissue that was adjacent to the histology sample.! Hematoxylin and eosin stained formalin-fixed paraffin-embedded microsections of uterus with attached placenta were assessed by pathologists blinded to PCR results. The grading scheme was based on percentage of affected endometrial tissue and the total number of inflammatory cells present in the lamina propria. The degree of vasculitis was assessed based on its distribution and severity within the endometrium. Non-infected controls did not have lesions.

Results

A total number of 679 uterine tissue sections randomly selected from 110 infected pregnant gilts were microscopically examined. Results showed that most of the uterine tissues (73%) had severe lymphohistiocytic endometritis and the lesions were very severe for an additional 10% of tissue sections. In two-thirds (67%) of the tissue sections, the vasculitis was present in <30% of vessels, but in nearly a third of the sections (29%), the vasculitis was in 30-70% of blood vessels. No signs of uterine infection or vasculitis were found in negative control gilts. Endometrial inflammation expressed as total number of inflammatory cell present in the lamina propria was positively related to distribution and severity of vasculitis in the endometrium (P<0.001; Multilevel Mixed-Effect Model, STATA). Given a 1 unit increase in average severity score of vasculitis, a total number of inflammatory cells in the lamina propria were more likely to be increased by 10 units. There were no significant relationship between endometrial inflammation and PRRS viral loads in uterus (P=0.4059; Multilevel Mixed-Effect Model, STATA). Also, percentage of vessels affected with vasculitis and average severity score of vasculitis were not significantly related to PRRS viral loads in the uterus (P=0.4147, P=0.6425, respectively; Multilevel Mixed-Effect Model, STATA).

Conclusions and Discussion

As part of the analysis of the fetal preservation and viral loads, there was no relationship between viral loads in the uterus and the fetal thymus, nor was there a relationship between fetal position in the uterus and viral loads or preservation[4]. Therefore, it is not a surprise that there was no relationship between the viral loads and the inflammation within the uterus. However, since one of the keys to understanding fetal losses during PRRSv infection may be a connection between the inflammatory process in the uterus and fetal death, further analysis is underway to look at viral antigens and macrophage markers in the tissues present, as well as apoptotis in the fetal implantation sites.

References

1. Holtkamp DJ, et al. 2013.. J Swine Health Prod 21: 72-84.
2. Christianson WT, et al. 1992. Am J Vet Res 53:485-488.
3. Karniychuk UU, et al. 2011. Microb Pathog 51: 194-202.
4. Ladinig A, et al. 2014. Variation in fetal outcome, viral load and ORF5 sequence mutations in a large scale study of phenotypic responses to late gestation exposure to type 2 Porcine Reproductive and Respiratory Syndrome Virus. *Submitted.*

O.095

Effects of high molecular poly-γ-glutamic acid on pigs challenged with PRRSV and its application on farm

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Introduction

Poly-γ-glutamic acid (γ-PGA) is a natural biopolymer produced by various strains of *Bacillus subtilis* during the process of fermentation. Previously various molecular weights of γ-PGA (~2,000 kDa) have been studied using laboratory animals to evaluate its potential applications based on its diverse characteristics (1). Common findings from previous studies were γ-PGA induces high responses in both humoral and cell mediated immunity on mouse models, and high molecular weight γ-PGA showed higher levels of immune response. Moreover, antiviral activity against highly pathogenic viruses using γ-PGA has been reported (2). The objective of this study is to investigate effects of high molecular weight γ-PGA (2,000 kDa) produced by *Bacillus subtilis sups. Chungkookjang* against 2 genetically different porcine reproductive and respiratory syndrome viruses (PRRSV), MN184 and JA142, and its application on PRRSV infected farm

Materials and Methods

γ-PGA 2% solution which contains γ-PGA (2,000 kDa) 20 mg/ml, was used for treatment. Total 12, 4 weeks old pigs free from PRRSV were purchased and randomly divided into 4 groups and challenged with JA142 virus. Three groups were treated with 5 ml of γ-PGA by IM at 3 days before and 0 or 3 days post challenge (dpc) and the remaining one group of pigs was served as challenge control without γ-PGA treatment (non-treated challenge control). Similarly, total 12, 4 weeks old, PRRSV-free pigs were purchased and randomly divided into 4 groups, then challenged with MN184 virus. Three groups of pigs were inoculated with 5 ml of γ-PGA by intramuscular (IM) route at 0, 3 and 7 dpc and the remaining one group of pigs was served as non-treated challenge control. Serum samples were collected from all of the pigs on a weekly basis and tested by RT-PCR, ELISA, and virus neutralization test to determine the levels of viremia, PRRSV nucleocapsid-specific antibody and serum virus neutralizing antibody (SVN), respectively. Body weight also was measured to determine average daily weight gains (ADWG) during the experiments. For farm application of γ-PGA, 10 sows from a farm consistently infected with PRRSV were selected. One hundred piglets born from the 10 sows were randomly divided into 2 groups, 50 piglets for each group, at weaning. Then one group was treated with 5 ml of γ-PGA and another group was served as a non-treated control. Serum samples were collected from both of the groups at 30, 40, and 60 days post γ-PGA treatment and analyzed by ELISA and RT-PCR to find differences between the groups.

Results

Pig groups treated with γ-PGA showed lower levels of viremia and higher levels of SVN antibodies and ADWG after challenged either with JA142 or MN184 when compared with the non-treated challenge control groups. Pig groups treated with γ-PGA also showed higher levels of cytokine responses (IFN-α, TNF-α and IL-2) after challenged with MN-184 as compared with the challenge control group. In the farm application, more number of pigs in the group treated with γ-PGA remained uninfected for longer time as compared with the non-treated group.

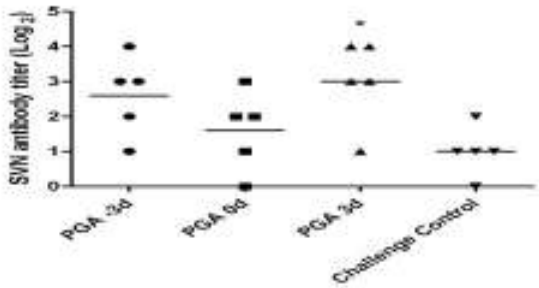

Figure 1. SVN antibodies induced against JA142 in pigs treated with γ-PGA at various time points as compared with non-treated pigs (challenge control).

Conclusions and Discussion

Treatment with high molecular weight γ-PGA increased the levels of ADWG, SVN antibody, and cytokine response, decreased the levels of viremia and delayed the onset of PRRS infection both in experimental challenge studies and field application. These results suggest that γ-PGA could be an alternative way to control PRRSV infection in various field situations.

Acknowledgments

GSBC, Suwon, Gyeonggi-Do, Korea 443-766

References

1. Davaatseren M. et al. Mediators Inflamm. 2013:982383
2. Lee W et al. 2013. Biomaterials 2013.08.067

O.096

Clinical characterization of a type 2 PRRSV causing significant clinical disease in the field in Denmark

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Introduction

PRRSV is the cause of significant reproductive and respiratory disease in swine worldwide. In Denmark, approximately 50 % of the herds are seropositive for PRRSV of either or both Type 1 or Type 2. In November 2010, a pig herd in the Northern part of Denmark experienced an outbreak with Type 2 PRRSV where the clinical impact appeared to be much more severe than usually reported from Danish Type 2 PRRSV affected herds. Due to the clinical observations of reproductive failure (stillborn & mummified) in sows and high mortality in piglets, it was speculated that a new, more pathogenic PRRSV strain had evolved in Denmark. The aim of this study was: 1) to make a clinical characterization of the virus isolated from the herd by comparing the infection dynamics of the virus isolated from the herd with an older Danish Type 2 isolate (DK-2010-10-13-1) by experimental infection in young pigs and 2) to assess the protective effect of a MLV Type 2 vaccine against this recent Danish Type 2 PRRSV isolate.

Materials and Methods

Samples from the PRRS clinical case collected in Nov 2010 were obtained and the virus, designated DK-2010-10-13-1, was isolated in Marc-145 cells using general cell culture procedures and sequenced in full. Twenty-eight 4-week-old pigs from a commercial farm with health status according to the Danish Specific Pathogen Free (SPF) system were randomly divided into one control group N=4 (group 1) and three experimental groups (groups 2-4) (N=8). The groups were housed in separate sections at biosafety 3 facility. Pigs in group 4 were vaccinated (2 ml i.m.) with Ingelvac® PRRS MLV vaccine (Boehringer Ingelheim Animal Health). Four weeks later, at post inoculation day (DPI) 0, pigs in group 1 were sham- inoculated intranasally (i.n.) with Eagle's MEM. Pigs in group 2 and group 3 were inoculated i.n. with DK-1997-19407B and DK-2010-10-13-1 isolates respectively. Pigs in group 4 were challenged with the DK-2010-10-13-1. The study was carried out in accordance with the Danish and EU regulations on the use of laboratory animals for research. Individual daily clinical scoring and individual rectal temps were recorded. Blood samples were collected from all pigs on DPI -28, 0, 3, 7, 10, 14, 21, 28, and 30 for PRRS RT-PCR and serology. Samples were collected from lungs at necropsy for histopathology and virus quantification by RT-PCR.

Results

All pigs remained healthy throughout the experimental period. At necropsy, only minor lesions were revealed. PRRSV was detected by real-time RT-PCR in all pigs inoculated (figure 1). The viremia peaked at DPI 7, and was significantly lower and of much shorter duration in the vaccinated group (group 4) compared to the unvaccinated animals (p= 0.04). Histopathology results are pending and will be presented.

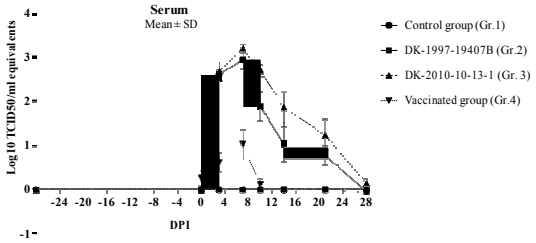

Figure 1. Viral load in serum quantified by real-time RT-PCR

Conclusions and Discussion

PRRSV isolated from a herd experiencing overt clinical signs and significant mortalities, failed to induce comparable signs in this experimental set-up but if the reproductive infection model have been used the signs may have been more like the signs seen in the herd. Full genome sequencing of the isolate revealed that the isolate was very similar to other Danish Type 2 isolates. Our data suggested that the severity of the disease in the field could be influenced by other factors than the virus and emphasize the need to aim further research for identifying virulence markers of PRRSV. In addition, the results demonstrated that vaccination with Ingelvac® PRRS MLV vaccine (Boehringer Ingelheim Animal Health) reduced viremia and viral excretion, thus being effective of limiting the impact of the DK-2010-10-13-1 case virus and as such would be expected to be effective in the control of an outbreak with this and related viruses. These findings are in accordance with the finding that the vaccine was used effectively in the control of the infected herd.

Acknowledgement

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O.097

Reduction of PRRSV transmission in vaccinated pigs

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Introduction

Because of long term shedding, PRRSv can easily persist within a population for years, and attempts to eradicate the virus often lead to disappointing results. Live modified vaccines have shown good results in reducing the clinical outcomes of the infection (Martelli et al., 2009). To be an appropriate tool for controlling an infectious disease, a vaccine should reduce viral shedding to such an extent that the basic reproduction ratio (R0) falls below the 1 threshold (Anderson and May, 1992). In this experimental study, the impact of vaccination was assessed on PRRSv transmission and compared with the R0 estimate in non vaccinated pigs.

Materials and Methods

The experiment involved 56 3-week-old SPF piglets without anti-PRRSv maternal antibodies. The transmission experiment was organized as 6 replicates of 2 inoculated in contact with 2 sentinel piglets in the vaccinated group with a similar setting in the non-vaccinated group. Vaccinated piglets received the commercial modified live vaccine Porcilis PRRS ID® (MSD) via the intradermal route at 3-weeks-old and challenged intranasally with 5.10^5 DCP_{50} of a genotype 1, subtype 1 PRRSv strain (PRRS/FR/29/24/1/2005), 31 days post-vaccination. Piglets were monitored individually (blood samples every 3 days) for 49 dpi, to measure viral load in serum using a quantitative real-time RT-PCR designed to distinguish the challenge of the vaccine strain. Transmission parameters were estimated through a Bayesian modelling framework relying on the likelihood of the observed probability of infection in contact piglets. Durations of latency and infectiousness were modelled by gamma distributions.

Results

Only one piglet was detected infected in vaccinated contact piglets whereas all piglets were infected in the non vaccinated group. The estimated transmission rate was 10-times lower in vaccinated (0.02 [95% CI 0.003-0.08]) than in non-vaccinated piglets (0.22 [0.12-0.37]). The duration of infectiousness was reduced: 12 days [9.8-14.5] versus 22.6 days [20.3-25] in vaccinated and non-vaccinated piglets respectively. Hence R0 estimate was significantly lower than one in vaccinated piglets (0.28 [0.04-0.96]) and considerably lower than R0 estimate in non-vaccinated piglets (4.97(2.71-8.36]).

Table 1. Detection of PRRS challenge virus in serum of unvaccinated and vaccinated sentinel piglets

		Day'post'challenge												
	Animal'#	D2	D4	D7	D10	D14	D17	D21	D24	D28	D32	D35	D38	D49
vaccinated'sentinels	4637													
	4656													
	4648													
	4654													
	4667													
	4700													
	4680													
	4705													
	4669								!	!	!	!	!	!
	4690													
	4668												!	!
	4692					pos$	pos							
unvaccinated'sentinels	4636		pos$	pos$	pos$	pos$	pos$		pos$					
	4660			pos$	pos$	pos$	pos$	pos$	pos$					!
	4641								pos$	pos$	pos$	pos$		
	4655										pos$	pos$	pos$	pos$
	4670		pos$	pos$	pos$	pos$	pos$	pos$	pos$			pos$		
	4701				pos$	pos$	pos$	pos$	pos$	pos$	pos$			
	4672				pos$	pos$	pos$	pos$	pos$	pos$				
	4699				pos$	pos$		pos$	pos$					
	4666			pos$	pos$	pos$	pos$	pos$						
	4693				pos$	pos$	pos$	pos$	pos$	pos$				
	4684				pos$	pos$	pos$	pos$			pos$			
	4686				pos$	pos$	pos$	pos$	pos$	pos$				

Conclusions and Discussion

These results showed the theoretical ability of such a vaccine to clear out the virus from an infected population. However, further work is needed to evaluate this effect in field conditions taking into account the possibility of vaccinating passively immune animals or an important delay between vaccination and infection, and transmission enhanced by herd practices and lack of biosecurity.

Acknowledgements

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References

1. Martelli, P et al. 2009. Vaccine 27, 3788-3799.
2. Anderson, R.M. et al. 1992. Infectious diseases of humans: dynamics and control. Oxford University press, 756 p.

O.098

Efficacy of Fostera® PRRS in pigs challenged with a heterologous PRRSV two weeks after vaccination

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Introduction

A characteristic of porcine reproductive and respiratory syndrome virus (PRRSV) infection is a relatively slow development of immunity compared to other pathogens. This has been associated with various immune-modulating properties of the virus, including an ability to suppress interferon alpha production, although this is strain dependent. Efficacy studies with modified live PRRSV vaccines typically use a minimum of 4 weeks between vaccination and challenge. Fostera PRRS (Zoetis) has been shown to be highly effective using this model (1). In the field, however, natural challenge may occur before 4 weeks post-vaccination. This study was conducted to test the ability of Fostera PRRS to protect pigs challenged with a virulent, heterologous Type 2 PRRS strain 2 weeks post-vaccination.

Materials and Methods

A summary of the study design is given in Table 1. At 3 weeks of age (day 0) 36 pigs were vaccinated with either placebo or Fostera PRRS. Pigs were housed by treatment in separate rooms and pens of 6 until challenge at Day 14 when they were re-housed, by treatment, in pens of 3 in mixed rooms but with empty pens between each occupied pen. Six additional pigs were used as sentinels for extraneous PRRSV infection and housed with the placebo group; these pigs were necropsied before challenge. Pigs were necropsied at 10 days after challenge and lung lesions scored by visual assessment and manual palpation. Clinical signs were monitored regularly and blood samples collected for PRRSV ELISA testing and interferon-α analysis (IFN-α). Work was conducted in compliance with national legislation and subject to review by the local Institutional Animal Care and Use Committee.

Table 1. Study design

Treatment	N	Vaccination	Challenge
Placebo	18	2 mL I/M at 3 weeks of age	2 mL intranasal & 2 mL I/M at 5 weeks of age of strain NADC20 at 5.6 $\log_{10}$TCID$_{50}$/mL
Fostera PRRS	18		

Results

The sentinel and placebo vaccinated pigs remained PRRSV negative during the pre-challenge period. The post-vaccination clinical signs and rectal temperatures were unremarkable. Following challenge a higher percent of placebo vaccinated pigs showed clinical signs than in the Fostera PRRS vaccinated group (Figure 1).

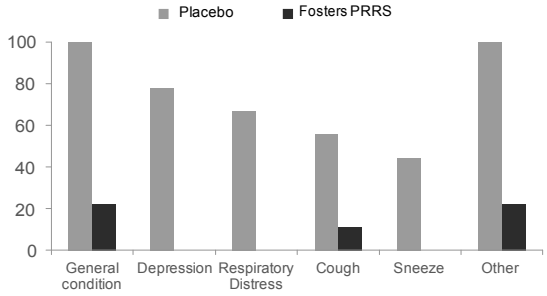

Figure 1. Percent of animals ever observed with clinical signs of any severity following challenge.

At vaccination and challenge mean weights were identical between treatments, but after challenge vaccinated pigs showed better weight gain and were significantly heavier at necropsy (P<0.001) (Table 2). At necropsy lung lesions in the Fostera PRRS vaccinated pigs were substantially and significantly lower (P<0.018) than in the placebo vaccinated pigs (17.5 percent of the lungs affected compared to 46.1%), although this reduction was less marked than seen in previous studies with a longer vaccination to challenge interval (1). Vaccination induced a rise in IFN-α, with concentrations being significantly elevated compared to controls just before challenge (10.15 v 0.85 picograms/ml, P=0.0001).

Table 2. Mean bodyweights (kg) at allotment (day -4), re-housing (day 13) and necropsy (day 24)

Treatment	Day -4	Day 13	Day 24
Placebo vaccine	6.3	12.1	13.6
Fostera PRRS	6.3	12.1	16.6

Conclusions and Discussion

The results demonstrate that Fostera PRRS administered at weaning gives substantial protection by 2 weeks post vaccination. The mechanisms could include the early stages of acquired immunity, but also innate immune mechanisms such as IFN-α, and possibly a reduction in the availability of susceptible macrophages for the challenge virus.

References

1. Calvert J G et al. 2012. Proc AASV: 195-198

O.099

Elimination of PRRSV type 2 from an acutely infected sow farm by using Ingelvac PRRS MLV in a load, close and homogenize model in a 2 site production

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Introduction

Load-Close Homogenize (LCH) methodology has been reported as a succesful tool to control and eliminate PRRSv in. This study evaluates PRRSv elimination from a 2 site production farm, acutely infected with PRRSV type 2, by using a Load-Close-Homogenize (L-C-H) model.

Materials and Methods

The case herd is a 1384 head sow farm, member of the SPF health declaration system, but infected with M-hyo and App-12. All piglets are weaned to an off-site, sectionized nursery. Two connected rooms (buffer 1 & 2) are regularly used for left over pigs if number of delivered pigs does not fit the nmbers in the emptied section.. The pigs are exported to Germany at 30 kg liveweight.In February 2013, the sow herd got infected with PRRSV type 2. In March 2013, 7 out of 20 sows were seropositive (IDEXX ELISA X3, Kjellerup DK). To initiate the L-C-H procedure, the small finisher site was emptied, cleaned and disinfected and was loaded with gilts needed for 200 days (30 weeks) (10 – 19 weeks of age) and then closed. The staff was educated to keep strict McRebel rules. The sow herd was mass vaccinated twice 4 weeks apart. During 8 weeks, all piglets were vaccinated at 7 – 10 days of age (doa). In November 2013, 60 PRRS negative SPF gilts were introduced as sentinels in the breeding unit on the sow site.

Load-Close and Homogenize timeline:

Week 12; All animals present on the sow site vaccinated with Ingelvac® PRRS MLV together with the gilts loaded in the off-site finisher barn.

Week 16; Whole herd mass vaccination repeated.

Week 18; Half of the loaded and vaccinated gilts moved to sow site

Week 12 – week 20; All newborn piglets vaccinated at 7-10 doa.

Week 23; Remaining gilts moved to sow site.

Week 45; 29 weeks after the second mass vaccination, 60 PRRSv naive sentinel gilts placed in the breeding unit.

Results

Oral fluid testing for PRRSV by PCR of weaned, non-vaccinated piglest in July and August, indicated that piglets were weaned negative. From October to December 2013, blood samples were taken regularly from 5 pigs from all sections in the nursery. Underweight pigs were kept up to 14 days extra in a buffer section to reach weight limit. The buffer was emptied regularly, washed and disinfected. All pigs from weaning to 6 weeks post wean were PCR negative for PRRSV on all samplings. In buffer1, some pigs were found PCR positive for PRRSv (vaccine strain) both in December and January, . Monthly testing of 20 gilts per month until December and after that10 gilts showed no seroconversion against PRRSV at any point of time.

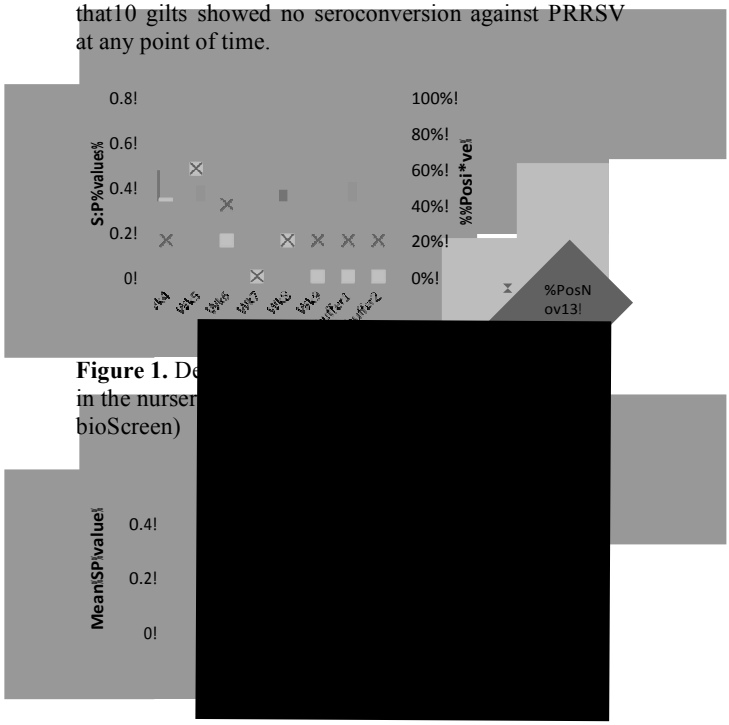

Figure 1. De
in the nurser
bioScreen)

Figure 2. mean SP ratio of 30 kg piglets and sentinel gilts

Conclusions and Discussion

PCR testing of oral fluid and blood samples confirmed that the sow unit weaned PRRSv negative piglets from July 2013 and onwards. PCR positive, but antibody negative pigs in one buffer indicated residual infection in this room. Theese findings document the importance of frequent testing of weaners to ensure that all PRRSv has been completely cleaned up. Weaning of PCR negative piglets and the antibody negative sentinels gives strong evidence that no PRRSv Wild Type circulated on the sow site after L-C-H. Hence, L-C-H proved to be an efficient tool for PRRSv elimination from an acutely infected farm.

References

1. Linhares DC, *et al. Prev Vet Med,* 2014. Submitted

O.100

Field efficacy of sow vaccination with AMERVAC® PRRS in a Vietnamese farm co-infected with genotype-I and genotype-II PRRSV

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Introduction

The emergence of highly virulent PRRS virus (HP-PRRSV) strains in China in 2006 (1,2) was a milestone in the epidemiology of PRRS in Asia. Nowadays, genotype-II strains of different virulence coexist with genotype-I strains in Vietnam (3,4). At present, AMERVAC® PRRS a genotype-I modified live vaccine (MLV) is licensed and marketed in many countries of Asia. Previously, AMERVAC® PRRS (HIPRA, Spain) has demonstrated efficacy in pigs experimentally infected with genotype-II HP-PRRS isolated from severe PRRS clinical outbreaks in Vietnamese pig farms (5,6). The present study was aimed to explore the field efficacy of AMERVAC® PRRS when applied in sows in a Vietnamese farm co-infected with different genotypes of PRRSV.

Materials and Methods

A 1200 sows farrow-to-finish farm located in Binh Duong province (Vietnam) was selected for these study after being diagnosticated positive to PRRS virus, for both genotypes (I and II), by PCR (7) and serology (CIVTEST®PRRS ES and AS, HIPRA, Spain). To evaluate the efficacy of AMERVAC® PRRS (VP-046 BIS strain; $\geq 10^{3.5}$ $TCID_{50}$/dose) we compared several reproductive and productive parameters (see table 1) of the farm 4 months before (from May to August 2012) and 6 months after (from November to April 2013) the onset of the vaccination of the breeding herd. Initially, vaccination program was established as 2 mass vaccinations of all sows of the breeding herd. First mass vaccination was applied in September 2012 and a second mass vaccination to all sows as well in October 2012. For both mass vaccinations we applied the commercial dose (2 ml, IM) of AMERVAC® PRRS.

Results

Vaccination of sows with AMERVAC® PRRS (*P<0.05*) improved significantly all the reproductive parameters evaluated in this study as it is shown in the following table:

Table.1 Reproductive parameters before and after sow vaccination.

Group	FR (%)	PBA	LM (%)	WP
BV	82.8	9.0	13.4	8.5
AV	89.2*	9.8*	9.7*	9.4*

BV: Before vaccination (average of monthly results from May to September 2012); **AV**: After vaccination (average of monthly results from May to September 2012); **FR**: Farrowing rate; **PBA**: Piglets born alive; **LM**: Lactation mortality; **WP**: Weaned piglets.
(*) subscripts indicate statistically significant differences (*P<0.05*) between BV and AV results, *T-Student test.*

Conclusions and Discussion

Vaccination of sows with AMERVAC® PRRS improved the reproductive performance of the farm co-infected with both PRRSV genotypes. Concretely, after 2 mass vaccinations of the entire breeding herd we observed an improvement of the farrowing rate and an increase of the number of born alive piglets. At the same time, vaccination could reduce piglet mortality rate during lactation, and therefore it also increased the number of weaned piglets. Despite the circulation of both genotypes of PRRSV in the farm, vaccination with a genotype-I PRRS MLV showed a wide efficacy reducing the impairing effects of PRRSV on reproductive parameters and productive performance during lactation. These results corroborate the results of previous studies where AMERVAC®PRRS showed clinical protection in front experimental infection of piglets with HP-PRRS Chinese-like strain isolated in Vietnam (5). Therefore, according to these results, we could consider AMERVAC® PRRS a very useful tool for the control of clinical reproductive PRRS in Vietnamese farms, regardless of the PRRSV genotype infecting the breeding herd.

References

1. Tian et al. 2007. PLoS ONE, 2: e526.
2. Li et al. 2007. Vet J, 174 : 577-584.
3. Nam et al. 2009. Arch Virol, 154: 629-638.
4. Amonsin et al. 2009. Virol J, 16: 143.
5. Roca et al. 2012. Vet J, 193: 92-96
6. Thanh et al. 2013. 6th APVS Congress Proceedings, OR59.
7. Martinez E. et al. 2008. Res Vet Sci, 85:184-193.

O.101

Herd diagnosis of non-infectious diarrhea in growing pigs

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Introduction

Treatment of intestinal disease, clinically evident as diarrhea outbreaks[1], account for the majority of the antibiotic consumption in the Danish swine industry.
Some of these treated diarrhea outbreaks have a non-infectious cause, including non-specific colitis[2,3].
Termination of antibiotic treatment in herds suffering from non-infectious diarrhea outbreaks could contribute towards a reduction in the consumption of antibiotics in the swine industry.
The objective of the study was to establish criteria for herd diagnosis of non-infectious diarrhea in growing pigs.

Materials and Methods

Data previously collected from diarrhea outbreaks in 20 Danish herds with pigs 10-70 days post-weaning were used to create a case-series of clinical diarrhea outbreaks, illustrating examples of diarrhea normally subjected to antibiotic treatment.
The data consisted of 16 euthanized pigs from each herd (n=320) selected within a single batch suffering from diarrhea. The pigs were subjected to necropsy and histopathological examination. Occurrence of *Salmonella* spp., *Escherichia coli*, *Brachyspira* spp. *Lawsonia intracellularis*, Rotavirus and Porcine Circovirus type 2 were investigated by immunohistochemistry, fluorescent in-situ hybridization and quantitative PCR testing (qPCR).
For each diarrhea outbreak the prevalence of pigs suffering from infectious intestinal disease was determined. Based on this case-series of diarrhea outbreaks criteria for identification and decision support in relation to termination of antibiotic treatment of non-infectious diarrhea were suggested. The treatment criteria were discussed with a panel of 5 veterinary experts in porcine health. All data analyses were performed using Stata IC 13.

Results

A prevalence <15% of pigs (diarrheic or not) within a room suffering from bacterial intestinal disease was selected as the limit where antibiotic treatment was not required.
Using this limit five (25%) outbreaks were classified as non-treatment-requiring and 15 (75%) outbreaks were classified as treatment-requiring. The non-treatment-requiring outbreaks had a within-outbreak prevalence of pigs with bacterial intestinal disease ranging from 0 to 6.4%. The treatment-requiring outbreaks had a mean within-outbreak prevalence of pigs with bacterial intestinal disease of 41% (range: 16-91%). *E. coli*-associated intestinal disease was demonstrated in 73% (n = 11), *L. intracellularis*-associated intestinal disease was demonstrated in 47% (n = 7) and *B. pilosicoli*-associated intestinal disease was demonstrated in 47% (n = 7) of the diarrhea outbreaks. No other bacterial intestinal pathogens were demonstrated.
The apparently best criteria for differentiation between treatment-requiring and non-treatment-requiring diarrhea outbreaks were a combination of clinical signs and qPCR testing of a pooled fecal sample.
The criteria for a treatment-requiring outbreak was an average of ≥1.5 diarrheic fecal pools on the floor of each pen in the room under investigation and a load of pathogenic bacteria ≥4.5 $\log_{10}$ per gram in a fecal pool consisting of 20 individual fecal samples. The pathogenic bacterial load was determined by the sum of *L. intracellularis*, *B. pilosicoli* and *E. coli* F4 and F18 bacteria per gram of feces determined by quantitative qPCR testing.

Conclusions and Discussion

The study demonstrated that criteria for herd diagnosis of non-infectious diarrhea in growing pigs could be established. Antibiotic treatment may be terminated in such herds. The suggested criteria should now be further evaluated under field conditions, exploring the effect of terminating the antibiotic treatment in herds identified to suffer from non-infectious diarrhea.

References

1. Hybschmann GK et al. 2011. Prev Vet Med 98:190-197.
2. Chase-Topping ME et al. 2007. Vet J 173:353-360.
3. Pedersen KS et al. 2012. Vet Q 32:45-49.

O.102

Sock-sample testing for herd diagnosis of non-infectious diarrhea in growing pigs

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Introduction

Treatment of intestinal disease is the major indication for antibiotic consumption in the Danish swine industry[1].
Termination of antibiotic treatment strategies in herds suffering from non-infectious diarrhea outbreaks could result in a reduction in the consumption of antibiotics.
A previous study has suggested criteria for herd diagnosis of non-infectious diarrhea, including a limit of 4.5 $\log_{10}$ pathogenic bacteria per gram feces in pooled samples[2].
An alternative to a pooled fecal sample could be a sock-sample obtained by walking around in pens subjected to examination. The sock-samples could potential provide an easy and cheap method for sampling feces for quantitative PCR (qPCR) testing.
The objective of the study was to evaluate agreement between qPCR results obtained by testing a pooled fecal sample and a sock-sample from a group of pigs.

Materials and Methods

Fecal samples were obtained from 43 outbreaks of diarrhea in different Danish herds.
From each of the diarrhea outbreaks, the herd veterinarian collected 20 fecal samples randomly from different pens containing non-medicated growing pigs (10-70 days post weaning). Approximately 10 fecal samples representing normal feces and 10 fecal samples representing diarrheic feces were collected. Each fecal sample consisted of approximately 2 g of fecal material obtained from freshly deposited feces.
From the same pens a sock-sample was obtained by walking through all pens wearing sock-covered boots (standard sock with known standard dry weight). First the boots were covered with a disposable plastic cover. Secondly the boots were covered with a clean standard sock. Then a walk through all pens within the room was performed by walking on the fecal contaminated slatted floor. In order to evaluate sock-sample repeatability the sampling was repeated using a new set of socks within 10 minutes.
All fecal and sock-samples were packed in a polystyrene box containing freezer packs and sent to the National Veterinary Laboratory, Copenhagen, where they arrived the following day. From each herd the 20 individual fecal samples were diluted to 10% in phosphate buffered saline (PBS) and equally pooled by weight in one sample (pooling of 10% PBS solutions). The wet sock-samples were weighed, suspended in PBS to obtain a 10% solution and processed in a Stomacher for 1 minute. Pools and sock-samples were subjected to qPCR testing for *Lawsonia intracellularis*, *Brachyspira pilosicoli* and *Escherichia coli* F 4 and F18.
The quantitative qPCR results were $\log_{10}$ transformed ($\log_{10}$ bacteria/g feces). All the qPCR results were dichotomized as being below or above 4.5 $\log_{10}$ bacteria per gram feces.
The pool and the first sock-sample from each outbreak were compared. The two sock-samples from each outbreak were compared. All comparisons were performed by calculation of agreement and Cohen's Kappa using Stata IC 13.

Results

A total of 860 individual fecal samples (43 pools were prepared) and 43 sock-samples were submitted from 26 veterinarians. Agreement between pools and socks were 88% (Cohen's Kappa = 0.63) for the dichotomized results, with 79% (n=34) of the pools and 81% (n=35) of the socks containing more than 4.5 $\log_{10}$ pathogenic bacteria per gram feces. Agreement between the two sock-samples within the same outbreak was 90% (Cohen's Kappa = 0.66) for the dichotomized results, with 81% (n=35) of the first socks and 86% (n=36) of the second socks containing more than 4.5 $\log_{10}$ pathogenic bacteria per gram feces.

Conclusions and Discussion

The study demonstrated an acceptable agreement between the qPCR results obtained from testing a group of pigs using either a pooled fecal sample or a sock-sample. Repeatability for the sock-samples was acceptable. The sock-sampling technique offers a very easy and cheap way to obtain fecal material from pigs for qPCR testing. We suggest using qPCR testing of sock-samples for herd diagnosis of of non-infectious diarrhea in growing pigs.

References

1. Hybschmann GK et al. 2011. Prev Vet Med 98:190-197.
2. Pedersen KS et al. 2014. Submitted for IPVS 2014.

Investigation of respiratory tract lesions at slaughter in a large pig production system

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Introduction

The British Pig Health Scheme which monitors carcass lesions at slaughter has shown that Enzootic Pneumonia like lesions (EPL) have been rising since 2010 to mid 2012 in the UK, at the same time, pleurisy levels have been increasing since 2008[1].

The increase in the diagnostic rate by the national laboratories of Swine Influenza (SI) since 2010[2] and changes in the enzootic pneumonia (EP) and PCV2 in 2011 control programs (started using mixed EP and PCV2 vaccines) drove the investigation to assess the involvement of *Mycoplasma hyopneumoniae* (Mhyo) and SI in the lesions seen at slaughter in the pigs produced in this system, no changes were made in the control of Porcine reproductive and respiratory syndrome (PRRS) and PCV2 piglet vaccination.

Material and Methods

During October and November 2013, consignments of pigs sent to slaughter were assessed in the abattoir, EPL were scored by assessing the antero-ventral lung consolidation following the method described by Goodwin *et al*[3]. Pleurisy was scored as mild (visceral pleurisy) or severe (parietal pleurisy). *Actinobacillus pleuropneumoniae* (APP) like lesions were also scored as acute or chronic. Blood samples were collected at slaughter (10 per consignment) and the samples were tested for M hyo, SI, APP and PRRS antibody titres with commercial ELISA tests. Consignments were considered positive if they had at least two positive samples. The resulting data was statistically analyzed using proprietary statistical software.

Results

During the study period, 33 consignments of slaughter pigs were assessed, this resulted in the examination of 2930 pig lungs, and the submission of 330 blood samples.

The blood samples demonstrated that 52%, 33%, 79% and 39% of the consignments were positive for PRRS, APP, Mhyo and SI. The serology showed a significant positive correlation between the presence of EPL > 4 at slaughter and Mhyo ELISA (0.443, p = 0.011), Figure 1. None of the other agents assessed through serology were correlated with EPL > 4.

PRRS was correlated with the presence of mild pleurisy at slaughter (0.365, p = 0.037), Figure 1.

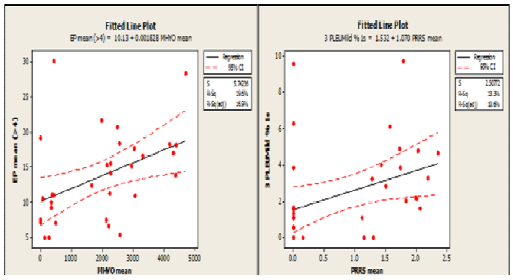

Figure 1. Regression analysis between EPL and Mhyo ELISA titres (left) and mild pleurisy and PRRS ELISA titres (right)

Conclusions and Discussion

In this study, the only disease in which humoral response was associated with an increase in the EPL was Mhyo, this is not surprising as this is the causal agent of EP. It sheds however some light regarding the importance of other agents in the respiratory complex affecting this producer in the finishing stage. This may have coincided with management changes (EP control) or / and increased SI prevalence in the UK, since SI was also correlated with EPL < 4 in this study.

The fact that APP was not correlated with pleurisy levels and only PRRS was associated with mild pleurisy levels was surprising and it warrants further investigation, particularly what APP serotypes may be circulating or present in this production system.

In conclusion this study demonstrates that M hyo is still a major cause of respiratory disease and continuous monitoring of control programs should be implemented to ensure a good control is in place.

References

1. Gomes C. *et al.*, (2013) Exploring BPHS lesions time trends: (July 2005 - December 2012). SRUC
2. Williamson S. *et al* (2013) Emerging Threats Quarterly Report 2012: (Q4)
3. Goodwin, R.F.W. et al. (1967). Vet. Rec. 81, 643-647

Three-in-one diagnostic approach for clinical evaluation of herds with respiratory problems

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Introduction

Mycoplasma hyopneumoniae (M hyo) is widely spread in commercial pig herds, and the infection is known to affect both growth and feed conversion of infected pigs. The negative effects are to a broad extent controlled by vaccination. To assure that pig producers get the best return of investment from vaccination, vets use different approaches to diagnose and evaluate respiratory problems in vaccinated and non-vaccinated herds.

In Denmark, the traditional way to evaluate a herd with respiratory problems is an extended slaughterhouse evaluation (USK) on lungs of slaughtered pigs. This method implies 2 main problems: First, the extent of the lesions at slaughter does not show the extent in newly infected pigs, as lesions heal up over time. Secondly, USK does not reveal the causative pathogen. Recently, a new method for evaluation of finishing herds with regard to M hyo infections was published (1). This method classifies herds as healthy, M hyo infected under control or M hyo infected out of control based on coughing index (CI) and M hyo seroprevalence in older finishers. Herds with high CI and low M hyo seroprevalence are suspected to be infected with other pathogens.

The purpose of this study was to evaluate the diagnostic method described by (1) against the traditional Danish approach: USK. To give a broader picture of the herds, analysis of oral fluid (OF) samples were included, too.

Materials and Methods

Practitioners provided contact data for finishing herds wanting an evaluation of coughing among finishers. Each herd had one visit from a veterinarian, when a batch of finishers had the age where coughing historically peaked in that herd. Three procedures were carried out:

1: Determination of CI in the relevant age group (CI section) and examination of blood samples from 20 pigs at least 18 weeks old for antibodies to M hyo by ELISA (Laboratorium for Svinesygdomme, Kjellerup, DK).

2: OF samples by rope in CI section and sections with pigs 2 weeks younger and 2 weeks older (pool of 4 pens/section). OF was examined by PCR for M hyo, SIV and PCV2 (BioScreen GmbH, Hannover, Germany).

3: Slaughterhouse examination (USK) of 20 lungs from pigs from the CI section. The USK results were given as prevalence of pigs with at least one M hyo like lesion and the mean extension of the recorded lesions (Laboratorium for Svinesygdomme, Kjellerup, DK).

Part 1 and 2 were done on the day of the visit, and part 3 was done when the CI section was slaughtered.

Results

The study included 37 herds, with 7-11 herds in each of the categories (table 1). For healthy herds and herds with M hyo out of control, prevalence and extension of M hyo like lesions were as expected, but in herds with M hyo under control or no M hyo, prevalence and extension of lesions was surprisingly high (fig. 1). In OF, a high level of PCV2 was found in 79% of non-vaccinated herds. M hyo was only found in herds with high M hyo seroprevalence, and SIV was not found in any samples.

Tab. 1. Vaccinations and level of PCV2 in OF in 37 finishing herds grouped by CI[a] and Mhyo seroprevalence

Category	No. herds	%vacc M hyo	%vacc PCV2	Circovirus >5log[b]
Healthy	9	22	11	56%
M hyo under control	7	71	14	57%
Diseased, other	10	30	50	50%
Diseased, M hyo	11	50	20	82%

[a]: Coughing index as described by (1). [b]: Level of PCR copies considered clinically relevant (BioScreen GmbH, Germany)

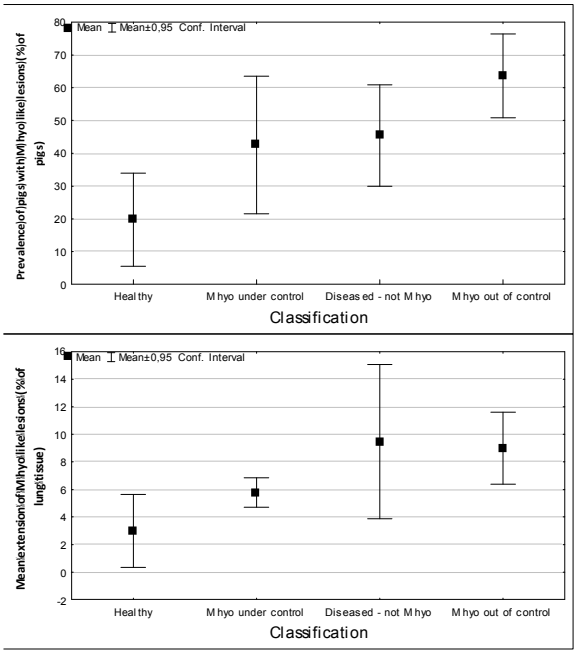

Fig. 1. Prevalence and extension of M hyo like lesions (catarrhal bronchopneumonia or cranio-ventral scars).

Conclusions and Discussion

Classification of herds by CI and M hyo seroprevalence was valuable for screening of herds. PCR analysis of saliva added information for PCV2, but not M hyo or SIV. USK could lead to misclassification of herds, revealing extensive M hyo like lesions also in herds without clinical symptoms or without M hyo infection.

Hence, screening of herds with respiratory problems should determine CI, M hyo seroprevalence in older pigs and PCV2 load in saliva in several age groups. Some herds will require further examination after the screening.

References

1. Nathues et al (2012): The vet Journal 193, 443-447

Behavioral aspects of oral fluid sample collection

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Introduction

The use of oral fluid specimens in research and diagnostics has been the focus of recent investigations. The majority of this work has focused on the detection of pathogen-specific antibody or nucleic acid in the oral fluid matrix (1, 2, 3). The specific objective of the present study was to evaluate the effect of the number of ropes provided in the pen on oral fluid sampling.

Materials and Methods

60 5-week-old pigs were divided into two groups of 30 (15 gilts, 15 barrows) in two pens of identical size and structure (Fig 1). For 9 days pigs were acclimated to the pen and familiarized to oral fluid collection using 2 cm (3/4 inch) 3-strand 100% cotton rope. During acclimatization and throughout the study, oral fluids were collected at approximately 7:00 a.m. The process of harvesting oral fluids and quantifying the sample is described in detail elsewhere (3).

The effect of the number of ropes provided on oral fluid sampling was evaluated for 20 days. Prior to initiating the study, the number of ropes (1, 2, 3, or 4) in the pen was randomized across the 20 days, with each "rope treatment" repeated 5 times. Observations were taken at both the group and individual level; that is, the whole pen (30 pigs per pen), and a subset of pigs (10 tagged pigs in each pen). The tagged pigs were chosen at random and marked with colored ear tags.

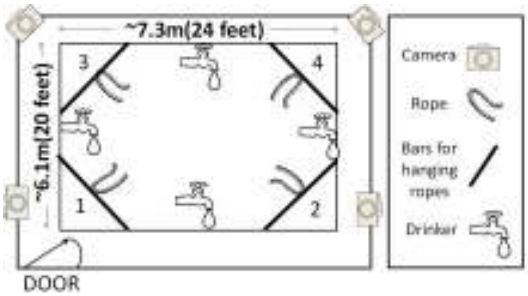

Figure 1. Pen arrangement for conducting the study

To collect oral fluid, ropes were hung from bars at the corners of the pens (Fig 1). Four cameras (Fig 1) synchronously took pictures at 2 second intervals throughout the 20 minute sampling period using an external computer trigger. Pictures were used to document pig behavior. "Chewing" was defined as a picture showing a pig's mouth closed around the rope.

Table 1. Oral fluid collection and pig behavior at the group level (2 pens of 30 pigs each)

No. of ropes in the pen	x̄ total oral fluid collected	x̄ pigs chewing	x̄ total pig minutes[1]
1	29.9 ml	5.0 pigs/min	99.6
2	39.0 ml	7.1 pigs/min	141.2
3	57.3 ml	9.1 pigs/min	181.5
4	79.2 ml	11.1 pigs/min	221.8

[1] Pig minutes = number of pigs chewing on a rope at each minute over the 20 minute observation period

Results

Mean sample volumes and pig participation data are shown in Table 1 (above). Participation by individually-observed pigs is shown in Figure 2 (below).

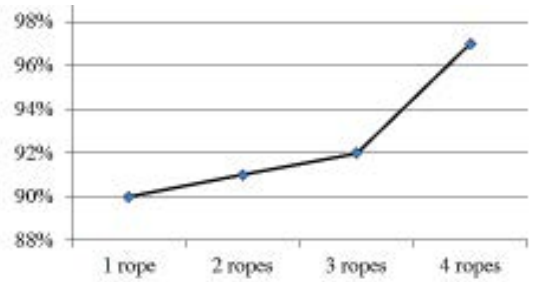

Figure 2. Mean % of individually identified pigs (n = 20) that chewed one or more times in 20 minutes by the number of ropes in the pen

Conclusions and Discussion

The total volume of oral fluid, the number of pigs chewing rope(s), and the total time that pigs chewed rope(s) increased as more ropes were provided. The behavioral aspects of oral fluid collection have only been addressed in one publication (4), i.e., many questions remain to be answered. Regardless, these data imply that there is an ideal pigs:ropes ratio that provides for optimal diagnostic sensitivity.

Acknowledgments

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References

1. Kittawornrat A et al. 2014. Vet Micro 168:331-339.
2. Mur L et al. 2013. Vet Micro. 165:135-139.
3. Olsen C et al. 2013. J Vet Diagn Invest 25:328-335.
4. Seddon Y et al. 2012. Vet J. 193:180-184.

Antibiotic reduction in Dutch swine veterinary practice – the practical approach

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Introduction

In Europe the use of antibiotics in food producing animals has been subject of intense debate for it's possible consequences for public health (1, 2). In the Netherlands 2009 is regarded as the year of reference and a 50% reduction of antibiotic use in food producing animals was issued for 2013 by the government (3). One of the tools of reducing antibiotic use in farms is a change in farm management and a focus on preventive disease control like vaccination. Veterinary clinic De Oosthof implemented a program for their clients to reduce the antibiotic use on pigfarms. The objective of this paper is to describe from a practical veterinary point of view what success factors contributed to the reduction of antibiotic use.

Materials and Methods

The program consisted of different projects that were implemented. For the evaluation and comparison of antibiotic use, the Dutch standardized method of defined daily dose per animal year (DDD) was introduced (4, 5, 8). The DDD for sow farms was calculated including the offspring until 10 weeks of age. DDD for finishing farms was calculated for pigs from 10 weeks of age to slaughter. With this method over more than 300 pig farms, all clients of De Oosthof, were analyzed during the program. In 2009 group sessions for farmers and farmworkers were organized explaining the need of antibiotic reduction and giving information on what different medicines do. The second step, also in 2009, was to visualize the antibiotic use on each farm by introducing DDD. This was calculated and presented at the individual farms to discuss the antibiotic use per farm. Because of doubts on water hygiene on farms and the risk for pig health (6) in 2010 an 'on farm drinking water hygiene awareness program' was started by doing microbial drinking water tests and advising on the subject. The same year a program was started to improve communication on the piglets sold for finishing; in the Netherlands in only 30% of the farms the sows and finishers have the same owner. By experience a change of ownership often causes a lot of miscommunication on the health status of the piglets. As part of changes in management the percentage of piglets receiving PCV2 vaccination increased during the period of the program from 20% to 65%. From 2010 onwards De Oosthof veterinarians advised continuously on biosecurity measures.

Results

The program resulted in a reduction of the antibiotic use in sow and finishing farms by 53% and 55 % respectively (2012 compared to 2009; figure 1). In 2012 at De Oosthof in sow farms the average DDD was reduced to 11.1 and in finishing farms to DDD 7.6. In the same year the Dutch average in sow farms was DDD 14.6 and in finishing farms was DDD 9.2 (7).

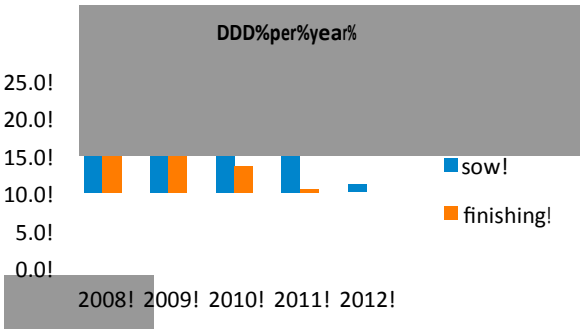

Figure 1. Antibiotic use in daily doses per animal year (DDD) for sow and finishing farms, 2008 - 2012.

Discussion

It not easy to point out the most successful intervention to reduce the use of antibiotics. It seems that the combination of 1) learning the farmers, 2) visualizing the antibiotic use per farm, 3) better communication on the piglets and 4) the continuous focus on reducing antibiotic use all together resulted in a change of mindset and frequently motivated a change of farm management. As a result the reduction of antibiotic use was more than 50% in a period of 3 years.

References

1. European Centre for Disease Prevention and Control, ARHAI, *ecdc.europa.eu*
2. Note 'CVO Conclusions on AMR' (2010) *ec.europa.eu*
3. *www.commissiewerner.nl*
4. Geijlswijk et al. (2009) *Tijdschrift voor diergeneeskunde 134:* 69-73.
5. Maran (2008) *www.cvi.wur.nl*: p18
6. Hutschemaekers et al.(1976) *Tijdschrift voor diergeneeskunde 101*:355-64.
7. SDa Report 'Usage of antibiotics in Livestock in the Netherlands in 2012' (2013) *www.autoriteitdiergeneesmiddelen.nl*
8. *www.antibioticawijzer.nl*

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Production and welfare effects of stopped antibiotic diarrhea treatment in nursery pigs with negative bacterial diagnosis

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Introduction

Reduction of antibiotic consumption in pig production is a political priority in Denmark. The major part of the antibiotic consumption in for pigs is prescriptions for treatment of gastrointestinal diseases post weaning[1].

Clinical decisions on antibiotic treatment are often based on inspection of diarrheic pools on the pen floor in "all in all out" managed nurseries. In order to diagnose the major bacterial agents involved in enteritis (*E. coli*, *L. Intracellularis*, *B. pilosicoli*), a PCR-based sock-sampling method was recently developed[2].

Surprisingly, a well-established bacterial diagnosis can is obtained in 75% of the diarrhea outbreaks, only. In order to reduce the antibiotic consumption it is therefore relevant to consider, whether antibiotic treatment is really indicated, when a bacterial cause of enteritis cannot be demonstrated. However, the production and welfare consequences of stopping antibiotic treatment in such cases are unknown.

The objective of the present study was to investigate average daily gain and animal welfare when stopping oral tiamulin treatment of diarrhea in nursery pigs with negative bacterial diagnosis of enteritis.

Materials and Methods

The study was carried out in a Danish nursery herd receiving weekly batches of 1000 pigs housed in rooms with 64 pens. Before the study the herd was tested by sock-samples, which revealed few diarrheic pools on pen floor and low levels of intestinal pathogens, which indicated a non-bacterial diarrhea outbreak.

A cohort study was performed in 3 nursery rooms from day 28 to 47 after weaning. Group A+ (213 pigs, 18 pens) received oral tiamulin 8mg/kg/day by water for five days at the start of a diarrhea outbreak. Group A- (210 pigs, 18 pens) were studied in parallel and did not receive antibiotic treatment. All pigs were weighed day 1 and 19 and clinical examined day 1 and 5. Eating and laying behavior and body condition scoring were recorded at day 5. Sock-samples were collected day 1, 5 and 19 for qPCR[3]. A multilevel mixed-effects linear model was used to investigate the treatment effect on ADG and occurrence of diarrhea using In Stata/IC 13 ® software. A multilevel mixed-effects logistic models were used to investigate the effect of treatment on eating and laying behavior at day 5.

Results

A low level of *E. coli* F18 was detected in 66 % (24) of the pens at day 1 before tiamulin treatment was initiated. At day 5 a low level of *E.coli* F18 was detected in all group A- pens and in 33 % (6) of the group A+ pens.

A 30-gram reduction in average daily gain (p=0,002) was found in the non-treated group when compared to the tiamulin treated group. The risk of having diarrhea after treatment was increased 2.5 times in untreated pigs (p=0,003)

Welfare indicators at day 5 including body condition score, eating behavior (p=0,193) and lying behavior (p=0,108) was not different between groups

Conclusions and Discussion

The demonstration of *E. coli* F18 in untreated pigs at day 5 indicates that the treatment effect on daily gain may in fact be due to treatment of a mild enteritis caused by *E. coli* F18. However, non-pathogenic *E. coli* F18 are considered common in Danish pigs. The 30 g increase in average daily gain could also be caused by a pure antibiotic growth promoting effect[4].

None of the observed welfare indicators (body condition, eating or laying behavior) were different between the two groups, suggesting that stopping treatment did not compromise animal welfare.

In conclusion the pigs under study lost 30 gram daily gain when not treated, while animal welfare was apparently not influenced.

Further studies of treatment stop in herds with diarrhea outbreaks in nurseries with negative bacterial diagnosis should be carried out.

References

1. Jensen et al: 2010, *Danmap 2010,* 23-40.
2. Pedersen at al: 2014, submitted for IPVS.
3. Ståhl et al.: 2011, Vet. microbiol. 151: 307-314.
4. Zimmerman: 1986, Jour. of Animal Science 62: 6-16

Reduction of antibiotics in The Netherlands; historical overview of three swine practices

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Introduction

The antibiotic use in animal husbandry and its possible influence on antibiotic resistance in the human population has become an increasing issue in Europe over recent years. In 2010, the Dutch government commissioned a reduction of 50 % in the use of veterinary antibiotics (using 2009 as reference year) over the next five years (1). In order to maintain the license to sell pharmaceuticals at veterinary clinics, this 50% reduction had to be achieved by 2013; demonstrating prudent and transparent use of antibiotics within the industry. The objective of this paper is to give an overview of the developments in the antibiotic use in 3 large swine veterinary clinics over the period of 2009-2013 in The Netherlands.

Materials and Methods

In 2010 there was no robust system for measuring the exact quantity of antibiotics used per species; only the total amount of veterinary antibiotics sold (in kilograms active substance) in The Netherlands was known. For benchmarking farmers and vets, a standardized method of defined daily dose per animal year (DDD) was introduced (2,3). By means of an independent database (Vetcis, introduced in 2010), an exact insight into the amount of antibiotics used per farm on a yearly base was determined. The 3 swine vet clinics in this paper represent approximately 45-50 % of the total swine population in The Netherlands. In cooperation with AUV (vet cooperation) the DDD for the swine farms in the 3 practices was calculated on a quarterly base. From historical data the DDD in the 3 practices was calculated retrospectively to the beginning of 2009. In this way quarterly DDDs were obtained instead of yearly figures. In the meantime, national guidelines for antibiotic use in swine were introduced. In the guidelines a category ranking for individual antibiotics is used (1th, 2nd or 3rd choice) based on narrow- or broad spectrum of activity, efficacy and human bacterial resistance; the aim being to stimulate the use of 1st choice antibiotics. The use of second or third choice antibiotics is only permitted following proper investigation and diagnosis, making it more difficult to prescribe these antibiotics to animals. From 2012 onwards, the independent institute SDa started their work as the veterinary medicine authority, also giving indicators for the maximum of antibiotic use on farms (4). These indicators are considered to be the targets for the maximum antibiotic use in Dutch pig industry until 2015.

Results

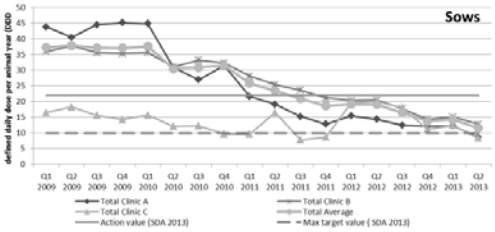

Figure 1. Development Defined Daily dose per animal year on sow farms for 3 clinics Q1 2009-Q2 2013.

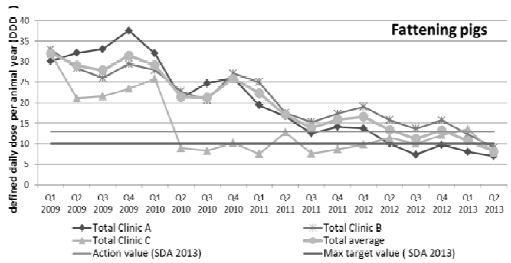

Figure 2. Development Defined Daily dose per animal year for fattening pig farms for 3 clinics Q1 2009-Q2 2013

Conclusions and Discussion

The total antibiotic reduction of 66% (Q1 2009 vs Q1 2013) on the swine farms in the 3 clinics far exceeded the government targets set in 2010. This reduction was achieved only through close cooperation between vets and farmers mainly by improving management systems, more prudent use of antibiotics, introducing vaccination strategies and the introduction of health programs. Independent of their different initial levels of use in 2009, the 3 practices are now all around the same level. Reduction in all 3 categories of antibiotics was observed. However the reduction in the 1st choice class was the most significant.

Acknowledgments

Henri Jans (AUV)

References

1. www.commisiewerner.nl
2. Geijlswijk et al. (2009) *Tijdschrift voor diergeneeskunde 134:* 69-73.
3. www.autoriteitdiergeneesmiddelen.nl
4. http://www.autoriteitdiergeneesmiddelen.nl/english

A preliminary study to assess lung lesions in slaughter pigs by using digital images analysis

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Introduction

Assessment of the lungs lesion to evaluate the incidence of pneumonia within a herd is commonly performed at slaughter[1]. Several techniques have been developed to grade the lungs lesion in slaughter pigs and visual evaluations of digital images of the lungs at slaughter may be an effective and practicable alternative [2].

The aim of this study was to compare the conventional method versus the digital images analysis to assess lungs lesion and determine the feasibility to use this last technique.

Materials and Methods

One hundred eighty two slaughter pigs coming from two multisite pig farms were used for this study. Conventional lung evaluation was carried out by using that technique previously described[3]. At slaughterhouse, lungs lesion score was calculated by one person and then a dorsal a ventral pictures were taken immediately for each lung by using a Olympus camera model VR350. For the image analysis, the percentage of lung area affected in relation to total lung area was calculated in every picture by using the open software Image J[4] (v.1.46r). Conventional (Co) and Digital (Dg) evaluation were performed by two veterinarian in a separate way. Data were arcsine transformed an a linear regression model was used to determine the correlation of conventional versus digital image evaluation. Reliability for both methods was carried out through a Anova analysis.

Results

One hundred forty lungs were evaluated. The remaining 42 lungs were discarded because of several causes, for example, identification number missing, artifacts image on the pictures like petechial hemorrhages or blood scattered over the lung surface which prevented an effective evaluation of the pictures (Fig 1), etc. Table 1 show the overall results. The average of lungs lesion was 8,78% ± 10,515. The mean for both technique was 9,08% and 8,47% for conventional and digital evaluation, respectively, with none statistical significant for both technique ($P \geq 0{,}018$). Furthermore, the linear regression showed a good fit for conventional versus digital evaluation (adjusted $R^2=0.9016$, $p<0.05$)

The data submitted for correlation coefficient study showed a very good correlation (0,9499), suggesting that is feasible to use image processing to assess lungs lesion from slaughter pigs . However, when we analyzed those data with minor lesion (<10%) the correlation was very low (0.2682).

Figure 1. A lung showing dotted lesion area.

Table 1. Results of the Lungs Lesion Evaluation

TECHNIQUE	$\bar{x}$	StDev	
Conventional (Co)	9,08	10,112	a
Digit Image (Dg)	8.47	10,930	a
Average	8,78	10,515	

Conclusions

Apparently there was a very good correlation between the two methods although with none different, however this digital tool allowed to reach similar results without the hassle and inconvenience of lungs inspection at slaughter. Therefore, digital image evaluation appears to be a potential and feasible alternative to evaluate lungs lesion from slaughter pigs

Acknowledgments.
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References

1. Thacker, EL and Minion FC. 2012. Diseases of Swine. 10th Ed. p.2850-2923.
2. Baysinger A, et al. 2010. In: Proc Congr Int Pig Vet Soc, p. 659.
3. Morrison RB, et al. 1985. Can Vet J. 26: 381-384.
4. Image J. http://rsbweb.nih.gov/ij/

O.110

Administering Meloxicam to postpartum sows reduces within litter variation of piglet IgG titres

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Introduction

The intake of colostrum is of vital importance for newborn piglets. Piglets are born with limited energy sources and limited immune protection (1). Therefore the intake of a sufficient amount of colostrum is a necessity for energy supply as well as for acquisition of passive immunity. The distribution of sufficient amounts of colostrum to all piglets presents a challenge for contemporary swine producers as sows give birth to a large number of piglets, while production of colostrum is limited in amount and time. The objective of this study was to determine the influence of injecting a single dose of the NSAID Meloxicam post-partum in farrowing sows on the variation of immunity status of their piglets.

Materials and Methods

The experiment was conducted on a 1400 head sow farm in The Netherlands. In one batch of farrowing sows a trial group (n= 12) receiving 6 ml of Meloxicam (Novem® 20, Boehringer Ingelheim) by intramuscular injection after farrowing, and a control group (n=18) that did not receive the treatment, were selected. Postpartum, all litters were adjusted to a size of 11 to 14 piglets. Both groups were housed in the same compartments and received the same amount and quality of care. On day 3, the litters of 9 sows from the trial group and of 10 sows from the control group were selected for blood sampling. Selection criteria included comparability in litter size and parity number, the presence of healthy litters and preferably unchanged or minimally cross fostered litters. On day 3 after birth, all piglets of these sows were sampled individually to determine their serological IgG status (Pig IgG ELISA, Bethyl Laboratories). The values were log transformed for statistical analysis. The standard deviation (SDEV) and coefficient of variation (CoV = SDEV / MEAN) of the individual logIgG values within litters were determined as a parameter for distribution of the colostral IgG's. Lower CoV means less variation in IgG values within a litter, showing a more equal distribution of colostrum among the piglets.

Results

As can be seen in table 1 the mean log IgG is higher in the Meloxicam group compared to the Control group (p=0.099). The corresponding original mean IgG titers of Meloxicam versus Control showed a difference of 93,7 ng/ mL versus 60,6 ng/ mL.

Table 1. Results of the analysis of individual piglet data on log IgG titres (linear mixed effect model with litter as a random effect and parity and treatment as fixed effects)

Treatment	n	MEAN	SDEV	CoV
Meloxicam	120	4,54	0,96	21,1%
Control	129	4,10	1,29	31,4

Figure 1 shows that the mean of within litter CoV in the Meloxicam is lower compared to the control group (p=0.051).

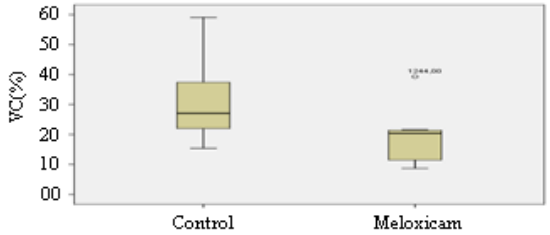

Figure 1. Box and Whisker plot of mean coefficient of variation (CoV) in the Meloxicam and control Group (Independent-sample T-test).

Conclusions and Discussion

Lower piglet mortality and higher growth in preweaning piglets after administration of Meloxicam to post-farrowing sows have been reported (3). In this experimental study a numerical advantage for two parameters was shown when sows are Meloxicam treated postpartum, compared to litters of non-treated sows: a higher mean log IgG titre and a lower within litter IgG titre variation. Treating post-partum sows with Meloxicam (Novem® 20, Boehringer Ingelheim) has been shown to improve the sow's welfare (2) and appears to improve the immune status of their piglets. Further study is needed to confirm the results of this study.

References

1. Le Dividich J, R.J. Herpin P. J Agr Sci 143, 469-485 (2005)
2. Mainau 2008. 20th IPVS Congress, Durban, 2008. P08.049
3. Revilla et al. 2006. IPVS proceedings p 475

Birth weight of piglets and litter characteristics in the Netherlands under practical circumstances

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Introduction

A low birth weight is associated with increased risk for mortality (1,3) and an increased time to reach market (2,3). The increase in fertility of reproductive sows the last decade, resulted in increasing litter sizes and consequently decreased birth weight (1,2). To gain insight in the situation of the litter characteristics under practical circumstances in the Netherlands, breeding farms were asked to register the birth weight of piglets at their farm in the period 2011-2012.

Materials and Methods

Breeding farms in the Netherlands were given the opportunity to register the birth weights by a standardized protocol. A digital weighing scale (Kern CH 15K20, accuracy 20 grams) was provided to weigh a minimum of 40 litters. All born piglets, live born (LB) and still born (SB) piglets, were weighed individually within 24h ours after birth. Sow parity, breed and the breed of the boar was registered as well. The data were analyzed and the farmers received a report with the litter characteristics. Birth weights between different parities were statistically analyzed with ANOVA and Bonferroni post hoc analysis using SPSS 15.0. Results of $p<0,05$ were considered as significant.

Results

In total 151 farms gathered data resulting in the birth weights of in total more than 112.500 piglets. As represented in figure 1, the birth weights are normal distributed with an average of 1340 grams with TB 14,77 piglets (LB 13.76, SB 1.02, 6,9% SB) and a mean parity of 4,02. LB piglets were heavier (1357 gram) compared to SB piglets (1093 gram) ($p<0,0001$). Birth weights differ significantly (at least $p<0,05$) between sows of different parity (figure 2). Gilts produce piglets with the lowest birth weights ($P<0,001$). Linear regression of the mean weight of the piglets decreases with 35 gram for each additional born piglet with a R-square of 0,97(figure 3).

Conclusions and Discussion

Increase in litter size results in reduced mean birth weight and this in agreement with other publications (2). Also the sows parity has a significant influence on mean birth weight with sows of parity 2-5 with the highest mean birth weights and gilts with the lowest birth weights. Live born piglets are heavier than still born piglets which also emphasizes the importance of a low birth weight variation. The results are a good representation of the birth weight distribution of piglets in the Dutch swine industry.

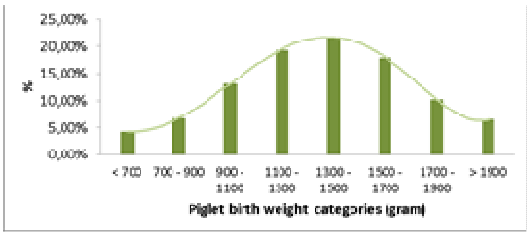

Figure 1: Normal percentual distribution of the birth weight of in total more than 112.500 piglets. The mean birth weight was 1340 grams.

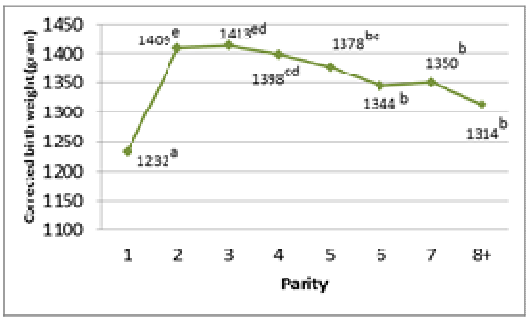

Figure 2:Birth weights of different sow parities. Results with different superscripts are significant (at least $p<0,05$)

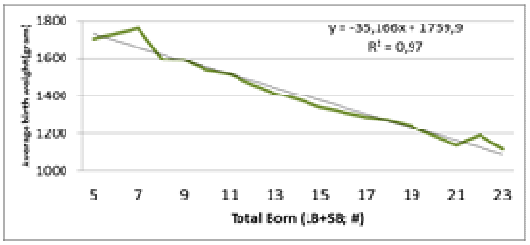

Figure 3: Relationship of littersize (LB + SB) and mean birth weight of the piglets. With each additional piglet born, the mean birthweight decreases with 35 gram (R-sq 0,97)

References

1. Wolf et al. 2008 Livestock Science 115 :2 195
2. Beaulieu et al. 2010 J. Anim. Sci. 88:2767
3. Opschoor et al. 2012 IPVS Korea RO-086

A program to control taeniosis-cysticercosis (*Taenia solium*) in Mexico Collaboration agreement SENASICA-UNAM

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Abstract

Taeniasis-cysticercosis is a disease linked to poverty. Presently its cycle persists mainly in those developing countries where people live under conditions of extreme poverty, without adequate education and medical attention. It affects human beings as the definite host, pigs being the most common intermediate host. Pig population in Mexico is around 15 million; of those at least 2.1 million are "rural" pigs that live in the marginated areas and roam about searching for food. Toilet facilities in those areas do not exist or are rudimentary, pigs having access to human feces, which, when coming from a *T. solium* carrier infect them with cysticercosis. By eating their insufficiently cooked meat, human beings become infected with *T. solium* and if they ingest eggs, they develop cysticercosis, often in the most serious form: neurocisticercosis. All these factors, in addition to the absence of controlled slaughterhouses and meat inspection result in the persistence of the zoonosis. A program to control the disease in Mexico is described, based on education and vaccination of pigs with a vaccine recently developed at UNAM. A team of Veterinarians visits the affected regions offering talks to children and adults, explaining the disease, measures to avoid the infection, and vaccinating pigs free of charge. At the beginning of the program the vaccine has to be applied 3 times with 3 months interval, thereafter it is administered once yearly. The combination of education and vaccination has given encouraging results. In the areas where the program has been in place for the last 4 year, the frequency of pig cysticercosis has been reduced from 14.25% to 0.00% people have understood the problem, they do not eat infected pork anymore and their hygienic habits have improved, refraining from defecating in the open, using better latrines and getting deworming treatment at least once a year. The main problems we have encountered are: in many communities, difficulty to communicate with people living in remote areas where they do not speak Spanish and the lack of roads for motor vehicles.

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Results on fixed-time-insemination in a sow herd with an afternoon-weaning-schedule in Germany

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Introduction

"Fixed-time insemination" (FTI) using the GnRH analogue Buserelin (Porceptal®) and a single AL has been recently launched by Merck Ltd (U.S.A.) as a novel breeding tool in gilts and sows with the purpose of inducing ovulation within a very narrow time frame allowing for a single fixed AI thereby reducing labor for heat checking and costs for semen. While good fertility results were achieved in recent studies with FTI compared to conventional breeding procedures[1,2], treatment and AI partially occurred at undesired daytimes or nights. The objective of this study was thus to slightly modify the existing FTI protocol with respect to timing in a batch-farrowing system with afternoon weaning with the goal of moving duties to more convenient workday hours.

Materials and Methods

Study was performed on a 2.200 sow farm with Danbred genetics and a weekly batch farrowing system in Saxony, Germany. The farm weaned slightly over 30 PSY. A total of 138 sows (parity 1 – 10; average: 3.8) were included. Sows were weaned on Wednesday 2 pm. Experimental sows (ES; n = 93) received 10 µg Buserelin (2.5 ml Porceptal®) IM on Sunday 9 am (i.e. 91 h post weaning), while control sows (CS; n = 45) remained untreated. Boar contact started Sunday AM, and then on an AM/PM schedule until Wednesday AM. ES were artificially inseminated (AI-ed) once on Monday 3 PM (i.e. 30 h post GnRH), while CS were AI-ed according to standing heat at least twice on an AM/PM schedule. Commercial semen from 7 was used for AI, and randomly allocated to ES and CS. All sows were scanned for backfat (BF) on the day of weaning, and a subset of 82 animals also prior to farrowing. All sows were transcutaneously ultrasounded to monitor follicular population as well as ovulation at five times: at weaning, at GnRH treatment, around AI (i.e. 30 h post GnRH) as well as 48 and 72 h post GnRH. CS sows were scanned at concurrent times. Animals were pregnancy checked by ultrasound on day 24 post AI.

Results

Mean BF at weaning was 1.46 cm (n = 138), and BF loss in lactation 0.32 cm (n = 82), without differences between groups. ES and CS had follicles of same size at weaning (i.e. 0.38 mm); it was similar at 91 h post weaning (i.e. at the time of GnRH treatment; 0.62 vs. 0.61 mm). Overall, ovulation was relatively synchronous, as most ES and CS ovulated within the observation interval of 72 h after the time of GnRH treatment. However, more ES had ovulated within 48 h then CS (92.5 vs. 77.7 %; P < 0.05; Table 1). One sow in each group failed to ovulate, either due to cystic ovaries (ES) or no follicle growth (CS). More ES than CS were tested pregnant (94.6 vs. 84.4 %; P < 0.05).

Table 1. Number (%) of sows with and without ovulations at different time intervals relative to the time of GnRH-treatment[1]

Group	Time after GnRH			Without
	30 h	48 h	72 h	Ovulation
	n (%)	n (%)	n (%)	n (%)
ES	10	76	6 (6.5)[b]	1 (1.1)
(n = 93)	(10.8)[b]	(81.7)[a]		
CS	11	24	9	1 (2.3)
(n = 45)	(24.4)[a]	(53.3)[b]	(20.0)[a]	

[1] Treatment occurred only in ES sows
Values with different superscripts differ significantly P < 0.05

Conclusions and Discussion

This study demonstrates that a FTI protocol as employed in this study works very well even on a well-managed farm with high production. The GnRH analogue Buserelin (Proceptal®) reliably induced ovulation in a vast majority of treated sows within 48 h post treatment, thus ensuring synchronous ovulation and enabling for only a single AI at a defined time with very high conception rates. Results are thus in line with those from previous FTI studies using a 83-89 h interval (instead of 91 h in this study) between weaning and GnRH treatment[1,2]. In conclusion, the FTI protocol as used in this study results into a synchrony of ovulation and high conception rates. Since farrowing results are yet not available, a final recommendation toward a broad use of that FTI protocol as employed in this study has to be postponed.

Acknowledgments

Farm crew members are acknowleged for the help during the study.

References

1. Martinat-Botté F et al. 2010. Theriogenology. 73(3):332-42.
2. Driancourt MA et al. 2013. Theriogenology 80:391-399.

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Vaccination audits: A systematic approach

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Introduction

Vaccination of pigs is one of the most important procedures performed in a swine operation. Vaccines are used to help protect and/or prevent a wide variety of diseases, which in turn increases the performance of the growing pig.[1] Vaccination of pigs can be an expensive cost to the farm. For example, vaccinating for porcine circovirus type 2 (PCV2) can cost $1.50-$2.00 per pig.[2] On the other hand, failing to effectively vaccinate pigs for PCV2 can cost anywhere from $3 to $30 per pig.[3] Therefore, it is critical that the farm staff vaccinates pigs correctly to get the best return on this investment. Each individual farm develops vaccination procedures to fit their particular situation. Implementation is often assumed but in reality, failure to correctly implement and execute vaccination programs is a common finding. One method to help ensure proper implementation is the use of structured, vaccination quality assurance audits. This project presents the findings of vaccination quality assurance audits performed by the first author during the summer of 2013.

Materials and Methods

In this study, fifteen audits were performed on a total of 30,535 pigs which were being vaccinated by the farm staff. A vaccination quality assurance audit form developed and provided by Merck Animal Health was used to capture the necessary information to evaluate the level of compliance with vaccination procedures. The form addresses the vaccine, farm staff, pig health statu s,barn information, vaccine preparation, vaccination process and post-vaccination procedures. In total, there are 60 items that are addressed during the audit. During each audit, about six percent of the total pigs being vaccinated were observed at the time of injection and a tally was kept of misplaced injections, reacting pigs and pigs with excessive bleeding at the site of vaccination. Most pigs had recently arrived into wean-to-finish barns at the time of vaccination.

Results

The audit data captured in the forms was entered into a spreadsheet. From there, the top five best practices and top five areas for improvement for the vaccination process were determined. The best practices identified included: 1) appropriate needle changing; 2) lifting every pig to give injections; 3) correct dosages of vaccines; 4) correct needle gauge and length; and 5) proper identification of vaccinated pigs versus unvaccinated pigs.

Areas for improvements included: 1) better needle placement for injections; 2) less tissue trauma from vaccination to decrease the amount of injection site bleeding; 3) more careful handling of the pigs; 4) placing thermometers in refrigerators; and 5) warming of vaccines as recommended.

Conclusions and Discussion

Vaccinating pigs is one of the most costly and time consuming, but yet highly rewarding, processes in a swine operation. Vaccination failure for PCV2 in 15% of a 2,000 head barn because of improper vaccination can cost anywhere from $900-$6,000.[3] Therefore, it is important for producers to ensure that vaccination protocols are being followed by the farm staff. The use of structured, form-driven vaccination audits provides an objective mechanism for identifying the strengths and weaknesses of the farm staff with regard to vaccination of pigs. In this study, we identified several areas for improvement that were common among the various swine operations and also identified several areas where protocols were being implemented correctly.

Aknowledgments

Thank you to the farm staffs that assisted with this project.

References

1. Thacker, E. "Vaccines How They Work, Why They Fail." The National Hog Farmer. N.p., 15 Apr. 2003. Web. 8 July 2013. <http://nationalhogfarmer.com-/mag/farming_vaccines_work_why>.
2. DeMint,J. "Five Key Changes to Modern Management Practices Necessary to Improve Profitability." The Pig Site. N.p., 10 May 2013. Web. 31 July 2013. <http://www.thepigsite.com/articles/4323- /five-key-changes-to-modern-management-practices-necessary-to-improve-profitability
3. Gillepsie, J, et al. Porcine Circovirus Type 2 and Porcine Circovirus-Associated Disease. J Vet Int Med 23.6 (2009): 1151-1163.
4. Horlen, K, et al. A Field Evaluation of Mortality Rate and Growth Performance in Pigs Vaccinated Against Porcine Circovirus Type 2. JAVMA 232.6 (2008): 906-912.!

Monitoring of a commercial fattening herd by means of the Pig Cough Monitor and oral fluid diagnostics

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Introduction

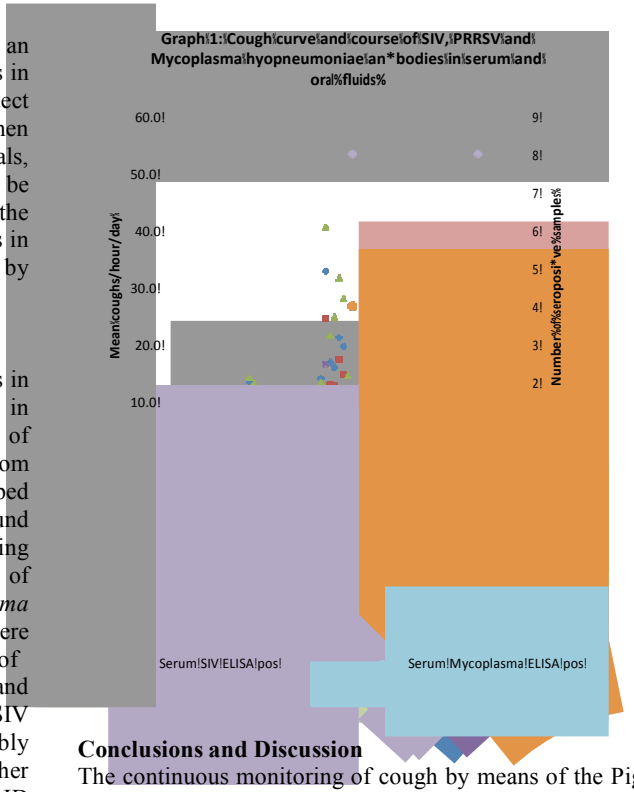
Pen-based **oral fluid** sampling has proven to be an efficient method for surveillance of infectious diseases in swine populations. Real time sound analysis to detect cough in pigs has been showing promising results when used experimentally and based on these laboratory trials, it could be demonstrated that cough in pigs could be identified correctly in 94% (1). The purpose of the current trial was to use pen-based oral fluid samplings in combination with monitoring cough in fattening pigs by means of the Pig Cough Monitor.

Materials and Methods

The trial was carried out in one batch of fattening pigs in a commercial herd in North-West Germany in spring/summer 2013. One batch consisted of approximately 100 pigs, which was placed in one room with 4 pens with 25 pigs each. Each pen was equipped with one microphone transmitting continuous sound signals for 4 months to the Pig Cough Monitor, resulting in 4 microphones per room. The herd had a history of circulating PRRSV, SIV and *Mycoplasma hyopneumoniae.* Pen-based oral fluid samplings were obtained each week as well as monthly serum samples of 2 animals per pen for a duration of 3 months and analyzed for presence of antibodies against PRRSV, SIV as well *Mycoplasma hyopneumoniae* in order to possibly link the presence of "cough" to infection with either pathogen. Samples were analyzed by means of the ID Screen Influenza A Antibody Competition ELISA (ID.vet, France), IDEXX M.hyo Ab Test and IDEXX PRRS OF Ab Test (IDEXX, Germany).

Results

The continuous recordings of the Pig Cough Monitor are summarized in a graph by indicating the mean counts of coughs/hour/microphone together with the number of postive samples for SIV, PRRSV and *Mycoplasma hyopneumoniae* antibodies in serum and oral fluids (see graph 1). As shown in previous studies (2) a mean coughing rate of 10 coughs/hour seems to be a baseline that is occurring in this farm irrespective of pathogen exposure as measured by the algorithm. The weekly pen-based oral fluids enabled a timely monitoring of respiratory pathogen exposure. As evident from graph 1 there was a small paroxysmal cough for 8 days (between July 3 and 11, 2013) that coincided with a seroconversion to SIV in both oral fluids and serum. Although the animals remained seropositive to SIV in oral fluids and serum until the end of the fattening period, the cough declined rapidly.



Conclusions and Discussion

The continuous monitoring of cough by means of the Pig Cough Monitor in a German fattening herd enabled an objective measurement of "respiratory health" or cough respectively. Infection of fattening pigs with SIV resulted in a paroxysmal increase of coughing for 8 days, which confirms the pattern of clinical disease described in literature. The pen-based oral sampling and subsequent lab analyses allowed a timely and animal friendly surveillance of herd health.
Further studies are needed to analyze the possibility to discriminate the different types of cough.

References

1. A.Van Hirtum and Berckmans D.; Automated recognition of spontaneous versus voluntary cough; Medical engineering & physics 24: 541-545 (2003)
2. Genzow et al, Course of cough in two batches of fattening pigs with different respiratory pathogen exposure, submitted for publication (2014)

Utilizing the Canadian Swine health intelligence network during the porcine epidemic diarrhea outbreak

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Introduction

The Canadian Swine Health Intelligence Network (CSHIN), a national swine surveillance network was utilized in many ways to assist detection and mitigation efforts during the Canadian outbreak of Porcine Epidemic Diarrhea (PED) in 2014.

Materials and Methods

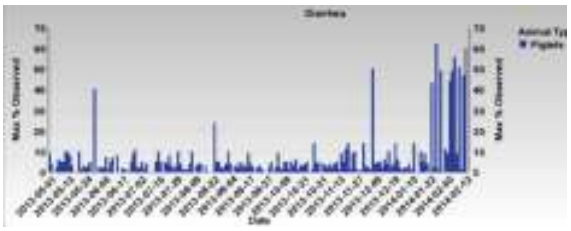
The CSHIN is made of two integrated networks: 1) a network for discussion, decision-making and communication and 2) a network for collection and analyses of syndromic data. Data from veterinary visits or communications with producers is submitted to the CSHIN database in near real time. Communication occurs among practitioners and other swine health specialists on a formal schedule thereby ensuring that all swine veterinarians are made aware of important health issues in their region. The network has been operating for almost two years functioning as an endemic and emerging disease surveillance and communication tool. During the PED outbreak the CSHIN was adapted to new uses and proved to be an effective tool for helping to control PED in Canada, Activities included:.

1. Maintaining routine activities such as reporting changes in swine health through quarterly reports following regional and national teleconferences, as well as monitoring for other emerging diseases
2. After PED was identified in the US, automated reports were designed for identifying suspect PED outbreaks. They reported diarrhea by animal type. All cases that exceeded a predefined threshold were investigated with a call to the practice to ensure data accuracy and discuss the case. Freedom from PED was established and maintained by the lack of veterinarians reporting high within herd diarrhea prevalence.
3. Once the Canadian outbreak started the CSHIN produced reports and regional maps of cases and positive environmental samples and distributed these across Canada
4. The CSHIN collected daily clinical signs by animal type from some of the cases to estimate the variability in the clinical expression of the disease.
5. The CFIA utilized clinical information collected by the CSHIN as part of a vaccine efficacy study
6. CSHIN produced and distributed a daily PED information update to the Canadian industry. Information included: maps, reports, charts, concise information about the disease, biocontainment and mitigation measures from affected regions as well as other initiatives that would help the industry.
7. CSHIN data was evaluated for agri-risk management and mortality insurance in this outbreak

Results

During the first month of the outbreak there were constant requests from industry stakeholders to receive information updates from the CSHIN. These came from practitioners, researchers, government staff, provincial and federal Chief Veterinarians, processors, transporters, pork boards and producers.

The within herd prevalence of diarrhea in piglets during farm visits (only the worst case is reported each day) is presented in Figure 1. Note the PED outbreak starting Jan. 22, 2014.



Regional maps reported the number of cases while ensuring the farm identities were kept confidential.

Conclusions and Discussion

During the outbreak the CSHIN was used for purposes beyond what it was originally intended. To facilitate these additional activities, flexibility will be added to the data capture, analytical and reporting capabilities. Syndromic surveillance combined with a formal communication network was an effective tool assisting with the outbreak response.

Acknowledgements

The CSHIN was a project of the Canadian Swine Health Board funded by Agriculture and Agri-Food Canada.

Practical application of the Pig Cough Monitor in a German fattening pig herd with PRDC

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Introduction

Acoustic monitoring of farm animals may serve as an efficient management tool to enhance animal health, welfare, and farm efficiency. The diagnosis of respiratory infections in pig fattening is a serious economic issue. Respiratory infections in pig fattening farms cause losses of millions of Euros per year (1). The objective of this trial was to investigate the use of a microphone-operated system (Pig Cough Monitor) to detect coughing in a fattening pig herd.

Materials and Methods

The trial was conducted in late autumn/winter 2012/2013 in a fattening herd in North-West Germany. One room with 4 pens with 25 pigs each was equipped with one microphone per pen transmitting continuous sound signals for 3.5 months to the Pig Cough Monitor, which is based on an algorithm that automatically detects and translates measured animal responses such as cough in real-time. The herd had a history of circulating PRRSV, SIV and *Mycoplasma hyopneumoniae.* Serum of twelve randomly selected pigs (3 per pen) was obtained once per month and analyzed for presence of antibodies against PRRSV, SIV as well *Mycoplasma hyopneumoniae* in order to possibly link the presence of "cough" to infection with either pathogen.

Results

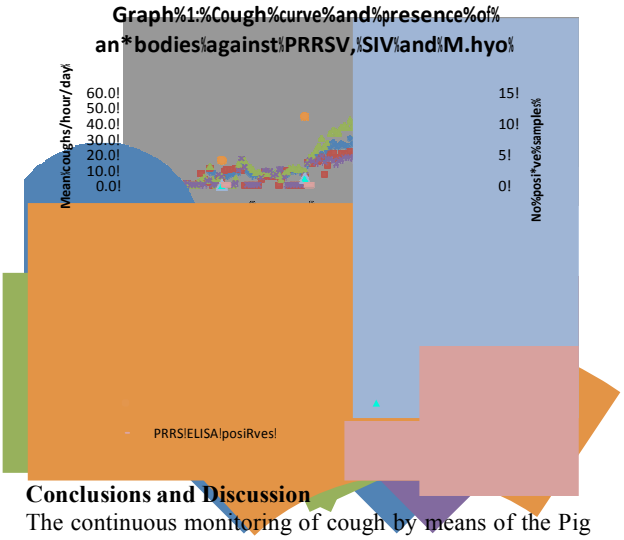
The installation of the equipment requires connection to the internet, which needed some extra work to smoothly record the continuous sounds. Moreover, one microphone broke for unknown reasons end of December 2012 so that for the last 6 weeks only recordings of 3 microphones are available.

The continuous recordings of the Pig Cough Monitor are summarized in a graph by indicating the mean counts of coughs/hour/microphone (see graph 1). Although there was one microphone per pen recording coughs, it is anticipated that there is some overlap of sounds, i.e. each microphone will have recorded also some coughs from the adjacent pens.

As evident from graph 1, coughing is a regular phenomenon in pigs to clear the airway of any irritant.

In this herd an average of 10-20 coughs/hour/pen seems to be the baseline for "cough" measured by the algorithm.

Pigs seroconverted first to SIV which was accompanied by a short and transient increase in coughing. Upon seroconversion to *M. hyopneumoniae*the frequency of coughing was rapidly and distinctly increased with resulting in up to 50 coughs/hour/pen. Interestingly, after the presence of antibodies against *M. hyopneumoniae*(Nov 30, 2012) it took 3 days until the cough frequency almost tripled. Moreover, when pigs started seroconverting against PRRSV (4.January 2013) the frequency of coughing was further increased with reaching up to 50 coughs/hour/pen.



Conclusions and Discussion

The continuous monitoring of cough by means of the Pig Cough Monitor in a German fattening herd enabled an objective measurement of "respiratory health". In contrast to other subjective measurement (e.g. individuals counting coughs for a short period of time), this systems enables an unbiased measurement of any deviation from normal respiratory pattern and could be clearly linked to infection with both *M. hyopneumoniae*and PRRSV.Based on our findings it seems that the time between seroconversion to *Mycoplasma hyopneumoniae* and onset of clinical relevant cough seems to short and less than previously reported.

Further research is needed on the cough pattern for each individual pathogen.

References

1. Hoy, S.: Zu den Auswirkungen von Atemwegserkrankungen auf die Mast- und Fruchtbarkeitsleistungen der Schweine. Prakt Tierarzt 2, 121–127 (1994)

Investigating biosecurity risks associated with the delivery of feed to swine farms

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Introduction

Biosecurity protocols reduce the introduction and spread of pathogens among swine farms. For pigs, biosecurity ensures market stability, maintains export opportunities, and controls spread of production and public health diseases. Salmonella, the second most common cause of bacterial foodborne illness, causes gastrointestinal illness. Pigs can be asymptomatic carriers of the bacteria and pork products are a known source of salmonellosis in humans. Salmonella have been isolated from pigs, boots, flies, rodents, bird feces, feed, feed-ingredients, and feed trucks. The objective was to identify management factors to reduce the risk of disease spread among swine farms through feed trucks.

Materials and Methods

The study was conducted in two phases - the first phase included a series of focus groups and key-informant interviews; discussions held with swine producers and feed company personnel explored the protocols currently in place that reduce the risk of disease spread through the delivery of feed. Participants were asked to identify possible management changes that could further reduce the risk, and to rate these ideas in terms of their effect on disease control, and feasibility based on implementation and economics. This information was used to structure the second phase of the study – a pilot study. The pilot study was conducted over 6 weeks in the winter of 2013, and included 40 feed truck drivers from 3 Ontario feed companies. Truck drivers filled out log sheets as they delivered feed to swine farms, and provided information about the prevalence of the identified biosecurity risks during the day-to-day delivery of feed. Drivers were randomly assigned to be in either the treatment or control groups. Drivers in the control group delivered feed as they normally do. Drivers in the treatment group were: i) asked not to enter the barn unless absolutely necessary, and ii) were provided with re-usable, washable rubber over-shoes and were asked to wear a clean pair at each farm when they felt safe doing so. Chi-squared tests were used to compare the usage of clean boots between drivers in the treatment and control groups.

In total, 40 drivers from 3 companies, delivering feed to 2202 farms over 6 weeks (in the winter) collected descriptive data on the factors and compared use of different boots.

Results

The factors followed by the proportion of farms and drivers where the factor was found were found as follows: Most farms were: keeping areas (driveway, feed bin and barnyard) clean of mud and manure (82%), and dead-stock (91%) was appropriately disposed of and not visible to the driver. Some farms had an outbuilding to deliver bagged feed (24%). Other factors that were identified that producers should work on were to provide farm boots and coveralls for the driver if the driver needed to go into the barn, ordering the correct amount of feed to decrease the numbers of deliveries (or frequencies of deliveries) of feed, and notifying feed mill of a disease outbreak on the farm. Feed truck driver factors included wearing a separate pair of clean, disinfected, dried boots (25%) and gloves (50%) at each farm that is visited (even if the driver does not go into the barn, remaining outside the barn (92%); washing the steering wheel (49%), floor mat (77%) and outside of the truck (32%) every 24 hours. From these results, it may be advisable to increase the frequency of washing the truck. Another factor identified was for the driver to know and follow the farm biosecurity protocols. Half of the time, the driver left the feed bill outside barn (54%). Drivers given disinfected rubber boots were more likely to wear these on farms (42%) than plastic disposable boots (4%) ($P<0.05$). These drivers were also more likely to wear disinfected rubber boots than the control drivers (11%) ($P<0.001$).

Conclusions and Discussion

Biosecurity is a responsibility that is shared among all members of the industry. Feed personnel were encouraged to know more about disease transmission.

Acknowledgments

Canadian Swine Health Board for funding, feed company personnel and producers for participation.

Intestinal disease in nursery herds part 1: epidemiology, antibiotic treatment and prevention

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Introduction

Treatment of intestinal disease in nursery pigs is the most important cause of antibiotic prescription in the Danish swine industry[1].
The objective of the study was to gain knowledge on epidemiology, antibiotic treatment and preventive strategies for intestinal diseases in nursery pigs.

Materials and Methods

A total of 28 full-time swine veterinarians were randomly selected, stratified by geographic area in Denmark. The primary sampling frame was nursery herds (>1000 head), where these veterinarians had prescribed antibiotics. The herds were selected and antibiotic prescription data were obtained from the Danish national database Vetstat.
From each veterinarian, two nursery herds were randomly selected for a questionnaire investigation concerning epidemiological, preventive, treatment and diagnostic aspects of diarrhea.
All data management and analysis were performed using MS Excel and Stata IC 13.

Results

In 2011, the 28 veterinarians had prescribed antibiotics for a total of 1074 herds with nursery pigs (>1000 head). Of these herds, 91% used antibiotics for intestinal disease, and 86% used antibiotics for intestinal disease applied in either food or water.
Of the herds using antibiotics in food or water, 83% treated intestinal disease at the batch level using clinical inspection criteria (including diarrhea) to determine the time-point at which treatment should begin. For the remaining 17%, antibiotic batch medication was used on a fixed day post-weaning in all batches of pigs.
A total of 43 herds were included in the questionnaire survey. In 81% of these herds, more than 50% of all batches were treated based on the occurrence of diarrhea at some point during the nursery phase of production (range for all included herds was 5-100%). In 30% of the herds, diarrhea was observed exclusively within the first two weeks post-weaning, while in the remaining 70% of the herds diarrhea occurred from week 3 to 8. In 63% of the herds, diarrhea occurred only in relation to changes in feed composition, in 21% diarrhea did not occur in relation to changes in feed composition, while the rest varied.
For 93% of the herds, the diarrhea problem had been present for more than one year, and in 51% of the herds the diarrhea problem had remained the same without any changes in relation to age and clinical signs.
With regard to treatment strategies, the veterinarians indicated that batch medication was used because an exclusive use of individual treatment was not practically possible. Further, batch medication was considered the most appropriate strategy from a disease control point of view. A few veterinarians indicated that lack of clinical skills among farm personnel was also a motivation for selecting batch medication.
The antibiotics used were dominated by pleuromutilins, macrolides and/or tetracyclines with treatment durations of between three and seven days.
In 53% of the herds, termination of antibiotic treatments had been attempted. In the majority of the herds, this had resulted in increasing mortality, uneven growth and/or unthrifty pigs.
In 89% of the herds, preventive control strategies had been attempted. These included *Lawsonia intracellularis* vaccination (9%), changes in feed composition and/or feeding strategy (51%), while 18% had tried various other interventions.
Results for laboratory testing, veterinary opinions on causes and diagnostic aspects are presented elsewhere in the IPVS 2014 proceedings.

Conclusions and Discussion

This study confirms that antibiotic batch medication of intestinal disease in mid to late nursery is very common in Danish herds. The dominant treatment strategy was batch medication using clinical inspections to decide when to treat. The primary clinical criterion was the occurrence of diarrhea, which is probably assessed by inspection of diarrheic pools on the pen floor.
Despite the application of different preventive measures, intestinal disease continued to be a problem in the majority of the investigated herds. This strongly suggests the need for more efficient preventive strategies.

References

1. Hybschmann GK et al. 2011. Prev Vet Med 98:190-197.

Intestinal disease in nursery herds part 2: diagnostic strategies used in practice, herd veterinarians' opinion on etiology and findings using quantitative PCR testing of sock samples

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Introduction

Treatment of intestinal disease in nursery pigs is the major cause of antibiotic consumption in the Danish swine industry[1]. *Lawsonia intracellularis* has been considered the most important cause of these treatments.
The objective of the current study was to explore the diagnostic strategies used in practice, to investigate veterinarians´ opinion on etiology and to investigate microbiological findings using quantitative PCR testing of sock samples in nursery pigs.

Materials and Methods

A total of 43 herds using antibiotic batch medication for treatment of intestinal disease in nursery pigs were randomly selected from 28 swine veterinarians, stratified by geographic area in Denmark.
A telephone interview was performed with all the veterinarians included in the study. During the interview, the veterinarians were asked questions concerning etiology, diagnostic work, treatment, prevention and other aspects of intestinal disease in each herd.
In each herd, the veterinarians collected a sock sample (pen sample obtained using a sock-covered boot) from a diarrhea outbreak as previously described[2]. The sock was subjected to quantitative PCR (qPCR) testing for *L. intracellularis*, *B. pilosicoli* and *Escherichia coli* F4 and F18 at the National Veterinary Institute[3].

Results

Testing of the sock samples by qPCR analysis demonstrated that *L. intracellularis* was detected in 60%, *B. pilosicoli* in 18%, *E. coli* F4 in 35% and *E. coli* F18 in 63% of the herds. A combination of infections was demonstrated in 56% of the herds. No infection was detected in 7% of the herds.
The telephone interviews demonstrated that the veterinarians generally considered diarrhea problems to have an infectious etiology. *L. intracellularis* was believed to be the most important infection (86% of the herds, 42% as mono-infection). *E. coli* was the second most important infection (51% of the herds, 5% as mono-infection) followed by Porcine Circovirus type 2 (7% of herds). *B. pilosicoli* was not considered to be involved in any herds. The veterinarians believed that a combination of the infections was causing diarrhea in 53% of the herds.
In 67% of the herds, the veterinarians believed that the etiology had not changed within the last year. In addition to the infections, other factors were believed to be involved in the diarrhea problems. The most important factor was feed composition, which was considered important in 35% of the herds. Other factors considered included temperature, humidity, housing conditions and pig density. Questions concerning diagnostic work in each herd revealed that, in 14% of the herds, demonstration of clinical signs, uneven growth and/or low feed efficacy was the only diagnostic work performed. Necropsy had been used in 23% of the herds, and laboratory examinations of intestines and/or fecal samples had been used in 33% of the herds. Serology for *L. intracellularis* had been used in 37% of the herds. In the herds using laboratory investigations, 64% of the laboratory submissions were more than one year old. In 42% of the herds, no laboratory investigations had been performed. The most important reason was that the veterinarian did not believe that a laboratory investigation would change the treatment or preventive intervention strategies in the herd. In contrast, in those herds where laboratory investigations had been performed, the results were considered important for treatment and prevention strategies. Laboratory investigations were in some cases performed to provide justification for the use of antibiotics.
Results for intestinal disease epidemiology, treatment and preventive strategies from the same herds are presented elsewhere in the IPVS 2014 proceedings.

Conclusions and Discussion

The results of the study indicate that different infections are involved in diarrhea in Danish nursery herds and that the etiology is more complex than believed in practice. In a large proportion of herds, no laboratory investigations have been performed, partly because of a low value of the diagnostic information in the opinion of the practicing veterinarians.

References

1. Hybschmann GK et al. 2011. Prev Vet Med 98:190-197.
2. Pedersen KS et al. 2014. Submitted IPVS 2014.
3. Ståhl M et al.: 2011, Vet. microbiol. 151: 307-314.

SSF: A web application for sample size and frequency calculation in repeated diagnostic testing

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Introduction

A pivotal part of disease surveillance is the repeated disease diagnostic testing to monitor the status of the disease. Despite their importance, little work has been done to address the common questions of sampling frequency as well as sample size for repeated testing. This paper introduces a web application called SSF (Sample Size and Frequency), built upon the Shiny web-application framework. SSF provides easy-to-use and instantly displayed calculation of sample size and frequency based on a custom-defined scheme of their own choosing.

Materials and Methods

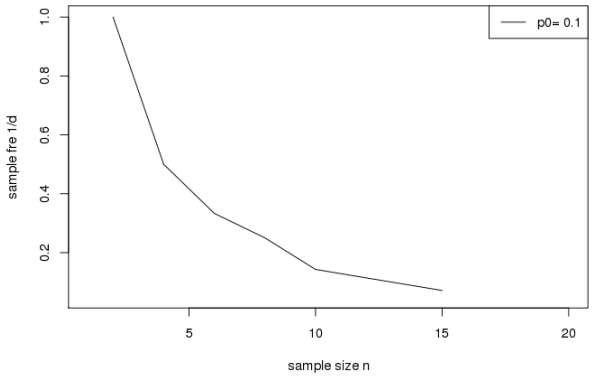
SSF is an application of Shiny, a framework for writing web-applications in the R language. SSF uses this framework to provide quick and convenient calculations of sample size and frequency for design of repeated diagnostic testing. For instance, when the user of SSF changes a value of parameter in setting, such as the prevalence, the result tables and plots will instantly update to reflect the new setting. SSF is currently hosted on the server provided by Iowa State University.

Similar to other Shiny applications, SSF consists of three primary UI components, the Configuration Panel, the Tab Panel, and the Results Panel, as shown in Figure 1. The Configuration Panel is located along the left-hand column. This panel allows for various parameters to be adjusted. The top of the right panel is the Tab Panel, which contains two tabs corresponding to table and plot, respectively. The bottom of the right panel is the Results panel, which will contain the results of the analysis depending on the tab and configuration options selected.!!

Figure 1. Screenshot of SSF, with tabular results.!

Results

The Configuration Panel allows users to input parameter values in calculation of sample size and frequency. The parameters include the prevalence, the desired detection time and the desired power of detection. For example, if the user specifies the parameter values for detecting a disease onset of prevalence 0.1 within 14 days with desired power 0.95, combinations of sample size and



!

Figure 2. Sample size vs frequency plot in result section.

Conclusions and Discussion

By utilizing the R language and the web application framework Shiny, SSF allows for a quick and convinient calculation of sample size and frequency in repeated diagnostic testing. It is flexible enough to allow investigation of a wide variety of different library schemes, peptide lengths, and library sizes. Still, the application is web-based and easy to use, making the barrier of entry for those outside the field of statistics very low.

Acknowledgement

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References

1. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria, 2013. url: http : //www. R-project.org/.Aufricht C et al. 1992.!Eur J of Clin Chem and Clin Biochem 30:81-83.

Course of cough in two batches of fattening pigs with different respiratory pathogen exposure

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Introduction

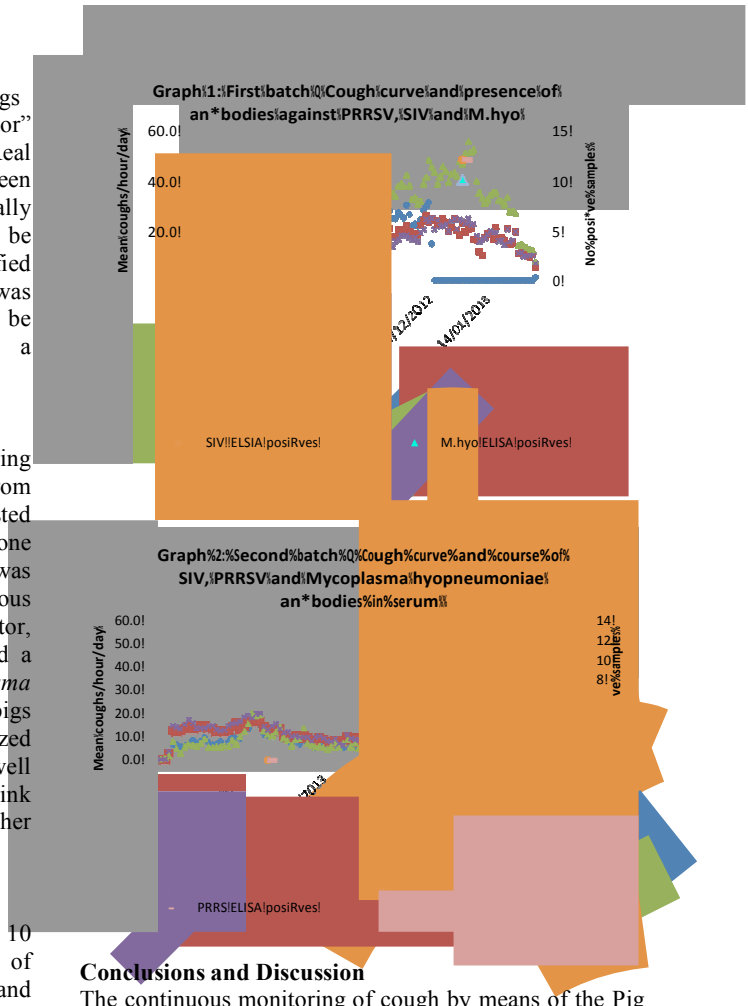
Algorithms have been developed to detect cough in pigs and recently the automated tool "Pig Cough Monitor" became available to monitor cough in pig farms. Real time sound analysis to detect cough in pigs has been showing promising results when used experimentally and based on these laboratory trials, it could be demonstrated that cough in pigs could be identified correctly in 94% (1). The purpose of the current trial was to investigate whether cough in fattening pigs could be related to a specific respiratory pathogen in a commercial setting.

Materials and Methods

The trial involved two subsequent batches of fattening pigs in a commercial herd in North-West Germany from autumn 2012 until mid 2013. One batch of pigs consisted of approximately 100 pigs, which was placed in one room with 4 pens with 25 pigs each. Each pen was equipped with one microphone transmitting continuous sound signals for 4 months to the Pig Cough Monitor, resulting in 4 microphones per room. The herd had a history of circulating PRRSV, SIV and *Mycoplasma hyopneumoniae.* Serum of twelve randomly selected pigs (3 per pen) was obtained once per month and analyzed for presence of antibodies against PRRSV, SIV as well *Mycoplasma hyopneumoniae* in order to possibly link the presence of "cough" to infection with either pathogen.

Results

In both batches of pigs a daily cough pattern between 10 and 20 coughs/hour could be observed irrespective of any exposure to respiratory pathogens (see graph 1 and graph 2). Both batches were exposed to SIV in the middle of the fattening period and PRRSV at the end of the fattening period. The first batch of pigs was exposed to infection with *M. hyopneumoniae*approximately 8 weeks after placement into the fattening (17 weeks of age), which was followed by a distinct increase in coughing reaching 50 coughs/hour/day. The second batch of pigs was not exposed to *M. hyopneumoniae*and had no distinct increase in the pattern of cough. Infection with SIV and PRRSV alone did not result in an increase of cough in the second batch, whereas a subsequent PRRSV infection of infection with *M. hyopneumoniae*seems to result in a further increase in coughing in the first batch.



Conclusions and Discussion

The continuous monitoring of cough by means of the Pig Cough Monitor in a German fattening herd enabled an objective measurement of "respiratory health". Infection of fattening pigs with *M. hyopneumoniae*resulted in a distinct increase of coughing. The underlying data suggest that an additional infection with PRRSV aggravated the cough induced by *Mycoplasma hyopneumoniae.* Further research is needed to investigate the co-infection with regard to the frequency and intensity of cough by using the Pig Cough Monitor.

References

1. A.Van Hirtum and Berckmans D.; Automated recognition of spontaneous versus voluntary cough; Medical engineering & physics 24: 541-545

Genotype and evolution of the PRRSV present in Mexico (2005-2013)

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Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) was first diagnosed in Mexico in the 90´s and readily spread among the nation´s pig population. Due to its repercussions in the productive chain, it's considered as the most important source of economic losses for the industry (1).

Materials and Methods

Based on the genetic analysis of 447 sequences (ORF5 gene) from clinical cases collected in several states of the country during 2005–2013, a dendogram was built (Figure 1) to verify viral evolution and the genetic patterns of the infection in the country (Vector NTI advance v11.5.1; Geneious Basic V 5.0).

Results

So far, 12 genetic clusters were determined based on our data analysis. The 1992 North American isolate type 2 (2) designated as VR2332 (GenBank-U87392) initially related with the first viruses isolated in Puebla, Jalisco and Sonora, evolved in 12 genetic clusters with a notorious geographic correlation.

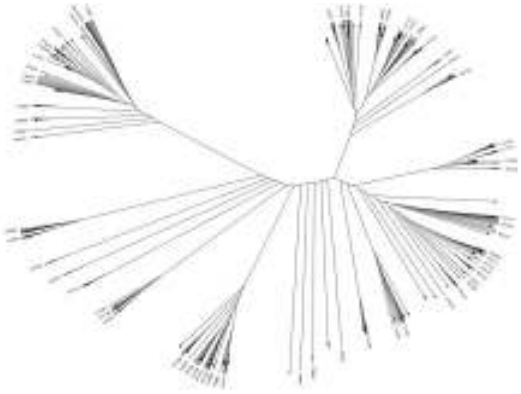

Figure 1. Dendogram of the evolution of PRRS virus in Mexico.

Conclusions and Discussion

Until now, we have not detected PRRS virus type 1 in Mexico in accordance with other authors (3). Mexican PRRS viruses have evolved separately from VR2332 since 2005 (Table 1). The evolution to 12 genetic clusters is also noticeable (Figure 2). Related isolates to VR2332 were a relevant factor in the genetic of PRRS Mexican viruses at the onset of outbreaks, but viral recombination and natural mutations excluded it from their genetic evolutive patterns. The live vaccine strain derived from isolate VR2332 was introduced before 2005 in Mexico, but now it has an important distant genetic homology (>10%) against 2007-2013 isolates (Table 1). According to our analysis, isolates from Jalisco are the origin of actual Mexican PRRS virus strains with 12 genetic clusters, and should be considered in all control programs in order to choose among the available commercial vaccines (MLV), a strategy based on the use of autogenous vaccines or a combination of both.

Table 1. Homology of MxPRRS viruses vs VR2332.

Year	Number of isolates	% homology (ORF5)
2005	5	96.6
2006	48	99
2007	25	89.5
2008	5	91.2
2009	49	88.9
2010	72	88.2
2011	70	86.9
2012	103	90.1
2013	70	88.1

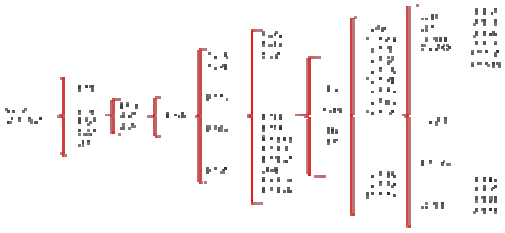

Figure 2. Evolution of PRRS virus in Mexico

References

1. Holkamp *et al*. (2011). BIVI PRRS ARC&E Seminar; iPRRS.
2. Collins J. J Vet Diagn Invest 1992; 4:117–263.
3. Sotomayor G.A. (2011). Determinación de muestras positivas al virus de PRRS con la técnica de PCR. Tesis professional, FMVZ, UNAM.

Economical impact of a PRRS area regional control project in Canada

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Introduction

Area regional control (ARC) projects have been shown to be a potentially effective strategy to control or reduce PRRS-associated losses within a region.[1] To be successful these projects need dedicated and committed producers, but they also involve an increase in health related costs. The purpose of this study was to evaluate if there is a positive return on investment for producers to get involved into a PRRS ARC project.

Materials and Methods

The study was conducted in the Monteregie region, in Quebec (Canada), where 47 of the 50 production sites were included. The project was initiated in June 2011. Collaborative and individual action plans were developed and implementation was mostly done by June 2012.

We used a tool to estimate the PRRS financial impact for farms in a region.[2] This tool estimates average productivity losses for PRRSv positives sites compared to PRRSv negative sites in financial numbers. It allows for adjustment of key financial parameters such as feed and hog prices. We looked at the evolution of the sites PRRS status in the region from June 2012 to June 2013. Sites using PRRS vaccine but with no clinical signs and where no field strain could be found by a repeated standardized sampling method were considered PRRSv negative for the purpose of the economical evaluation.

Expenses related to PRRS control were separated into costs and investments. Administration of the ARC project, the time of producers, staff and veterinarians involved, vaccines and diagnostic procedures were accounted as «costs». Expenses not done on a continuous basis like herd closure, biosecurity related improvements, transition to a 4 week batch farrowing system and complete depopulation-repopulation of continuous flow nursery and finisher sites were included into «investments». The estimated revenue was compared to the estimated cost. The difference between the revenue and the cost was calculated to obtain the overall profitability. All numbers are in Canadian dollars and the key financial parameters used were the average for the study period for the region.

Results

17 of the 31 sites that were PRRS-positive for a field strain in June 2012 succeeded in the elimination of that strain by the end of June 2013 (Table 1). The tool estimated that PRRS was costing producers of the region about 2 M$ per year in June 2012 compared to 1 M$ per year in June 2013 (Table 2). Both annual costs and investments were estimated to be 300 000 $ per year. The overall profitability was thus of 700 000 $. This profit could be used to reimburse the investments in about 5 months.

Table 1. Proportion of PRRSv positive sites in the Monteregie ARC project at the beginning and at the end of the evaluated period

	PRRSv positives sites[1]	
	June 1st, 2012	June 30th, 2013
Sow Nursery and/or Finisher	5	3
Farrow to Finish	20	5
	6	6

[1]Sites were considered negative if only a PRRS vaccine strain could be recovered.

Table 2. Annual budget for the Monteregie ARC project , June 30th, 2013

Annual benefit	1 000 000 $
Annual cost	300 000 $
Profit (Benefice – cost)	700 000 $
Investments	300 000 $
Profit:cost ratio	3,3
Time to pay investment (month)	5,1

Conclusions and Discussion

The purpose of the study was to determine if this PRRS ARC project was profitable for participating farms, and to help producers decide what to do with the future of the project. The financial numbers used for PRRS impact, costs and investments are estimates, so one should be cautious not to over-interpret these numbers. However, the strong positive outcome allows to conclude that this ARC project very likely produces more benefits than costs for farms of the region and this, only in one year of application of PRRS control action plans.

Acknowledgments

CDPQ and Cle-Monteregie project, Qc, Canada.

References

1. Waddell J. 2010. GA Young Swine Conf., 54-59.
2. Morin M et al.. 2014. Production and economic aspects of pig production sites involved in PRRS area regional control projects in Canada. Qc: CDPQ.

Evaluation of biosecurity measures and management variables as risk factors for infection of growing pigs that are negative at placement with PRRSV

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) causes significant productivity losses in all stages of production, including substantial losses in the growing pig even when the pigs are negative for the PRRS virus (PRRSV) at placement (1). Lack of understanding of key risk factors for lateral virus introduction has limited the industry's ability to effectively and economically implement bio-security measures to reduce PRRSV introduction into growing herd premises.

The objective of this study was to identify risk factors for lateral introduction of PRRSV in growing pigs that are PRRSV negative at placement.

Materials and Methods

Cohorts of finishing and wean-to-finish pigs that were PRRSV negative at placement and received no subsequent vaccination for PRRSV were enrolled. Status at placement was confirmed by diagnostic testing or by the PRRSV status of the breeding herd(s) from which the pigs were sourced. The pigs were sampled via venipuncture and tested by ELISA for antibodies to the PRRSV prior to marketing to determine the outcome variable (negative or positive). A positive outcome was used to confirm the introduction of PRRSV during the growing period. Information regarding biosecurity and management practices at each growing pig premises was collected using the PRRS Risk Assessment for the Growing Pig Herd offered through AASV's Production Animal Risk Assessment Program (PADRAP). The group lasso algorithm of Meier *et al.* (3) was used to perform the variable selection following the procedure described by H. Lin *et al.* (2).

Results

There were 149 groups of pigs included in the analysis raised on 100 premises within 7 pig production systems located in Iowa and Minnesota. The majority (129) of the groups were finisher groups with the remainder (20) being wean-to-finish groups. Farm size ranged from 175 head to 8,000 head. With regards to ownership of sites, 94 percent were owned or leased by a contract producer who

provided the labor but did not own the pigs and the remaining 6 percent were owned or leased by a production system which also provided the labor. Nearly 31% (46) of the groups became PRRSV positive prior to marketing with the other 69% (103) remaining PRRSV negative.

Fourteen of the 135 variables in the final model had non-zero variable coefficients and were therefore identified as being meaningful for determining whether a group of negative at placement growing pigs will become PRRSV positive prior to marketing. These variables included: Age spread of pigs in finishers; number of finisher spaces; Number of finishing pigs per employee; Fill time to form a group of pigs; winter air quality in the nursery phase; number of visits per year by trucks hauling standard weight market pigs; Disinfectant use on manure removal equipment and vehicles; Sanitation procedure for non-maintenance service personnel; Pig density within 1 mile of site; Distance to nearest PRRSV positive swine farm; Having significant market, slaughter plant, or collection point traffic on the public road nearest to the site; Distance to nearest field where manure from another site is applied; Topography; Disposal of dead animals.

Conclusions and Discussion

The findings in this study provide objective information about biosecurity and management variables associated with groups of growing pigs becoming infected with the PRRSV when the pigs are negative at placement. These results will help practitioners make informed decisions regarding implementation of biosecurity and management practices to reduce risk of PRRSV infection in growing pigs.

Acknowledgments

Boehringer Ingelheim Vetmedica, Inc Health Management Center, Ames, Iowa 50010 USA

References

1. Holtkamp D. 2013. J Swine Health Prod. 21(2):72-84.
2. Lin H. etal. 2012. J of Applied Stat, 40(4): 736-46.
3. Meier L. etal. 2008. J of Royal Stat Soc: Ser B (Stat Method). 70: 53-71.

Outbreak and elimination of PRRSV from Switzerland in 2012/2013 - collaboration of Swiss Animal Health Authorities and QIAGEN Leipzig

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Abstract

We describe an outbreak of PRRS in Switzerland, introduced into a Swiss PRRSV negative population by boar semen and how a good collaboration of the Swiss authorities together with a reliable supplier of high quality veterinary diagnostics helped to eradicate the disease in only seven weeks.

The Swiss pig population is 1.5 Mill. and free of PRRS. Import of live pigs into Switzerland is only permitted after quarantine. Previous to 2013 up to 32,000 doses of boar semen per year were imported without restrictions.
Boar facilities tested approximately 10% of boars every 4 weeks using ELISA and PCR from serum. In addition boars delivering semen for Switzerland were tested every 2 weeks in between by PCR from semen samples.
November 27th 2012, PRRSV was detected in a German boar facility delivering boar semen samples to Switzerland. One blood sample and one semen sample from two boars were PRRSV positive. The outbreak was confirmed by the detection of PRRSV in up to 90% of the boars in one boar-stable. The Swiss authorities were informed by the German boar station on November 28th. By that time 26 Swiss farms had received boar semen from the infected boar facility in the last 2 weeks, 5 of those farms received semen samples from the two boars which were initially tested as PRRSV positive. All 26 farms and all 61 contact farms were put on quarantine on November 29th.
The sudden, overnight requirement of PRRSV PCR for 87 farms was handled by the Swiss Institute of Virology and Immunology (IVI) in collaboration with QIAGEN Leipzig. Because of a proven performance of the virotype® PRRSV PCR in evaluation studies in Switzerland and international ring trials, protocols for blood, boar semen, and oral fluid testing in place and a long term scientific and business relationship with IVI, QIAGEN Leipzig was the preferred supplier for this outbreak.
The virotype PRRSV PCR was developed in collaboration between QIAGEN Leipzig and the German Friedrich-Loeffler-Institute (FLI). During development, emphasize was given to broad detection of EU and NA strains, particularly the newly derived Eastern European strains, such as Lena, combined with an easy-to-use assay protocol.
An urgent order for 5000 PRRSV reactions was received on Friday, November 30th. QIAGERN Leipzig managed to ship the PCR kits needed the same day over night and delivered to IVI on December 1st at 2 a.m.
In total in a seven weeks' time frame over 15,000 tests (ELISA and PCR) were conducted, wherein over 7200 PCR. Samples were collected by veterinarians, vet students, and employees of the Swiss Pig Health Service. The testing was conducted in 3 Swiss laboratories. The costs for sample collection, diagnostic reagents, and testing service were estimated at € 1 Mill.
On January 11th, the PRRS free status of Switzerland was confirmed and restrictions lifted.
The following new regulations, for importing boar semen into Switzerland, are now implemented by the Swiss authorities:
The foreign boar station must have an EU approval and be free of Aujeszky. Testing for PRRSV must be performed on blood and semen samples by PRRSV PCR and ELISA. Swiss farms using fresh semen, are not permitted to sell pigs for 4 weeks. This ban is lifted if blood samples from such farms are tested PRRSV negative 4 weeks after using such semen samples. Frozen semen can be used on Swiss Farms only three months after sampling. During this monthly examination or PRRSV must be done on the original boar station.

Conclusions

Virotype PRRSV PCR kit is officially approved by the German and Swiss authorities for PRRS control. The PCR assay allowed to eradicate the 2012 PRRS outbreak and will be used to reliably prevent PRRSV from entering Switzerland.

References

1. Report of the 2013 International Proficiency Testing Scheme (PTS) for Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) RNA detection in porcine serum and tissue samples, GD B.V. Animal Health Service Ltd.

Prevalence of PRRSV, swine influenza virus and *A. pleuropneumoniae* in Northern Ireland and its implication in post-mortem lesions

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Introduction

Routine surveillance is an essential component in protecting animal health, production efficiency and public health. The aim of this study was to find, for the first time, the prevalence and geographical distribution of PRRS (Porcine Reproductive and Respiratory Syndrome), SIV (Swine Influenza Virus) and APP (*Actinobacillus pleuropneumoniae*) infection in Northern Ireland and its possible implication in developing lesions in the respiratory and cardiovascular system.

Materials and Methods

Thirteen thousand and ninety two pigs from 142 producers were examined in the slaughterhouse in May and June 2011. This sampling represented 95% of the pig producers in Northern Ireland in 2011 (1). Lungs were scored for pneumonia consolidation using the Muirhead 55 point scoring system (2). Lesions of pleurisy, pericarditis and necrotising pneumonia were also recorded. Eight blood samples were collected in the bleeding area from each producer and analysed for PRRS (ELISA, IDEXX), APP (ELISA, IDEXX) and SIV (ELISA, IDvet) in AFBI, Stormont (Belfast). Statistical analysis was carried by using association between variables. Fisher's Exact Test was used to screen for an association between the presence of lesions and positive ELISA responses at the 20% significance level. ELISA responses found to be significant were further investigated individually and in combination as significant risk factors (P-value: 0.05) in a two-factor interaction binary logistic regression model. The odds ratio for increased risk of positive ELISA responses was estimated.

Results

Prevalence of lesions and PRRS, APP and SIV are shown in Table 1.!!

There is a significant association between pleurisy and PRRS positive (P-value: 0.014), SIV positive (P-value: 0.03) and APP positive (P-value: 0.004) using a Fisher´s Exact Test. The regression model indicated positive PRRS and SIV and APP responses significantly increased the risk of pleurisy. Herds positive to ELISA PRRS and SIV and APP were respectively 2.34, 2.86, and 3.68 times (odds ratio) more likely to be pleurisy positive than if the herd was negative to these infectious agents. There were no significant two factor interactions, suggesting that being infected with two or three of the pathogens studied did not disproportionately increase the risk of pleurisy and that pathogens act independently. There was no significant association for severity of pleurisy, enzootic pneumonia, necrotising pneumonia and pericarditis lesions between positive or negative pigs to these three pathogens. Finally, there is no significant difference when including two factor interactions, suggesting that being infected with two or three of the pathogens studied does not proportionately increase pleurisy. They act independently and not in combination.

Table 1. Prevalence and number of cases of PRRS, SIV, APP and post-mortem lesions in the respiratory and cardiovascular system.

	Prev.	Pl.	Pl.≥10 %	EP	EP >2.0	NP	Per.
PRRS	*52* (36.6 %)	*45* (86.5 %)	*22* (42.3 %)	*47* (90.5 %)	*11* (21.1 %)	*6* (11.5 %)	*34* (65.4 %)
SIV	*58* (40.8 %)	*51* (87.9 %)	*26* (44.8 %)	*51* (87.9 %)	*13* (22.4 %)	*9* (15.5 %)	*44* (75.9 %)
APP	*102* (71.8 %)	*84* (82.3 %)	*49* (48%)	*78* (76.5 %)	*21* (20.6 %)	*14* (13.7 %)	*65* (63.7 %)

Prev.= Prevalence *Pl.*= Pleurisy *EP*=Enzootic Pneumonia *NE*=Necrotising Pneumonia *Per.*=Pericarditis

Conclusions and Discussion

The results of the present study prove for the first time the prevalence of PRRS, APP and SIV in Northern Ireland. The geographical distribution of the infection is concentrated in pig dense areas. The results of this study indicated the association of PRRS, APP and SIV infection and pleurisy. However, contrary to (3), there was no association of PRRS infection and bacterial bronchopneumonia. According to our findings, these three infectious agents act independently and not in combination.

Acknowledgments

Pig ReGen, Karen Burns (AFBI, Belfast), MSD, Merial, IDEXX.

References

1. DARD 2013 http://www.dardni.gov.uk/december-agricultural-survey-historical-data.
2. Muirhead M et al 1997. In Managing Pig Health and the Treatment of Disease.5M Enterprises Ltd., Sheffield, U.K., 9:309.
3. Zimmerman J et al 2012. PRRS. In Diseases of Swine. 10th ed. Eds Wiley-Blackwell. Iowa. p469.

Epidemiological evaluation of PRRSV prevalence and the effectiveness of vaccination on farms served by one veterinary practice in an area of The Netherlands with a dense pig population

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Introduction

PRRSv causes severe economic losses in the pig industry all over the world. The cost of a PRRS outbreak in sows in The Netherlands varied from €59 to €329 /sow[1]. Since the risk of PRRSv infections is very high, the systematic control of PRRS is necessary in sow herds in order to reduce these losses. Biosecurity and target MLV vaccination of sows and piglets can reduce the incidence of weak and premature piglets caused by PRRSv [2,3]. The veterinarians of the Peelhorst Veterinary Practice serve farms in the most densely pig populated area of The Netherlands. The aim of this study was to investigate the effectiveness of PRRS vaccination by comparing prevalence of PRRSv in the nursery and benchmark the vaccination rates, PRRSv prevalence and genotypic variation in the Peelhorst practice with previously published data from The Netherlands.

Materials and Methods

Between 12th January and 19th April 2012, blood samples were taken on 42 different multiplying farms each from two batches of five piglets at weaning and two batches of five 10-week old piglets. The samples were tested individually using the IDEXX 3x herd check PRRSv ELISA, and PRRSv isolation was carried out on a pooled sample from each batch. If there were positive isolates, one isolate per age group was sequenced.
Details were listed with respect to the sows and piglets vaccinated and the vaccines used. Existing PRRSv epidemiological data were used for comparison [2,4,5].

Results

1. PRRS vaccination rates

	Sows		Piglets	
	Peelhorst	Netherlands(5)	Peelhorst	Netherlands
PRRS vaccination:	2012	2004;2007	2012	ResPigs2012
total	78%	80%	19%	15%
Porcilis PRRS	74%	76%	19%	15%
KV	4%	4%	0%	0%

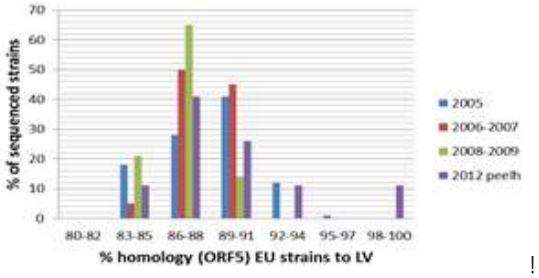
2. PRRS prevalence

PRRSv prevalence The Peelhorst	at weaning	two pools pos.	piglets 10 weeks	two pools pos.
Total	29% (12/42)		67% (28/42)	
no sow vaccination	33% (3/9)	67% (2/3)	67% (6/9)	100% (6/6)
sow vaccination	27% (9/33)	33% (3/9)	67% (22/33)	70% (9/22)

3. PRRSv genotype prevalence

	Peelhorst	Netherlands((5)
EU PRRSv	97,5% (39/40)	86%
US PRRSv	2,5% (1/40)*	14%
DV PRRSv	10% (4/40)**	

*≥98% homolog VR2332 **only in recent vaccinated piglets



!

Conclusions and Discussion

-PRRS sow vaccination rates were in line with the Dutch average reported in 2010, while piglet vaccination rates were slightly higher than average.
-The PRRSv prevalence in recently weaned piglets was lower at both farm and batch level in farms with PRRSv-vaccinated sows, which agrees with published reports about the protective effect of vaccination [2,3]. The effect was less pronounced in the nursery units, where the prevalence was lower only at batch level in farms with vaccinated sows . Of the PRRSv-free weaned piglets, 53% (16/30) were infected in the nursery units. Since colostrum offers no long-term protection to piglets, optimizing internal biosecurity measures is very important for maintaining their virus-free status.
-The Peelhorst prevalence of PRRSv genotype 2 was much lower than the Dutch average, may be due to the fact that the related vaccine strain has not been used in the practice for a very long time. The DV stain was only found in recently vaccinated animals which makes it more suitable for control strategies and, eventually, for eradication schemes [3,6]. Comparison of the ORF 5 PRRSv strain sequences with previously isolates in The Netherlands does not reflect further genetic drift of EU-PRRSv field strains or selection of more heterologous strains due to sow or piglet vaccination.
-A possibly PRRSv free status or at least a low infection pressure was attained by 26% of the Peelhorst practice served farms in early 2012. PRRS vaccination is an effective tool to reduce PRRSv prevalence in piglets and, when used together with optimal biosecurity, increases the percentage of PRRSv free batches or herds.

References

1. Nieuwenhuis N.et.al.,2011: Proc.ESPHM, p.94-95
2. Cruijsen A.L.M. et.al.,2011: Proc.SERPD, P. 155
3. v Groenland G. et.al.,2010: Proc.IPVS, Oral 240
4. Wellenberg G. et.al.,2010: Proc.IPVS,Poster.554
5. Geurts V.N.A.M. et.al.,2012: Proc.ESPHM, P194
6. Grosse Beilage E.,2009: Prev.Vet.Med. vol.92 p.31-37

Costs for having and not having PRRS

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Introduction

PRRS is a pig disease causing large economical losses. Sweden has been free from PRRS, which since 1998 has been documented by a control program. However, in 2007 the country was hit with PRRS. PRRS was promptly eradicated [1, 2], and the control program was redesigned. The present report discusses the cost for controlling and documenting freedom from PRRS and relates that to costs for having the disease.

Materials and Methods

The Swedish Board of Agriculture has calculated the total costs for controlling PRRS [2]. To better visualise that cost, it was divided with the number pigs slaughtered per year in the country (3 million pigs).

Results

The initial control program 1998 - 2007

Level I. 20 pigs in nucleus and multiplying herds were antibody-tested at least once a year.

Level II. Boars were antibody-tested twice before entering and once before leaving AI-stations (financed by the boar studs as a part of QA-work).

Level III. 20 pigs in each of 50 randomly chosen production sites were annually antibody-tested.

Level IV. Antibody-testing on clinical suspicion of PRRS (financed from "the epizootic budget").

The official program financed Level I and III of the program at a cost of 83,000 € per year [3], corresponding to 0.03 € per pig produced (**Table 1**).

Costs for eradicating PRRS in 2007

The 5th of July in 2007, a level III-herd tested positive for PRRSV. Within 5 days, 140 other herds were tested, and within 14 days the battle was over. In total, seven herds in two clusters were identified and stamped out [1, 2] at a total cost of 5.5 million € [3], which corresponded to 1.80 € per pig produced. Of these, affected farmers were compensated with 2.9 million € [3], and 1.9 million € were used for sample collection, veterinary aid and analysis [3]. The work required to again declare the country free from PRRS took four months and 0.7 million € into account [3] (0.23 € per pig produced).

Costs for PRRS in affected herds

When PRRS was diagnosed, the number of weaned pigs per litter had declined from 10.7 to 9.1 and the returners increased to 25%, corresponding to a loss of 90 € per sow and year! The herds were stamped out, but assuming an increase with 10 days to market weight and a mortality from weaning to slaughter of 5% the total losses would have corresponded to 443 € per sow and year, which in turn corresponded to 20 € per pig produced in PRRSV-affected herds [4].

The extended control program from 2007 and onwards

The control program was redesigned following the outbreak. Levels II and IV remained unaltered.

Level I was changed, and 8 pigs in nucleus and multiplying herds and in multisite units (sow pools) were tested at least twice a year.

Level III was altered to annual testing of 3 pigs from each production site - collected at slaughter.

The official program financed Level I and III of the program at a cost of 267,000 € [3], which corresponded to 0.09 € per pig produced.

Table 1. Costs for controlling, eradicating and having PRRS

Period	Measure	Cost per pig produced
1998-2007	Initial Control Program	0.03 € National level
2007	**Outbreak**, *cost for eradication*	1.80 € National level
2007	**Outbreak**, *cost due to PRRS*	20.00 € **Affected herds**
2008-2014	Extended Control program	0.09 € National level

Conclusions and Discussion

With freedom from PRRS, the costs for controlling and documenting appeared high at a national level, but were in reality negligible when calculated as cost per pig slaughtered - even when the control program was extended with the aim to improve the early signals. The comparably low cost for eradicating the disease [3] was explained by an early detection of the disease and an immediate and successful initiating of the eradication measures [1, 2], Still the cost in affected herds were most significant, and exceeded those of controlling freedom from disease with a factor of 222 (20.00€ versus 0,09€).

References

1. Wallgren et al. 2008. Proc. IPVS 20:53.
2. Carlsson et al. 2009.!Transbound Emerg Dis 6:121-31.
3. Viske et al. 2009. Sw Board Agricult. Rep 2009:4 132 pp.
4. Wallgren. 2011. Proc. Int. symp. Emerg & Re-emerg Dis. 6: 340

PRRS economic impact simulation tool for regional control and eradication projects in Canada

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Introduction

In the last few years, many area regional control & elimination (ARC&E) projects against porcine reproductive and respiratory syndrome virus (PRRSV) were initiated in Canada. In order to assist the decision making process of ARC&E steering committees together with promoting these initiatives, a PRRS cost simulator was designed as part of a broader Canadian PRRS study.

Materials and Methods

The cost evaluation is based on productivity losses for the three stages of production (farrowing, nursery and grow-finish). Production data together with PRRS status was collected from 205 Canadian production sites (1), situated in seven different ARC&E zones from three provinces (Québec, Ontario and Alberta). Information covered 78 sow units (42,727 sows), 658 batches of nursery piglets (1,066,213 piglets) and 720 batches of finishing pigs (828,449 pigs). Approximately 75% of sow units and 66% of piglet and pig batches tested PRRS positive. The production data covered a two year period extending from April 2010 to March 2012.

To take into account the variability of the impacts of PRRS on either a herd or a batch, the performance data (Table 1) was split into three types of impacts: no discernible impact, moderate and severe impact. For sow herds and pig groups testing negative for PRRS, production parameters are the same as herds in the category PRRS positive with no discernible impact.

Table 1 Production parameters of the economic simulation tool according to PRRS impact

	PRRS impact		
	None	Moderate	Severe
Piglets weaned/sow	27.20	24.70	23.70
Nursery mortality	1.5%	2.5%	4.3%
Nursery FC[1]	1.46	1.53	1.64
Finishing mortality	2.5%	3.7%	6.3%
Finishing FC[2]	2.60	2.71	2.90

[1] Feed conversion adjusted to a 6-25 kg weight gain.
[2] Feed conversion adjusted to a 25-120 kg weight gain.

Price data was collected from two public sources and covered the period of 2009-2011. Production results and price data were presented for validation to five of the participating ARC&E committee members (which included producers, veterinarians and economists).

A spreadsheet was developed with these parameters. Losses due to PRRS could then be estimated by comparing the margin of sales over feed costs to what the margin would be if all the places tested negative to PRRS (2).

Results

The economic impact was estimated using the specific information for each zone, i.e. number of animal places and PRRS impact. Losses for these zones ranged from C$ 416,000 to C$ 2 million. On average, for producers struggling with moderate or severe PRRS, losses were projected at C$ 73 per sow place in farrowing units, C$ 9 per piglet place in nurseries and C$ 31 per pig place in grow-finish units (see Table 2).

Table 2 Losses due to PRRS for five ARC&E zones

Losses for barns with moderate or severe PRRS outbreaks (C$/place/year)						
Zone	1	2	3	4	5	Average
Farrowing	-90	-102	-98	-69	-7	-73
Nursery	-10	-6	-9	-11	-9	-9
Grow-finish	-23	-35	-35	-32	-30	-31

Some zones showed lower losses than the rest, e.g. farrowing units in zone 5. Farrowing barns testing positive in this precise zone showed either no or only moderate impacts of PRRS infection; no severe case was recorded in farrowing barns for the period studied.

Conclusions and Discussion

Production losses can vary considerably from one zone to the other depending on the severity of PRRS outbreak. This validates the need to estimate costs individually for an ARC&E zone, for a better evaluation of the potential returns of a control or eradication measure. For Canada, losses due to PRRS outbreak can be estimated from C$ 116 million per year (if 35% of Canadian production sites test PRRS positive), to as high as $C 219 million per year for Canadian hog producers (if 66% of Canadian production sites test PRRS positive).

Acknowledgments

The authors would like to thank the Canadian Swine Health Board and the Canadian Association of Swine Veterinarians for their financial support.

References

1. Klopfenstein, C., et al. 2014. Clinical, Technical and Economic aspects of pig production sites involved in PRRS control projects in Canada. Québec : CDPQ.
2. http://www.cdpq.ca/outils-d-aide-a-la-decision/$imul-prrs.aspx?lang=en-CA

A. pleuropneumoniae – The Australian story

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Introduction

Actinobacillus pleuropneumoniae, formerly known as *Haemophilus parahaemolyticus* and *H. pleuropneumoniae*, is the causative agent of porcine pleuropneumoniae[1]. There are currently a total of 15 serovars of *A. pleuropneumoniae*, with serovar 1, 5, 7 and 15 being believed to be the dominant serovars in Australia. To check whether this is still accurate, the prevalence of *A. pleuropneumoniae* serovars in diagnostic isolates submitted to our laboratory over 11 years are reported. The study also examined the hypothesis that serovar 5 isolates had limited genetic diversity. Finally a multiplex PCR was developed to serotype the dominant serovars.

Materials and Methods

Over the period from February 2002 until April 2013 a total of 336 isolates of *A. pleuropneumoniae* were submitted. The isolates were serotyped via gel diffusion or indirect haemagglutination. We then compared the serotyping results in time period from February 2002 until December 2011 with the period of January 2012 until April 2013.

Genotyping of the serovar 5 isolates was performed with the enterobacterial repetitive intergenic consensus (ERIC) PCR. A total of 27 isolates, originating from 11 farms from three Australian States, were analysed with the ERIC PCR.

In an Australian Centre for International Agriculture Research funded project we developed a multiplex PCR for the identification and serotyping of *Actinobacillus pleuropneumoniae* serovars 1, 5, 7, 12 and 15. All 15 reference strains and 411 field isolates (394 from Australia, 11 from Indonesia, five from Mexico and one from New Zealand) of *A. pleuropneumoniae* were tested with the multiplex PCR. The specificity of this multiplex PCR was validated on 26 non-*A. pleuropneumoniae* species.

Results

Over the ten years of 2002 -2011 submissions of serovar 1, 5, 7, 12, 15 and non-typable accounted for 94% of all submissions. Serovar 15 (35%) was the most dominant serovar, followed by serovar 7 (26%), then serovar 5 (19%) and then serovar 1 (9%) and non-typables (4%). Serovar 12 was only 1% of the submissions. In the later period (2012 till 2013) 1, 5, 7, 15 and non-typable accounted for 100% of the submissions. In this period the most dominant serovar was serovar 7 (42%), followed by serovar 15 (22%), serovar 12 (16%), serovar 5 (11%), serovar 1 (4%) and non-typable (4%).

The new multiplex PCR gave a species-specific band with all 411 *A. pleuropneumoniae* isolates and agreed with conventional serotyping for all serovar 1 (46 field isolates), 5 (81 field isolates), 7 (80 field isolates), 12 (16 field isolates) and 15 (117 field isolates). Out of the 25 non-typable field isolates examined only two did not yield a serovar band in the multiplex PCR.

When determining the genotype of serovar 5 isolates in the period from 2003 to 2013, all 27 isolates, originating from 11 farms from three Australian States, displayed the same genetic fingerprint.

Conclusions and Discussion

This data has to be taken with caution as the results are based on diagnostic submissions. While recognizing this need for caution, the data suggest a shift in serovars in the period 2012 onwards with serovar 12 submissions from outbreaks being more prominent than in previous years. Single serovar 12 isolates were seen in 2007 and in 2008 and only two isolates were seen in 2011. However from 2012 onwards, a total of 7 serovar 12 isolates have been encountered. There was also an apparent increase in serovar 7 compared to the previous years.

It is apparent that the main serovars in Australia are serovar 1, 7, 5, 12 and 15 and that a molecular diagnostic assay able to recognize all these serovars would be advantageous. This multiplex PCR for serovar 1, 5, 7, 12 and 15 is species specific and capable of recognizing the serovar of diverse field isolates.

The data obtained when exploring the clonality of the *A. pleuropneumoniae* isolates of serovar 5 suggests that the isolates are a single clone and of one origin

Acknowledgments

We like to thank the Australian Centre for International Agriculture Research for their funding support

References

1. Gottschalk, M. and Taylor, D.J. 2006 In Diseases of Swine eds. Straw, B.E et al. pp.563-576. Ames, Iowa: Blackwell Publishing

O.133

Differences in the clinical outcome of an *A. pleuropneumoniae* serotype 2 and serotype 7 infection comparing incubation time and distribution pattern of lung alterations

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Introduction

Actinobacillus pleuropneumoniae (*App*) is one of the most important bacterial lung pathogens in swine. So far 15 different serotypes of *App* with different pathogenicity are described. It is described that incubation time can be quite variable due to amount of inoculated bacteria (1) and that lung lesions vary in accordance with the clinical course of disease. In several experimental infection studies the impression occurred that differences in incubation time and lung lesions (especially the distribution pattern of the lung lesions) differed not only because of inoculated numbers of *App* or course of disease but also as a function of the serotype, leading to serotype specific clinical and pathomorphological patterns. This study is a retrospective analysis of data from different *App* infection studies that were conducted under the same conditions.

Materials and Methods

Four experimental infection trials (120 piglets, aged 7 weeks) were included in this study. 56 piglets were infected with *App* Serotype (S) 2 and 64 animals with *App* Serotype 7 in a standardized aerosol infection model (2). All animals were tested negative for *App* before being included into the studies. Half of the animals were infected with $1x10^2$ (low), the other half with $3x10^3$ (high) *App* cells per litre aerosol. After infection the animals were examined in two hour intervals. Rectal temperature and clinical symptoms were noted. The incubation period was defined as the time until the first significant increase of rectal temperature above 40.2°C or the appearance of the first clinical symptoms. Overall 78 piglets were euthanized on day 7 post infection (p.inf.), 28 piglets on day 20 p.inf, 14 animals died or were euthanized before due to the severity of symptoms. Lung lesions were scored by a lung lesion score (3). Numbers of separated lung lesions per animal and average size of the lung lesion were counted.

Results

Regarding the known influence of infection rates on the course of porcine pleuropneumonia the influence of the infection dose on the incubation time was repeatable in serotype 2 (mean low: 8.19h, mean high: 4.48h; p: <0.0001), this influence was not repeatable for serotype 7. For serotype 7 only an influence of the dose of infection could be shown for the number of lung lesion (mean low: 2.67, mean high: 4.09; p: 0.028). There were no statistical differences for the different necropsy dates (day 7 vs. day 20 p.inf.) regarding number or size of lung lesions for serotype 2 (p-number: 0.39; p-size: 0.355), for serotype 7 it could be shown that there were a decrease of the numbers of lung lesions between the two days (mean 7days p.inf.: 4.08, mean 20days p.inf.: 2.62; p: 0.001). In addition to these differences within one serotype class clear statistical significant differences in incubation time (mean S2: 6.17 hours (h), mean S7: 23.66h, p: <0.0001), number of lung lesions (mean S2:10.19, mean S7: 3.77, p: <0.0001) and number of triangles per lesion (mean S2: 2.61, mean S7: 6.98, p: <0.0001) between the two serotypes were discovered.

Conclusion and Discussion

It seems that there is, in addition to the known influence of infection rate and course of disease, also a distinct influence of the involved serotype on incubation time and on the pattern of the lung lesion distribution. The incubation time of serotype 7 infections seems to be three to four times longer as the incubation time of serotype 2 infections. Animals infected with serotype 2 develop a significantly higher number of smaller lung lesions than animals infected with serotype 7. In further studies it would be interesting to investigate the responsible mechanisms of these differences as they can not only be explained by the known virulence factors of *App*.

References

1. Gottschalk 2012, in Diseases of Swine, Wiley-Blackwell publishing, 653-665.
2. Jacobsen et al.: 1996. Vet Microbiol 49: 159-168.
3. Hannan et al. : 1982, Res Vet Sci 33 : 76-88.

O.134

Improvement of surveillance of atrophic rhinitis in pigs by using qPCR on oral fluid samples from individual and grouped pigs

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Introduction

Atrophic rhinitis (AR) in pigs is caused by toxigenic strains of *Bordetella bronchiseptica* (Bb) and *Pasteurella multocida* type D (PMT). Ante mortem AR surveillance is usually carried out by direct detection through bacterial culture, PCR or antigen detection in nasal swabs (NS).[3] Oral fluid (OF) testing offers an opportunity to easily collect herd-level disease data on a periodic basis, and has been evaluated for monitoring various bacterial and viral diseases of swine.[4] To our knowledge, there is no previous report on the use of OF for the detection of Bb and PMT on commercial farms. Therefore, this study was conducted to determine if OF samples collected from pigs housed individually or in groups contain Bb and PMT, capable of being detected by qPCR.

Materials and Methods

Two assays originally developed as conventional PCRs[1,2] for the detection of Bb (*flaA* gene) and PMT (*toxA* gene), were adapted to real time format with SYBR Green I detection and melting curve analysis (qPCR). Analytical sensitivities and specificities were assessed using reference strains of Bb and PMT, along with a panel of viral and bacterial respiratory pathogens of pigs, respectively.

Pen samples came from 46 pens (20 to 25 pigs, 4-16 weeks old) in 10 growing-finishing farms, and consisted of one OF and one pool of 4-7 NS per pen (4-6 pens per herd). Individual samples consisted of 32 OF and NS collected from the same number of adult sows in two breeding-gestation units.

Herds with at least one sample testing positive in the qPCR were considered positive to Bb or PMT, as appropriate.

Results

The PCR assays were successfully adapted to the qPCR format, allowing the detection of Bb and PMT in a sensitive and specific way, with no cross-reactivity to other pig pathogens, and detection limits of 1-10 ufc/ul.

Of the 10 growing-finishing units, seven were positive to Bb, and one to PMT. The two breeding-gestation units were negative to PMT, and one positive to Bb. Positive qPCR results for each target and sample type are summarized in Table 1. In all cases both NS and OF samples tested positive in different proportions (data not shown). Only one sow tested positive to Bb in both OF and NS; none tested positive to PMT.

Table 1. *Bordetella bronchyseptica* and *Pasteurella multocida* qPCR positive oral fluid and nasal swabs, collected from pigs in 46 pens and 32 sows in individual stalls.

	Pen Samples[1] (n=46)		Individual Samples (n=32)	
	Bb	PMT	Bb	PMT
OF	23/46	3/46	1/32	0/32
NS	24/46	2/46	1/32	0/32

OF= oral fluid; NS= nasal swab; Bb= *B. bronchyseptica*; PMT= *P. multocida*; [1]Samples were taken simultaneously from pens of 20-25 pigs: One OF and 1 pool of 4-7 nasal swabs were obtained from each pen;

Conclusions and Discussion

This study demonstrates that pig OF may contain genetic material of Bb and PMT, which is detectable by qPCR. The detection rate was similar in OF and NS from the same animal or group of animals. These results suggest that OF could be used for monitoring of AR in pigs, reducing labor and animal stress during sample collection, and providing valuable information about the health status of breeding and growing pigs in regards to AR.

The findings also suggest that there may be a significant proportion of pigs free from Bb infection, although it is considered a normal inhabitant of the respiratory tract in pigs. However it is noteworthy that the small number of animals tested may have had an influence on the results presented. Nevertheless, these findings encourage to carry out further studies using the methodology described, along with herd production data, to look for possible links between the prevalence of Bb and PMT in OF and the incidence of the disease.

Acknowledgments

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References

1. Hozbor D et al. 1999. Res. Microbiol. 150: 333−41.
2. Kamp EM et al. 1996. J Vet Diagn Invest 8:304-09.
3. OIE Terrestrial Manual. 2012. Chapter 2.8.2.
4. Prickett JR et al. 2010. Anim Health Res Rev 11:207-16.

O.135

Update on *H. parasuis* serovars isolated from pathological samples in Northern Italy

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Introduction

Haemophilus parasuis is the causative agent of porcine fibrinous polyserositis, arthritis and meningitis (Glässer's disease) but it is also a commensal bacterium commonly isolated from the upper respiratory tract of pigs in conventional farms. The infection can be controlled by antibiotic treatment and vaccination. Available vaccines are based on certain *H. parausis* serovars, therefore, knowledge of the serovars distribution is essential in order to evaluate the possible benefits of vaccination (1).

Materials and Methods

From 2007 to 2013 a total of 198 *Haemophilus parasuis* strains were isolated by bacteriological examination from diseased pigs submitted for diagnostic investigations to Istituto Zooprofilattico Sperimentale of Lombardia and Emilia Romagna (IZSLER). The isolates were serotyped by agar gel diffusion test (GD) using rabbits' hyperimmune reference antisera against serovars 1,2,4,5,10,12,13,14 and autoclaved antigens. The choice of the antisera used was performed considering the prevalence of different virulent serovars described in other European Countries (1). The strains have been divided into two groups depending on whether they were isolated from cases of systemic disease (polyserositis, arthritis or meningitis) or they only were isolated from the lower respiratory tract. The cases of systemic disease have been evaluated considering the frequency of isolation of *H. parasuis* from different samples (lungs, pericardial exudates, brain, joints and pleural exudates).
For each serovar, when the number of isolates were greater than 10, the difference between the proportion of *H. parasuis* isolated from pigs with systemic disease and with catarrhal to purulent bronchopneumonia (Bp) only was tested by two proportion z-test, one tail with $P<0.05$ by using Intercooled Stata 7.0 software (Stata Corporation, College Station, TX, USA).

Results

In our study serovar 4 was the most prevalent (26.7%) followed by serovar 13 (20.2%) and serovar 5 (8.6%), while 30.8% of the isolates could not be assigned to a serovar (non-typable isolates) (Table 1). The frequency of isolation of serovars 4, 13 and 12 from swine with systemic disease was significantly higher than the frequency of isolation of these serovars from swine without systemic disease ($P<0.01$). The frequency of isolation of serovar 5 and non-typeable strains from swine with and without systemic disease was similar. The isolation of *H. parasuis* from pigs with systemic disease was perfomed from lungs (62%), pericardial exudate (40%), brain (21.2%), joints (8.7%) and pleural exudate (2.5%).

Table 1: *H. parasuis* serovars prevalence and gross lesions observed (n.s. not significant; n.t. not tested).

Serotype	%	Systemic disease %	Bp %	p
4	26.7	81.25	18.75	<0.01
13	20.2	64	36	<0.01
5	8.6	54.5	45.5	n.s.
12	5.5	87.5	12.5	<0.01
2	3	66	44	n.t.
14	3	75	25	n.t.
1	1	100	0	n.t.
10	0.5	100	0	n.t.
15	0.5	100	0	n.t.
Nt	30.8	51.7	48.3	n.s.

Conclusions and Discussion

This study reports an updating of the prevalence of *H. parasuis* serovars in Italy. Serovar 4 is the most prevalent, followed by serovar 13 while the proportion of isolates belonging to serovar 5 was lower. The results obtained showed that the distribution of serovars in Italy is very similar to that recorded in other Countries (1,2).
In this study, the frequency of isolation of serovars 4, 12 and 13 from swine with systemic disease was significantly higher than the frequency of isolation of these serovars from pigs affected by bronchopneumonia only. The occurrence of isolation of serovar 5 from swine with and without polyserositis was similar. Other studies showed that serovars 2, 5 and 13 were isolated from cases of systemic disease only (3), while Angen et al.(2004) reported a higher prevalence of serovar 4 in cases without systemic disease. The reasons of these conflicting results could be related to the considerable genetic variability of *H. parasuis* and to the well-known differences in virulence of the strains within the same serovar.

References

1. Angen Ø. et al. 2004. Veterinary Microbiology 103, 255-258.
2. Turni C. and Blackall P.J. 2005. Veterinary Microbiology 106,145-151
3. Olvera, A. et al. 2007. Vet. J.174, 522–529.

O.136

Impact of early antibiotic treatment on *H. parasuis* disease, seroconversion and resistance to challenge

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Introduction

Exposure of young pigs to a low dose of virulent *H. parasuis* (HPS) reduced mortality due to Glasser's disease[1]. Factors that disrupt colonization patterns of HPS at weaning, such as use of antibiotics, can alter the dynamics of HPS infection[2]. However, the effect of antibiotics on the development of an immune response is contradictory[3,4] and requires further investigation. In this study we seek to evaluate the impact of early antibiotic treatment on HPS disease and resistance to HPS challenge.

Study design

Sixty 3-week-old conventional pigs were either treated with enrofloxacin 3 days prior (ABT/EXP), or 3 days after (EXP/ABT) controlled exposure with a virulent HPS strain at day 0. Controlled exposure took place using 10^6 CFU/ml of virulent live HPS Nagasaki strain, intranasally. Antibiotic treatments were done using one dose of 7.5 mg/kg injectable enrofloxacin (Baytril® 100). Group EXP was exposed to HPS only. Group ABT received only enrofloxacin. The positive control group (CHA) was non-exposed, untreated and challenged. The negative control group (NEG) was untreated, non-exposed and non-challenged. Nasal swabs were collected on days -3, 0, 2, 3, 5, 7 and 18 for HPS isolation. ELISA was used to measure IgG in serum collected before and after controlled exposure and at necropsy. All groups, except the NEG, were challenged on day 21 with 10^8 CFU/ml of virulent HPS Nagasaki strain, intranasally. Pigs were monitored for clinical signs daily. After challenge, surviving pigs were euthanized and necropsied on days 25 or 35. At necropsy, a complete pathological investigation was conducted.

Results

The virulent HPS strain was isolated from the nose of at least half of the pigs in the EXP, ABT/EXP and EXP/ABT groups after controlled exposure. Clinical signs of disease were observed on EXP and ABT/EXP groups starting 4 days after controlled exposure. Four pigs from each group were treated with enrofloxacin and recovered. Two other pigs from each of these groups were euthanized due to severe clinical signs and one pig had fibrinopurulent polyarthritis. Virulent Nagasaki HPS was isolated from several systemic sites from all four pigs, indicating acute septicemia. Pigs from EXP/ABT group remained healthy until challenge.

Pigs in the EXP and ABT/EXP groups remained healthy after challenge. Pigs from CHA, EXP/ABT and ABT groups presented fever starting 36 hours after challenge. Three pigs from ABT and one pig from CHA groups were euthanized before the first necropsy due to severe clinical signs and all of them presented fibrinopurulent polyserositis and polyarthritis. Four days after challenge, up to five pigs from all groups were selected for necropsy based first on clinical signs and then randomly. Virulent HPS was isolated from systemic sites from all diseased pigs. Seroconversion was observed in pigs from groups EXP and ABT/EXP after controlled exposure, which also survived challenge without any clinical signs or lesions at necropsy. Pigs from the EXP/ABT did not seroconvert after controlled exposure. After challenge, seroconversion was observed on surviving pigs from groups ABT and CHA.

Conclusion and Discussion

Virulent HPS was able to colonize the nasal mucosa of at least half of the pigs in controlled exposed groups, and even at low dose, was also able to cause disease in 60% of the pigs from groups EXP and ABT/EXP. Interestingly, disease in ABT/EXP group started 3 days after onset of disease in group EXP, which might reflect a decay of enrofloxacin levels in that group and delay in infection. Enrofloxacin treatment was successful to limit disease and HPS Nagasaki colonization in pigs that became ill after controlled exposure (groups EXP and ABT/EXP) and pigs that received treatment right before the onset of disease (group EXP/ABT). Seroconversion after controlled exposure was variable between and within groups, but all exposed pigs in groups EXP and ABT/EXP survived challenge, independently of seroconversion. Early enrofloxacin treatment (before clinical signs of disease) in group EXP/ABT interfered with protection against challenge.

This study showed that antibiotics can be a powerful tool to modulate bacterial colonization. The results of this study stress the importance of timing in the administration of antibiotics for the control and treatment of bacterial infections in swine.

Acknowledgments

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References

1. Oliveira S et al. 2004. JSHP, 2:123-128.
2. Macedo N et al. 2014. CJVR, 78:17-22.
3. Griffin A et al. 2009. J. Immunol. 183:1263-1270
4. Johanns TM et al. 2011. Microb. Infect. 13:322-33.

Effects of different antimicrobials in weaned piglets experimentally infected with *H. parasuis*

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Introduction

Over the last decades, *Haemophilus parasuis* (HPS) evolved to one of the economically most important pathogens in swine industry (1). To control clinical HPS infections antibiotics are routinely used (2). The aim of the present study was the evaluation of different antimicrobials (enrofloxacin, tulathromycine, tildipirosin) against HPS serovar 5 infection in weaned piglets.

Materials and Methods

In total, 60 weaned piglets at three weeks of age were randomly allocated to five treatment groups. After seven days of acclimatization, the pigs were either intratracheally infected with HPS serovar 5 (10^8 CFU) or received physiological saline instead (table 1). After infection (p.i.), rectal temperatures were measured in a two hours interval for 48 hours p.i. and once daily afterwards. Test products were administered once 24 hours p.i. (table 1). Daily clinical investigations were used to calculate a total clinical and a joint score. Piglets were weighed at arrival, at the day of infection and at the end of the study to calculate average daily weight gain (ADWG). Seven days p.i., all pigs were euthanized and necropsy was performed. At necropsy, changes on the serosal surfaces (severity, extension) were scored using a serositis score and swabs from the peritoneal and pleural cavity were collected for PCR and bacterial culture.

Table 1. Groups, infection, treatment

group	# of pigs	intratracheal infection	treatment
A	12	phys. saline	no therapy
B	12	10^8 CFU HPS serovar 5	no therapy
C	12	10^8 CFU HPS serovar 5	Tulathromycine 2.5 mg/kg
D	12	10^8 CFU HPS serovar 5	Tildipirosin 4.0 mg/kg
E	12	10^8 CFU HPS serovar 5	Enrofloxacin 7.5 mg/kg

Results

HPS infection induced only mild clinical signs revealing no significant differences between groups in the clinical score. Significantly higher body temperatures (BT) were detected p.i. in pigs from the infected groups compared to control pigs at least at one study day ($p<0.05$). Two hours after treatment, significantly lower BT ($p<0.05$) were found in groups D and E compared to group C and six hours after treatment in group D compared to group E, respectively. Joint scores were significantly lower in treatment groups D and E than in group C ($p<0.05$) (fig. 1). The highest ADWG was reached by group C (275.00 g/d), followed by groups E (268.45 g/d), A (233.93 g/d), D (225.00 g/d) and B (219.05 g/d) (fig. 2). Regarding the serositis score, only mild lesions were evident displaying no differences between groups. HPS isolation succeeds in 3 cases (group B) and 7 out of 12 group B pigs tested positive by PCR. All other pigs tested negative.

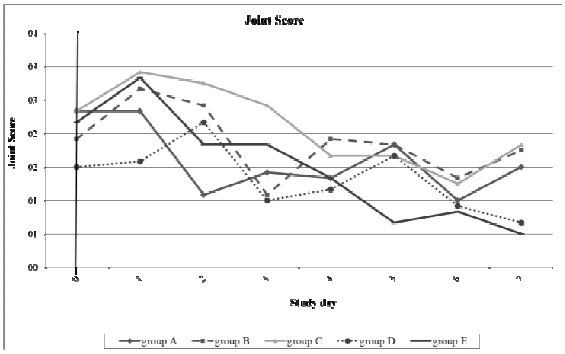

Figure 1. Mean joint scores of the five treatment groups

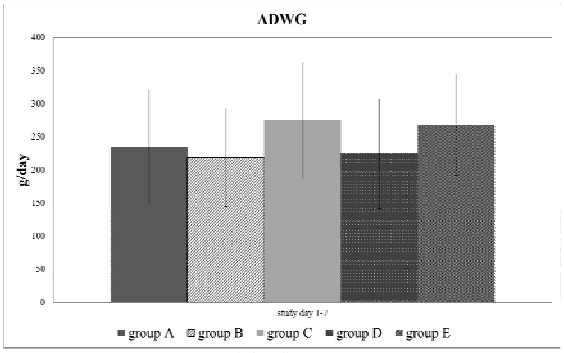

Figure 2. Mean ADGW of the five treatment groups

Conclusions and Discussion

The results demonstrate the efficacy of all antimicrobials used combating HPS serovar 5 infection. However, data indicate no superiority of a particular treatment, because differences between infected, not-treated (group B) and non-infected pigs (group A) were small. Nevertheless, HPS colonization of the internal organs was successful and isolation succeeds only from group B swabs. Re-isolates tested for resistance demonstrate the same sensitivity pattern for all antimicrobials used as the challenge strain. The positive effect of some antimicrobials presented by a slightly higher ADWG (not significant) in groups C and E compared to groups A and D, might be due to the fact that piglets originated from a conventional farm harboring various pathogens additionally to the HPS challenge strain.

References

1. Aragon V 2012. Diseases of Swine 10th, Ames, Iowa, 760-769.
2. Aarestrup FM et al. 2004. Vet Mircobiol 101:143-146

Characterization of *S. suis* isolates recovered between 2008 and 2011 from diseased pigs in Brazil

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Introduction

Streptococcus suis is a major agent of meningitis, septicemia, and other diseases in pigs and is also a zoonotic agent (Gottschalk, 2012). A total of 35 serotypes have been described and most studies on virulence, pathogenesis of the infection and protection have been carried out with serotype 2. Important differences in virulence also exist among strains of *S. suis* serotype 2. Therefore, the objective of the present study was report the distribution of serotypes in Brazil. Serotype 2 strains were further studied for the presence of virulence markers *sly* (suilisin) , *efp(extracellular factor), MRP(muramidase released protein) and hemolysin..*

Materials and Methods

A total of 117 field strains were collected from January 2008 to December 2011 from diseased pigs, originated from seven states in Brazil that had been submitted to IPEVE Laboratory. Tissue specimens were cultured, identificated and serotyped by co-agglutination test as previously described (Gottschalk et al., 1993). Amplification of the *sly* and *epf* genes was performed by PCR and positive strains were further tested in an hemolysis assay and MRP expression by Western-blotting, as previously described (Fittipaldi et al., 2011).

Results

Table 1 shows the distribution of different serotypes during the 4-year study. Serotype 2 were found in 33 different herds and states of Brazil. In this study, a majority of serotype 2 strains studied (70,4%) belonged to phenotype groups profile MRP^+, *epf*+ *sly*+ hem+ and 22,2% presented a profile MRP^+ epf^-, sly^-hem- .

Table 1. Distribution of serotypes of ***S.suis*** isolated from diseased pigs in Brazil, between 2008 and 2011

Serotype	Number of isolates	%
2	*54*	*46,2%*
1/2	*2*	*1,7%*
3	*10*	*8,5%*
4	*3*	*2,6%*
7	*10*	*8,5%*
8	*2*	*1,7%*
9	*4*	*3,4%*
11	*1*	*0,9%*
12	*2*	*1,7%*
24	*1*	*0,9%*
30	*1*	*0,9%*
*NT **	*25*	*21,4%*
Auto aglutinating	*2*	*1,7%*

* Non typable

Conclusions and Discussion

This study confirmed the high prevalence of serotype 2 strains, followed by serotype 3 and 7. A high number of non typable strains (21,4%) were founded. The most prevalent phenotype MRP^+, *epf*+ *sly*+ hem+ were usually associated with high virulent group and can be important for dissemination of diseases for human being.

Serotyping remains a valuable tool used by veterinary practitioners and diagnosticians to understand the epidemiology of a particular outbreak and/or to increase the possibility of success of a vaccination program within a herd (Gottschalk, 2012). The role and pathogenesis of the infection caused by these strains remain to be elucidated.

Acknowledgments

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References

1. Gottschalk, M. Et al, 2012. Diseases of Swine. Blackwell Publishing, Ames, Iowa, USA, 841-855.
2. Gottschalk, M., Higgins, R., Boudreau, M., 1993. J. Clin. Microbiol. 31, 2192-2194.
3. Gottschalk, M., Lebrun, A., Wisselink, H., Dubreuil, J.D., Smith, H., Vecht, U., 1998. Can. J. Vet. Res. 62, 75-79.
4. Fittipaldi, et al., 2011. Emerg. Infect.Dise. 17, 2239-2244.

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Use of oral fluids for detection of *Ascaris suum* eggs

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Introduction

Infection with ascarids (*Ascaris suum*) causes production performance issues[1] (decreased gain, reduced feed conversion, liver condemnations, secondary respiratory disease) resulting in economic loss.[2] There have been implications that prevalence of internal parasites in swine is quite high, particularly on finishing sites.[3-4] Antemortem diagnosis of ascariasis in pigs has been limited to fecal flotation and serodiagnostics.[5] Research has shown that oral fluids can be used to detect PRRSv, SIV, PCV2, *Mycoplasma hyopneumoniae* and *Lawsonia intracellularis*.[6-7] The objective of this study was to determine if ascarid eggs could be detected in an oral fluid collection, and how this method might compare to fecal flotation.

Materials and Methods

A finishing site with a history of ascarid infestation was selected for this study. In an effort to adjust the prevalence of infestation within the population on this site, one barn was treated with fenbendazole (Safe-Guard EZ Scoop, Merck & Co., Inc., Whitehouse Station, NJ) and another was left untreated. Both barns were monitored monthly, collecting paired fecal composites (FC)[8] and oral fluids (OF) from 15 pens in each barn at each sampling point. A total of six sample collections were conducted from the time of placement to the time of marketing. Fifteen milliliters of oral fluids were collected from each pen and were centrifuged for 10 minutes to form a pellet at the bottom of the conical centrifugation tube. The supernate was poured off, and the pellet was redistributed in a concentrated sugar solution (sp gr 1.27) for flotation. Fecal samples were processed using a modified sugar flotation protocol.[8] Positive samples were defined as having at least 1 ascarid egg. Diagnostic tests were compared using Kappa analysis and percent agreement. No "gold standard" exists for antemortem ascaris diagnosis, so sensitivity of each method was calculated based on the overall pen result (positive by either fecal or oral fluid).

Results

One-hundred seventy three paired samples were obtained for evaluation. There were 68 positive OF samples and 55 positive FC samples. These samples had a 79.2 percent agreement, and a Kappa value of 0.551 (95% CI 0.423, 0.678). OF samples had a sensitivity of 86.3% (95% CI 76.3, 92.6), and FC had a sensitivity of 68.8% (95% CI 57.3, 78.4).

Conclusions and Discussion

This study has shown that *Ascaris suum* eggs can be detected in oral fluids. High agreement results were observed between the traditional fecal flotation of pen fecal composites, and modification of flotation protocol using oral fluids. This could be a useful sampling method for detection of ascariasis in swine, as a resourceful utilization of the solid pellet. This can serve as a more convenient and sensitive sampling method that could easily be included in routine diagnostic testing of swine populations, such as growing pigs and replacement gilts in gilt development or isolation units.

Table 1. Comparison of oral fluids and fecal pen composites for the diagnosis of *A suum*.

Oral Fluids	Fecal Composites		
	Negative	Positive	
Negative	44	25	69
Positive	11	93	104
	55	118	173

Kappa = 0.551 (0.423, 0.678), *P*=0.003
% Agreement = 79.2
Oral Fluid sensitivity* = 0.863
Fecal Composite sensitivity* = 0.688
*Sensitivity against overall pen result

Acknowledgements

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References

1. Greve JH. Internal Parasites: Helminths. In: Diseases of Swine. 10th ED. 2012.
2. Stewart TB *et al. J Anim Sci*. 1998;66:1548-1554.
3. NAHMS Swine Study. USDA APHIS. 2006.
4. Shepherd *et al.* IPVS. 2010:807.
5. Vlaminck *et al. Vet Parasitol.* 2012;189:267-73.
6. Iowa State Vet Diag Lab. 2013.
7. LaVigne *et al*. AASV. 2013;71-73.
8. Pittman JS *et al. J Swine Health Prod.* 2010;18:249-252.

System wide survey of *Ascaris suum* eggs in sows housed in penned gestation facilities

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Introduction

The large roundworm, *Ascaris suum*, continues to be a concern for swine producers and most operations routinely treat breeding animals with anthelmintics, not only to address the health benefits of the breeding animals but more importantly to avoid vertical transmission from dams to their offspring. The survey reported here was initiated to guide decisions for converting from a semi-annual, whole herd deworming program to deworming sows approximately 30 days prior to each farrowing. With both programs, gilts are treated prior to entry into the breeding herds. Fenbendazole (Safe-Guard®, Merck Animal Health, Summit, New Jersey) applied in the feed was used for both programs. In addition, the operation had unexpectedly discovered ascarid infections in several finishing sites and a group of young replacement gilts.

Materials and Methods

Fecal (n = 1909) and oral fluid (n = 134) samples were collected from 61 sites. Three sites contained only gilts in isolation, 15 sites contained sows only and 43 sites contained gilts in isolation or pre-breeding, and sows. In all sow sites, animals were housed in small pens with approximately 6 per pen. The sites were grouped by flow which refers to where gilts are obtained from and where the offspring goes to after weaning. Samples were classified by one of three production stages. "Gilts" refers to gilts prior to breeding in isolation or after entry to the breeding herd. "Gestation" refers to pregnant females; most of these samples were collected within a month prior to farrowing. "Lactation" refers to farrowed females; most of these samples were collected near the time of weaning. From each stage at each farm, fecal samples were collected from 10 (predominate number) or more animals. In 11 farms, oral fluids were collected from the pens of the same animals that fecal samples were collected from. The Modified Wisconsin Sugar-Centrifugal Flotation Method was used to detect ascarid eggs. For the oral fluids, a 15 mL sample was centrifuged, the supernatant was removed and the pellet was handled similar to a fecal sample.

An individual sample was considered positive if any ascarid eggs were observed. A production stage within a farm or a farm overall was considered positive if any individual sample was positive. Chi Square analysis was done to determine factors that influence whether an individual animal, a production stage within a farm or a farm overall was positive.

Results

The prevalence by production stage for individual animals or by farm is presented in the table below. Both fecal sample and oral fluid results are included. Overall, the positive rate for fecal samples was 4.2% and for oral fluids, 5.2%. Seventeen sets (a production stage within a farm) contained both fecal samples and oral fluids; 13 agreed (1 both positive and 12 both negative) and 4 sets had conflicting results (2 fecal positive and oral fluid negative, and 2 fecal negative and oral fluid positive).

Factors that could potentially influence whether an individual sample was positive include farm, flow or production stage. Gilts and Gestation stages had higher than expected positive animals while lactation had fewer. Factors that influence whether a production stage was positive included for: Gilts, Flow but not Farm; Gestation, Gilt status (Odds ratio = 12.8) but not Farm or Flow; and Lactation, Gestation status (Odds ratio = 27.0) but not Farm, Flow or Gilt status.

Production Stage	By Individual		By Farm	
	No.	% Pos	No.	% Pos
Gilts	706	5.9	46	13.0
Gestation	665	5.7	58	19.0
Lactation	672	1.0	54	7.4
All	2043	4.3	61	23.0

Conclusions and Discussion

Overall, the rate of ascarid infection based on detecting eggs in feces or oral fluids was judged to be low and suggests that the previous, semi-annual deworming program with Safe-Guard was successful overall. The rate in this survey is similar to a previous survey conducted in a system from the same region of the US where 25% of sow herds were found to be positive. Follow up testing of positive farms along with downstream testing of offspring and upstream testing of replacement gilts is in process.

Acknowledgments

Thank you to the operation's service and laboratory staffs for assistance with the sample collection and shipment.

References

1. Shepherd G, et al. 2010. Proceedings of AASV Annual Meeting, Phoenix, Arizona, pp. 341-342.

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Impact of fenbendazole on shedding of *Ascaris suum* eggs in naturally infected gestating sows

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Introduction

The Large Roundworm of swine, *Ascaris suum*, is prevalent worldwide, even in modern swine production.[1-2] Preventive treatment of *A suum* is common practice in the swine industry and with many protocols, sows are treated with anthelmintics prior to farrowing to prevent transmission to their offspring.[3] The timing of treatment prior to farrowing can be quite variable among protocols and may not correctly reflect the time required for cessation of egg shedding, which may allow contamination of farrowing facilities and transmission of ascarid eggs to offspring. The objective of this study was to determine the time to negative (TTN - cessation of shedding of *A suum* eggs) and percent negative (PCTN) of naturally infected sows following treatment with various levels of fenbendazole (FBZ) (Safe-Guard EZ Scoop, Merck Animal Health, Summit, New Jersey).

Materials and Methods

Five studies in three herds (A, B, C1, C2 and C3) were conducted. Feces, collected per rectum, of gestating sows were evaluated using the Modified Wisconsin Sugar-Centrifugal Flotation Method for the presence of ascarid eggs.[4] Samples were considered positive if at least one *Ascarid* egg was observed. Positive sows were randomly assigned to treatment (TX1, 2 or 3) or control (CNT) groups. Using the manufacturer included scoop (~545.5 mg FBZ per scoop) treated sows were administered; 1 scoop for 1 day (TX1), 1 scoop for 3 consecutive days (TX2) or 3 scoops for 1 day (TX3), as a feed top dress.[5] On day 0 and periodically thereafter (varied by study), fecal samples were collected, with a new clean glove per rectum, from sows. TTN was defined as the number of days to the first negative fecal sample, and PCTN as percent of animals negative by end of study.

Survival analysis (log-rank test) was used to compare TTN between groups. Censoring was established for subjects that fell out of the study or reached the end of the study without cessation of shedding. Chi-square with Fisher's Exact Test was used to compare PCTN. Significance between treatments was determined at α=0.05. (SAS® Enterprise Guide 5.1, Cary, NC, USA).

Results

Average TTN ranged from 8.9 to 13.1 days in TX# and 13.4 to 28.2 in CNT. (Table 1) There was a significant difference between TX# and CNT, but not between TX# in all studies. PCTN for TX# ranged from 90 to 100, CNT ranged from 0 to 30 percent. (Table 1).

Conclusions and Discussion

Based on the results from these studies, FBZ remains an effective anthelmintic for *A suum*. It is recommended that sows be treated 14 days prior to movement into a clean farrowing facility when using FBZ for the treatment of *A suum*. This timing should reduce environmental contamination and vertical transmission.

Table 1. Average days (range) to cessation of shedding and percent negative of *A suum* eggs after treatment with various levels of FBZ.

Study	Days*	CNT	TX1	TX2	TX3
A	20	18.0[a]	13.1[b]	11.0[b]	---
		(18-20)	(10-16)	(4-18)	
		25.0[c]	100.0[d]	100.0[d]	
B	14	14.9[a]	9.3[b]	10.6[b]	---
		(10-14)	(8-14)	(8-14)	
		0.0[c]	100.0[d]	90.0[d]	
C1	31	22.3[a]	11.7[b]	11.3[b]	---
		(10-31)	(10-14)	(8-31)	
		30.0[c]	100.0[d]	91.7[d]	
C2	37	28.2[a]	11.5[b]	13.1[b]	---
		(14-37)	(4-22)	(4-22)	
		21.4[c]	96.6[d]	100.0[d]	
C3	21	13.4[a]	9.5[b]	8.9[b]	9.8[b]
		(10-21)	(6-14)	(6-14)	(8-14)
		28.6[c]	100.0[d]	100.0[d]	100.0

* Duration of study in days
[a,b] values within row with different superscripts are significant ($p<0.05$) – Log-rank test
[c,d] values within row with different superscripts are significant ($p<0.05$) - Fisher's Exact Test

Acknowledgments

Farm staff where the studies were conducted.

References

1. Greve JH. Internal Parasites. In: Diseases of Swine. 10th ED. 2012.
2. Pittman *et al*. 21st IPVS. Vancouver, Canada. 2010:806.
3. NAHMS Swine Study. USDA APHIS. 2006.
4. SG-SW-Swine Monograph, Intervet Technical Bulletin. 2007.
5. Batte EG. *Vet Med/Small Anim Clin*. 1978;73:1183-1186.

Impact of fenbendazole on embryonation of *Ascaris suum* eggs in naturally infected gestating sows

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Introduction

The Large Roundworm of swine, *Ascaris suum*, is prevalent throughout the world and results in reduced growth performance and liver condemnations.[1] The major epidemiological characteristics of *A suum* are the direct life-cycle, fecundity of the adult female and the highly resistant nature of the eggs in the environment.[1] Reduction of environmental contamination through sanitation and anthelmintic use are the basis for successful *A suum* control programs. *A suum* eggs are not initially infective when shed and require a period of development from ova to infectious larvae. Benzimidazoles have been shown to be ovicidal to numerous helminths, including fenbendazole (FBZ) on eggs of *A suum*.[2] The objective of this study was to determine embryonation rates of *A suum* eggs shed from naturally infected sows following treatment with various levels of FBZ (Safe-Guard EZ Scoop, Merck Animal Health, Summit, New Jersey, USA).

Materials and Methods

Three separate studies were conducted (A, B, C). Fecal samples from gestating sows were evaluated using the Modified Wisconsin Sugar-Centrifugal Flotation Method for the presence of ascarid eggs.[3] Positive samples were defined as those with at least one ascarid egg. Positive sows were randomly assigned to treatment (TX1, 2 or 3) or control (CNT) groups. Using the manufacturer included scoop (~545.5 mg FBZ per scoop) sows were administered FBZ at: 1 scoop for 1 day (TX1), 1 scoop for 3 consecutive days (TX2) or 3 scoops for 1 day (TX3), as a feed top dress.[4] Large volumes of feces were collected at days 0, 2, 4, 6 and 8 days post treatment (dpt), depending on the study, for evaluation of embryonation. *A suum* eggs were isolated from feces through a series of filtration steps and cultivated in 50ml cell culture flasks in 0.1 N H_2SO_4 in the dark at room temperature for 60 days.[5] Embryonation rates were determined by evaluating 100 eggs at 100X magnification under a light microscope and counting the number embryonated (fully developed larvae) or unembryonated (failure to divide, arrested development, abnormal division) (Figure 1). Percent embryonation (PCTEMB) was expressed as the number of embryonated eggs per 100 eggs for each sample.

Chi-square analysis with Fisher's Exact Test was used to compare embryonation rates between TX# and CNT. Significance between treatments was determined at α=0.05. (SAS® Enterprise Guide 5.1, Cary, NC, USA).

Results

Average PCTEMB ranged from 90.3 to 99.3 for CNT, and in TX# ranged from 47.0 to 74.5 on day 4, 26.6 to 48.6 on day 6, and 29.3 to 38.2 on day 8. (Table 1)

Conclusions and Discussion

Based on these studies, FBZ is ovicidal for *A suum* at three treatment levels and effect on embryonation appears to begin as early as 4 dpt and last at least to 8 dpt. In addition to reducing environmental contamination through decreased shedding of *A suum* eggs, FBZ further reduces the environmental infectious load by reducing embryo development.

Figure 1. Images of *A suum* eggs after 48 days of incubation. Left: unembryonated with unequal division, Right: fully embryonated with viable larvae.

Table 1. Percent embryonation of *A suum* eggs shed from sows after treatment with various levels of FBZ.

Study	DPT*	CNT	TX1	TX2	TX3
A	8	95.4[a]	29.3[b]	30.5[b]	---
B	0	92.9[a]	92.5[a]	85.0[a]	---
	8	95.6[a]	29.4[b]	38.2[b]	---
C	0	98.6[a]	97.8[a]	94.0[a]	97.1[a]
	2	99.1[a]	99.0[a]	93.1[a]	95.9[a]
	4	99.3[a]	74.5[a]	70.9[a]	47.0[b]
	6	90.3[a]	48.6[b]	28.6[b]	26.6[b]

*DPT = days post treatment

[a,b] Values in rows with different superscripts are significant (P<0.05)

References

1. Greve JH. Internal Parasites: Helminths. In: Diseases of Swine. 10th ED. 2012.
2. Friedman PA *et al. Biochim Biophys Acta*. 1980;630:271-278.
3. SG-SW-Swine Monograph, Intervet Technical Bulletin. 2007.
4. Batte EG. *Vet Med/Small Anim Clin.* 1978;73:1183-1186.
5. Boes J *et al. Vet parasitol.* 1998;75:181-190.

Treponema spp. in porcine skin ulcers

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Introduction

It is well recognized that spirochetes can reside in various skin lesions in pigs (1). Recently, spirochetes from a shoulder ulcer, ear necrosis and gingiva were isolated and identified as *Treponema* spp. (2, 3). The objectives of this study were to further investigate the occurrence and diversity of *Treponema* spp. in skin lesions in pigs, to study the appearance of lesions, localization of treponemes and to investigate a possible association between *Treponema* spp. from gingiva and porcine skin ulcers.

Materials and Methods

Scrapings, swabs and biopsies from skin lesions and swabs from gingiva were collected from pigs in 19 Swedish herds Apr 2010 to Dec 2011. Herds, animals, sampling and laboratory methods have been described in detail (4, 5, 6, 7). An overview of used methods is given in table 1. In addition, staining with hematoxylin and eosin (HE) for histopathology was performed. Sanger sequencing and high-throughput sequencing (HTS) was carried out on a subset of samples (4, unpublished).

Table 1. Origin and number of samples investigated and found positive for *Treponema* spp. by each method.

	Origin, No. positive/ No. investigated				
Method	G[a]	SU[b]	EN[c]	BL[d]	FU[e]
	n=139	n=52	n=57	n=5	n=4
PhC[f]	nt[j]	22/48	12/57	1/5	2/4
Culture	7/139	4/52	1/57	0/5	0/4
ISR2[g]	13/134	27/52	26/57	0/2	3/4
WS[h]	nt	34/51	12/54	0/5	2/2
FISH[i]	nt	36/51	32/54	2/5	2/2

[a]Gingiva, [b]Shoulder ulcer, [c]Ear necrosis, [d]Body lesion, [e]Facial ulcer, [f]Phase contrast microscopy, [g]Intergenic spacer region 2-based PCR, [h]Warthin Starry silver staining, [i]Fluorescence *In Situ* Hybridization, [j]Not tested

Results

Spirochetes were detected in all types of skin ulcers and in all herds. *Treponema* positive samples by each method are shown in table 1. Sanger sequencing of ISR2 revealed three main phylotypes; one unknown, *T. pedis* and *T. parvum* (4, 5). Isolates of *T. pedis* and *T. parvum* were obtained, as well as one phylotype most similar to *Treponema* sp. OMZ 840 (5). Identical ISR2 sequences from ulcers and gingiva were found. Histopathology and FISH showed that a majority of the investigated lesions were of chronic nature. Treponemes were located deep between necrotic surface debris and vital granulation tissue. Other bacteria were mostly present on the surface of the ulcers. *Treponema* spp. constituted the main part of bacteria present in the ulcers. Further studies by FISH and HTS suggested *T. pedis* as a dominating species in the examined samples.

Conclusions and Discussion

This study showed that bacteria of genus *Treponema* are frequently present and abundant in ear necroses and shoulder ulcers in pigs, and are also present in facial ulcers and other ulcers on the body. The number of treponemal phylotypes in ulcers and gingiva were higher than previously reported (2, 3, 4). The same treponemal phylotypes can reside both in ulcers and gingiva. In addition, identical ISR2 sequences from both sites may indicate spreading from mouth to skin ulcer. We also detected phylotypes that we did not succeed to culture or species identify. The difference between the results using culture-independent methods compared to culture clearly illustrates the difficulties in isolating treponemes. The histopathological findings of both shoulder ulcers and ear necroses were in agreement with earlier descriptions of similar lesions (8, 9). The predominance of *T. pedis* in results from FISH, ISR2-PCR and HTS of the investigated lesions were in concordance. The role of *Treponema* spp. as part of the etiology of ear necrosis and shoulder ulcers still needs to be clarified, but our results point towards an important role of treponemes in chronic and severe skin ulcers in pigs.

Acknowledgements

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References

1. Cameron, R., 2012. Diseases of swine. 10th Ed. 256-257.
2. Pringle, M. et al., 2009. Vet. Microbiol. 139: 279-283.
3. Pringle, M. et al., 2010. Vet. Microbiol. 142: 461-463.
4. Karlsson, F. et al., 2013. Vet. Microbiol. 165: 402-409
5. Svartström, O. et al., 2013. Vet. Microbiol. 166: 617-623
6. Karlsson, F. et al., 2012. Proc. IPVS. Vol 2: 776
7. Karlsson, F. et al., 2014. Proc. ESPHM. Poster 243.
8. Richardson et al., 1984. Vet. Pathol. 21: 152-157
9. Jensen, H.E., 2009. Vet. Rec. 165: 171-174

Midge bites in Northern Irish pigs

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Introduction

Following a long winter, weather conditions improved in the middle of May 2013 which resulted in warm and sunny days. This favored insect proliferation. Within 4 days of these improved conditions, the first clinical signs of dermatitis were detected during a routine farm visit in 4 pig units.

Materials and Methods

Clinical investigation consisted of a farm visit on Friday, 17th May 2013 and two visits to the slaughterhouse on Monday, 20th May 2013. Further follow up visits were carried out in May and June. Aerial traps were placed in the finishing pens of four different affected farms for 2 weeks. Live specimens of arthropods feeding on the pigs were found and submitted to AFBI Newforge Lane (Belfast) for identification.

Results

Photographic evidence of ante-mortem and post-mortem dermatitis lesions is shown in Figures 1 and 2.

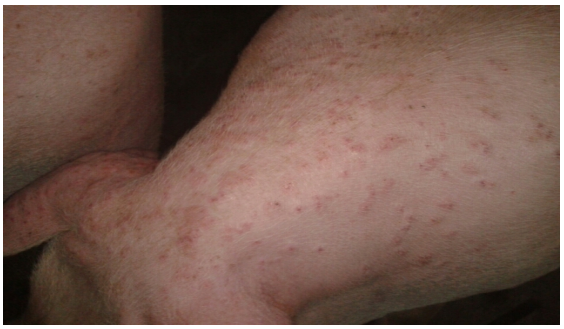

Figure 1. Ante-mortem midge bite lesions.

Sixty two producers were detected with dermatitis caused by midge bites in the slaughterhouse. The prevalence of dermatitis ranged from 30% to 100% of the pigs slaughtered per batch. One producer had 651 kg condemned as a consequence of these skin lesions.

The lesions were multifocal, slightly raised and hyperaemic with 0.5 to 2 cm in diameter widespread all over the body/carcass. Pruritic discomfort was detected in live animals. The incidence of this condition reduced within 3 weeks of the initial outbreak.

The species of insects trapped with the aerial traps were *Musca domestica*, *Drosophila melanogaster*, wood/widow-gnats (family Anisopodidae) and *Culicoides obsoletus*. The live specimens collected were identified as *Culicoides obsoletus*.

One farmer developed the same pruritic dermatitis lesions in the arm and legs.

Conclusions and Discussion

Reports of biting midges in pigs are rare in the literature (1). The importance of parasitic arthropods to pig production depends of the geographical location and the production system used. The direct economic impact of biting midges on pig production has not been studied. Skin blemishes from insect bites at slaughter may lead to unnecessary trimming or even condemnation (2), as seen in this case.

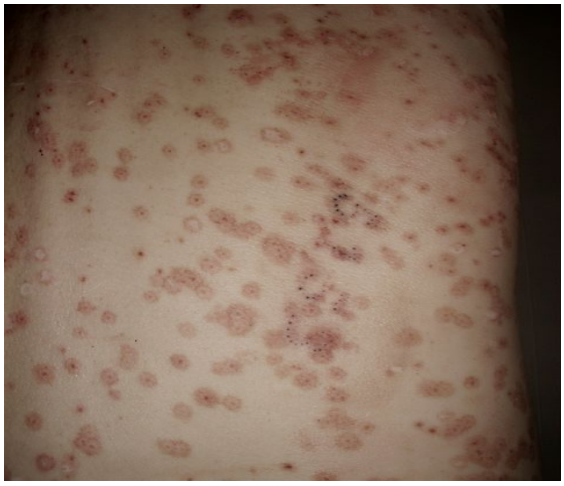

Figure 2. Post-mortem midge bite lesions.

All the affected units contained finishing pens with natural ventilation and no insect screen protection. Control methods of prevention for further occurrence involve slurry treatment with insecticides in order to kill larval stages, regular slurry removal, tidying the surrounding area of the farms, application of insecticides in order to kill adult stages and fitting insect screen netting in the pig houses. The improper use of these products may produce residues in the tissues causing contamination of pork.

Acknowledgments

Sam Clawson (AFBI Newforge Lane, Belfast), William Gilmore and Niall McAuley (DARD).

References

1. SAC 2011. Vet Rec. Nov 12, 520-521.
2. Greve J et al 2012. In Diseases of Swine, 885.

O.145

Hernia umbilicalis - prediction by ultrasound

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Introduction

Hernia umbilicalis is one of the most frequent developmental defects in pigs. Many investigators have hypothesized that hernias are inherited, although there is dispute about the mode of inheritance (1). Omphalitis has also been claimed to cause umbilical hernia in pigs (2), and associations between the presence of umbilical abscess and herniation have recently been seen (3). The purpose of this preliminary study was to determine whether the development of an umbilical hernia could be predicted by the use of ultrasound.

Materials and Methods

The study was carried out at Groenhoej, a research facility owned by The Pig Research Centre.
Groenhoej receives 7 kg piglets every week from one production farm with SPF status. Only the female piglets were used in this study in order to avoid problems with the prepuce in the scanning process. The clinical investigation included a visual and palpatory examination for abnormalities (diverticulum, lump or crust) of the umbilicus before the ultrasound scanning was performed using a MyLab One scanner with a 4 – 10 MHz convex probe. By putting the five-week-old piglet on its back with its head in a tube, it was possible to get a good ultrasonic response of the structures within the umbilicus. No sedation was used. The first ultrasonic view was longitudinal followed by a transverse view. Depending on the structures seen within the umbilicus, pictures or videos were taken, and the piglet was ear tagged. The piglets without any ultrasonic abnormalities were returned to the pen without any remarks or ear tags.

Picture 1. Ultrasound examination of the umbilicus of a five-week-old female piglet.

Later, when the 30 kg pigs were moved to the finisher unit, they were examined for umbilical hernia. If a pig showed signs of umbilical hernia, it was moved to a sick pen with soft flooring. Twice during the finishing period, all the female pigs were examined for umbilical hernia. Also, the slaughterhouse records were checked for remarks concerning hernia.

Pigs that had developed an umbilical hernia were euthanized at 45 to 100 kg, and a full autopsy was performed at the Laboratory for Pig Diseases. Each autopsy report included several pictures showing the external and internal abnormalities of the umbilical hernia.

Results

A total of 496 pigs were examined, and 14 of these developed an umbilical hernia. At five weeks of age, eleven of these were declared ultrasonic positive for developing a hernia later on in life. Three pigs were said to be ultrasonic negative but developed a hernia. Table 1 also shows that 52 pigs were false positive.

Table 1.

	Umbilical hernia +	Umbilical hernia -	$\bar{x}$
Ultra +	11	52	63
Ultra -	3	430	433
$\bar{x}$	14	482	496

The overall prevalence of hernia was 2.8%. However, in the two groups ultra +/- it was respectively 17.7% and 0.7%. The data show a test sensitivity of 0.8 and a specificity of 0.9. The 14 autopsies showed that eight pigs had intestines in the hernia, while six did not; instead, they had cysts of different sizes in the hernia. Only one pig had a non reponibel hernia.

Conclusions and Discussion

Ultrasound can be used to predict if a 7 kg piglet will develop an umbilical hernia later on in life. In this study, ultrasonic positive pigs were 30 times more likely to develop a hernia than the ultrasonic negative pigs.
Nine of the 11 ultrasonic positive pigs had external abnormalities (diverticulum, lump or crust) of the umbilicus at five weeks of age. A close examination of the umbilicus at weaning could therefore be helpful in controlling the problem in many herds.

Acknowledgments

Peter J. Rasmussen and employees at Groenhoej

References

1. Angus K, Young GB. Vet. Rec. 1972; 90:245-247
2. Becker HN. Diseases of swine. 6th ed. Ames, Iowa State University Press 1986; 861-862
3. Pfeiffer M, Ritzmann M, Heinritzi K. Tierärztl. Umschau 61; 2006; 376-379

Efficacy of vaccination against Glässer's disease in the control strategy of porcine respiratory diseases complex (PRDC) in large production system

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Introduction

Haemophilus parasuis (Hps) is the etiologic agent of Glässer's disease and part of the porcine respiratory diseases complex (PRDC) (1). The goal of this study was to improve the control of PRDC through Glässer's disease vaccination and estimation two vaccination protocols (sows and pigs).

Materials and Methods

The study was conducted in a large production multisystem (17,000 sows) which included four farrow-to-finish farms that were positive to PCV2, EU PRRSV, M. hyo, Aujeszky's disease (PRV), APP and Hps. All pigs in the system included this trial were vaccinated against PCV2 and M. hyo with FLEXcombo® at 21 days old and APP with commercial bacterin at 32 and 62 days old as routine. The reproductive herd was vaccinated with EU PRRS MLV, PRV (gE negative MLV) and APP. According to a serological study (BioChek ELISA test kit, Holland) by the end of fattening most of pigs demonstrated antibodies against the OppA protein of Hps which is released by macrophages of the immune system of the pigs in the case that the Hps is causing clinical disease. Traditionally on necropsy many dead pigs demonstrated pneumonia and polyserositis. In this trial were compared two Hps vaccination protocols, for sows and pigs. On farm "A" in two treated barns 2183 pigs were vaccinated with one dose (2 ml) one-shot Hps vaccine (Ingelvac HP-1®, Boehringer Ingelheim) from 14 to 21 days old. On farm "B" the trial included 4821 pigs from sows which were vaccinated with Ingelvac HP-1® at four weeks before farrowing. Pigs from control groups in both farms and treated group of farm "B" not vaccinated (NV) against Hps. Control groups were kept under same management conditions as treated pigs, in different rooms on the same buildings and site (side-by-side). The Chi-square test was applied to analyse the results.

Results

The trial results for both farms show in tables and figure.

Conclusions and Discussion

This results demonstrate good efficacy of Hps vaccination with Ingelvac HP-1® as additional measure for control of primary respiratory pathogens in common strategy of PRDC control. Vaccination against Hps provided more market pigs than NV control groups. Obviously, the choice of the Hps vaccination programme to depend on the specific situation in the farm.

Table 1. Results for farm "A" (Hps vaccinated pigs)

Parameters	HP-1	NV control	X^2
Nursery (28-87 d)			
Number of pigs, n	2183	2290	
Died, %	1.37%	1.75%	1.03
Finishing (88-170 d)			
Number of pigs, n	2153	9769	
Died, %	0.98%	1.48%	3.57
Cull, %	1.07%	1.82%	6.34*
Total losses, %	2.04%	3.31%	9.69*

* The results are significantly different.

Table 2. Results for farm "B" (Hps vaccinated sows)

Parameters	HP-1	NV control	X^2
Nursery (28-87 d)			
Number of pigs, n	4821	7395	
Died, %	1.70%	2.08%	2.29
Cull, %	0%	0.14%	
Total losses, %	1.70%	2.22%	4.01*
Finishing (88-170 d)			
Number of pigs, n	2009	4944	
Died, %	2.29%	1.90%	1.02
Cull, %	1.14%	2.71%	16.16*
Total losses, %	3.43%	4.61%	4.96*

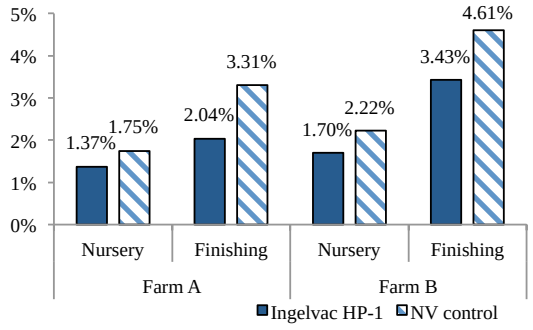

Figure 1. Total losses of pigs in nursery and finishing sites in Hps treated and NV control groups.

References

1. Aragon V et al. 2012. Glässer's Disease. In Diseases of Swine, 10th ed. Wiley-Blackwell: 760-769.

O.147

Comparative field study of Porcilis® M Hyo ID Once, an intradermal *M. hyopneumoniae* vaccine and Ingelvac Mhyo

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Introduction

Different vaccination protocols are used worldwide against *Mycoplasma hyopneumoniae* (M.h) in pigs. They have a common indication: reduction of lung lesions due to M.h infection.
The objective of this study was to compare two vaccines with a different vaccination route (intramuscular and intradermal).

Materials and methods

For this multicentric, comparative, contemporary, controlled and randomized trial, five farms were selected in Western France. Per farm, 2 consecutive batches were included to evaluate two M.h vaccines administered at 3 weeks of age: an intramuscular vaccine (Ingelvac® M Hyo (Ing)), and an intradermal vaccine (Porcilis® M Hyo ID Once (Porc) with IDAL® injector).
Among the farms, 4, 7 and 21 batch managements are represented, with 4 farms of 150 productive sows and 1 farm of 670 sows. The presence of M.h, PRRS, Influenza (SIV), and *Actinobacillus pleuropneumoniae* (App) was serologically tested on 10 pigs at the end of fattening in each farm. With respect to season and batch effects, 1 farm was found negative for M.h, another for SIV, and 1 for App that was also positive for PRRS.
A total of 2593 piglets were randomized into 2 groups at the age of 21 days. This represents 400 to 700 piglets per farm. Groups were tattooed at inclusion. No changes were made to management and animals of the 2 groups were comingled.
At slaughter, a total of 2282 carcass weights were obtained, and 1405 lungs scored for lesions. The number of these data per farm was proportional to the number of piglets included. Carcass weights were used to calculate an average daily weight gain (ADG). Pneumonia lesions were scored on a scale of 24[1]. Pneumonia scars and pleuritis were scored as present or not.
ADG was tested with an ANOVA. The prevalence of lung lesions was analyzed with a Mantel-Haenszel test. Scores of pneumonia were analyzed by a non-parametric test.

Results

The results are summarized in Table 1.
The main criteria tested in this study (ADG, pneumonia lesion prevalence) were not different between groups. Among the secondary criteria, significantly fewer pigs had lung lesion scores >5 and more pigs had lung lesion scores ≤2 in Porc Group. This was mainly due to a higher lesion score in one farm: mean scores were 3.7 and 2.7, respectively, in Ing and Porc groups whereas in other farms scores ranged from 0.2 to 1.2.

Table 1: Summary of observations

	Ing	Porc	p
ADG (g/d)	607	609	0.070
% lesion-free lungs	61.1	64.6	0.117
% mild pneumonia lungs (scores 0, 1 and 2)	83.9	87.6	0.013
% severe pneumonia lungs (score > 5)	6.2	4.0	0.013
Pneumonia score of all lungs	1.2	1.0	0.114
Pneumonia score of affected lungs (score > 0)	3.0	2.7	0.305
Prevalence of pneumonia scars	4.3	4.1	0.980
Prevalence of pleuritis	1.8	3.0	0.149

Discussion

In general, the farms and animals in this study had good respiratory health, compared to other French swine farms. Although the lung lesion observations were indicative of a mild M.h. infection, a significantly higher % of Porc pigs had mild pneumonia lesions compared to Ing pigs, and a significantly lower % of Porc pigs had a severe (>5) pneumonia score than Ing pigs. In addition to the small differences in the observed parameters between vaccines, the farmers appreciated the ease and safety of intradermal vaccination with IDAL® injector and appreciated to not have to manage needles. Overall, IDAL makes vaccination of pigs easier and improves the quality of injections, resulting in better vaccination compliance.

References

1. Madec F. and Kobisch M. (1982) JRP, 14, 506-412

Characterization and differentiation of *M. hyorhinis* and *M. hyosynoviae* field strains for Tailor-Made® vaccine production

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Introduction

Both *Mycoplasma hyosynoviae* (Mhs) and *Mycoplasma hyorhinis* (Mhr) can cause serious arthritic diseases in eight to thirty weeks old pigs and pre-weaning young pigs respectively. During the past three years the isolation of Mhs and Mhr strains from field cases that were submitted to MVP Laboratories for Tailor-Made® vaccine production has increased dramatically. Since there are no commercial Mhs and Mhr vaccines on the world market, Tailor-Made® vaccines have been used more often to prevent big economic losses due to serious lameness problems in both young and older pigs in the US. However, it was not unusual to isolate multiple strains of Mhs and Mhr in an affected pig herd. In order to design a better bivalent vaccine it became a necessity to characterize and differentiate field strains using molecular approaches.

Materials and Methods

100 Mhs strains and 150 Mhr strains that were isolated at MVP Laboratories were examined for species identification by PCR assays[1], soluble protein profiling by mini-gel electrophoresis[2], mycoplasma-specific antigen structure recognition by vaccinated pig's serum using immunoblotting, vlp (variable lipoprotein) gene detection by PCR assays for Mhr[3], and PCR-RFLP fingerprinting for Mhs[4].

Results and Discussion

150 mycoplasma strains isolated at MVP Laboratories from pigs that had polyserositis, polyarthritis, pneumonia, and conjunctivitis were identified as Mhr by PCR assay. 100 Mycoplasma strains isolated from lung, joint, tendon, and leg muscle of older pigs with lameness were identified as Mhs by PCR assay. The protein variability of Mhr and Mhs were detected by SDS-PAGE and Colloidal Blue staining and a dendrogram could be created to show the intra-species divergence among field strains. The variable lipoprotein system of a Mhr strain determines its capacity for surface variation and its ability to evade the host immune system. Table 1 shows the distribution of the five most commonly seen vlp gene patterns among the 150 Mhr strains that were isolated from different organs. Table 2 shows the PCR-RFLP patterns and antigen structures of five Mhs field strains that were isolated from an infected pig herd. Results obtained from this study indicate the existence of intra-species heterogeneity among Mhs field strains as well as Mhr field strains. The molecular approaches developed in this study will not only offer an effective epidemiological analysis of porcine mycoplasma field strains but also help swine veterinarians to select the right strains for the production of Tailor-Made® vaccines to control the lameness problem in both young and old pigs.

Table 1. Five most commonly seen patterns of vlp gene family among Mhr strains

	Vlp gene family						
	vlpA	**vlpB**	**vlpC**	**vlpD**	**vlpE**	**vlpF**	**vlpG**
1	+	–	–	+	+	–	+
2	+	+	–	+	+	+	+
3	+	–	+	+	–	+	+
4	–	–	–	+	+	+	–
5	+	–	+	+	+	+	+

Table 2. Characterization and differentiation of five Mhs strains isolated from an infected pig herd using Western blot and PCR-RFLP pattern

	Mhs–specific antigen (kD)								PCR-RFLP pattern
	125	**115**	**100**	**95**	**93**	**83**	**72**	**48**	
1	--	+	--	+	+	--	+	+	**A1E0**
2	--	+	+	+	--	+	+	+	**A1E2**
3	+	--	+	+	--	--	+	+	**A0E0**
4	--	+	+	+	--	+	+	+	**A1E3**
5	--	+	+	+	+	+	+	+	**A3E2**

References

1. Lauerman. L. H. et al. 1998. In Nucleic acid amplification assays for diagnosis of animal diseases. AAVLD Press, 41-48.
2. Lin. B. C. et al. 2011. Proc AASV Ann Meet. Phoenix, Arizona. 2011:231-236.
3. Citti. C. et al. 2000. J. Bacteriol. 182:1356-1363.
4. Kokotovic. B. et al. 2002. Vet Microbiol. 85:221-231.
5.

Safety and efficacy of a novel live vaccine for *M. hyopneumoniae*: Vaxsafe® MHP

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Introduction

Vaxsafe® MHP is a live attenuated vaccine intended for the protection against *M. hyopneumoniae*(Mh) infection in pigs. Vaxsafe® MHP has been developed in two formats: wet frozen (WF) and freeze dried (FD). A number of *in vivo* studies have been completed which assessed safety, efficacy and colonization of Vaxsafe® MHP in target animals following intra-nasal administration. In this paper two regulatory based studies will be presented.

Materials and Methods

Study 1: Six-week-old healthy Mh free commercial pigs were used in this study. A dose of $10^{9.3}$ ccu/mL of Vaxsafe® MHP (WF) was tested for safety. The regulatory guidelines for live vaccines stipulate that safety of a 10 times (10X) overdose should be tested. The dose of $10^{9.3}$ ccu represents a significant overdose of the vaccine . This study also investigated efficacy of Vaxsafe® MHP (WF) at doses of $10^{5.7}$, $10^{6.9}$ and $10^{8.0}$ ccu/mL. Positive and negative control groups were included. At 22 days post vaccination (DPV), all groups except the negative control group were challenged by intra-nasal spray with Mh wild type strain.

Study 2: Three-week-old healthy Mh free commercial pigs were used in this study. A dose of $10^{5.0}$ ccu/mL of both the WF and FD formats of Vaxsafe® MHP were tested for efficacy. A group of pigs given a commercial inactivated vaccine as well as positive and negative control groups were also included in this study. Groups vaccinated on DPV-0 as well as the positive control group, were challenged by intra-nasal spray on two occasions (DPV-24 and -67) with Mh wild type strain. Post mortems were conducts at DPV-102. Safety of Vaxsafe® MHP (FD) was also investigated at a dose of 10^{8} ccu/mL. This dose was at least 10-fold higher than the expected maximum release titre (MaxRT) of the vaccine.

Results

Study 1: The safety profile indicated that despite the high overdose, Vaxsafe® MHP did not result in any deaths. Analysis of data revealed that no significant difference in body temperature and weight gain existed between the vaccinated (safety group) and negative control group. At post-mortem, 2/10 pigs had a small lung lesion (not significant ($P>0.05$) compared with the negative group). Also, the lesions did not translate to any adverse effect on weight gain nor on any clinical signs. Tracheal colonization was investigated by taking swabs from the upper, middle and lower regions of the trachea at post-mortem (DPV-59). Swabs were only collected from the safety (unchallenged) group. Therefore, Mh detected would be due to Vaxsafe® MHP. Results obtained from PCR analysis showed that Vaxsafe® MHP was present in the trachea of 60% of pigs in this group. This indicates that Vaxsafe® MHP is capable of colonizing the respiratory passages of vaccinated pigs for at least 8 weeks post vaccination.

In the efficacy part of the study 4/10 pigs in the unvaccinated, challenged control group presented with significant lung lesions (~30% of the lung). No pigs in either the $10^{5.7}$ or the $10^{6.9}$ ccu/dose vaccinated groups showed any lesions. In the $10^{8.0}$ ccu/dose vaccinated group, 1/10 pigs presented with a minor lung lesion (~7% of the lung). PCR analysis of nasal swabs taken at DPV-21 prior to challenge showed presence of live Vaxsafe® MHP in 60% to 70% of pigs from each of the vaccinated groups.

Study 2: The unvaccinated challenged group presented with lung lesions in 100% of pigs with a lung lesion score (planimetric method) of 9.2%. At a dose of $10^{5.0}$ ccu lung lesions were detected in 30% (WF) and 10% (FD) of vaccinated pigs post challenge with average lesion scores of 0.7% and 1.7% respectively. Presence of lung lesions in the commercial inactivated vaccine group was detected in 60% of pigs with an average lesion score of 8.5%. A direct comparison between the commercial inactivated vaccine group and the $10^{5.0}$ ccu/dose Vaxsafe® MHP group showed a significant reduction in both the incidence of lung lesions (60% to 10%) and in the severity of lung lesions (8.5% to 1.7%), in the Vaxsafe® MHP vaccinated group. The 10^{5} ccu/mL dose of Vaxsafe® MHP (FD) provided a protective index of 90% (incidence) and 81% (severity) of lung lesions. The commercial inactivated vaccine provided a protective index of 40% (incidence) and only 8% (severity) of lung lesions. All indices were calculated in comparison to the positive control group.

Conclusions and Discussion

Administration of an overdose of Vaxsafe® MHP did not result in pig mortalities, any adverse clinical signs, nor any significant increase in the incidence or severity of lung lesions compared to the negative control group. A comparative study with a commercial inactivated vaccine showed Vaxsafe® MHP to offer a higher level of protection following challenge. Overall, these two studies indicate that Vaxsafe® MHP to be a safe and efficacious live vaccine against Mh infection.

Evidence for association of emerging parvoviruses in pigs with cases of PCV-associated disease

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Introduction

Parvovirus infection in pigs (PPV) is typically associated with reproductive failure and increased numbers of mummified fetuses in breeding age females. Recently several PPVs have been found to circulate in the global pig population (1). To differentiate the known PPV from the emerging PPVs, a consecutive numbering system was implemented. PPV1 is the PPV strain associated with reproductive failure whereas little is known about the pathogenic potential of the other PPVs designated as PPV2, PPV3, PPV4 and PPV5. The objective of this project was to investigate the prevalence of PPVs in cases of porcine circovirus associated disease (PCVAD) in the USA collected from 1998 to 2013.

Materials and Methods

Archived lung tissues (n=164) and serum samples (n=588) were tested with several PPV-type specific PCR assays. In addition, the presence of specific genotypes of porcine circovirus type 2 (PCV2) was determined by ORF2-based differential PCR assays. Among all samples, 280 samples were and 478 were not associated with PCVAD cases. The data was organized by time intervals and summarized by PCVAD pattern occurrence including 1998-2005=sporadic; 2006-2007 severe; 2008-2011=sporadic, PCV2 vaccine; 2012-2013 increased PCVAD, PCV2 vaccine).

Results

As expected, PCV2a was the only PCV2 subtype identified prior to 2006, PCV2b was first detected in samples from 2006 and mPCV2b was first detected in samples from 2012. Interestingly, PPV2 and PPV3 were first detected in 1998, PPV4 was first identified in 2001, and PPV5 was first identified in 2006 (Table 1).

Table 1. Distribution of PPVs in different time intervals from 1998 to 2013. The highest percentage of each PPV type in a given time interval is in bold font.

Time interval	PPV1	PPV2	PPV3	PPV4	PPV5
1998-2005	**11**	**46**	4	3	0
(n=86)	**12.8%**	**53.5%**	4.7%	3.5%	
2006-2007	10	21	7	1	8
(n=121)	(8.43%	17.4%	5.8%	0.8%	6.6%
2008-2011	3	11	4	2	3
(n=56)	5.4%	19.6%	7.1%	3.6%	5.4%
2012-2013	38	189	**49**	**17**	**13**
(n=469)	8.1%	40.3%	**10.4%**	**3.6%**	**2.8%**

The results indicate that there has been since 1998 and continues to be a high prevalence of PPV2 viremic pigs (27.4%, 161/588). Among the 274 PPV2 positive pigs, 45.9% (126) were also positive for PCV2 indicating a slight correlation (r=0.1287, p=0.0005). The obtained data for all PPVs on lung tissues are summarized in Figure 1.

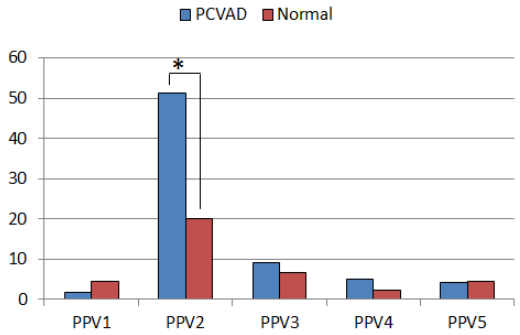

Figure 1. Association of PCVAD and PPVs in lung tissues (% of cases positive for PPV). There is a significantly higher proportion of PCVAD cases positive for PPV2 compared to non-PCVAD cases ($p<0.05$).

Conclusions and Discussion

It is currently unknown if the emerging PPVs are associated with any disease conditions in pigs; however, as PPVs are lymphotrophic they may affect immune responses resulting in increased susceptibility to other diseases. Studies to better understand the pathogenesis of these PPVs are needed and are underway.

Acknowelegements

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References

1. Xiao et al. 2013. Infect Genet Evol 16:369-376.

O.151

PCV2 infection in pigs in Vietnam

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Introduction

Porcine circovirus type 2 (PCV2) is the major swine pathogen for the diasease with Porcine circovirus associated (PCVAD) including post-weaning multisystemic wasting syndrome (PMWS). PCV2 can infect to any age and has been found in serum, plasma, faecal and tonsillar swabs (Caprioli A. et al, 2006). PCV2 may circulate in pigs which were suggestive or not of PMWS (R. Larochelle, 1999). The infection status of PCV2 in pig herds influence the effectiveness of virus transmission control. The objectifs of the study was to investigate the PCV2 infection patterns and to determine the suitable sample type for PCV2 diagnosis by PCR, and that could be used for the PCV2 control in the field.

Materials and Methods

The 107 samples (lymph node, serum and feces) collected from 91 pigs with or without the clinical symptomes causing by PCV2 in Vietnam for PCV2 diagnosis by PCR. The samples were analysed by PCR using specific primer pairs PF1 5' TTGCTGAGCCTAGCGACACC 3' and PR1 5' TCCACTGCTTCAAATCGGCC 3' for PCV1, and PF2 5' CCGCACCTTCGGATATACTG 3' and PR2 5' TAGGTTAGGGCTGTGGCCTT 3' for PCV2, described by (Larochelle et al., 1999) with products size expected is about 349 bp and 263 bp for PCV1 and PCV2, respectively.

Results

The PCR results showed 64/91 pigs that was positive with PCV2 and only 1/91 that was positive with PCV1. The frequency of positive samples was 86.49 %, 71.43 % and only 55 % in pigs with clinical PMWS, PDNS, and normal, respectively. The prevalence of PCV2 infection was 57.14 % in nursery , from 65 to 100 % in weaning, from 68.42 to 100 % in growing and 55.56 % in sow. The frequency of positive samples with PCV2 in feces was 69.39 %, more than those in serum (59.18 %).

Conclusions and Discussion

Puvanendiran (2008), had found the positive samples with PCV1 in 2.5 % (20/800). With 64/91 pigs (70.33 %) that was positive with PCV2, the results of the study was not far from those of J. Long et al., 2009, in China, with 61.3 % (117/191).

Table 1. Frequency of PCV2 DNA positive by clinical symptomes

Clinical symptomes	Normal n=40	Wasting n=37	With skin lesions n=14
PCR positive	22 [(a)]	32[(b)]	10[(ab)]
%	*55 %*	*86.49 %*	*71.43 %*

The analysis showed the significative difference (P < 0,05) of frequency of positive samples with PCV2 DNA between groupes of pigs with or without clinical symptomes. Shibata et al., 2003, had demonstrated PCV2 DNA by PCR in the whole blood, nasal swabs and feces from the field samples. The results obtained in the study showed that the feces samples seems be is more important epidemical material than the serum to demonstrate the transmission of PCV2 between the animals. The lymph node is the most valuable sample types for the PCR diagnosis of PCV2 infection (Caprioli và ctv (2006), but taking the feces sample is most suitable than other to diagnosis of PCV2 infection in the field, in particular in the case of the sows.
In the field, PCV2 infection could occur in any stage of ages of animal and could be detected in several biological samples. However the feces is considered most suitable to epidemical investigation of PCV2 infection status.

Acknowledgements

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References

1. Caprioli A. et al., 2006. *Research in Veterinary Science*, 81 (2006) 287–292.
2. Larochelle R. et al., 1999. *Journal of Virological Methods*, 80 (1999) 69–75.
3. Long J., 2009. *Report of Shanghai veterinary research institute*, (7): p1-5.
4. Puvanendiran S. et al., 2008. *American Association Of Swine Veterinarians*: 131-135.
5. Shibata I. et al., 2003. *J. Vet. Med. Sci*., 65(3), 405-408.

O.152

Utilization of colostrum for evaluating the PCV-2 status of sow herds

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Introduction

The role of vertical transmission of porcine circovirus type 2 (PCV2) in the overall ecology of this potentially devastating disease is not well-defined. Several studies have demonstrated that PCV2 can be transmitted from dam to offspring in utero and while the pigs are suckling via contact with the dam or ingestion of colostrum or milk.[1] More commonly, pigs become infected with PCV2 in the late nursery/early finishing phase of production and vaccination of young pigs ahead of this exposure has been very successful. The impact of early infection before or during the period of vaccination is less clear. A recent study indicated that viremia at the time of vaccination impaired growth and resulted in a persistent viremia.[2] The study reported here evolved from a suspected lack of expected efficacy case in growing pigs that were vaccinated with Circumvent® PCV M. A detailed investigation indicated that exposure and subsequent infection of the pigs was occurring at the sow farm during the period of vaccination. Sows, suckling pigs and recently weaned pigs were viremic. In addition, colostrum was found to contain PCV2. The sow herd was then vaccinated twice, 4 weeks apart with Circumvent® PCV M and the performance of the offspring improved immediately. Colostrum was still positive at 2 months after the sow herd vaccination but was negative by 4 months. Based on this investigation, all other managed sow farms (n = 17) were evaluated for the presence of PCV2 in colostrum.

Materials and Methods

For the initial survey, 30 colostrum samples were collected from each sow farm: 10 first litter gilts, 10 parity 2-3 sows and 10 parity ≥4 sows. The samples were collected while the dam was farrowing or within 12 hours after farrowing but most often after the birth of 2-3 pigs. The samples were collected in standard blood tubes and kept refrigerated until shipment. The samples were tested for PCV2 by PCR at the Iowa State University Veterinary Diagnostic Laboratory. Results are reported as the cycle time (CT); >37 is considered negative and the lower the cycle time, the more PCV2 is present. At the lab, the samples were pooled with 5 samples per pool by parity (6 tests per farm). After the survey was completed, the decision was made to vaccinate several of the sow herds. Follow-up testing was done at 8 of the sow farms using the same sampling and testing protocol.

Results

The following table summarizes the findings of the initial survey. The farms tended to fall into four categories as described in the table below. At the time of the survey, none of the sow farms were experiencing reproductive problems. However, the offspring from two farms (one high and one moderate) were experiencing PCV2 related disease in finishing and the offspring from two other farms (one high and one moderate) were experiencing slow starting pigs in the nursery. Improvement in performance was observed after sow herd vaccination and follow-up testing found PCV2 was no longer present in colostrum by 3-6 months later.

Initial Classi-fication	No. farms	No. (%) Positive Pools	Lowest Pool CT	Avg. CT Pos. Pools
High	3	5-6/6 (>85%)	28.2	32.4
Moderate	3	2-3/6 (33-50%)	31.2	34.8
Low	5	1/6-7 (14-16%)	32.1	34.8
Negative	7	0/6 (0%)	>37	>37

Conclusions and Discussion

The data reported here is part of a larger study that is investigating the dynamics of PCV2 infection in sow herds, especially the infection status of incoming gilts. This case study demonstrates the potential use of colostrum for elucidating the PCV2 status of sow herds to help better understand the role of early pig infection and to guide decisions regarding sow herd vaccination.

Acknowledgments

Thank you to the farm staffs that assisted with collecting the colostrum samples.

References

1. Shen H, et al. 2010. Prev Vet Med. 97 (3-4):228-236.
2. Thacker B, et al. 2013. Proc AASV Annual Meeting, San Diego, CA, p. 217.

Investigations to better understand the clinical importance of PAstV infection in pigs

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Introduction

Many astrovirus (AstV) species are associated with enteric disease in a variety of hosts including humans and pigs (1). In pigs, five genotypes of porcine AstV (PAstV) have been recognized; however, little is known about the importance of PAstV including their pathogenic potential. In recent years several AstV species have been associated with extra-intestinal manifestations and there are indications that systemic AstV infection also occurs in pigs (2). The objective of this study was to investigate the PAstV nucleic acid prevalence rates in fecal samples in US pigs and to characterize the main genotypes. In addition, a PAstV serology assay was developed to further investigate prevalence rates.

Materials and Methods

Five-hundred-and-nine fecal samples were randomly chosen from routine diagnostic cases submitted during 2011-2012 to the Iowa State University Veterinary Diagnostic Laboratory. In most cases the samples were from pigs with a history of diarrhea. These samples originated from 255 farms located in 19 U.S. states. Two different PAstV genotype specific real-time PCR assays were developed and utilized to test all samples (3). Selected PCR positive samples were further sequenced and sequences were aligned with other AstV sequences available through GenBank. Moreover, infectious clones of PAstV genotype 3 and 4 were constructed and tested *in vitro*. The capsid of PAstV4 was expressed in a plasmid and the resulting protein was utilized as coating antigen in an ELISA.

Results

All of the five known PAstV genotypes 1-5 were found to circulate in pigs in the USA (Table 1). Coinfection of a single pig with two or more PAstV genotypes was frequently observed. A high overall prevalence of 63.9% (325/509) of PAstV RNA positive samples was detected, with 97.5% (317/325) of the PAstV RNA positive pigs infected with PAstV4. Further genomic sequencing and characterization of the selected isolates revealed low sequence identities (49.2% to 89%) to known PAstV strains indicating the presence of novel subtypes of PAstV2, PAstV4 and PAstV5. The first complete genome of a PAstV3 isolate was obtained and showed identities of 50.5% to 55.3% to mink AstV and the novel human AstVs, whereas identities with other PAstV genotypes was 38.4% to 42.7%. Phylogenetic analysis revealed that PAstV1, PAstV2 and PAstV3 were more closely related to AstVs from humans and other animals than to each other, providing evidence of possible cross-species transmission in the past and the zoonotic potential of these PAstVs. Infectious PAstV clones were constructed and used to transfect ST cells, PK15 cells, and IPEC J2 cells. Preliminary results indicate that *in vitro* transfection was successful (increasing RNA levels in infected cells through serial passage). The serology assay was found to be able to detect PAstV4 seroconversion in pigs after exposure to PAstV4 positive fecal material.

Table 1. Prevalence of PastV genotypes 1, 2, 3, 4 and 5 in fecal samples obtained from U.S. pigs.

Age group	PAstV1	PAstV2	PAstV3	PAstV4	PAstV5
Suckling	3/79	4/79	3/79	21/79	3/79
Nursery	14/181	19/181	1/181	134/181	21/181
Grow-finish	11/148	4/148	0/148	107/148	1/148
Mature	0/7	1/7	0/7	2/7	0/7

Conclusions and Discussion

Knowledge about the prevalence, clinical significance and molecular characterization of PAstVs is still limited. The difficulty in propagating PastVs in cell culture has limited progress in advancing knowledge on virulence and pathogenesis. Our group has made progress in developing a PastV infectious clone for use in experimental inoculations and an update will provided. In the present study, we found a high prevalence rate of PastV in U.S. pigs with PastV4 as the main genotype (3). The clinical relevance of PAstV infection in pigs remains unclear as healthy pigs can harbor PAstV and clinical symptoms have been reported mainly in piglets that are also infected with other enteric pathogens such as rotavirus, coronavirus and calicivirus (4). Future studies will reveal if PAstV, which appears to be ubiquitous in the global pig population, is capable of inducing lesions and disease in pigs.

Acknowelegements

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References

1. Bosch et al. 2011. Virus Taxonomy 9:953-959.
2. Brnić et al. 2013. Infect Genetit Evol 14:258-264.
3. Xiao CT et al. 2013. J Gen Virol 94:570-582.
4. De Benedictis et al. 2011. Infect Genet Evol 11:1529-1544.

Serological investigation of porcine pseudorabies on farms in Jiangxi province and nearby regions in China

Dedicated to the Memory of Xianjin Yang

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Introduction

Porcine pseudorabies (PR), also known as Aujeszky's disease, is an infectious disease caused by pseudorabies virus (PRV) in pigs. Although PR has been eradicated in many pig-producing countries [1], a pandemic outbreak of this disease appeared throughout China in 2012, especially in the north, causing high loss in suckling piglets. ELISA for testing of PRVgE antibody is a convenient way to detect wild-type PRv infection because it can distinguish wild virus from vaccine strain and it has been widely used in many countries where PR is still epidemic.

The objective of this study is to demonstrate the PR prevalence in pig farms in Jiangxi Province and nearby region from 2010 to 2012 using the PRVgE ELISA and relating it to the recent outbreaks.

Materials and methods

A total of 14808 serum samples were collected (6842 samples from sows; 3284 samples from piglets; 4082 samples from growing pigs; 600 serum samples from boars) from January 2010 to December 2012 from farms in Jiangxi Province and nearby region. PRVgE antibody was tested by using ELISA kit from IDEXX and positive rate of wild-type PRv was calculated.

ResultsThe positive rate of PR-gE antibody in Jiangxi and nearby region in 2010, 2011 and 2012 were 10.06%, 32.88% and 40.57% (table1), which showed an obvious increase of wild-type PRv infection during these years.

Table1 PRV-gE antibody positive rate (2010-2012)

	2010	2011	2012
All samples	2991	5204	6713
Positive samples	301	1711	2724
Positive rate %	10.06	32.88	40.57

It was also found that the positive rate in piglets was much higher than sows, and the positive rate for Boars increased sharply after 2011. The positive rate in growing pigs has also increased from 2010 to 2012 (picture 1).

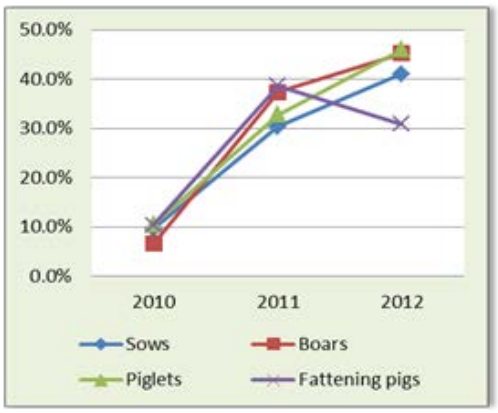

Figure 1. Positive rate of PR-gE antibody in different herds (2010-2012)

Conclusion and Discussion

For many years, the prevalence of this disease was relatively low with only sporadic cases. The outbreak of PR in 2012 caused high loss in many Chinese farms and many farmers and veterinarians were confused. The epidemiology of PR in Jiangxi province and nearby region from 2010 to 2012 was shown via serological investigation, which suggested that as the positive rate of wild-type PRv was getting higher, the incidence of outbreak of this disease also increased. This study also indicates that serological investigation for PR is important not only in the eradication program but also in the prevention of the reoccurrence of PR on swine herd.

Reference

1. Mettenleiter TC et al (2008), Animal viruses: molecular biology. Page 531.

O.155

The African swine fever virus rp30 ELISA detects antibody in serum and/or oral fluid specimens

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Introduction

African swine fever virus (ASF) is of major concern because of its high mortality rate, its severe economic impact, and its recent rapid geographic expansion. Prevention and control of ASFV is complicated by the absence of effective vaccines. Serology is useful for ASFV diagnosis and surveillance because antibodies are a definitive indication of infection and are detectable for a prolonged period of time (1).

An earlier study showed that ASFV antibodies could be detected in oral fluids (2) and suggested that oral fluids could serve as a suitable specimen for ASFV surveillance. The long-term aim of this line of research is to continue the development of an ASFV antibody ELISA. Several ASFV proteins have been identified as highly antigenic, e.g., p72, p30 and p54, and others (3). In this study, a recombinant p30 (rp30) polypeptide was selected evaluated in an indirect ELISA format.

Materials and Methods

Plasmid containing the p30 gene was over-expressed in *E. coli* and the recombinant His-tagged fusion p30 polypeptide was purified from extracts of *E. coli* using nickel-affinity chromatography. ELISA conditions, e.g., rp30 coating/blocking conditions, sample/conjugate dilutions, buffers, and incubation times were optimized for antibody detection in serum and oral fluid specimens. Serum and oral fluid antibody-positive samples were generated by experimental inoculation of 9 pigs with an attenuated ASFV isolate (NHV) that produces chronic infection. Paired oral fluid and serum samples were sequentially collected from individual pigs over days post inoculation (DPI 0, 6, 12, 15, 19, 26, 33, 40, 47, 54, and 61) using methods previously described (4).

ELISA specificity was evaluated using samples of known ASFV-negative status, i.e., 200 oral fluid and 200 serum samples submitted for routine diagnostic testing at the Iowa State University Veterinary Diagnostic Laboratory from swine herds in the U.S.

Results

The mean ASFV rp30 antibody ELISA ODs for serum and oral fluid are shown in Figure 1. IgG antibody was detected by DPI 12 in both serum and oral fluid specimens. The evaluation of known ASFV negative field samples showed specificities of 99.5% and 100% for serum and oral fluid samples, respectively.

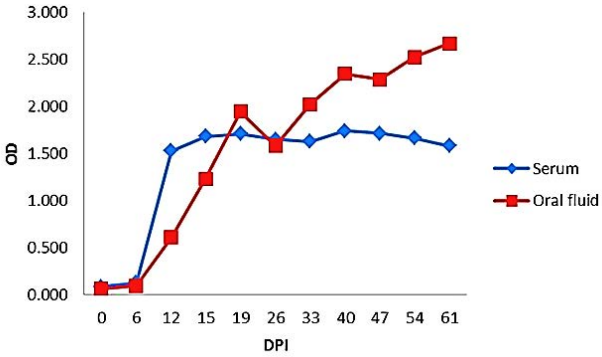

Figure 1. Mean optical density for serum (♦) and oral fluid (■) specimens collected over time from 9 pigs inoculated with ASFV isolate NHV

Conclusions and Discussion

A preliminary assessment of the ASFV rp30 antibody ELISA showed that the test detects ASFV antibodies in either oral fluid or serum samples, yet is highly specific for both specimen types. In fact, both serum and oral fluid samples from the same pig(s) could be run on the same plate simultaneously. Given the increased surveillance efficiency provided by oral fluid sampling (5) and the ability to corroborate results using serum samples, the ASFV rp30 antibody would be a highly useful under conditions that warrant ASFV surveillance.

Acknowledgments

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References

1. Sánchez-Vizcaíno JM, Arias M. 2012. African Swine Fever *in*: Diseases of Swine, pp 396-404.
2. Mur et al., 2013. Vet Microbiol 165: 135–139
3. Cubillos et al., 2013. Virus Res 173: 159-167.
4. Kittawornrat et al., 2010. Virus Res 154:170-176.
5. Olsen et al., 2013. J Vet Diagn Invest 25:328-335.

Detection and isolation of PorPV-like in Mexican bats

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Introduction

Bats have been implicated in numerous new emerging infectious diseases, through its role as important reservoirs for many viruses that have the ability to cross species barriers and infect humans and/or domestic and wild mammals (*1*). In the American Continent, retrospective analysis showed the presence of two different new paramyxoviruses, from the genus *Rubulavirus*, one of them is Mapuera virus (MprPV) isolated from the salivary glands of an apparently healthy a fruit bat (*Sturnira lilium*) in Brazil in 1979 and the other is the Porcine rubulavirus (PorPV) a novel paramyxovirus has been identified as the causative agent of the disease in pigs named "blue eye disease" (BED) that emerged spontaneously in Mexico in the early 1980s *(*2,3*)*. Sequence and genome organization comparison indicated that PorPV-LPMV is closest related to MprPV and phylogenetic studies suggested that the PorPV-LPMV could have been transmitted from an unknown wild reservoir *(4)*. This work was aimed to determine if bats could be a virus host and/or reservoir of PorPV in nature.

Material and methods

A total of 20 bats (four *Pteronotus parnellii* (insectivorous)*;* twelve *Artibeus jamaicensis*-frugivorous; two *Desmodus rotundus*- hematophagus and four *Balantiopteryx plicata*-insectivorous) were caught from two different refuges N. 18° 42´ 32.4" W 99° 14´ 14.1" at an altitude of 1004 MASL; and N. 18° 46´ 14.33" W 98° 51´ 55.95" located at 1403 MASL at locations in a Central Zone of Mexico (Morelos), where spontaneous outbreaks of BED occur. Captured bats were collected under SEMARNAT DGVS permit FAUT-0211. All animal research was performed in accordance with the ethical guidelines of the Animal Care and Ethical Committee of the *CENID-MA-INIFAP* in Mexico City. The Serum samples were tested by serum-virus neutralizing analysis to PorPV and immunoperoxidase monolayer assay. For virus isolation, frozen brain tissue samples were homogenized approximately 10% (w/v) in minimal essential medium and inoculated onto MDCK monolayers. The cell cultures were observed for 96 h for presence or absence of cytopathic effect (CPE) and analyzed by immunofluorescence test. Total RNA was extracted using TRIzol LS reagent according to the manufacturer's protocol. Primers for RT-PCR amplification were designed to amplify a conserved 613 bp region of PorPV-HN gene of (GenBank accession: S77541) *(3)* and compared with previously published sequences of HN of PorPV uploaded in the GenBank. PCR gel products were purified and sequenced in both directions using an ABI Prism 3100 DNA sequencer gene analyzer. Multiple sequence alignments were performed using the ClustalW v. 1.8.

Results

Serological evidence revealed the presence of neutralizing antibodies to PorPV between 1:20 to 1:1280 in eight of the bats (two *Pteronotus parnellii;* three *Artibeus jamaicensis;* two *Desmodus rotundus* and one *Balantiopteryx plicata.* Fragments of the HN gene were amplified from fifteen brain tissue samples by RT-PCR and from four of them; PorPV antigen was detect in infected cell cultures by immunofluorescence analysis (two *Artibeus jamaicensis* and two *Desmodus rotundus*. The sequence analysis of PCR products showed that, over the 600 nucleotide region analyzed, all the sequences isolated from bats displayed a 99.7% amino acid sequence identity to the original PorPV-LPMV strain and a 100% identity to the antigenic variant PAC4, suggesting that they belonged to the same strain.

Conclusion and Discusion

In this study, we report the first isolation of a Porcine rubulavirus-like as describe above and the presence of a fragment of PorPV-RNA in bats, that was isolated from brain and infected cell cultures. These findings suggested that bats could act as a reservoir of PorPV in nature. Detection of antibodies against this virus in different specimens of bats will be highly important to our further understanding of PorPV evolution, mechanism of cross-species transmission and pathogenesis in different animal species.

Acknowledgments

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References

1. Calisher *et al* 2006. Clin Microbiol Rev. 2006; 19:531-45.
2. Moreno-Lopez *et al*. Arch Virol. 1986; 91:221-31.
3. Wang *et al*.2007. Arch Virol. 152:1259-71.
4. Svenda M. [Doctoral thesis]. 1998. SLU. Uppsala, Sweden.

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Epidemiological survey of three diarrhea related viruses in China east coast from 2012 to 2013

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Introduction

Porcine epidemic diarrhea virus (PEDV), porcine transmissible gastroenteritis virus (TGEV) and porcine rotavirus (PROV) are three major viral agents to cause suckling pig diarrhea. Before 2011, these pathogens had been in endemic circulation in Chinese farms for more than 20 years, after last outbreak in beginning of 1990s. The new outbreak of sever newborn piglets diarrhea started from early spring of 2011 in China, with mixture infection of TGEV and PEDV. However, PEDV occupied the predominant pathogen in 2011, confirmed by RT-PCR diagnosis. It causes average 60-100% mortality in the herd infected within 7 days old.

Materials and Methods

All fecal and intestine samples are collected from field cases with PEDV-like diarrhea out break for east coast of China during 2012-2013. RT-PCR diagnoses were conducted to detect virus gene, targeting on PEDV M gene, TGEV M gene and PROV group A vp7 gene, separately.

The epidemic data were extracted from Hangzhou Beta Veterinary Diagnostic Laboratory (Beta VDL) database and further analyzed below.

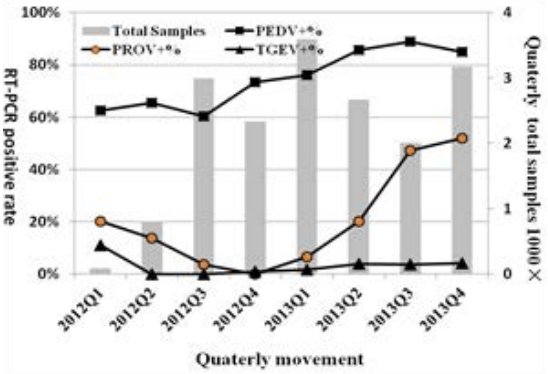
Results

Generally, PEDV is still the major pathogen in past 2013 to cause new born pig viral diarrhea. TGEV and PROV take the position as minority. However, RT-PCR positive rate of all three viruses climbed in 2013, comparing to 2012. Interestingly, PROV infection appeared much more wide in 2013 (41.44% in average), four times higher than 2012.

Table 1. General statics of viral diarrhea infection from 2012-2013 along east coast of China

Samples		PEDV	TGEV	PROV
2012	Samples tested	5283	702	231
	Positive rate	65.70%	0.85%	7.79%
2013	Samples tested	9357	1422	666
	Positive rate	83.01%	3.16%	41.44%

As shown in fig 1, in past 8quaters, PEDV field infection kept climbing trend, from 62.5% (2012Q1) peak to 88.8% (2013Q3). TGEV remained in very low infection rate, despite seasonal changing. Although PROV infection is quite common historically in pig farms, this virus infection performed very different pattern in 2013. The RT-PCR positive rate upraised rapidly from 2013Q1, and peaked at 2013Q4. This information strongly warmed the industry to watch this virus more closely in coming year.



Conclusions and Discussion

PEDV, TGEV and PROV are still major viral pathogens infect suckling pigs. Among three of them, PEDV is still the predominant cause in field cases. However, PROV showed much wider infection, along with PEDV outbreaks. This trend may be related to over-use of feed-back in the field to shut-off PEDV outbreak.

Molecular detection of emerging strains of PEDV in Peru

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Introduction

Porcine epidemic diarrhea (PED) is an enteric disease caused by a virus belonging to Coronoviridae family, and named porcine epidemic diarrhea virus (PEDV). PED is considered an acute, highly contagious enteric disease, characterized for aqueous diarrhea, vomiting and severe dehydration, which could reach to 100% of mortality in under 7 days old piglets (1,2,3). PEDV has been considered an exotic disease for Peru, because there was no evidence of its presence. However, five herds from Lima suffered from watery diarrhea, vomiting and severe dehydration in piglets; mainly in under twenty-one days old, where it caused almost 100% of morbidity and mortality, and it was suspected to be caused by PEDV. Therefore, the objective of this study is to report the presence of porcine epidemic diarrhea virus (PEDV) in Peruvian pig farms.

Materials and methods

It was collected 25 watery feces and/or intestines from piglets between five and twenty-one days old, belonging to five herds with clinical sings suspected of being infected by PEDV in northern Lima, Perú. We use fast immunochromatographic test (Bionote, Korea) in order to establish a first diagnostic for PEDV. Positive samples were processed using a specific real time RT- PCR for detection of S gene of PEDV in an in-house protocol of Laboratory of Microbiology and Parasitology of Faculty of Veterinary Medicine from National University of San Marcos. Positive control was provided kindly by Dr. Miguel Quevedo (SENASA – Peru) obtained from USDA-APHIS, USA. We have used RNA of transmissible gastroenteritis virus Holland strain (TGEV) kindly provided by The Swedish National Veterinary Institute (SVA) and classical swine fever virus Alfort - 187 strain (CSFV) kindly provided by Dr. J. Pasick from Canadian Food Inspection Agency, and they were used as negative controls.

Briefly, Stool and intestinal samples were used to chromatographic test (Bionote, Korea) according to the manufacturer's instructions. For RNA extraction, samples were diluted 1:5 (v/v) in water, and Viral RNA was extracted using commercial membrane-silice based method (Qiamp Viral RNA, USA). After RNA isolation, we performed reverse transcription reaction, using Superscript™ III first-strand kit (Invitrogen, USA). For real time PCR, 2µl of cDNA was used to amplify a 101pb segment of S gene carried out in a specific PCR test. The primers used were PEDV FW: 5'GCACTTATTGGCAGGCTTTGT 3' and PEDV RW: 5'CCATTGAGAAAAGAAAGTGTCGTAG 3'.

Results

It has been found 23/25 (92%) of positive samples to PEDV using fast immunochromatographic test and confirmed by real time RT-PCR. Positives samples showed a threshold between 13 and 21 cycles. A specific melting curve (Tm) of 77.7°C was shown in samples and positives controls. There was no evidence of amplification and melting curves in TGEV and CSFV negative controls.

Conclusions and Discussion

PEDV has been considered an exotic enteric disease to Peru, because there was no evidence of its presence until now. This abstract represent the first documented report of the PEDV presence in Peru, using immunochromatografic test and confirmed by real time RT-PCR. There was outbreak of PEDV in USA, causing high mortality and morbidity in piglets since the beginning of May 2013. Nowadays, we are sequencing these samples in order to define the origin of virus and its genetic characterization. Preliminaries studies are showing that there is strong genetic relationship between Peruvian and North American strains.

Acknowledgements

The study was funded by National University of San Marcos.

References

1. *Kim O, Chae C. 2000.* In Situ Hibridization for the detection and localization of porcine epidemic diarrhea virus in the intestinal tissues from naturally infected piglets. *Vet Pathol 37:62–67.*
2. *Chen Jianfei et al., 2008.* Molecular characterization and phylogenetic analysis of membrane protein genes of porcine epidemic diarrhea virus isolates in China. Virus Genes 36: 355 – 364.
3. *Chen Jianfei, et al., 2010.* Molecular epidemiology of porcine epidemic diarrhea virus in China. Arch Virol 155: 1471 – 1476.

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Real time RT-PCR comparison to ensure accurate detection of PEDV and TGEV

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Introduction

Porcine epidemic diarrhea virus (PEDV) is major cause of severe diarrhea and dehydration in pigs. Belonging to the *Coronaviridae* family, PEDV is an enveloped, positive-sense, single-stranded RNA virus with a genome size of approximately 28kb. The first detection of PEDV was reported in 1971 from England while Japan, China, South Korea, and Thailand also have reported PEDV infections. The United States first detected PEDV in May 2013 (1-5). The veterinary diagnostic laboratories quickly development sensitive and specific real time RT-PCR (RRT-PCR) assays to detect PEDV in a variety of porcine and environmental samples. In this study, we compared the PEDV-TGEV multiplex RRT-PCR assay developed at the University of Minnesota (UMN) to a commercial TGEV-PEDV multiplex RRT-PCR assay.

Materials and Methods

Porcine intestinal samples, fecal samples, fecal swabs, oral fluid samples, and environmental samples are routinely submitted to University of Minnesota (UMN) Veterinary Diagnostic Laboratory for enteric pathogen testing. Sample homogenates were extracted with the MagMax 96 Viral RNA Isolation Kit (Thermo Scientific), according to manufacturer's instructions. The commercial TGEV-PEDV multiplex RRT-PCR assay was preformed, according to manufacturer's instructions whiles the UM RRT-PCR assay utilized the Path-ID Multiplex One-Step RT-PCR kit (Thermo Scientific, according to manufacture's instructions.

Results

Porcine oral fluids (n=39), intestinal homogenates (n=107), fecal (n=136), fecal swabs (n=47), feedback (n=12) and environmental samples (n=55), totaling 396 samples, were compared with the UMN TGEV-PEDV multiplex RRT-PCR and the commercial TGEV-PEDV multiplex RT-PCR assays. The UMN TGEV-PEDV multiplex RRT-PCR assay had lower Ct values compared to the commercial TGEV-PEDV multiplex RRT-PCR assay. The UMN TGEV-PEDV multiplex RRT-PCR assay detected 53 more positive PEDV samples (oral fluids (n=6), intestinal homogenates (n=9), fecal (n=6), fecal swabs (n=13), and environmental samples (n=19)) compared to the commercial TGEV-PEDV multiplex assay. The additional positive PEDV samples as indicated by the UMN TGEV-PEDV multiplex assay, but negative by the commercial TGEV-PEDV multiplex assay, were confirmed positive by a secondary UMN PEDV RRT-PCR assay, which targeted the N gene. The UMN TGEV-PEDV multiplex RRT-PCR assay detected 11 more positive TGEV samples (intestinal (n=4) and fecal (n=7)) compared to the commercial TGEV-PEDV multiplex RRT-PCR.

Conclusions and Discussion

The UMN TGEV-PEDV RRT-PCR assay had superior performance over the commercial TGEV-PEDV multiplex RRT-PCR assay. Accurate detection of PEDV and TGEV in clinical samples is important to minimize the spread of these two viruses. The role of the clinical diagnostic laboratories is to provide high sensitivity and specificity assay to help prevent and control pathogens and many assays must be evaluated before choosing the best assay to support the swine industry.

Acknowledgments

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References

1. Chaset, D et al. 1978. Res Vet Sci. 1978 Sep;25(2):255-6.
2. Martelli, P et al. 2008. Vet Rec. 2008 Mar 8;162(10):307-10.
3. Park, SJ et al. 2011. Arch Virol. 2011 Apr;156(4):577-85.
4. Pensaert, MB et al. 1978. Arch Virol. 1978;58(3):243-7.
5. Song, D et al. 2012. Virus Genes. 2012 Apr;44(2):167-75.

Evaluation of time and temperature sufficient to inactivate porcine epidemic diarrhea virus in swine feces on metal surfaces

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Introduction

Porcine epidemic diarrhea virus (PEDV) was first described in England in 1971 (2). On May 17, 2013 PEDV was isolated for the first time from swine in the United States and spread quickly across much of the country (3). The route of entry of PEDV into the US is still unknown, but contaminated livestock trailers represent a significant risk (1) for movement of the virus between and within US swine herds.

The objective of this study was to investigate time and temperature combinations sufficient to inactivate PEDV in swine feces on metal surfaces similar to what is found in livestock trailers after fecal and other organic matter have been manually removed. Combinations of time and temperature evaluated represent options for trailer decontamination that are possible when washing and disinfecting are not possible.

Materials and Methods

Eight groups representing different combinations of time and temperature were evaluated. Five ml of undiluted PEDV-positive feces (or negative feces for the negative control group) was spread evenly on the bottom surface of a 15.24 cm by 15.24 cm aluminum tray with 2.54 cm sides, made to replicate a trailer floor. Following treatment as outlined in Table 1, the feces was re-collected from the tray, diluted and passed into PEDV-naïve 4-week old pigs via oral-gastric tube. These pigs served as a bioassay to detect the presence of infectious PEDV. Pigs were monitored for clinical signs consistent with PED and fecal swabs were collected on days 3 and 7 post-challenge. Swabs were tested via PEDV RT-PCR. The individual pig was the experimental unit and each treatment group contained 4 replicates.

Results

PEDV swine bioassay results were analyzed using Fisher's Exact test (SAS® Enterprise Guide 5.1, Cary, NC, USA) for all groups simultaneously as well as pairwise comparisons of all groups. Overall, treatment was found to have a significant effect on PEDV status (p=0.0335). More specifically, the 63C-10M and 20C-7D groups were each found to be significantly different than the Positive Control group (p=0.0286). No other group comparisons were found to be significantly different from one another using p<0.05 as a cutoff for significance.

Table 1. Summary of swine bioassay PEDV results by treatment group.

Treatment Group	Temperature & Time applied	Percentage of PEDV positives (out of 4)
Negative Control	No heat, no time	0% (0/4) [a]
Positive Control	No heat, no time	100% (4/4) [b]
71C-10M	71°C (160°F), 10 minutes	0% (0/4) [a]
63C-10M	63°C (145°F), 10 minutes	25% (1/4) [a, b]
54C-10M	54°C (130°F), 10 minutes	25% (1/4) [a, b]
38C-12H	38°C (100°F), 12 hours	50% (2/4) [a, b]
20C-24H	20°C (68°F), 24 hours	25% (1/4) [a, b]
20C-7D	20°C (68°F), 7 days	0% (0/4) [a]

Groups with different superscripts indicate statistically significant differences ($p \leq 0.05$)

Conclusions and Discussion

These results suggest that it may be possible to inactivate PEDV in the presence of feces by heating trailers to 71°C for 10 minutes or by maintaining them at room temperature (20°C) for at least 7 days. No other combinations of time and temperature were shown to be effective at inactivating PEDV.

The investigators do not propose that this is a preferred alternative to thoroughly washing, disinfecting, and drying trailers. Rather, this work demonstrates the value of possible alternatives when proper washing and disinfection cannot be accomplished as a means to reduce the risk of transmitting PEDV between groups of animals.

Acknowledgments

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References

1. Lowe, J. 2013. NPB #13-216.
2. Odham J. 1972. Pig Farming (Suppl Oct):72-73.
3. Stevenson GW. 2013. J Vet Diagn Invest. 25(5):649-54.

Evaluation of Stalosan® F disinfectant powder to inactivate porcine epidemic diarrhea virus when applied to commercial hog trailers

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Introduction

Porcine epidemic diarrhea virus (PEDV) was first described in England in 1971 (2). On May 17, 2013 PEDV was isolated for the first time from swine in the United States and spread quickly across much of the country (3). The route of entry of PEDV into the US is still unknown, but contaminated livestock trailers represent a significant risk (1) for movement of the virus between and within US swine herds.

The objective of this study was to investigate the efficacy of using Stalosan® F disinfectant powder (Vitfoss, Gråsten, Denmark) to inactivate PEDV in swine feces on metal surfaces similar to what is found in livestock trailers after fecal and other organic matter have been manually removed.

Materials and Methods

A Stalosan® F treatment group and positive and negative control groups were evaluated. Five ml of undiluted PEDV-positive feces was mixed with 2mL of porcine reproductive and respiratory syndrome virus (PRRSV) SDSU 73 with a final concentration of $3x10^5$ TCID50 per ml. For the negative control group negative feces was mixed with 2 ml of sterile saline solution. The mixture was spread evenly on the bottom surface of a 15.24 cm by 15.24 cm aluminum tray with 2.54 cm sides, made to replicate a trailer floor. Trays from the negative and positive control groups were covered with a sealed lid to prevent contact with Stalosan® F disinfectant powder during treatment. Aluminum trays were then placed in various locations on the floor and walls within a commercial hog trailer. Stalosan® F disinfectant powder was then blown throughout the trailer with an electric leaf blower at a rate of 81 grams per $meter^2$ and allowed to contact the trays in such a way as determined by the natural movement of the powder through the trailer. During the one hour period of contact time, the trays were removed from the trailer and placed indoors at room temperature (20°C). Following treatment the feces was diluted with 10 ml of saline, re-collected from the tray, and 4 ml was removed for other use. The remaining mixture (~6-8 ml) was passed via gastric tube into PEDV-naïve 4-week old pigs. The individual pig was the experimental unit and a single pig corresponded to a single tray. Each treatment group contained 8 replicates. Pigs were monitored for clinical signs consistent with PED and fecal swabs were collected on days 3 and 7 post-challenge. Swabs were tested via PEDV RT-PCR.

PRRSV was added to the aluminum trays to simultaneously evaluate the ability of Stalosan® F to inactivate PRRSV; however the positive control bioassays failed to become positive.

Results

PEDV swine bioassay results were analyzed using Fisher's Exact test (SAS® Enterprise Guide 5.1, Cary, NC, USA) as pairwise comparisons of each group. Treatment with Stalosan® F disinfectant powder was found to be no different than positive controls ($p=1.000$) and significantly different than negative controls ($p<0.001$).

Table 1. Summary of PEDV swine bioassay results by treatment group.

Treatment Group	Description of Treatment	Percentage of PEDV positives (out of 8)
Negative Control	No Stalosan® F contact	0% (0/8) [a]
Positive Control	No Stalosan® F contact	100% (8/8) [b]
Stalosan® F Treatment	One hour of Stalosan® F contact time	100% (8/8) [b]

Groups with different superscripts indicate statistically significant differences ($p \leq 0.05$)

Conclusions and Discussion

These results suggest that under the conditions of this study Stalosan® F disinfectant powder did not inactivate PEDV.

Acknowledgments

Funding for this study was provided by National Pork Board checkoff dollars and Vitfoss, Gråsten, Denmark. In-kind support provided by Audubon Manning Veterinary Clinic, Audubon, Iowa, USA and Choice Genetics, West Des Moines, Iowa, USA

References

1. Lowe, J. 2013. NPB #13-216.
2. Odham J. 1972. Pig Farming (Suppl Oct):72-73.
3. Stevenson GW. 2013. J Vet Diagn Invest. 25(5):649-54.

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Oral/nasal inoculation of four-week-old pigs with PEDV: tissue tropism, shedding, carriage, antibody response and aerosol transmission

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Introduction
Porcine epidemic diarrhea virus (PEDV) has recently emerged in the US, the purpose of this investigation was to determine tissue localization, shedding pattern, virus carriage, antibody response, and aerosol transmission of PEDV following oral/nasal inoculation of 4-week-old feeder pigs.

Materials and Methods
Experimental Animals: Thirty-three PEDV naive 3-week-old feeder pigs obtained from a high health commercial source were allowed to acclimate for one week prior to inoculation. The study was conducted under BSL2 containment at the Biosecurity Research Institute at Kansas State University.
Twenty-three Group-A pigs were inoculated with the PEDV challenge material. Five Group B pigs were not inoculated, but were co-mingled with inoculated Group A animals approximately 6 hours post inoculation (PI). Five aerosol transmission Group-C pigs were not inoculated, but were housed in a separate pen in the common animal room as Groups A and B.
Challenge: The challenge material was a pool of gut derived intestinal contents that has been used as "feedback" inocula for controlled exposure of a sow herd in a commercial swine production unit; it was negative for PRRS and PCV and a PEDV nucleic acid "CT titer" of 22. Challenged animals (Group A) were inoculated at 4 weeks of age via the oral and intranasal routes with 5 ml of inocula per route. Following inoculation, the animals were observed daily for clinical symptoms. Nasal and fecal swabs and serum samples were collected prior to challenge and days 0-7, 9, 14, 21, 28, 35 and 42 post inoculation (PI). Pen oral fluid samples were also collected at the same time points for Groups A/B and the aerosol control Group C.
PEDV shedding was monitored by real-time PCR of fecal and nasal swab samples and oral fluids. Serum samples were collected in order to monitor viremia and antibody response. Fresh and formalized tissues were collected from randomly selected Group A pigs at days 0, 2,4,7,9,14,21,28, 35 and 42 PI in order to monitor tissue tropism of the virus and histopathology.

Results and Discussion
Experimental data indicate the following:
Mild clinical signs appeared 2 days post inoculation and resolved by 8 days post inoculation.
Fecal and nasal swabs were PCR positive in the inoculated group at 48 hours post inoculation.
Peak fecal shedding occurred 5 to 6 days post challenge and was significantly higher than nasal swabs.
Most group A & B animals were negative for fecal or nasal swab testing at 21 days post inoculation, some animals shed virus as long as 35 days PI.
Productive transmission did not appear to occur in the aerosol control group in spite of the fact that PEDV nucleic acid could be detected in the nares of some of those animals and oral fluids.
Room environmental samples were collected at 14 days post inoculation-the data demonstrate that viral nucleic acid was abundant on the walls, pens and food bins on both the inoculated and aerosol control areas in the challenge room.
Due to the possibility of a false positive PCR reaction, questionable samples were retested and the reaction products were sequenced to determine if the product was PEDV specific. All questionable reactions demonstrated the presence of PEDV viral nucleic acid.
PEDV viremia was clearly detected in 3 of the 5 contact controls and 9 of the 22 inoculated animals. No detectable viremia was detected in any of the aerosol control animals.
The raw data suggest that there seems to be a correlation with viremia and extended duration of shedding either fecal or nasal.
Serological data (IFA) show that pre-inoculation samples are negative and that there was significant seroconversion in the all of the inoculated and contact control animals.
There is no evidence of seroconversion in the aerosol control group in spite of the clear demonstration of PEDV nucleic acid in nasal and oral fluid samples.
The IFA data was in complete agreement with an E. coli expressed NP ELISA (96 well format) and the fluorescent microsphere immuno assay that are being developed.
Tissue blocks were sent to Dr. Madson at ISU for PEDV immunohistochemistry (IHC) evaluation. The only samples that tested positive for the presence of viral antigen were tissues from the GI tract. Turbinates, trachea, lung, bronchial lymph nodes, spleen, and other visceral tissues were all negative for PEDV as evaluated by IHC.
A complete set of serum samples have been provided to 5 laboratories (~1,200 samples) for assay development/standardization. In addition, 3 complete sets of oral fluid samples and tissues samples have been provided to other laboratories.
Virus isolation attempts on frozen intestinal tissue samples that have previously been provided to three laboratories is still underway—sequential passage attempts have been unsuccessful thus far.

The experimental results demonstrate that aerosol transmission did not occur in this study. These results seem to be in conflict with reports from the field that implicate aerosol transmission, but lack confirmation via bioassay. Factors like disinfectant and ultraviolet inactivation of PEDV, sensitivity of the indicator animal (nursing pigs vs weaned pigs) and infectious dose as a function of route of exposure need to be investigated in order to gain insight into modes of transmission of PEDV.

Understanding PEDV timeline of exposure based on clinical findings

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Introduction

Porcine Epidemic Diarrhea virus (PEDV) was diagnosed in the United States in 2013. While PEDV has been in other countries prior to 2013, it was not in the United States. Over the year since the first clinical case, a large amount of research has been done to develop diagnostic tests and to determine how the virus is being moved throughout the country. One area of question is how the virus is introduced into a farm. In this study, twelve farrow to wean farms were identified and classified either as low or high risk based on farm traffic and were environmentally sampled daily for 30 days during the winter months. During this time, five farms were diagnosed with PEDV. The findings of the sampling are presented in this abstract.

Materials and Methods

Environmental samples were collected from 12 farms over a period of 30 days using the Swiffer® collection method. In brief, a Swiffer was placed in a Ziploc® bag with 30 mL of PBS. The farm manager placed on latex gloves, squeezed the excess fluid from the Swiffer® and then swiped the designated area. The Swiffer® was then placed back into the Ziploc® bag and squeezed three times with the PBS in the bag. The bag was then turned upside down and the liquid was squeezed out of the cloth. The liquid was then dispensed into a sterile tube and frozen. When a farm was diagnosed with PEDV, the samples were submitted to Iowa State Diagnostic Laboratory (Ames, IA, USA) for PEDV testing via PCR.

The areas tested on each farm were: 1. Shoe change area, 2. Lunch bag change table and UV window, 3. Dirty side of shower, 4. Lunch table and fridge handle, and 5. Inside the fridge.

The findings of the samples were reported as the percentage of samples positive based on a CT value of <37 or suspect (CT 37-39.9).

Results

Three of the five farms that contracted PEDV were originally classified as low risk. Two of the 5 farms were filtered farms. Of the 5 farms that developed PEDV, 4 of the 5 farms were found to have either suspect or positive samples 3 days prior to the onset of clinical signs. The other farm did not have positive findings until the day of the clinical symptoms (Table 1).

Conclusions and Discussion

The findings of the survey demonstrate that in this case, the fomites/food belonging to the animal caretakers do not appear to be the source of contamination in these cases. The fact that multiple testing areas within a farm are testing positive/suspect prior to actual clinical symptoms indicate that the virus is present on-site at low levels approximately 48 hours prior to identification of clincal symptoms and appears to be moving throughout the facility before it reaches a threshold for clinical disease presentation.

Even though the virus can cause clinical signs 12-24 hours post-exposure, these findings demonstrate the challenge in identifying the source of contamination in farrow to wean farms as it appears to be a delay of 24-48 hours post-introduction before clinical signs are detected by animal caretakers.

Table 1. Findings of environmental swabs prior to clinical symptoms.

		Days Pre-Clinical Signs				
		-3+	-3	-2	-1	0
Boot Room Floor	Negative	5	4	2	3	3
	Suspect			1	1	
	Positive					1
Lunch Exchange Table /UV light	Negative	5	4	2	1	2
	Suspect			1	2	1
	Positive				1	1
Shower Room Floor	Negative	5	3	1	2	2
	Suspect	1	1	2	1	
	Positive				1	2
Lunch Table/ Door Handle	Negative	5	4	2	2	2
	Suspect			1	1	1
	Positive				1	1
Inside Refrigerator	Negative	5	4	1	1	2
	Suspect			1	2	
	Positive			1	1	2

O.164

Kinetics of humoral immune response (IgM, IgA, and IgG) to porcine epidemic diarrhea virus (PEDV) in experimentally infected pigs

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Introduction

PEDV has been a significant challenge to the US swine industry since its introduction in early 2013 (1). The virus is a member of *Alphacoronavirus* along with TGEV, another significant viral enteric pathogen of swine. These 2 viruses are genetically and antigenically distinct from each other. PEDV infection cannot be clinically and histologically differentiated from TGEV infection. Etiological diagnosis can only be established on the basis of laboratory methods.

A key to preventing the spread of PEDV is stringent movement control of animals and fomites contaminated with the virus combined with surveillance. Serology can be a vital tool to aid in the control and for further understanding of disease epidemiology. Anti-PEDV antibodies have been detected in sera from swine with naturally occurring or experimentally induced PED by indirect ELISA (2), IFA test (4), and serum-virus neutralization test (2,3). However, the kinetics of the antibody response against PEDV has not been described and commercial ELISA tests currently available have proven unreliable. The following study was conducted to assess the ontogeny of humoral immune response in pigs after experimental infection with PEDV.

Materials and Methods

Fifty six 3-week old pigs negative for PED, TGE and PRRS viruses were inoculated with a PEDV isolate (USA/Iowa/18984/2013) (4) via gastric gavage. The pigs were maintained for 76 days post inoculation (dpi) and monitored for clinical signs and fecal shedding of the virus with periodic necropsy for histological evaluation. Serum samples were collected on day 0 and every 7 days for antibody testing using ELISA.

The same PEDV isolate was propagated in Vero cell, pelleted, processed and used as whole virus antigen for ELISA plate preparation. ELISA conditions such as coating conditions, reagent concentrations, incubation time and buffer compositions were optimized for simultaneous detection of anti-PEDV IgM, IgA, and IgM antibodies.

Results

Clinical diarrhea started at 3 or 4 dpi and subsided by 10 dpi. Fecal shedding of PEDV was detected in 100%, 88%, 42% and 0% on 7, 14, 21, and 28 dpi, respectively.

Anti-PEDV IgM, IgA, and IgG responses over time after experimental inoculation are shown in Figure 1. A short-term, low-level IgM response was detected first which was followed by a strong IgA and a moderate IgG response. Both IgA and IgG antibodies started to gradually decline after 3 or 4 weeks post inoculation.

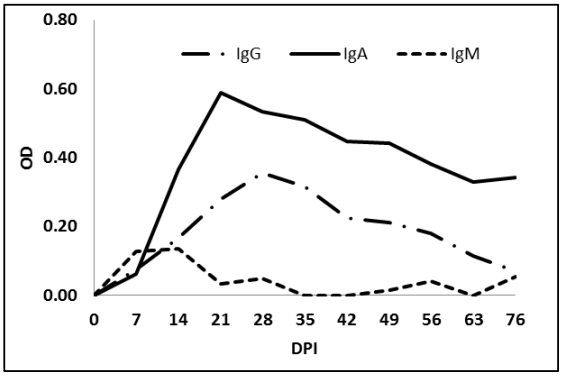

Figure 1. Kinetics of anti-PEDV IgM, IgA, and IgG responses (net OD) in weaned pigs after experimental oral inoculation with PEDV isolate

Conclusions and Discussion

After experimental infection, pigs developed all major isotypes of virus-specific antibody when tested by a whole-virus based ELISA, suggesting that serology is applicable to monitoring pigs for PEDV exposure. IgA antibody may be a better choice for monitoring the humoral immune response. It should be noted that anti-PEDV antibodies may not last long after exposure.

Acknowledgments

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References

1. Stevenson et al., 2013. J Vet Diagn Invest 25:649-654.
2. Oh et al., 2005. J Vet Sci 6:349-352
3. Hoang et al., 2013. Proc CRWAD Abstr #142.
4. Hoang et al., 2013. Genome Announc 1(6):e01049-13.

PEDV: immunity following feedback

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Introduction

Porcine epidemic diarrhea virus (PEDV), a coronavirus related to transmissible gastroenteritis virus (TGEV), appeared suddenly in the Unites States in April, 2013. Epidemic sow herd outbreaks, characterized by severe diarrhea, vomiting, and high mortality in nursing pigs for several weeks, continue to spread the disease. PEDV appeared in Canada in February, 2014, and multiple outbreaks have already been reported despite extensive efforts to prevent and control the disease. In the absence of vaccines for PEDV in the US, producers have used feedback of infected intestinal tissues to induce immunity. However, there is little information on the efficacy of various feedback protocols on induction of immunity, including duration of the serological response and mucosal antibody production.

Materials and Methods

Recombinant protein antigens were produced by cloning and bacterial expression of PEDV capsid, and the S1 and S2 fragments of envelope glycoprotein.

Purified proteins were used to coat wells in microtiter plates and conditions for coating blocking, and testing were optimized for detection of anti-PEDV antibodies in serum, colostrum, milk and feces by ELISA.

Feedback materials were titered for PEDV by qPCR and fed to pregnant sows in multiple doses over one week with or without a second administration about 3 weeks later.

Samples were obtained at intervals to weaning from sows and piglets at weaning. Infection was assessed by qPCR and immune response by anti-capsid ELISA.

Results

Validation of the PEDV ELISA was performed on 448 serum samples from positive populations and 280 samples from negative populations. The resulting S/P ratios are shown in Figure 1. Using a cutoff absorbance value of 0.5, the sensitivity and specificity of the assay both were 0.99.

Feedback administrations to sows at about 80 days of gestation performed one time or repeated after 3 weeks both produced uniform seroconversion at 3 weeks. There was no significant effect of a second administration. In one study, the At 6 to 8 weeks post-administration, average antibody levels had returned to baseline levels in one study, and litters had no detectable serum antibodies at 10 days of age. In another study, serum antibody levels were a mixture of negative and positive, and about half of the litters contained pigs with anti- PEDV antibodies in serum.

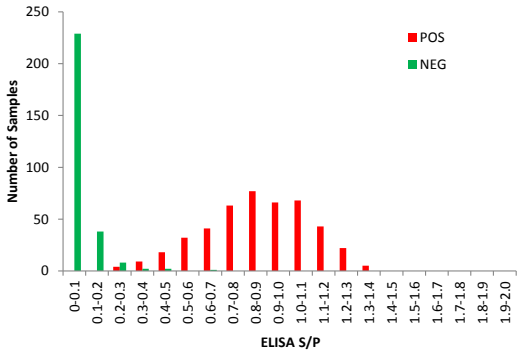

Figure 1. Distribution of S/P ratios of positive and negative sera used to validate the PEDV ELISA.

At no time were anti-PEDV IgG or IgA antibodies detected in milk (colostrum was not collected), or feces of sows, or in feces of piglets.

Conclusions and Discussion

Feedback of infected gut material is an effective method for inducing anti-PEDV immune responses in adult sows. However, anti-capsid antibody ELISA has not demonstrated reproducible transfer of maternal immunity to piglets and has not shown the presence of secretory antibodies in feces. It is generally assumed that secretory IgA is key to protection against enteric pathogens. Thus, it is possible that effective PEDV immunity is hard to induce in sows, or to transfer to piglets; or that the anti-capsid ELISA does not detect a protective response. We do not yet know which possibility is correct, but have found that total IgA is abundant in feces, and that serum antibodies mixed in with feces is readily detected. Hence, negative fecal ELISA means that the specific antibodies are not present.

Overall, we conclude that PEDV infection induces an immune response that may be short-lived and may not provide solid protection to sows or piglets.

Acknowledgments

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Modeling influenza A transmission dynamics in a growing pig herd with waning immunity

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Introduction

Swine influenza A virus has become a major pathogen, causing respiratory disease in swine, particularly growing pigs. Waning immunity against influenza A infection after infection has been poorly understood. Therefore, the objective of this study was to create a model of the effect of waning growing pig immunity on susceptible, infected, and immune population proportion over the 20 weeks of the wean-to-finish period.

Model and Assumptions

Simple deterministic Susceptible-Exposed-Infectious-Recovered (SEIR) models were constructed (Fig1) for a wean-to-finish pig population. One infectious weaned pig was introduced once into a barn totaling 1000 pigs with 5.3% mortality (6). The transmission rate β was 2.18 (1) and the exposure period was 1.9 days resulting in a rate of κ=0.526 day^{-1} (2, 4). 0.5% of infected piglets dying from influenza infection was assumed (f=0.005). Three waning immunity scenarios of mean half-life were simulated (π=0.0, π=0.05, and π=0.10, of piglet populations recovered day^{-1} becoming susceptible for scenario 1, 2, and 3 respectively). The coding was written in *R* (v3.0.2).

Results

The results of the simple deterministic SEIR model were presented in Fig. 1. After the 40th till the 140th day post influenza A virus introduction, the proportions of susceptible growing pigs remaining in the herd were 10.8%, 10.6%, 10.6% (scenario 1, 2, 3 respectively) (Fig1). However, the proportions of infected growing pigs remaining in the herd were 5.4%, 18.9%, 27.0% (scenario 1, 2, 3 respectively). Susceptible growing-pig proportions for three scenarios were similar over a course of Influenza A virus dissemination (Fig 1). In contrast, infected growing-pig proportions for three scenarios were different in proportions. In addition, among infected pig subpopulations, the pig proportion without waning immunity (π=0.0) had sharply increased since the 16th till the 30th day post introduction but consistently increased with both a 5% immunity wane (π=0.05) and a 10% immunity wane (π=0.10) (Fig 1).

Conclusions and Discussion

In conclusion, the waning rate of wean-to-finish pig immunity affected only the proportion of infected pigs, but not the proportion of susceptible pigs. Among infected pig subpopulations, pigs without waning immunity had sharply increased infections after 16th till the 30th day post introduction (in total 15 days), then became stable with Influenza A virus transmission in the wean-to-finish barn. In human diseases, waning immunity has interfered with vaccination to create more subclinical infections (3). Le (5) demonstrated that rates of waning immunity against PRRS might vary due to a different status of PRRSv infection on a farm by time to exposure or strains of virus (5). With higher waning immunity, there are more infectious pigs and higher area exposure to continue outbreaks. Conversely, lower rate of waning immunity may control the spread of swine influenza virus, reduce the number of infectious pigs and lessen area spread.

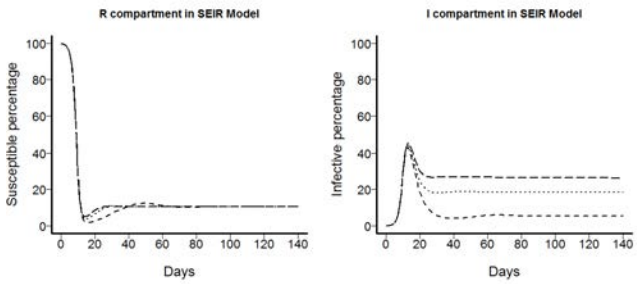

Figure 1. Comparison of susceptible (left), and infected (right) growing pig proportions for 3 scenarios (dashed, dotted, and long-dash lines with immune pigs becoming susceptible at rates of 0.0%, 5.0%, 10.0% day^{-1} for scenario1, 2 and 3, respectively)

Acknowledgments

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References

1. Allerson, M. et al. 2012. Vaccine. 31:500-505
2. Brauer, F.J. et al. 2010. Math. Epi. 321-347
3. Glass,K. , B.T. Grenfell. 2004.Vaccine. 22: 4110–4116
4. Longini, I.M. et al. 2004. Am J of Epi. 60:53-68.
5. Le H. T. 2011 PhD thesis.
6. Straw, B. E. et al. 1983. JAVMA. 183:452-455.

Influenza A virus infection and transmission in pigs after weaning

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Introduction

Influenza A virus (IAV) has a segmented genome, negative sense RNA, with 8 gene segments that translate up to 12 different proteins. Influenza infections are worldwide distributed in many animal species, including humans and its epidemiology within and between species is very complex and not completely understood[1]. The pig has been identified as a mixing[2] vessel for IAV, but little is known about evolution, prevalence and incidence of IAV in endemically infected swine populations. The objective of this study was to characterize IAV infection and transmission in pigs during the post-weaning period in order to identify determinants that may be associated with IAV persistence in swine populations.

Materials and Methods

A cohort of 132 pigs out of 2,200 3-week old pigs were randomly selected and individually identified at arrival to a commercial wean to finish facility. Pigs originated from a breeding herd known to be IAV infected according to diagnostic laboratory procedures. Nasal swabs were collected from each pig at arrival, and approximately on a weekly basis for 15 weeks. Each sample was tested for IAV by RT-PCR. The prevalence of IAV infections and the risk of re-infection for each animal were compared between weeks. Additionally serum samples were collected at arrival and every 4 weeks there after, and tested for antibodies to IAV by ELISA. Results were compared between weeks using a paired t-test, and considered statistically significant at $p<0.05$. Finally, a set of positive samples were conveniently selected by week and tested by RT-PCR to establish the virus subtype (H1N1, H1N2, or H3N2) that was circulating after weaning in the selected population.

Results

Six pigs out of 132 (4.5%) tested positive to IAV on arrival. The overall period prevalence was 75%, with a weekly prevalence ranging between 0% and 39.4% ($p<0.05$). We identified two incidence peaks of IAV, at 2 and 7 weeks post weaning with a prevalence of 39.4% and 24.5% in each peak respectively. Twenty nine percent of the pigs that tested positive became re-infected based on PCR results obtained positive in non-consecutive weeks, suggesting that there might be pig to pig transmission even among previously infected pigs. In regards to serological results, the mean and standard deviation of the sample to negative ratio (S/N) at weeks 0, 4, 8 and 12 were 0.550 (0.227), 0.695 (0.186), 0.191 (0.109), 0.224 (0.1426) respectively ($p<0.05$) indicating a decay on the maternal immunity followed by the subsequent seroconversion due to active infection. Finally, we identified by RT-PCR the same IAV subtype (H1N1) for at least 6 weeks after weaning, and an H3N2 virus was detected after week 5 indicating that the transmission and maintenance of IAVs after weaning is complex and dynamic.

Conclusions and Discussion

In conclusion our results indicate that IAV can be maintained in growing pig populations at a low prevalence and that infection can occur even among previously infected immune pigs. We speculate that persistence at the population level is in part because of the ability of pigs to become re-infected due to genetic differences observed at the virus level and the ranging levels of immunity found in the pigs. Additionally, we demonstrate that the prevalence of IAV in endemically infected populations can change significantly between weeks after weaning.

Acknowledgments

National Pork Board (NPB), Minnesota Super Computing Institute (MSI) and the BioMedical Genomic Center of the University of Minnesota (BMGC)

References

1. Taubenberger JK, Kash JC. Influenza Virus Evolution, Host Adaptation, and Pandemic Formation. Cell Host & Microbe. 2010;7(6):440-51.
2. Hass J, Matuszewski S, Cieslik D, Haase M. The role of swine as "mixing vessel" for interspecies transmission of the influenza A subtype H1N1: A simultaneous Bayesian inference of phylogeny and ancestral hosts. Infection Genetics and Evolution. 2011;11(2):437-41.

Temporal genetic characterization of influenza A viruses in swine breeding herds

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Introduction

Neonatal pigs and replacement gilts play an important role in maintaining and transmitting influenza A virus (IAV) in swine populations. These sub-populations may also play a key role on creating and maintaining IAV diversity over time in endemically infected breeding herds. The objective of this study was to describe the temporal dynamics of IAV infection in breeding herds and to evaluate the role of different sub-populations on the introduction and maintenance of IAV over time.

Materials and Methods

Five herds with history of IAV infection were conveniently selected in the Midwest USA and monitored for a period of 12 months. Only sow farms with the on-site gilt development unit (GDU) and off-site weaned commercial pigs were selected. At each farm three animal sub-populations were sampled on a monthly basis: 3 week-old suckling piglets, gilts that had been on-site for more than 4 weeks (gilts) and gilts that have been on site less than 4 weeks (new gilts). From each sub-population, 30 individual nasal swabs were collected on a monthly basis for a total of 12 months. All nasal swabs were tested for IAV by RT-PCR targeting the M gene of IAV, and positive samples were used for virus isolation on MDCK cells. Virus isolates obtained from all farm were sub-typed by gene sequencing based on their main antigens hemagglutinin (HA) and neuraminidase (NA) and compared throughout the study period.

Results

Two hundred and four (4.9%) out of 4188 samples collected across all farms tested positive. Suckling piglets tested positive at least once in all farms. The percentage of positive samples by farm on a 12-month period ranged between 1 and 11%. However the range of positive samples among suckling pigs, gilts and new gilts was 2.2 – 7.8, 0 -5.4, and 0 to 26.9 % respectively. One hundred and twenty five viruses were isolated from 204 positive samples and 118 of these viruses were sub-typed as single infections (Table 1) and 2 as mixed infections (more than 1 IAV subtype isolated from the same sample, data not shown).

Conclusions and Discussion

We found that IAV infection within and between subpopulation in endemically infected breeding herds is very dynamic and not constant throughout time. The most common IAV subtypes circulating in swine populations (H1N1, H1N2 and H3N2) were found in all farms except in farm 2 where only H1N1 and H3N2 viruses were isolated. Moreover multiple IAV subtypes were isolated from the same subpopulation at the same time, indicating that pigs at different stages within the breeding herd could potentially be mixing vessels for swine IAV. New gilts could be naive to resident viruses in the breeding herd or bring new strains when they are introduced into the GDU. Suckling piglets are born naive to IAV and can acquire maternal immunity to IAV; however they can become infected with IAV in the farrowing units and maintain IAV infection for long periods of time at the population level. Our results indicate that the diversity of IAV in endemically infected breeding herds is significant. Furthermore phylogenetic characterization of these viruses is needed to understand the mechanisms and populations that allow the virus to persists for long periods of time in breeding herds.

Table 1. Number of virus isolates and subtypes by farm identification (ID), animal subpopulation and month. Farms 3 and 4 were enrolled on November 2011 and farms 1, 2 and 5 on January 2012.

ID	Subpopulation	Month	Number of Isolates and Subtype
1	Piglets	1	1 H3N2
		2	3 H3N2
		3	6 H1N1, 1 H1N2, and 1 H3N2
		11	1 H1N1 and 1 H3N2
	Gilts	NA	NA
	New-gilts	NA	NA
2	Piglets	7	2 H1N1 and 2 H3N2
	Gilts	NA	NA
	New-gilts	NA	NA
3	Piglets	1	3 H1N1, 15 H1N2 and 2 H3N2
	Gilts	4	2 H1N1 and 10 H3N2
	New-gilts	5,	6 H1N1 and 3 H3N2
		6	3 H3N2
		10	5 H1N1 and 22 H3N2
4	Piglets	2	1 H1N1 and 3 H3N2
	Gilts	5	3 H1N2
	New-gilts	NA	NA
5	Piglets	1	6 H3N2
		2	6 H3N2
		11	1 H1N1 and 1 H3N2
	Gilts	NA	NA
	New-gilts	NA	NA

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Implementation of a structured PRRSV control program in a large specialized swine veterinary practice

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Introduction

The swine industry all over the world suffers large economic losses due to the reproductive and respiratory disorders caused by PRRSv. The need for control and eradication programs has become more urgent since the report of the changes in PRRS pathogenesis in respect of replication in the nasal mucosa of some genotype 1 strains which can influence the potential aerial spread [1].
At farm level there are reports from Vogelmayr and v.Groenland of successful PRRSv control /eradication using biosecurity measures and targeted vaccination with an EU-MLV (DV) vaccine. In Sonora, Iowa and in the northern part of the Netherlands, regional control programs have been initiated with varying success.
A unified approach to diagnosis, monitoring, finance, biosecurity measures and their implementation, as well as openness/transparency between farmers, vets and consultants, are major issues before PRRS can be successfully controlled.
As the 17 certified swine vets of Lintjeshof cover a substantial number of pig farms in The Netherlands, many of these pre-requisites are easily accomplished, so successful PRRSv control programs can be achieved at a practice level which includes a certified lab facility.
The objective of this endeavor is to establish a PRRS control program at practice level including monitoring of farm status [4], prevalence and changes in technical and economic performance. In this study we implemented the program steps on a large farm where PRRSv circulated in the nursery units.

Materials and Methods

The PRRS control program steps are: **1.** Alignment of vets regarding: PRRS diagnosis and monitoring (in sows, gilts, piglets), interpretation of laboratory results, uniform biosecurity checks[3] and the formulation of targets, suitable vaccines and the timing of PRRSv vaccination. (Targets: SOWS: a PRRS stable or negative sow herd (Cat III or IV)[2] -GILTS: virus protection and prevention of introduction of PRRSv into sow herds.- PIGLETS: reduction in PRRSv prevalence and spread towards sows and prevention of clinical signs, economic losses and reduction in antibiotic use).
2. Developing simple communication tools in respect of clinical PRRS and its financial impact, diagnosis, control via biosecurity measures and target vaccination, and the monitoring of their effects. **3.** Diagnosis+monitoring via uniform cross-sectional sampling with serological and pooled PCR testing [4] (incl. sequencing or DV testing).
4. Reports of changes in PRRSv status and Category, including analyses of possible risk factors.
-The control program was reviewed in a farm with PRRSv-related respiratory problems in two nursery facilities using cross-sectional sampling for serology, PCR and sequencing. The category of the sow herd [2], the status of the nursery (defined by the percentage of PRRSv-positive nursery units including genotyping) was determined. The effect of an adjusted piglet vaccination with an EU-MLV via IDAL was evaluated by assessing the percentage of PRRSv-positive units after 3 months.

Results

-Farm (program test)		nursery facility	
date	**cat sows**	**1: % pos units**	**2: % pos units**
18-07-2013	II	37,5% (3/8)	50%* (3/6)
1-11-2013	II	0% (0/8)	0% (0/6)

* 98% ORF5 homology VR2332

- Implementation PRRS control program:
Step 1, 2 and 3 have already been implemented. The certified Merefeld laboratory, originated within the Lintjeshof practice, performs the serology and PCR tests Sequencing of positive PCR tests will be carried out by an external laboratory. Biosecurity will be assessed in a manner similar to the use of the ResPig tool [3].

Conclusions and Discussion

-Structural PRRSv control at a farm level is possible via biosecurity optimization and targeted vaccination with an effective PRRS vaccine. This was demonstrated in our farm were an US PRRSv strain was controlled and possibly eradicated from the nursery units.
-A R_0 <1 of the vaccine strain and a discriminating test (PCR or sequencing) is very helpful in achieving and monitoring control/eradication programs so that the PRRSv status of vaccinated herds can effectively be assessed via serological and virological testing.
-Already published scientific data (Groenland, Holtkamp biosecurity scoring)are helpful to formulate and evaluate practical PRRSv control programs.
-The implementation of a control program is easier with a large specialized veterinary practice than with a high number of practices, authorities and consultants which need to agree on the various pre-requisites beforehand. The financial impact of PRRS and the financial evaluation will support the preparedness to participate.
Further results will be reported in the near future.

References

1. Nauwynck H.,2013 MSD-AH HQPC Rome
2. Holtkamp D.J.,2010 IPVS. Oral proc.p.265
3. Geurts V.N.A.M.,2011 Safe pork conf. Oral p59
4. v Groenland G.J et.al.,2010 IPVS Oral proc.p272

Evaluation of cyclonic air collectors for detection of PRRSV; variation & repeatability within and between collectors

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Introduction

Airborne transmission of PRRS virus is a documented risk to swine herds (Dee et al., 2009a). While liquid cyclonic collectors have been used to evaluate said risk, (Dee et al., 2009b, Pitkin et al 2009) whether variability exists across these instruments has not been described. The objective of this study was to measure variability among cyclonic collectors currently used in the field for their detection of aerosolized PRRS virus.

Materials and Methods

Three concentrations of Ingelvac PRRS® MLV vaccine (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) were prepared: 10^3 $TCID_{50}$/L, 10^5 $TCID_{50}$/L, and 10^7 $TCID_{50}$/L (Dee et al., 2009b). A sample of each vaccine dilution was submitted for PRRSV RT-PCR to confirm concentration. A dual chamber model (Dee et al., 2009b) was used to aerosolize diluted vaccine. Six cyclonic air collectors (Midwest Micro Tek, Brookings, SD) routinely used in the field (manufacture date 2008-2013) were selected for this study. Prior to daily use, all collectors were swabbed to verify the absence of residual virus. To reduce the risk of physical obstacles, 3 collectors were placed in the recipient chamber and locations within the chamber were systematically changed for each replicate. The remaining 3 collectors were then substituted and tested. Sterile PBS (5 mL) was added to the bowl of each collector as a means to collect airborne particles. A cold fog mister was used in the donor chamber to aerosolize the prepared concentrations, beginning with the 10^3 $TCID_{50}$/L solution, followed by 10^5 $TCID_{50}$/L and 10^7 $TCID_{50}$/L. Each concentration was aerosolized for 5 minutes at a static pressure of 0.15" H_2O. After completion of each 5 minute sampling period, a 3 mL aliquot of PBS was tested for the presence of PRRSV RNA by RT-PCR. Ten replicates of each concentration were performed across each of the 6 collectors. Collectors were cleaned between replicates using published methods (Dee et al., 2009). Logistic regression was used to compare the number of PRRSV PCR positive samples at each concentration according to device as well as by the interaction of collector and concentration.

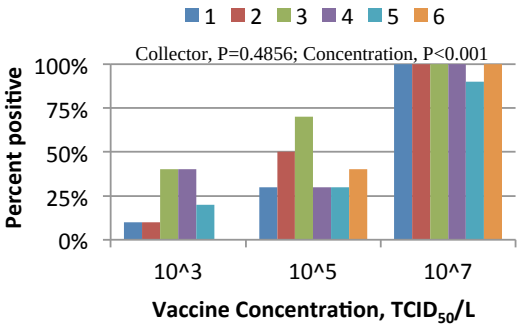
Results

A summary of the PRRSV PCR positive samples detected across collector and dilution is provided in Figure 1.

There was no significant interaction (P=0.7298) between collector and vaccine concentration. There was no statistical difference (P=0.4856) in the number of positive air samples detected by the 6 collectors across concentrations. There was a significant difference (P<0.0001) in the ability the air collectors to detect a positive sample at the different vaccine concentrations.

Conclusions and Discussion

Under the conditions of this study, no significant differences were detected between the 6 air collectors in their ability to detect aerosolized PRRV. However, the ability to detect a positive air sample decreased across all 6 collectors as concentration of aerosolized PRRSV was reduced. These data indicate that the sensitivity of these instruments is affected by concentration. This type of instrumentation has provided good historical data as it pertains to the detection of PRRSV in air but improved technologies are needed that will increase the sensitivity of aerosol detection of PRRSV.



[i]Analyzed using logistic regression. Interaction NS, P=0.7298

Figure 1. Number of PRRSV PCR positive samples by air collector and concentration

References

1. Dee S et al. 2005. Vet Rec 156:501-504.
2. Dee S et al. 2009. Vet Res 40:39.
3. Pitkin A et al. 2009. Vet Microbiol 136: 1-7.

Cost per kg of live pig obtained under the Cost Efficiency and Competitiveness Information System (SICEC) of livestock activities in Mexico

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Introduction

In order to make available to interested parties of the Mexican livestock activity, regular, reliable and of easy access information on livestock sectors in Mexico, the Ministry of Agriculture and the National University of Mexico (UNAM), designed and operate an information system (SICEC in Spanish). In light of the results obtained, SICEC proves to be a reliable system that complements the few sources of economic and technical information existing. This short article includes both the cost of production per kg of live pig sold, and the participation of the main inputs in total production costs.

Materials and Methods.

The information used for this research was obtained from the 2012 SICEC pig poll. A sample of 208 farms from 8 states of Mexico were selected from the "Padrón Nacional Ganadero" (the largest registry in the country) and interviewed in the 2012 SICEC poll.(1) The selection was randomly made in each of the following four stratums: 50 to 100 sows, 101 to 200, 201 to 500 and 501 or more sows. Out of that sample, 132 farms were selected for congruent information, and additionally a fifth stratum of less than 50 sows was included for analysis due that some farms had fewer animals. Finally, after removing farms producing piglets and introducing information filters, the costs of 82 farms in the states of Jalisco, Guanajuato, Aguascalientes, Tlaxcala and Veracruz were analyzed. The cost of production per kg of live pig sold (C/kg) was calculated considering the costs of food, health, labor, equipment, facilities, services, reproduction and replacement. (All costs are in Mexican pesos of 2012).
Comparison of means of the five stratum was performed using ANOVA with SPSS19 ® software.

Results

The mean of C/kg was $21.48 and the mean and confidence intervals for the mean in each stratum are shown in Table 1. When comparing the means between layers no significant difference was found (p=0.39). Breaking down the C/kg, the feeding costs represents the highest percentage with 81.26% followed by labor and health with 4.5% and 3.73% respectively.

Table 1. Cost per kg of live pig sold (C/kg).

Stratum sows	N	Mean	Std Error	Mean Cofidence Interval 95%	
				Lower	Upper
(x < 50)	4	23.5	3.7	17.4	29.5
[50 , 100]	30	22.2	1.3	19.4	24.9
[101, 200]	25	20.6	1.5	17.0	24.2
[201, 500]	18	21.2	1.7	18.2	24.3
(x > 500)	5	21.1	3.3	15.9	26.3
Total	82	21.48	0.8	19.9	23.1

Conclusions and Discussion

The costs reported in this paper are similar to those reported the C/kg of $20.9 technified companies in Latin America in 2012(2), and of those reported by FAO for Mexico in 2011 of $20.3 (3).
The C/kg average of $21.48 is a $1 greater that average selling price of live pig kg in 2012 (4). This reflects the great challenges that had to face the Mexican pork industry in 2012.
The failure to find significant difference in costs of production between stratum denotes the absence of scale economies (5), although a trend to lower costs in favour of larger farms can be noticed, these should be analised in further researches done in larger sample sizes.
In conclusion, the SICEC is a system that provides reliable elements for decision-making at farm and public policy levels. Therefore it must be strengthened to expand the sample size.

Acknowledgments

Coordinación General de Ganadería SAGARPA

References

1. SICEC 2012 available in http://www.sicec.unam.mx/index.php/portal
2. Pig Improvement Company 2012. Análisis de la industria porcina en latinoamerica PIC2012 vol 8
3. FAO 2011. http://faostat3.fao.org/faostat-gateway/go/to/download/P/PP/Shttp://
4. Servicio de Información Agroalimentaria y Pesquera SIAP 2012. http://www.siap. gob.mx/ganaderia-resumen-nacional-pecuario
5. Heywood J et al. 2012. Economics Letters 117:566-568.

Insemination and heat detection cost in Spanish sow farms

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Introduction

When we consider time consuming in a farm, gestation becomes from 40% to 60%Insemination and heat detection are by far the most time consuming task for people in sow farms (1). Often when the cost of performing one insemination is evaluated, the emphasis is only on semen cost, whilst the importance of labor cost is overlooked.

The aim of this study is to evaluate the labor cost of insemination and heat detection.

Materials and Methods

Forty (40) Spanish sow farms representing almost 70,000 sows, were included in this study to evaluate the time spend per sow on insemination and heat detection. Time Spend on heat-checking and insemination per sow and per day (TSD) was calculated considering the total daily time spend on these activitiesdivided by the number of sows per batch. Cost per hour (CH) was calculated by dividing the total annual cost by the total number of working hours per year. Cost per insemination and heat detection (CIHD) was calculated taking into account the cost per hour (CH) divided by 60 minutes and then multiplied by TSD.

CIHD = (CH/60) x TSD

Finally Gestation Labor cost per pregnant sow (GCPS) was calculated considering a farrowing rate of 84.5 % (2) and 5 days expend doing Heat detection and insemination.

Farms were divided in three different groups according to size; 300-1000 sows as small (SF) (n=13), 1000 to 2000 as medium (MF) (n=12) and higher than 2000 as large (LF) (n=15).

Results

Farm size affects the time that people spend per sow perday: larger farms have less time, 7.15 to 7.25 minutes, compared to small farms 9.12 minutes (almost two minutes difference). At the same time, cost per hour is lower in larger farm systems than in smaller ones, ie 9.1 Euros versus 9.8 euros respectively. These two characteristics result in a higher labour cost per insemination and heat detection per sow in small farms, 1.46 Euros, compared to medium ones, 1.12 Euros, and large ones, 1.08 euros. As a summary Gestation labor cost per pregnant sow (GCPS) varies betwen 8.43 Euros to 6.24 Euros. (See Table 1)

Table 1. Insemination and heat detection cost per sow and farm size

	TSD (minutes)	CH (Euros)	CIHD (Euros)	GCPS (Euros)
SF <1000	9.12	9.8	1.46	8.43
MF 1000-2000	7.25	9.3	1.12	6.47
LF >2000	7.15	9.1	1.08	6.24

Conclusions and Discussion

For every pregnant sow on Spanish farms a minimum of 6.24 Euros is expended. Large farms (LF) and Medium farms (MF) are more efficient in terms of time spend doing heat detection and insemination. On the other hand, small farms (SF) tend to pay better than large farms and medium farms (LF,MF). The cost difference for insemination and heat detection (CIHD) between large (LF) and small farms (SF) is 0.42 Euros per sow. The fact that Large Farms have more inseminations compared to small farms make them more efficient in terms of time and cost.

References

1. G. Martel J.-Y. Dourmad a, B. DedieuLivestock Science Volume 116, Issues 1–3, July 2008, Pages 96–107
2. BPEX, Agriculture and Horticulture Development Board 2011 Interpig Report

Critical factors influencing the economic feasibility of smallholder pig farming in Western Kenya

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Introduction

Pigs are important livestock for many smallholder farmers in Sub-Saharan Africa because they form a source of financial savings, grow quickly, can be marketed at 8-10 months and provide income in times of need such as for school fees, medicine, social events (weddings) or when there are food shortages. Feed accounts for 80% of the cost of raising a pig therefore, traditionally, pigs run free to scavenge food. But this causes problems. Pigs dig and causing soil erosion and crop damage and acquire zoonotic diseases such as *Taenia solium* that causes tapeworm and epilepsy in people. The objective was to assess how season, average daily gain (ADG), opportunity costs of farm-grown feeds, pig weight and butcher price variation impact the economic potential of semi-intensive pig rearing.

Materials and Methods

The study was conducted in the rural Busia District, in the Western Province, Kenya where most people live via subsistence agriculture. Farmers feed their pigs combinations of farm-grown, free, and purchased feeds (Mutua et al., 2012). The feeds most frequently given to pigs were ugali (ground maize with or without millet cooked in oil and water) (88% of farmers), kitchen leftovers (83%), omena (small dried local fish) (78%), sweet potatoes (75%), sweet potato vines (65%), cassava (57%), brewers' waste (48%), maize (33%), fish innards (30%), and less frequently vegetables, mango, avocado and banana peels (Mutua et al., 2012). We developed an algorithm to emulate growing a pig from weaning weight to market weight for every combination of weaning month (January to December), opportunity cost of feeding pigs farm-grown feeds (100%, 75%, 50%, 25%, 0%, and 0% of the market price with no free feeds available; where 100% = full market price, whereas 50% = half market price), and ADG (80 to 180 grams per day in 25 gram increments). At each pig weight and for each combination of factors, the algorithm used a linear program to determine the least-cost feed ration and feeding cost required for feeding a pig to gain one kilogram. The incremental feeding cost and the cumulative feeding costs were tracked. For each pig weight the cumulative feeding cost and piglet purchase price were added together and compared to the price that could be received if the pig was sold to a local pig butcher. Nutrient content of locally available feeds and the suggested restrictions within a pig diet were resourced from Feedipedia, 2012; NRC, 1998; NRC, 2012. Free feeds included banana leaves and peels, cassava foliage and peels, grass, and sweet potato vines.
Prices were presented in Kenyan shillings at an exchange rate (June 2009) of 1 USD ~ 78.74 KES.

Results

Median pig weights at the slaughter were 23 kg and 38 kg for low and high seasons based on demand for pork. Farmers who sold a pig in the previous year did so because they needed money for school fees (26%), funeral or health expenses (21%), specified household problems (17%), purchase school uniform (15%), farm inputs (9%), food for the family (9%), or the pig was ready for market (2%).
Feed cost to raise a pig to 30 kg were affected by ADG, opportunity cost of feed, and weaning season by 982 Ksh, 947 Ksh, and 379 Ksh respectively. If ADG is too low, feed is used for daily maintenance rather than growth. Variable butcher prices and seasonal differences in pig prices varied the 30 pig price by 744 Ksh and 225 Ksh respectively. Most common feed items in least-cost diets were small dried fish, cooked ground maize, whole maize, millet, cassava foliage, sweet potato vines, bone meal, avocado, and mango.

Conclusions and Discussion

Farmers have higher profits if they feed pigs to reach higher average daily gains, have lower opportunity costs of feeds, or effectively bargain with butchers. To make a profit, farmers must have access to some 'free' feed and/or access to feed at 50% of market price.

Acknowledgments

University of Guelph for funding and pig butchers, farmers, and village elders for participation.

References

1. Mutua F K, 2012. Tropical Animal Health and Production, 44(6), 1157-1162.
2. Feedipedia, 2012. http://www.feedipedia.org.

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Medication costs in French pig farms: Evolution and herd typology

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Introduction

The reduce of medication costs is a major challenge to optimize production costs and to reduce antibiotic used (1). The medication costs from the French technical-economic database allow an annual monitoring of the use of antibiotics and other drugs (2). The herds characteristics links with the drugs levels can be also studies.

Materials and Methods

The evolution of medication costs over the last 10 years (2002-2012) in French pig farms was analysed. Medication costs collected in the national technical-economic database (GTE) were analysed in two types of herds: farrow-to-finish herds (n>1475 farms) and fattening herds (n>349 farms). The total medication costs and sub-categories were considered:

-Preventive = vaccine + livestock management product;

-Curative = orally-administered medication + antibiotic and anti-inflammatory injection.

A herd typology with an ascendant hierarchic classification based on the medication costs in 2012 was also done to analyze the impact of some farm characteristics and technical and economic results from the GTE (area, farm size, sow productivity, standardized margin, etc.).

Results

In farrow-to-finish herds (figure 1), the total medication costs decreased significantly by 1.2 €/100 kg of carcass (-18%) between 2002 and 2012, in relation to the decrease in orally-administered medication (-0.75 €/100 kg of carcass, -39%), in antibiotic and anti-inflammatory injections (- 0.46 €/100kg of carcass, -40%) and also in livestock management products (-0.41 €/100 kg of carcass, -27%). During the same period vaccination costs increased (+0.14 €/100kg of carcass, +6%). Over those 10 years, the levels of curative medication decreased by 40% (-1.21 €/100 kg of carcass) and were lower than that of preventive medication.

Medication costs for fattening herds also decreased significantly by 0.95 €/100 kg of carcass (-29%) between 2002 and 2012, in relation to the decrease of orally-administered medication (-0.85 €/100kg of carcass, -46%) and antibiotic and anti-inflammatory injections (-0.35 €/100 kg of carcass, -62 %). However, livestock management products and vaccines remained stable. Over the 10 years, the level of curative medication decreased by 50%.

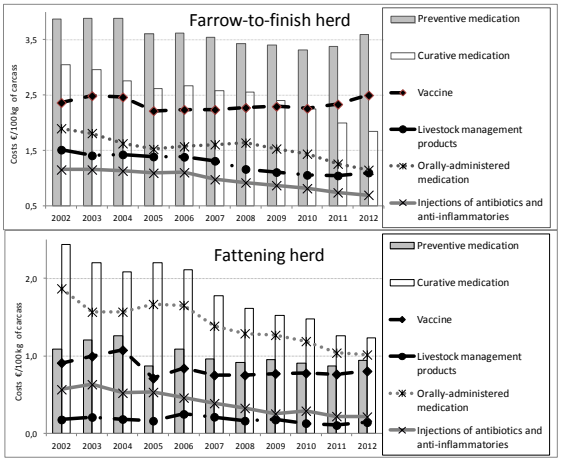

Figure 1. Evolution of medication costs in farrow-to-finish and fattening herds

Herd typology identified four groups of farms with quite significant profiles of medication costs. The influence of the geographical area, the impact of farm size and the relationship between levels of medication costs and some technical and economic criteria were shown in farrow-to-finish herds. Age at weaning, home-mixed feed, number of batches, the purchase of gilts or self renewal and growth performances (ADG and FCR) are not significantly associated with medications costs. In fattening herds only the geographical area was linked to medication costs.

Conclusion

The decrease in health costs, associated with a decrease in the use of curative treatments and an increased use of vaccines, meets the expectations of society. This is due to improvements in the health status of farms, linked to connection with the development of vaccinations and the awareness of the need to reduce antibiotic use.

Acknowledgments

This study was financially supported by INAPORC. Special thanks to farmers and technicians for their participation in the French Technical and Economic database.

References

1. EcoAntibio 2017 Plan, 2012.
2. Corrégé et al., 2013. J. Rech. Porcine, 44, 55-60

Individual Pig Care (IPC): A digital tool to improve pig management and optimize antibiotic use

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Introduction

In modern pig farming, the use of antimicrobials is of vital importance in order to maintain animal health and welfare (1,2). However, the use of antibiotics is under scrutiny due to its possible involvement in selection for resistant bacteria and the spread of resistance genes (3). Therefore responsible antibiotic use and its monitoring are one of the greatest challenges faced by modern pig production. On the other hand, competivenes and animal welfare demand an effective and economical control of diseases. To achieve both of these objectives, IPC was developed. IPC is based on daily keen observation of pigs, early detection of husbandry and health problems and prompt and accurate reaction to them, based on fast and effective digital data collection and processing.

Materials and Methods

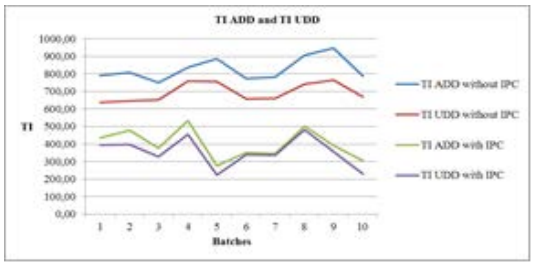
The study was conducted on a nursery farm. In total 5798 pigs were involved. Antibiotic use was monitored in five batches, during four rearing cycles. During the first two cycles (2848 animals), pigs were managed, working with the traditional method commonly used on the farm. In the following two cycles (2950 animals) the IPC protocol was used. The IPC method includes daily observations and reporting in a methodical manner. Parameters evaluated included respiratory-, enteric-, lameness-, neurological-, biting- and unspecific signs. Every sick pig was scored from A to D, depending on the severity of the disease. With IPC daily observation of individual pigs and associated quantification of symptoms resulting in eventual individual injection of piglets, was implemented instead of prophylactic and metaphylactic group treatment. To evaluate antibiotic consumption before and after IPC was introduced, the first ten batches were compared with the batches which used IPC. To quantify the antibiotic use, the treatment incidence (TI) was calculated, both based on the animal daily dose (ADD) and the used daily dose (UDD).

Results

During rearing cycle 3 and 4 (followed up with IPC) mainly 'A' animals were scored. Most animals were scored and treated for gastrointestinal signs during the first 10 days after weaning (38 % of total diseases), unspecific clinical signs (37 %) and ear and tail biting towards the end of the nursery period (17 %).

In the batches working with IPC, antibiotic use was significantly lower than in de batches working with the traditional method. The average TI ADD and TI UDD values were 826.8 ± 66.0 and 693.9 ± 53.3 in the group without IPC, compared to 399.5 ± 68.0 and 354.1 ± 84.3 in the group with IPC ($p<0.05$) (t-test for averages) whereas production parameters such as average daily gain and mortality were comparable in both groups. The treatment incidences for the different batches are shown in figure 1.

Figure 1. TI ADD and TI UDD of the 10 batches without IPC vs. the 10 batches with IPC



Conclusions and Discussion

Antibiotic use was reduced significantly by accurate and consistent individual observation and treatment of pigs. Identifying and quantifying disease symptoms at an early stage resulted in a drastic reduction of the number of group treatments and hence total antibiotic consumption. This study demonstrates that it is possible to optimize health management and to reduce the consumption of antibiotics, using IPC.

References

1. Mateu E., Martin M. 2001. Why is anti-microbial resistance a veterinary problem as well? Journal of Veterinary Medicine 48:569-581.
2. McEwen S.A., Fedorka-Cray P.J. 2002. Antimicrobial use and resistance in animals. Clinical Infectious Diseases 34:93-106.
3. Schwarz S., Chaslus-Dancla E. 2001. Use of antimirobials in veterinary medicine and mechanisms of resistance. Veterinary Research 32:201-225.

Use of Analysis of Means (ANOM) for statistical evaluation of production data

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Introduction

Production data comes in many forms. Data such as farrowing rate (FR) and pigs born alive/litter (PBA), are often normally distributed and, as such, may be analyzed with statistical methods such as ANOVA (followed by the appropriate test for means differences). An alternative statistical method, the ANOM, provides a more visually based method. It's simple visual format is easy to evaluate for lay staff accustomed to viewing SPC charts (1). This paper demonstrates the use of ANOM to analyze before-after data from a PRRS control project.

Materials and Methods

Production data was collected as part of a PRRS virus control project described in more detail elsewhere (2). Phase three marked a return to maize as the cereal grain utilized (from wheat in phase 2).

Briefly, sow herds were loaded with replacement gilts, closed, and Ingelvac PRRS® MLV (Boehringer Ingelheim Vetmedica, Inc., St Joseph, MO) applied twice 30 days apart, then quarterly thereafter. The benchmark period was the 18 month period prior to the initiation of the project. Each phase represented 12 – 18 month periods of a systematic approach to PRRS control. Data was provided by the production system in spreadsheet form, validated, and evaluated using a statistical process control tool, ANOM (Minitab 7.0). Pigs born alive per sow farrowed (PBA/SF, Figure 1) and growing pig (nursery phase) average daily gain (Figure 2) are presented.

Results

The ANOM method indicated statistically significant improvements in reproductive (Fig. 1) and growing pig performance parameters (Fig 2). The ANOM demonstrated significant differences in PBA/SF from the grand mean for each phase; the base line below, and Phases 1-3 above. For ADG, ANOM demonstrated all but Phase 2 (where the predominant change was wheat substituted for maize, with a subsequent reduction in feed intake) were different than the grand mean (baseline below, Phase 1 and 3 above; Phase 2 within control limits).

Conclusions and Discussion

ANOM and more traditional statistical methods can each identify significant changes in parameters. ANOVA requires a two step-approach to first detect if any individual group means were different than others, then a second test to reveal group to group relationships. The ANOM uses a single step to identify differences in means compared to a grand mean of all study groups.

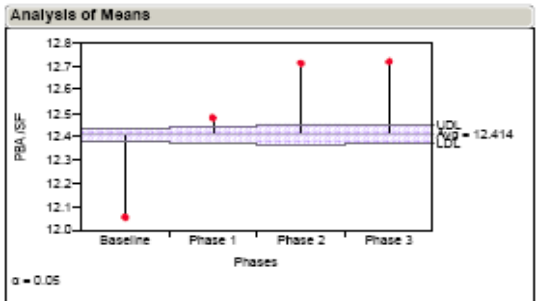

Figure 1. ANOM analysis of PBA/SF.

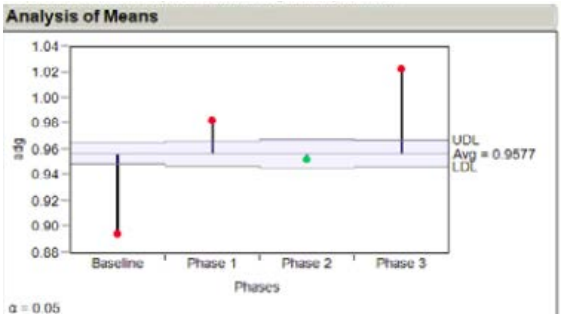

Figure 2. ANOM of ADG

A key point to remember is that the ANOM compares group performance to a grand mean of all data, rather than comparing groups directly to one another, as with ANOVA. Period vs overall mean comparisons are more conservative, limited only to the grand mean and not between individual groups (e.g. Fig 2; 1).

Both ANOVA and ANOM are suitable options for production data analysis. The ANOM procedure identifies means that are significantly different and offers a simple graphical representation of group means. Due the visual representation of the data, ANOM can be a useful method to easily identify improvement changes and assess practical differences.

References

1. Wheeler D et al. 1992.
2. Oropeza A, et al. Proc IPVS 2012, p.

Economic model to evaluate projects of pathogen elimination from breeding herds

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Introduction

Infectious diseases decrease productivity and increase cost of production resulting in lower competitiveness (1). Producers and veterinarians need tools for making informed decisions with regards to pathogen elimination (2). Some cases are a clear cut decision; however, when dealing with *Mycoplasma hyopneumoniae* infections, the decision of implementing a pathogen elimination plan is not straightforward. Therefore, the purpose of this study was to develop an economic model to translate the expected performance improvements into economic value and compare those to costs of a *M hyopneumoniae* elimination program. In this case eliminating the bacteria was worthwhile from an economic standpoint.

Materials and Methods

The economic model used in this study had 4 steps:

1. Calculation of costs associated with the elimination program. These included antibiotic therapy, labor supplies, breeding interruption and a lost profit margin of $50 per piglet not weaned (Fig 1).
2. Performance improvement.
   a. This system owned all finishing spaces. The premium this system had for better ADG was estimated considering that pigs would reach market weight faster, and thus it could make more turns/year, as demonstrated in figure 2.
   b. Feed conversion was estimated to improve by 0.04, therefore, (0.04)*(122 Kg from wean to finish)*($0.18 average feed price per kg)*(70,000 finishers per year).
   c. After eradication, the farm would save costs attributed to vaccination and medication.
3. The costs were compared to benefits of the program in a 5-year return of investment analysis considering a discount rate of 12%.
4. Final project value was calculated considering a success rate of 80%. The cost of failure was considered the total project cost (Fig 1) without benefits (Fig 2).

Results

Figure 1. Costs associated with the health program

Additional drug therapy		
Sows	R$	200,000
Piglets	R$	-
Facilities decontamination		
Labor	R$	960
Supplies	R$	5,000
Other costs		
Temporary interruption (6 weeks) of breedings ($50 lost margin per piglet not weaned)	R$	431,530
TOTAL	**R$**	**637,490**

Figure 2 – Estimated benefits of the project

1-	**Improvement of Average Daily Gain (ADG):**			
	After eradication project, pigs will grow faster to market weight			
	Days at wean-to-finish	Expected improvement on ADG (g/day)	Total extra gain per head (Kg)	Extra revenue/head (@$1.50/live Kg)
	155	10	1.55	$ 2.33
	Extra feed consumed due to extra Kg gained:			
	Extra Kg	F:G	Ave. feed price	Extra feed cost / hd
	1.55	2.40	$ 0.18	$ 1.86
	Additional margin per head:			$0.47
			Total $ extra/year (70,000 finishers)	**R$ 32,550.00**
2-	**Feed to Kg gained (Feed conversion)**			
	F:G improvement opportunity			**0.04**
	Gained Kg during wean-to-finish	(6 to 128 Kg)		**122**
	Average feed cost			**$ 0.18**
			Savings on feed cost:	**$ 0.88**
			Total annual feed cost savings (70,000 finishers)	**$ 61,488.00**
3-	**Savings with vaccinations**			
	Number of weaned pigs (vaccinated pigs):			76,087
	Cost of vaccinations per pig:			$ 0.60
			Total savings per year	**$ 45,652.17**
4-	**Savings on medications**			
	Cost of medications for pathogens to be eradicated			
			Total saved per year ($1.50 per pig):	**$ 105,000.00**
Grand total				

	Annual benefits	Year 1	Years 2-5
1	Better ADG	$32,550.00	$32,550.00
2	Better F:G	$61,488.00	$61,488.00
3	Savings on vaccines	$45,652.17	$45,652.17
4	Savings on medications	$105,000.00	$105,000.00
	Total	**$244,690.17**	**$244,690.17**

Figure 3. Cost-benefit analysis

5-year model for Benefit-Cost analysis

Discount factor (%) 12

	Benefit			Costs			Benef - Costs	Benef - Costs
Year	Gross Benefit	Discount factor	NPV* Benefit	Gross costs	Discount factor	NPV* Costs	Gross	NPV
1	$ 244,690	0.89	$ 218,473	$ 649,090	0.89	$ 579,545	$ (404,400)	$ (361,072)
2	$ 244,690	0.80	$ 195,066	$ -	0.80	$ -	$ 244,690	$ 195,066
3	$ 244,690	0.71	$ 174,166	$ -	0.71	$ -	$ 244,690	$ 174,166
4	$ 244,690	0.64	$ 155,505	$ -	0.64	$ -	$ 244,690	$ 155,505
5	$ 244,690	0.57	$ 138,844	$ -	0.57	$ -	$ 244,690	$ 138,844
		PVB=	$ 882,053		PVC=	$ 579,545	$ 574,360	$ 302,508

*Net present value	$ 302,508
Benefit/Cost ratio	1.52
Internal rate of return	48%

Figure 4. Payoff table including chance of success

	NPV	p	($) (p)
Chance of success	$ 302,508.23	0.80	$ 242,006.58
Chance of failure	$ (637,490.49)	0.20	$ (127,498.10)
Project value			**$ 114,508.49**

Assuming 80% success chance, this project was economically feasible, as it resulted on a $144,508 net gain. In case of success, the net annual gain would be $ 244,690, which represented $3.26 per marketed pig.

Conclusions and Discussion

This economic model is a tool to help producers/ veterinarians decide whether or not pathogen elimination is an option. It takes into account project costs, translates expected improvements on productivity on economic figures, which allows making benefit over cost analysis including chance of success or failure.

References

1. Neumann, et al. 2005. J Am Vet Med As. 227 (3):385-392
2. Dijkhuizen, et al. 1995. Prev Vet Med. 25 (2):135-149.

O.178

Effect of sow lameness on herd throughput

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Introduction

Sow lameness is a common cause of not only early removal of sows but also lower productivity of sows that remain in the herd (1). This productivity decrease can be seen as lower quality of progeny, decreased likelihoods of pregnancy and higher rates of preweaning mortality (2). However, it is not only sow productivity that should be a concern. Lame sows, when culled, are often not replaced in a timely fashion or they cluster in time so that the number culled exceeds gilt supplies. This could decrease the likelihood of meeting target herd output. The objective of this study was to predict the overall farm-economic effects of sow lameness, including the effects on herd output characteristics and value.

Materials and Methods

An economic model was built to simulate production during one breeding cycle. Data from 52 weekly breeding groups in a herd with an overall prevalence of approximately 20% lameness in sows at breeding was used. Performance was estimated (separately for lame and non-lame animals) for weaned sows, returned sows and gilts.

Production outputs were estimated based on expected performance and piglets were valued based on quality at weaning, dam parity and level of output. Lameness was predicted to affect not only retention likelihoods but also the quality of progeny from lame classes of sows.

Production was simulated using @Risk version 6.1 (Palisade Corporation, Ithaca, NY). The model was run for five different levels of lameness, varying from 20% to 0%.

Outputs of 30,000 iterations of breeding cycles, run for each level of lameness, include gross margin over feed and replacement costs per breeding group, likelihood of meeting target output and pigs per sow per year.

Results

Expected pigs weaned per mated female per year increased from 25.07 to 25.43 as lameness was reduced to 0%. Figure 1 shows the increase in expected number of acceptable piglets weaned and likelihood of meeting the production target as lameness is decreased. Figure 2 shows the cumulative distributions of expected margin as lameness is reduced.

Conclusions and Discussion

While reducing lameness increases productivity as measured by the expected number of pigs per sow per year, the effect of lameness is better illustrated by examining the output of the herd. The ability to meet production targets is a function of not only increasing the number of pigs weaned but also decreasing the variation in output. Moreover, the effect of decreasing lameness is not only an effect of the consistency of the production of pigs but also an increase in the quality of the pigs weaned.

This model underestimates the effects of lameness on herd dynamics and variability and continues to be refined as we understand and can predict more about the effects of lameness not only on the sow productivity, but also on the dynamics of the herd, particularly in replacement behavior and stability of breeding groups. These aspects will only add to the benefits of lameness control shown here.

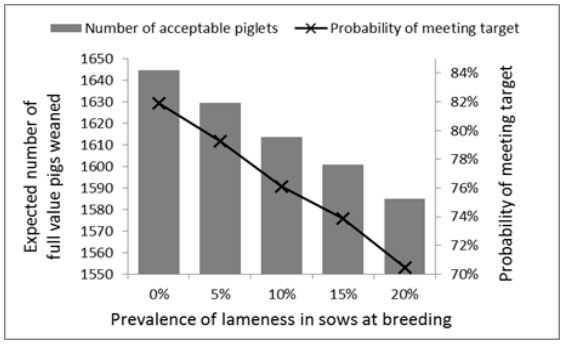

Figure 1. Expected number of acceptable piglets weaned and likelihood of meeting target when lameness is reduced in a swine herd

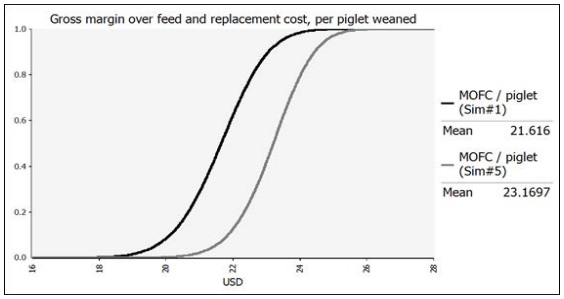

Figure 2. Cumulative distributions of margin over feed and replacement net costs when lameness is reduced from 20% (darker line) to 0%

References

1. Anil A et al. 2009. JAVMA 235, 734-738
2. Heinonen M et al. 2013. Livestock Sci 156: 2-9

Indoor climate for fatteners during summertime in presence and absence of chill

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Introduction

Rearing pigs to market weight in temperatured zones has focused on maintaining comfortable temperatures during wintertime. However, also in temperatured zones risk for side effects due to Temperature-Humidity Indexes (THI) above 25 may arise in summertime [1]. Therefore the aim of this study was to evaluate the potential of running the heating system backwards during the summer with the aim to accomplish a more comfortable indoor climate.

Table 1. Study periods during the years and mean climatic conditions during the study periods

	2010	**2012**	**2013**
Study period	May-July	July-Sept	June-Aug
Outdoors**			
Temp (°C)	19.6±5.4	17.3±4.4	20.2±3.1
Humidity (%)	48.3±11.5	74.2±14.3	55.0±9.2
THI	**18.1±3.9**	**16.7±3.3**	**18.8±2.3**
THI-max	**22.8**	**22.7**	**21.6**
Indoors			
Temp (°C)	24.0±2.8	23.3±1.5	23.6±1.7
Floor temp (°C)	26.2±1.7	25.3±1.4	26.3±0.8
Humidity (%)	49.4±9.6	70.0±8.2	56.2±5.5
THI	**21.4±2.3**	**21.9±1.3**	**21.4±1.5**
THI-max	**24.8**	**25.1**	**23.3**
Indoors; 16.00-19.00			
Temp (°C)	25.1±3.5	24.3±1.7	24.6±2.2
Floor temp (°C)	26.3±1.8	25.2±1.4	26.5±0.7
Humidity (%)	47.6±8.9	65.8±9.4	54.3±7.3
THI	**22.1±2.7**	**22.4±1.4**	**22.1±1.7**
THI-max	**25.5**	**25.8**	**23.9**

** = mean of whole day (from 07.00 to 19.00)

Materials and Methods

The study was made in an integrated herd with a module stable for fatteners with a natural mechanic ventilation system (www.nyborghuse.dk). Each unit had two pens sized 19.6 m^2, out of which 5.7 m^2 was a dunging area located outdoors. Each pen housed 20 pigs (0.98 m^2 per pig). Pigs entered the stable at 12 weeks of age and were reared to market weight all in-all out. Temperature and relative air humidity were monitored and Temperature-Humidity Indexes (THI) were established.

Summer batches from three years were compared. In 2010, no attempt to chill was made. In the summer of 2012 a heat exchanger with a high effect (Alfa Laval 70 kW; Δt = 10 °C) was installed and connected to the water-based heating system of floors, running from the 18th of July in 2012 and from the 28th of May in 2013.

Table 2. Mean temperatures of water in the heating system during 2013

	Into stable	From stable	*Difference*
Am (°C)	24.2±0.8	26.2±0.8	*2.0±1.1*
Pm (°C)	24.7±1.0	26.3±0.5	*1.6±1.0*

Results

The mean outdoor temperatures ranged within 3°C between the three years. The summer 2012 was coldest and the outdoor THI was lowest that year. Despite this, the mean indoor temperatures and THI-values were equal.

In 2013, the water from the heat exchanger increased with 2.0±1.1 °C from entering to leaving the fattening stable at 08.00 and with 1.6±1.0 °C at 16.00, indicating an absorbance of heat from the concrete floor (Table 2). The mean floor temperature was 1°C lower in 2012 compared 2012, but equally high in 2013 as in 2010 (Table 1).

The mean THI-levels were below the critical value of 25 during all years. However maximal THI values above 25 were denoted in 2010 and 2012, but not during 2013. The mean indoor temperatures were constantly 1°C higher in the late afternoon. Thus, also the highest THI-values were recorded in the afternoons (Table 1).

Conclusions and Discussion

As the top THI-values obtained in 2010 and 2012 exceeded 25, the results confirmed the risk for heat stress of pigs also in temperatured zones. Chilling floors could be a way to deal with this, but the results obtained indicated the need for high efficacy of such systems - which we think we had. Obviously the concrete floors accumulate heat, which probably also makes the time point of initiating chilling critical and we will further scrutinise the impact of that. Despite the failure to document a true decrease in floor temperature, a possible positive effect was indicated since THI-values above 25 not were recorded in 2013.

References

1. Zoric et al, 2012. Sw Vet J. 64, (1) 11-21.

O.180

Electronically controlled feedback dosing of gestating sows and gilts on electronic sow feeding stations

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Introduction

Feedback of feces (placenta, etc.), as a source of infective material extracted from the same farm, and administered to sows during gestation, has long been described as a crude method to induce infection and immunity to certain diseases[1]. This management has proven useful for diseases that do not have protective commercial vaccines.

Feedback might have various drawbacks and is not necessarily consistent in results; however, it is not the focus of this paper to discuss this. One of the problems related to feedback is its implementation. For feedback to be useful, it is basic to administer the infective material consistently at the right time of gestation, and during a long enough period of time. This job is difficult and time consuming, which frequently is a cause for failure in efficacy.

With Electronic Sow Feeding Stations (ESFS), the possibility of administering feedback to individual sow using the SEFS a modified paint spary system was evaluated.

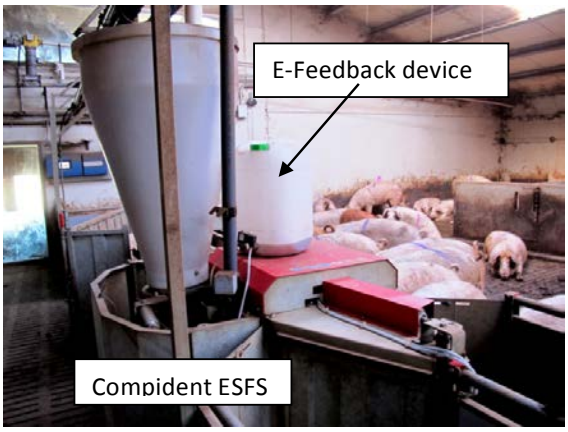
Materials and Methods

An E-Feedback device was developed by Optimal Pork Production, to adjust to the Compident® ESFS (Schauer Agrotronic GmbH). with the purpose of automatic dosing of feedback to sows and gilts, simplifying labor to a minimum. The design was evolved from prototype 1 to prototype 2, to ensure; a) ease of use, b) automatic recording of treated sows, c) minimal movable parts, d) low cost e) automatic down load of recorded information.

The E-Feedback device is composed of: 5 lt. Plastic bin. Electovalve, and a plastic hose. The device is connected to the paint spray system of the feeding station. This means that it´s use can be programed by the ESFS software. Information of sows to be treated, length of treatment, and dose, was entered in the software.

Rotavirus induced piglet diarrhea and gut content, was diluted to a liquid solution following the recommendations of the farmers veterinarian. It was later filtered to avoid clogging up of the valve. The feedback liquid was administered for 5 days to pregnant gilts and sows from day 80 to 85 of gestation, at a dose of 50 ml per sow/daily, at their first feed.

The authors were only involved in the design and testing of the E-Feedback device, not on the health related aspects of the feedback itself.



Results

The E-Feedback device was tested for a period of twenty two months in two large scale commercial farms in Spain.

The device managed to deliver efficiently the product for all the length of the treatment. When the bin was empty, the software delivered the following alarm: “empty paint can”.

Dosing treatments were completed in all cases, and consumption of the feedback was at the rate predicted.

Efficacy of feedback was variable.

Conclusions and Discussion

A summarized over all evaluation by users was:

All staff involved were trained in the use of ESFS.

Dosing management was greatly aided and simplified by the E-Feedback device.

Care should be taken to avoid clogging if the valve and outlet hose. Filtering of the mix is strongly recommended.

At the end of the treatment, staff should regularly evaluate the adequate flow of the liquid, since the pressure tends to diminish within the plastic bin.

Acknowledgments

Albesa-Ramadera Farm staff., Piensos Costa S.A. Fraga, Spain. Group Batallé S.A. Gerona, Spain.

References

1. A.D. Leman,B.E. Straw, W.L Mangeling, S.D. Allaire, D.J. Taylor..Diseases of swine seventh edition. 1992. P. 815-816.
2. J. Vansicle**.,** Refining feedback., www.nationalhogfarmer.com. Aug.15, 2012.

Design of an evaluation sheet to estimate efficiency of biogas production in swine farms digesters in Yucatán, México

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Introduction

Effluent management is an important issue for New Zealand's rural sector. It can be a source of unpleasant odours and also a substantial contributor to farm greenhouse gas (GHG) emissions, particularly methane, which is 21 times more potent than carbon dioxide.

The collection and combustion of methane has been identified as a sensible means for reducing agricultural GHG emissions. Where factors like scale and location allow for it, additional benefit can also be derived - for example, the combustion of recovered methane in a generator for producing on-site heat and electricity.

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Effluent management is an important issue for New Zealand's rural sector. It can be a source of unpleasant odours and also a substantial contributor to farm greenhouse gas (GHG) emissions, particularly methane, which is 21 times more potent than carbon dioxide.

The collection and combustion of methane has been identified as a sensible means for reducing agricultural GHG emissions. Where factors like scale and locationallow for it, additional benefit can also be derived - for example, the combustion of recovered methane in a generator for producing on-site heat and electricity.

Effluent management is an important issue for the rural sector, especially swine farms. It can be a substantial contributor to farm greenhouse gas (GHG) emissions, particularly methane, which is more than 20 times more potent than carbon dioxide (1,2). The collection and combustion of methane has been identified as a sensible means for reducing agricultural GHG emissions. Several biogas plants with digesters are available to dispose swine manure (3). Methane as a byproduct may be combusted to produce heat and electricity.

Under the Kyoto Protocol and its most important tool, the Clean Development Mechanism, the Local Pork Producers Livestock Association of Merida (LPPLAM) installed 74 anaerobic slurry digesters, using public founding. It has been reported to produce approximately 130,000 m3 of biogas / day, equivalent to 78 000 m3 of methane with an estimated GHG emission reduction is 428,600 t CO_2 (4). However, this program has not been evaluated and no one knows what the environmental, energy and economic benefits may generate.

Therefore, the objective of the present study was to design an evaluation instrument to determine the impact on energy transformation and on the environment, due to the installation of biogas digesters in Yucatán, México.

Materials and Methods

On site visits to a random sample of swine farms listed in the LPPLAM was carried out, in order to observe the housing, management and the biogas operation system. Visits were carried out during the first semester of 2013. Emphasis was made in the following aspects:

Location, source of water, type of animals, size of the farm, type of biogas plant, use of fermented byproducts, energy generation.

Data was analyzed and compared to the original requirements requested by the Mexican authorities at the time the financial aid was given to the farms.

Results

Seven variables were detected to be of the most importance in order to evaluate the effect of the use of slurry fermenter on energy transformation and on the environment:

1. Farm characteristics.
2. Use of water
3. Biodigesters, type and functionality
4. Biodigesters, biogas production and utilization.
5. Use of digestate.
6. Engines, pumps and other equipment.
7. Inputs and outputs of the digester

Conclusions and Discussion

The design of the evaluation sheet in shown in Table 1.

Table 1. Contents of the evaluation sheet design to estimate the efficiency of the use of biodigesters (BD).

VARIABLE	Number of items	Acti-vity
Farm Type	10	1
Use of water	11	1
BD type	19	1
BD product	10	2
Digestate	10	1
Equipment	10	2
In and out	9	3
Total	79	

[1]Questions made on site
[2]Measurements taken on farm such as: biogas production, electricity production, among others
[3]Lab analysis on slurry samples before and after treatment

The use of this evaluation sheet may provide a diagnosis of the functionality of the biodigester in swine farms, and may be able to determine which unit operation can be more efficient to increase the energetic income of the system. From the environmental point of view, this diagnose may improve its quality detecting mal functions that may damage soil and water resources.

Acknowledgments

Fundación Produce Yucatán, A.C.

References

1. Sejian V et al. 2013. Environmental Stress and Amelioration in Livestock Production, Pp 469-499.
2. Solomon S et al. 2008. Proc. of the National Academic of Sciences of the USA. 106:1704-1709.
3. W/o author. 2009. Biogas, an introduction. Fachagentur Nachwachsende Rohstoffe e.V. (FNR) Hofplatz 1 · 18276 Gülzow · Germany
4. http://www.porcicultoresmerida.com.mx/

Enhancing immune system by nutrigenomics in swine

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In the last 15 years, swine production have focused in meeting the demands of an always demanding consumer, craving for a tasty and fat-free meat that is conceived as good for his well-being. Lately, this list has grown to include an antibiotic-free product from a good-cared animal that also promotes the consumer´s health. Promoting health through nutrition is a science with a new name and not so new data that probes that selected nutrients stimulate gene responses.

Nutrigenomics is a field of nutrition and medicine that investigate the effect of nutrients in human beings' genome, targeting diseases as obesity, diabetes, cardiopathies, cancer and others; in order to improve human health (4). In farm animals, Nutrigenomics has been used mainly to promote health by nutrient stimulation of the innate immune system, with the aim to reduce vaccination and antibiotic use. Lean accretion is also an area for this relatively new science, and chromium and selenium are the most studied nutrients for stimulation of genes related to protein deposition in the muscle cell.

Once the immune response mediated by specific antigens is onset, nutrient utilization is redirected to functions related with antibody and defense cells production. In any pathogenic challenge the immune system undergoes an inflammatory response characterized by the release of pro inflammatory cytokines (IL-1β, IL-6, tumor necrosis factor α and other inflammatory mediators. These molecules are known for reducing appetite and growth; increasing body's nutrient mobilization, and altering loads of metabolic processes (2). In addition, there is an immunosuppressive effect by the releasing of anti-inflammatory cytokines (IL-10) (3). Hence, the immunosuppressed animal will show a reduced growth due to its incapacity of dealing with the aggressor.

In the intestinal lumen an active immune response affects growth by reducing the absorption of nutrients by mechanisms such as an increase in the secretion of mucines that reduced the interaction nutrient-microvilli.

Therefore preventing the activation of the acquire immune system is one of the goals of Nutrigenomics in farm animals. After the ban of the antibiotic growth-promoters and the introduction of microorganisms and their constituents, as substitutes; an interesting effect was observed. Yeast *(Saccharomyces cerevisiae)* wall components known as mannan-oligosaccharides (MOS), have the property of stimulating the animal´s immune system. This effect is mediated by gene stimulation positively altering the cellular, inflammatory and antimicrobial immune responses; through pathways such as toll-like receptor, interferon signaling, and retinoic acid inducible protein receptors (9).

The MOS have also demonstrated a modulatory effect on the intestinal immune system by up-regulating genes such as toll-like receptor 3, interferon regulatory factor 7, and the suppressor of cytokine signaling 1. MOS also alters the RNA intestinal content improving the activity of some intestinal enzymes (maltase, leucine, aminopeptidases and alkaline phosphatase) (7). MOS also increase the production of short-chain fatty acids, reduced epithelial cell apoptosis, and stimulate the activity of the intraepithelial lymphocytes (5, 11).

References

1. Brennan K et al. 2009. Poult Sci 89(Suppl 1):650.
2. Che M et al. 2011. J Anim Sci 89:2592–2602.
3. Che M et al. 2011. 27th Int Symp Alltech: 1-15.
4. Ghornade V et al. 2011. Vet Res For 2: 147-155.
5. Halas V et al. 2012. Animals 2: 261-274.
6. Hooge D et al. 2011. Int J Poult Sci 10: 819-824.
7. Mooran C. 2004. 24th Int Symp Alltech: 283-296
8. Xiao R et al. 2010. IPSF Atlanlta, GA, 76. Poster.
9. Xiao R et al. 2010. Poult Sci 90(E-Suppl. 1).
10. Xiao R et al. 2011. PSA 100th Annual Meeting. Poster.
11. Yitbarek A et al. 2012. Poult Sci 91:1105–1112

Effect of *Bacillus subtilis* C-3102 (Calsporin™) on the general performance of commercial sows in México

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Introduction

The use of antibiotic feed additives is decreasing due to the concern of residues in animal tissues intended for human consumption, and the increased bacterial resistance to human therapeutic antibiotics. Direct-fed microbials (DFM) are one of the more promising alternatives to antibiotic feed additives due to their multiple beneficial effects on the intestinal structure and function (1,2,3,4). The health and performance of the piglet is highly dependent of the sow health status, and the sow´s microbial balance at gut level will affect how the piglet grows. Therefore, the objective of the present study was to evaluate the effect of the supplementation of a DFM based on *Bacillus subtillis* C-3102 (Calsporin™ 600 MM Mx), on the general performance of sows under commercial conditions in México.

Materials and Methods

In a commercial farm in the center of México, 48 individually allocated multiparous hybrid sows, were selected and randomly divided in two groups. The control group (23 sows) received a regular diet and feeding program for gestation and lactation phases; and the DFM group (25 sows) received the regular diets along with the DFM (Calsporin™ 600 MM Mx), as follows: 1.0 kg/ton of feed during gestation, and 0.5 kg/ton of feed during lactation. All animals were kept under similar conditions. The response criteria were: number of live-born piglets, number of died-born piglets, number of mummified piglets, litter weight at birth and weaning, litter average daily weight gain, and mortality. The data were analyzed based on the used design, using a T Student test, of the Software SPSS.

Results

Even though no statistical differences were found between treatments ($p>0.05$); the supplementation of the gestation and lactation diets with the DFM improved the performance of the sows. We found: a 12.4% increase in the the number of live-born piglets, a 43% reduction in the died-born piglets, a 7% increase in the litter weight at birth, and 9% in the litter weight at weaning (Table 1).

Conclusions and Discussion

Microbial gut balance is key for animal performance, under commercial conditions many factors could influence the health and productivity of the sow and its litter; the use of a DFM based on *Bacillus subtilis* C-3102, as an additional dietary factor, sowed an interesting trend for improving the general performance of multiparous sows. Because similar responses have been observed in other places with the evaluated DFM (3,5), the industry should continue the evaluation and progressive adoption of this DFM as a practical tool for improving the sow and piglet performance under commercial conditions.

Table 1. Performance of multiparous sows fed with or without a DFM based on *Bacillus subtilis* C-3102, during the gestation and lactation phases.

Response criteria (1)	Treatments		*P* value
	T1 (Control)	T2 (Calsporin)	
Live-born piglets	9.57	10.76	0.07
Die-born piglets	0.65	0.28	0.08
Mummified piglets	0.30	0.24	0.87
Litter weight at birth (kg)	14.43	15.46	0.28
Litter weight at weaning (kg)	56.35	61.52	0.28
Litter ADWG	2.04	2.11	0.52

[1]Average values form 23 litters (control group) and 25 litters (Calsporin™ group).

Acknowledgments

To the commercial farm staff , Puebla, México.

References

1. Starke IC et al. 2013. Beneficial Microbes 4(4):345-356.
2. Taras D et al. 2006. J Anim Sci 84:608-617.
3. Hoodge D et al. 2004. J Appl Poult Res 13:222-228.
4. Cui C et al. 2013. Genet Mol Res 12(2): 1766-1776.
5. Marubashi T. 2010. Proc 21st IPVS Congress. 1020

Growth performance and bone characteristics in finishing pigs fed or not phytase at different calcium to phosphorous ratios

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Introduction

Excessive P in livestock wastes is a growing concern throughout the world; also on a worldwide basis P represent an economic concern being normally the 2nd most costly nutrient in swine diets. To reduce diet cost formulation and the use of mineral P, Phytase enzymes are normally used. In contrast, Ca sources are relatively cheap and, thus often included in excess, resulting in negative impacts on swine performance as it can prevent adequate P, energy and protein digestibility, and are associated with impaired Phytase activity, and altered bone mineralization. The objective of the present study was to determine the interaction between 2 Ca:P dietary ratios and the inclusion or not of a Phytase, on finishing pigs' growth performance and bone responses as different methodologies are applied.

Materials and Methods

A total of 78 barrows (initial BW= 51.9±4.4kg) were fed during the last 63d of the finishing period to assess the response to Ca:P ratios set at 1.2:1 and 2:1 and the factorial addition or not of a Phytase (HiPhos, DSM México) at 750 units/kg of feed. The experiment design was a RCB in a 2×2 factorial arrangement, being blocks pigs from 2 consecutive farrowing groups. During the 63d period, pigs were fed three 21d feeding phases, all calculated to satisfy nutrient requirements (NRC, 2012), except for those including Phytase, which where formulated to a lower (0.1 units %) digestible P content. Upon completion of the experimental period, pigs were commercially slaughtered and each pair of the 3rd metatarsal, 3rd metacarpal and the fibula were collected. Fibulas were subjected to a bone breaking strength test using a TX2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY). Adhering tissue was collected after autoclaving at 110C/1h, bones from the right side of the animal were defatted (fat extractor, ANKOM XT10), after that left and right bones were dried at 105C/24h and ashed at 600C/24h. Data was analyzed using the Mixed Models, and means are presented as the least squares means (SAS v9.2, Cary, NC, USA).

Results

There were no differences (P>0.2) due to Phytase and there were no interactions between Phytase and Ca:P ratios (Table 1). However, there was a negative effect (P<0.04) in response to the higher Ca:P ratio in daily weight gain (ADG) and feed efficiency (G:F), at similar feed intake (P>0.2). No differences were detected in ash density of the 3rd metacarpal (45.8±1.43%) or the 3rd metatarsal (43.1±0.99%) bones. Fibula ash was 2.8% higher when Phytase was used (P<0.1), conversely percent defatted fibula bone ash (DFFA) was higher (P<0.11) when diets were formulated to a better Ca:P ratio (1.2:1). The fibula breaking strength (BBS) was similar (P>0.75) for treatments and not affected by Ca:P or Phy.

Table 1. Growth performance and fibula bone ash of finishing pigs fed 63d with different Ca:P ratios, with or without Phytase.

Phyt1	0	750	0	750		P<	
Ca:P	1.2:1	1.2:1	2:1	2:1	EEM	Ca:P	Phy
ADFI 2	3.04	2.93	2.96	2.89	0.079	0.43	0.24
ADG 2	0.98	0.94	0.91	0.9	0.020	0.01	0.28
G:F 2	0.33	0.32	0.31	0.31	0.006	0.03	0.76
Fibula ash, %	51.4	53.2	51.3	52.4	0.82	0.54	0.07
DFFA3	56.6	56.7	54.4	55.6	0.95	0.10	0.51
BBS, 4	32.8	32.6	32.5	34.1	1.85	0.75	0.71

[1]Phytase Units/kg of diet. [2] In kg. [3] Defatted fibula ash. [4] Bone breaking strength, kg

Conclusions and Discussion

Higher Ca (Ca:P ratio) gives no benefit in productive performance or bone ossification, moreover Ca excesses may be negatively affect these response criteria. Instead, Phytase could effectively correct digestible P apportion and resulted in improved fibula ash, independently of the Ca:P ratio. These results suggest that the effects of a Ca in excess are in absorption and resorption and that, It is apparent that in Ca sufficiency, bone growth is P dependent. Fibula bone ash was a more practical and sensitive indicator of the mineral nutritional status of finishing pigs than the 3rd metacarpal or metatarsal bones.

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Stimulation of feed intake of lactating sows using different additives

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Introduction

Intensive production and the genetic improvement of farm pigs have resulted in low feed intake in both growing and breeding animals. In the later, diet contribution to milk synthesis is becoming less important (1,2), with a consequent body mass loss during lactation. Management strategies such as wet feeding work partially, but these and the stimulation of feed intake by the animal´s own appetite can be a better alternative. The objective of this study was to evaluate the effect of four additives on feed intake and its effect on weight loss, backfat depth, wean to estrus interval, litter weight at weaning, average daily gain and pre wean mortality.

Materials and Methods

Ninety lactating sows and their litters were selected and fostered according to piglet birth weight, forming homogeneous litter groups of 11 piglets, within 24 h postfarrow. The sows and their litters were distributed in a random block design with parity as the blocking factor. Sows and piglets were assigned to five experimental treatments (18 sows/treatment), which consisted in different feed additives with claiming appetite-stimulant roles. Control (C, basal diet); Phytogenic 1, commercial additive powder of essential oils obtained from oregano, anise, citrus peels and fructooligosaccharides (F1, 1 kg/ton); Phytogenic 2, commercial additive made from extracts of anise, aloe passionflower and other compounds (F2, 1 kg/ton); Phytogenic 3 commercial additive based on natural plant extracts (F3, 5 kg/ton); and dried Plasma (PL, 5 kg/ton). The lactation length was 26 days and feed was offered three times a day. Variables measured were: sow feed intake; in the sow: body condition: backfat at P2 (RENCO Lean Metter ®), weight loss during lactation, wean to estrus interval. In the litter: litter weight at birth, after foster and at weaning, average daily gain (ADG); and prewean mortality.

Results

No statistical differences (P>0.05) were observed for the sow feed intake, backfat loss, weight loss, wean to estrus interval from the control group. Differences (P <0.05) were observed for the weaning weight between F3 (7.35 ± 0.24 kg) treatment and F1 (5.69 ± 0.27 kg), F2 (5.57 ± 0.27 kg) and PL treatments (5.65 ± 0.24 kg). C group (6.85 ± 0.27 kg) and F3 (7.35 ± 0.24 kg) were similar (P > .05). ADG was different (P <0.05) between F3 (0.236 ± 0.007 kg / day) and treatments F1 (0.176 ± 0.007 kg/day), F2 (0.178 ± 0.018 kg/day) and PL (0.182 ± 0.006 kg/day). Daily gain in C (0.008 kg / day) group and F3 (0.236 ± 0.007 kg / day) were similar. No significant differences (P>0.05) were observed for the prewean mortality of the piglet (Table 1).

Table 1. Effect of the feed additives on sow performance during lactation and performance of the litter.

Treatments*	Control	F1	F2	F3	PL	P
Total feed intake, kg	117.22 ± 5.77	108.17 ± 5.91	104.27 ± 5.67	127.55 ± 5.42	102.80 ± 5.11	.429
Daily feed intake, kg	5.02 ± 0.23	4.84 ± 0.28	4.70 ± 0.44	5.28 ± 0.12	4.68 ± 0.30	.589
Weight loss, %	6.18 ± 0.91	8.16 ± 1.41	8.63 ± 1.42	3.89 ± 0.45	11.24 ± 1.72	.463
Backfat loss, mm	2.54 ± 0.33	1.75 ± 0.25	2.00 ± 0.36	2.58 ± 0.41	2.00 ± 0.21	.330
Wean estrus interval, d	5.22 ± 0.38	4.11 ± 0.11	4.20 ± 0.29	5.50 ± 0.51	4.78 ± 0.28	.446
Wean weight, kg	6.85 ± 0.265^{ab}	5.69 ± 0.265^{a}	5.57 ± 0.265^{a}	7.35 ± 0.237^{b}	5.65 ± 0.237^{a}	0.126
ADG, g	216 ± 8^{ab}	176 ± 7^{a}	178 ± 18^{a}	236 ± 7^{b}	182 ± 6^{a}	0.006
Prewean mortality, %	16.2 ± 2.26	14.6 ± 2.64	20.2 ± 5.53	11.7 ± 3.35	7.9 ± 4.31	0.521

*Treatments: Control (basal diet), F1: Phytogenic 1 (1 kg/ton); F2: Phytogenic 2 (1 kg/ton); F3: Phytogenic 3 (5 kg/ton); PL: dried Plasma (5 kg/ton).

[a,b] Superscripts within same row indicate statistically significant differences (P < 0.05).

Conclusions

Although feed intake was not improved by treatments, other performance parameters were improved by the use of phytogenic additives.

References

1. Bergsma *et al*. 2008. J Anim Sci 86:1067-1080.
2. Schenckel *et al*. 2010. Livest Sci 132:165-172.

Osteochondrosis prevalence and severity after supplementing gestation, lactation and growing pig diets with trace minerals complexed with amino acids

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Introduction

Osteochondrosis (OC) is a developmental disease that is a significant cause of lameness in finishing pigs and reduced longevity in breeding swine (1). Lesions occur in both weight-bearing cartilage in joints (articular) and growth plate (physeal) cartilage of growing pigs (2). Cartilage defects are caused by failure of the bone directly beneath the cartilage (endochondral bone) to form properly (3). Pigs become lame after cartilage defects progress to the point where clefts in the weight-bearing surface expose underlying subchondral bone, or when growth plates fracture.

Materials and Methods

Sixty-four Landrace/Large White sows were blocked by parity (P1, P2-P5, P6+) and randomly allotted into two treatment groups: 1) Control (C): Gestation and lactation diets with inorganic trace mineral (TM) supplementation only (ZnO, 150 ppm; $MnSO_4$, 50 ppm; $CuSO_4$, 16.5 ppm) or 2) Treatment (T): Partial substitution of inorganic TM with amino acid complexed TM (Zn, 50 ppm; Mn, 20 ppm; Cu, 10 ppm: Availa® Sow, Zinpro Corporation, Eden Prairie, MN). Progeny gilts (n=280) were randomly allotted at weaning within sow treatment and parity blocks and assigned to eitherGroups fed C or T diets (as described in the sow diets) from weaning until examination.

Group #	1	2	3	4
Sow diets[a]	C[b]	C	T[c]	T
G/F diets[d]	C	T	C	T

[a]Fed to sows from breeding through weaning;
[b]C = control diet (inorganic trace minerals only);
[c]T = Treatment group (partial substitution of inorganic TM with TM-amino acid complexes);
[d]Gilts fed from weaning until sacrifice at 12 or 24 wk.

Twenty gilts from each group were euthanized at 12 wk for evaluation of histological lesions. One distal humerus and femur from each pig was harvested, fixed in formalin, serially sectioned, radiographed and examined histologically for OC lesions. Fifty gilts per treatment were marketed at 24 wk at a commercial slaughter plant; distal humerus, distal femur and talus were scored for severity of gross OC lesions using published scoring criteria. Data were analyzed using general linear regression of Wilcoxon rank-sums.

Results

Histologic OC lesions were prevalent in both distal humerus (100%) and femur (92%) samples at 12 wk. Of 14 histologic and radiographic lesion categories, two were significantly different by sow diet treatment, but with opposite effects (Figure 1). Gross lesion prevalence and severity were low at 24 wk. The average severity of grossly visible lesions was slightly higher in the femur but slightly lower in the humerus in pigs from sows fed diets with a partial substitution of amino acid complexed TM (Figure 2).

Figure 1. Mean cartilage lesion area, % of total articular cartilage area

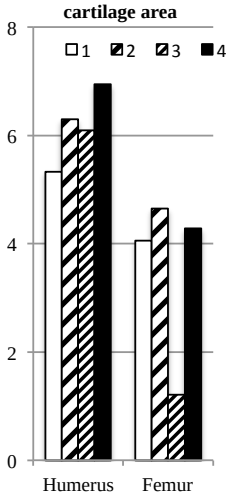
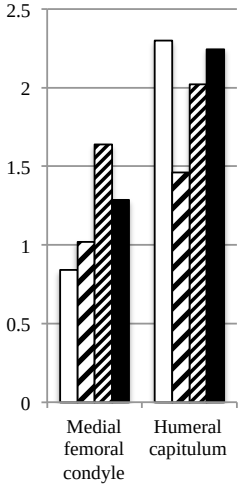


Figure 2: Average gross osteochondrosis lesion severity



Conclusions and Discussion

Partial substitution with amino acid complexed TM in diets fed to sows and growing pigs did not significantly alter OC lesion scores in pigs from a herd that displayed an overall low prevalence of lesions at market weight.

References

1. Grondalen 1974
2. McCoy *et al.* 2013
3. Yretrus, *et al.*, 2007

Effect of lactate infusion on clinical signs in finishing pigs

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Introduction

A finishing farm, that practice liquid feeding for their pigs, reported extreme stiffness of their pigs when they stand up and started walking. Although adverse housing conditions could not be excluded as obvious cause, dissection of pigs with characteristic signs did not reveal pathology related to trauma or osteochondrosis. Occasionally, high blood lactate levels were found in stiff pigs, and it was unclear whether that was muscle related or gut derived.
Lactate has two stereoisomers: L-lactate and D-lactate. L-lactate is produced by cells under anaerobic conditions and is dehydrogenated to pyruvate in the lever. D-lactate is produced in low quantities by the microflora in the gut or is absorbed from the feed. As D-lactate is metabolized very slowly, D-lactate may accumulate in the body and diffuse to the brain and result in neurologic signs(1). In man, this complication is known from the short bowel syndrome. In an earlier study, calves, infused with a high dose of D-lactate, showed neurologic signs and muscle stiffness(2).
This study was designed as a proof of principle whether high doses of D-lactate, administered intravenously, induced neurologic disorders or stiffness comparable to clinically observed field cases.

Materials and Methods

Nine pigs, without muscle stiffness or hobbling gait, were selected from the herd that experience extreme stiffness in finishers. The selected pigs were approximately 12 weeks of age and pen mates of pigs that were developing stiffness. Pigs were transported to our facilities for experimental procedures, that were carried out in consent with the experimental animal ethical committee. A catheter (Cavafix; 12G) was inserted into the lateral ear vein under general anesthesia (ketamine and azaperone). After recovery (24 hr), 3 pigs were intravenously infused with D-lactate (1, 2, or 3 g/kg BW, isotonic), 3 pigs were intravenously infused with L-lactate (1, 2, or 3 g/kg BW) and 3 pigs were given an equal volume of a 0.9% saline solution intravenously. Infusion time varied between 10 and 20 minutes, and the infused volume varied between 1.0 and 3.0 l (31.5 g lactate/l). The pigs were monitored for 2 hours and scored every 10 minutes for consciousness, drowsiness, ataxia, stiff gait. Blood samples were taken at -5,5,15,45,75,105 and 1400 minutes after infusion. Samples were immediately stored on ice, centrifuged at 150G for 10 minutes, and plasma harvested and stored at -20°C until use for analysis.
The D-lactate concentration was determined spectrophotometrically after enzymatic conversion of D-lactate to pyruvate and L-lactate was determined by spectrophotometric technique.

Results

There were no aberrant signs observed except that one pig that had received 2 g/BW of D-lactate was ataxic after 24 hours. Minor muscular fasciculations or transient rapid breathing was observed in some of the pigs, but unrelated to lactate dose given. One pig, that was infused with physiologic saline, developed serious lameness within 24 hours, and was euthanized.
The serumlactate concentrations are shown in Graph 1, where the upper three lines are from L-lactate. The half-life times of both L-lactate (64 min) is about twice as low as from D-lactate (119 min).

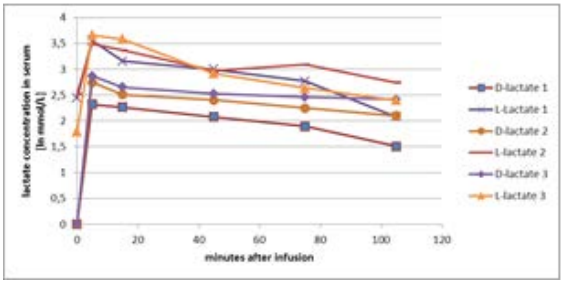

Figure 1. Development of serumlactate concentrations

Conclusions and Discussion

1.Pigs are very tolerant with regard to intravenous infusion of lactate. No clinical neurologic disorders or gait stiffness were observed because of the infusion with D-lactate or L-lactate. It is unlikely that the clinical sigs of the finishing pigs can be explained by the uptake of lactate.
2.Half-life times of L-Lactate and D-lactate were different, probably because of the different metabolic pathways.

References

1. Uribarri et al., Medicine, Vol 77(2) 1998, Pp 73-82
2. Lorenz et al., Vet. Rec. Vol 156 (13) 2005, Pp 412-41

O.188

Improvement performance of finishing pigs with ractopamine and chromium yeast in diets

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Introduction

Currently, consumers' preference and habits regarding pork reveal a greater consumption of prime cuts with a greater amount of lean meat and less fat. In view of this new demand, researchers have sought additives as modifiers of the animal metabolism that provide an increase in deposition of lean meat and reduction of the fat content of pig carcasses. The additives ractopamine and chromium are noteworthy, acting as modifiers of the animal metabolism by redirecting nutrients, thereby improving the muscle growth rate and consequently modifying the proportion of protein and fat[1]. Information on the effects of ractopamine in diets for pigs is widespread[2,3]. The use of chromium in the diet as a modifier of pig performance and carcass shows inconsistent results, however. Moreover, little is known about the synergistic effect of ractopamine and chromium. The objective of this study was to evaluate nutritional plans (NP) without and with chromium yeast (CrY) combined and not combined with ractopamine (Rac) in diets for finishing pigs corrected with amino acids (AA) on the performance of these animals.

Materials and Methods

Ninety-six commercial hybrid barrows with initial body weight (BW) of 75.07 ± 3.9 kg were distributed into a completely randomized blocks design with six treatments, eight blocks with one replicate and two animals per experimental unit. The experimental diets were formulated so as to meet or exceed the nutritional requirements of the animals[4]. The experiment consisted of two periods: period 1, with 14 days, when the animals from treatments 1, 4 and 5 consumed the control (C) diet and animals from treatments 2, 3 and 6 consumed a diet with CrY; and period 2, with 28 days, in which treatments 1 and 2 were subdivided into the 6 treatments, composing the NP: P1 = C/C; P2 = CrY/AA; P3 = CrY/Rac+AA; P4 = C/CrY; P5 = C/CrY+AA; and P6 = CrY/Rac+AA+CrY. When the CrY and Rac were used, they were added at 400 ppb and 10 ppm, respectively. The evaluated variables were final BW (Kg); fasted BW, (Kg), average daily gain (ADG, Kg/day), average daily feed intake (ADFI, Kg/day) and feed:gain (F:G). The effects of the NPs were analyzed using a mixed effect two-way ANOVA model followed by Tukey-Kramer adjusted t-test (SAS® Enterprise Guide 4.3, Cary, NC, USA).

Results

Greater final BW and fast BW ($P<0.001$) were observed in the animals fed CrY/Rac+AA+CrY, which was similar to that obtained with CrY/Rac+AA and higher than the other NPs. Pigs subjected to NP CrY/Rac+AA+CrY had a greater ($P<0.001$) ADG than those on the other NPs, whereas NP CrY/Rac+AA provided an intermediate response. No effect of the different NPs was observed on the ADFI ($P>0.05$) of the pigs. Pigs fed the NPs CrY/Rac+AA+CrY and CrY/Rac+AA had better F:G ($P<0.001$).

Conclusions and Discussion

Based on the results obtained with the NP CrY/Rac+AA and in the literature, Rac improves pig performance. The greater final and fast BW and the 8% increase in ADG with the use of CrY/Rac+AA+CrY in relation to the intermediate results obtained with CrY/Rac+AA revealed the synergistic effect of the use of CrY in combination with Rac on perrformance of pigs. In conclusion, the use of NP with CrY and Rac causes additive and synergistic improve on performance of finishing pigs.

Table 1. Growth performance of pigs

	Final BW	Fast BW	ADG	ADFI	F:G
P1	123.49bc	120.42bc	1.15bc	3.22	2.80a
P2	123.36bc	120.14bc	1.14bc	3.30	2.90a
P3	128.17ab	125.24ab	1.25ab	3.16	2.53b
P4	122.29bc	118.84c	1.12bc	3.24	2.89a
P5	121.04c	118.09c	1.09c	3.10	2.84a
P6	131.34a	127.49a	1.35a	3.39	2.50b

[a,b,c] - Means followed by different letters in the same row differ ($P<0.05$) by the Tukey-Kramer test.

Acknowledgments

Alltech INC and Alltech Brazil, CNPq, FAPEMIG, INCt.

References

1. Rodcliffe JS et al. 2004. Porkworld 22:50-54.
2. Stahly TS and Bark LJ. 1991. Swine nutrition 103-117.
3. Marinho PC et al., 2007. Revista Brasileira de Zootecnia 36: 1791-1798.
4. Rostagno HS et al. 2011. Tabelas Brasileiras para aves e suínos.

O.189

Formulating feed to the Standardized Total Tract Digestible Phosphorus (STTDP) requirement prevents productive failure, as long as the calcium to phosphorus ratio is correct

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Introduction

The adoption of the Standardized Total Tract Digestible Phosphorus (STTDP) by the NRC (1) has created doubts and some imprecisions to effectively satisfy the P requirements. However, accounting for endogenous excretion results in a more precise estimate of the true requirement; aside, the Ca to P ratio (Ca:P) may alter P absorption and resorption, conducing to lessened productive efficiency (2). From a previously determined STTDP demand, for this population of pigs at 30 kg of body weight (4.53 g/kg of diet), the requirement was validated from different ingredients composition estimates and challenged by the addition of graded supplementary Ca.

Materials and Methods

A total of 80 pigs (32 gilts and 48 barrows) were used from 30.9±3.380 kg; pigs were individually housed and randomly assigned to pens and Treatments by litter of origin, sex, and previous productive performance. Treatments were 8 diets formulated from (4) low P (LP) ingredients (sorghum and soybean meal) or (4) by the inclusion of canola meal (20%) as a higher P density ingredient (HP); all diets were similar in STTDP, but different in total P (LP, 0.56 vs 0.67% HP) and phytic phosphorus (0.23, LP vs 0.30%, HP). Four levels of total Ca were imposed (at the expense of sorghum grain) from $CaCO_3$ to result in 4 calcium to total P levels (Ca:P): 1.0:1; 1.5:1; 2.0:1 and 3.0:1, for the LP or HP diets. Digestible amino acids (SID lysine, 1.28%) and Net Energy (2.5 Mcal/kg) were the same for all diets. Data were statistically analyzed for a RCBD (blocks=2 similar buildings), in a 2 (LP or HP) × 4 (Ca:P ratios) factorial arrangement. In a 42d period average of daily feed intake (ADF), body weight gain (ADG) and feed efficiency (G:F) were measured. Data were analyzed using the General Linear Procedures of SAS (v9.2) and planned comparisons were those for the factorial arrangement.

Results

Table 1 summarizes results as the least squares means for the interaction (Ca:P levels within LP and HP diets). While ADF was similar (P>0.27) for all Treatments, ADG was reduced (P<0.02) by the Ca:P ratios: 0.93, 0.87, 0.90 and 0.85kg/d for the 1.0:1, 1.5:1, 2.0:1 and 3.0:1 ratios, respectively. Notably above the 1.5:1 Ca:P ratio, G:F was negatively affected (P<0.01): 0.40, 0.40, 0.38 and 0.37kg of gain/kg of feed, for the 1.0:1, 1.5:1, 2.0:1 and 3.0:1 ratios, respectively. Provided that diets meet the STTDP requirement, these results suggest that, independent of total P (LP or HP), the optimum total Ca:P ratio is at, or below 1.5:1.

Table 1. Productive performance in response to dietary total P density and different Ca:P ratios (Leas Squares Means, n = 10).

Ca:P ratio	Variable			
	Initial wt., kg	ADF, kg	ADG, kg[a]	G:F, kg[b]
Low-P density				
1.0:1	30.67	2.340	0.957	0.410
1.5:1	31.43	2.166	0.884	0.409
2.0:1	31.71	2.368	0.904	0.382
3.0:1	31.36	2.317	0.854	0.369
High-P density				
1.0:1	30.86	2.267	0.897	0.396
1.5:1	30.65	2.245	0.865	0.387
2.0:1	31.08	2.322	0.891	0.385
3.0:1	30.06	2.247	0.839	0.374
SEM	0.906	0.059	0.028	0.010

[a]Average daily gain, major effect of Ca:P ratios (P<0.02).
[b]Feed efficiency , major effect of Ca:P ratios (P<0.01).

Conclusions and Discussion

If diets are appropriately formulated to the STTDP requirement, independently of the type of ingredients, diets will precisely satisfy the demand, preventing expenditures and damage to the environment. Diets of higher P density, because of a lower P digestibility, may be efficiently managed by controlling the total Ca:P ratios. These results emphasize that consequences of a Ca excess may be on lessening productive performance, particularly feed efficiency.

References

1. NRC. 2012. Nutrient Requirements of Swine.
2. Stein H et al. 2011. J. Anim. Sci. 89:2139-2144.

The taste of water: The influence of temperature and additives

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Introduction

Many pigfarmers use the drinking water system to apply medication or additives to improve pig health. The effects of additives on water palatability are often ignored because pigs are not given a choice source of drinking water. When water palatability is impaired, pigs actually may drink less, thereby also impairing feed intake and growth[1]. With respect to drinking water constituents guidelines for pigs are mainly based on possible adverse effects for pig health and not on water palatability. Furthermore, it is known that pigs have a different taste than man[2]. In this study we will investigate the palatability of two water temperatures and two commercially available additives by measuring water consumption of weaned piglets.

Materials and Methods

Four types of drinking water (A = Control (11°C, pH= 7.3), B = Warm water (33°C) and two waters with a commercially available organic acid compound (C, pH=3.7) and additionally with essential oils (D, pH=3.6) were evaluated. A repeated latin square design was used in four groups of 22 weaned pigs (7 wks old) with the four water types on four subsequent days from 13:00 – 18:00hr. In the first hour, pigs were provided with one type of water in a closed drinker, after which it was changed for another in the 2nd, 3rd and 4th hour. The order of supplied water types differed each day to prevent confounding by learning. After the hour had passed the water consumption was measured. The data were analysed with univariate and multivariate linear regression models. The best multivariate model was chosen after a stepwise backward procedure.

Results

The pH and water temperature can be found in Table 1, the water consumption in Fig 1.

Table 1. pH and water temperature.

water type	A	B	C	D
pH mean	7.3	7.2	3.7	3.6
pH range	7.0-7.8	6.7-7.8	3.5-3.8	3.4-3.7
T start	10.7	33.4	10.4	10.4
T end	16.2	26.2	16.2	16.3

The mean water consumption was 0.21 L/pig/hour (range 0.08–0.39). Mean water consumption did not differ between pens, but was affected by day, time of day and type of water. On the last day and during the first hour on each day more water was consumed.

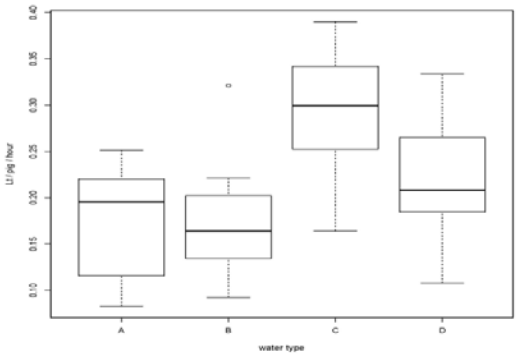

Figure 1. The water consumption per type

Water consumption was not affected by temperature, but water C was consumed more than the other three water types ($p<0.001$) and water D was consumed more than control ($p<0.05$).

Conclusions and Discussion

The organic acid compounds did not adversely affect the palatability of the drinking water in this study. Whether an increase of water uptake is seen in the long run remains unclear as pigs get used to the water taste. Furthermore, water types were not equal in sodium content which is the main driver for thirst and has certainly confounded our study results. Equaling sodium contents however would affect palatability too.
The non-invasive setup used in this trial proved possible to test water palatability preference under field conditions. Recommendations for future trial designs will be presented.

Acknowledgements

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References

1. Brooks, PH et al. 1989. Pig Vet. J. 23 Pp 51-66.
2. Glaser, D et al., 2000, Food Chemistry 68, Pp 375-385

Metabolism of the masked mycotoxin deoxynivalenol-3-β-D-glucoside in pigs

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Introduction

Deoxynivalenol (DON), one of the economically most relevant mycotoxins worldwide, causes numerous adverse health effects in pigs, like anorexia, emesis, reduced weight gain or immunomodulation (1). Plants can metabolize DON through enzymatic conjugation to glucose, which results in the formation of the masked mycotoxin deoxynivalenol-3-β-D-glucose (D3G; 2). D3G escapes routine detection, but might be hydrolyzed in the digestive tract of mammals, thus increasing the total DON burden of an individual. Due to a lack of *in vivo* data, D3G has so far not been considered in the regulatory limits for cereal-based foods and feeds (3). The aim of our study was to contribute to the risk assessment of this masked mycotoxin by first-time determination of its metabolism in pigs.

Materials and Methods

In a repeated measures design, male crossbred piglets (7.1 ± 0.8 kg b.w., n=4) received water, D3G (116 µg/kg b.w.) and the equimolar dose (0.25 µmol/kg b.w.) of DON (75 µg/kg b.w.) per gavage on days 1, 5 and 9 of the experiment, respectively. In addition, D3G was administered intravenously (15.5 µg/kg b.w.) on day 13. After each of the treatments, animals were separated and urine and feces were collected for 24 h. Excreta were analyzed for DON, D3G and the known DON-metabolites deoxynivalenol-3-glucuronide (DON-3-GlcA), deoxynivalenol-15-GlcA (DON-15-GlcA) and deepoxy-deoxynivalenol (DOM-1) by LC-MS/MS. The animal experiment was approved by the institutional ethics committee and the national authority according to § 8ff of Law for Animal Experiments, Tierversuchsgesetz – TVG (GZ 68.205/0236-II/3b/2012).

Results

After oral application of DON and D3G, 84.8 ± 9.7% and 40.3 ± 10.0% of the administered toxin dose were detected in urine, respectively (Figure 1). In piglets orally dosed with D3G, the masked mycotoxin itself as well as DON, DON-3-GlcA, DON-15-GlcA and DOM-1 were found in urine. Urinary D3G accounted for only 2.6 ± 1.4% of the given dose. Following intravenous application of D3G, 102.4 ± 5.2% of the administered dose were recovered in urine, with the vast majority being excreted in form of D3G.

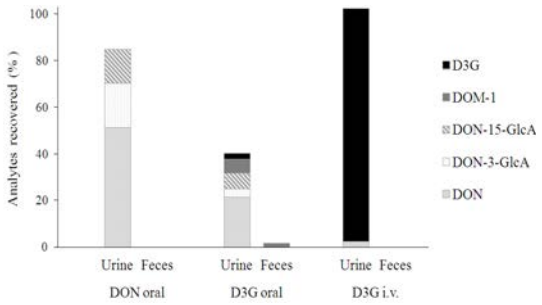

Figure 1: Excretion of DON, D3G and their metabolites in excreta of toxin exposed piglets. *Data are expressed as equivalent percentages of the given dose.*

Conclusions and Discussion

We could demonstrate that ingested D3G is nearly completely hydrolyzed in pigs, but only partially absorbed. Cleavage of D3G predominantly occurs in the digestive tract, while the toxin seems to be rather stable after systemic absorption. Compared to its parent toxin, the oral bioavailability of D3G and its metabolites is reduced by a factor of 2, approximately. Yet unidentified fecal metabolites could account for the lack of total recovered D3G. To sum up, the toxicological relevance of D3G regarding an increase of the total DON load in pigs seems to be limited.

Although this study provides important insights into the metabolism of D3G *in vivo*, it should be emphasized that D3G might exhibit biological activity on its own. Future studies should address possible emetic effects of the masked mycotoxin as well as its influence on intestinal gut health.

Acknowledgments

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References

1. Pestka J 2007. Anim Feed Sci Technol 137:283-298.
2. Berthiller F et al. 2013. Mol Nutr Food Res 57:165-186.
3. JECFA 2011. http://whqlibdoc.who.int/trs/WHO_TRS_959_eng.pdf

The effect of a feed additive containing herbs on early *Ascaris suum* migration

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Introduction

Ascaris (A.) suum causes porcine ascariasis, a wide spread disease that interferes with the health and performance of pigs and results in reduced feed-to-gain ratio and liver condemnation associated with important economic losses. Usually, ascariasis can efficiently be treated with conventional synthetic drugs, but organic farmers rely on alternative approaches such as phyto-therapy to achieve worm control (5). In a pilot study, a commercial herb-mixture was tested in pigs naturally infected with *A. suum*. The effect of these herbs was striking in that no hepatic milkspots in treated animals at the time of slaughter were observed. This finding led to the hypothesis that these herbs inhibited migration of the larvae from the intestine to the liver (1). Herb-All WORM® is a plant-based food supplement (2) that has a similar composition as the herb-mixture used in the pilot study mentioned above. The product was proposed to have a significant effect on the invasion of most known nematodes of the digestive tract (3). The aim of the present study was to determine in two independent experiments the anthelmintic effect of Herb-All WORM® in a murine model for early-stage *A. suum* infections.

Materials and Methods

Each of the two experiments was performed with 24 eight weeks- old, C57BL6, female mice. They were divided into four groups (G1-G4). Mice in G1, G2 and G3 were infected intragastrically with 2,500 (experiment 1) or 3,000 (experiment 2) infective *A. suum* eggs per animals, respectively. Starting one week prior to the infection and continuing throughout the entire infection experiment, the control group G1 was fed with conventional mouse feed pellets, while group G2 was fed with mouse feed pellets supplemented with 2g Herb-All WORM® per kg feed and group G3 received mouse feed pellets supplemented with 150 mg fenbendazol per kg feed. The mice of the negative control group G4 were not infected and were fed with conventional mouse feed pellets. Seven days post infection all mice were euthanized and necropsy was performed. Lungs, liver, small and large intestine of each animal were collected. Liver and lung lobes were trimmed and one part of each was fixed in 10% formalin, while the other halves were sliced with a scalpel and larvae were counted upon collection in a modified Baermann system (24h-incubation at 37.5°C) (3). Fixed samples were routinely processed and stained slides were analysed by a board certified veterinary pathologist.

Results

Mean counts of the larvae in lung samples from the two experiments are shown in Table 1. In both experiments, the mean larval count was highest in the untreated infected group G1. Animals from Herb-All WORM®-treated group G2 exhibited reduced infection intensity but a statistically significant difference was observed only in experiment 1. In contrast, no larvae were detected in lung tissue from animals of G3 that had been treated with conventional anthelmintic drug fenbendazol. While livers in infected animals from experiment 1 were overall negative, hepatic tissue of G1 and G2 in experiment 2 exhibited three and five larvae, respectively. In both experiments, intestinal samples scored negative. The histopathological examination of liver and lung tissue of all groups confirmed the results of the larval counts.

Table 1. Mean larval count of the lung samples

	Groups			
Experiment	1	2	3	4
1	17.3	2.6*	0	0
2	249	187	0	0

*Statistically significant difference to G1 (p=0.05)

Conclusions and Discussion

In our murine *A. suum* infection model, treatment with Herb-All WORM® reduced the larval burdens in the lung. The results suggest that there is a detectable effect on the migration of *A. suum* larvae. Despite such encouraging results, the efficacy of these herbs is not comparable to that of synthetic anthelmintic drugs, hence they cannot be replaced. Nevertheless, this commercialized herbs mixture may be a valid prophylaxis or as supplement to conventional treatment of porcine ascariasis with synthetic drugs.

Acknowledgements

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References

1. Gasser A et. al 2012; masterthesis
2. http://www.lifecirclenutrition.com
3. Lewis R et al. 2006. Parasitol 132(Pt 0):289-300.
4. van Krimpen MM et al. 2010. Vet Parasitol 168:269-77.

The effect of a processed calcium montmorillonite on multiple toxins *in vitro* and *in vivo*

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Introduction

It has long been known that certain clay minerals can decrease the effect of polar mycotoxins, such as aflatoxin, on livestock. However, these minerals were found to be less effective against the non-polar toxins such as zearalenone, leading to the general belief that clay minerals could not bind mycotoxins other than aflatoxin. However, if a mineral was found that could be processed in such a way that its properties could be made to bind these additional toxins it would be valuable in prevention of mycotoxicosis in livestock. In vitro work (De Boer et al., 2012) had shown that this was indeed the case that a unique, modified, mineral did decrease the availability of multiple mycotoxins. The next step was to try this in vivo. Bier et al., 2011, reported decreased effects of fumonisin B_1 in swine when a processed calcium montmorillonite was added to the diet. Therefore, the objective of the present studies was to determine if the unique mineral would also work against zearalenone mycotoxicosis in swine.

Materials and Methods

Sixty weaned pigs were used in two experiments (EXP) to evaluate the effects of a processed mineral on gilts fed zearalenone (ZEA) contaminated diets. In EXP 1, 24 gilts with an average initial weight of 10.4 kg were fed four treatments: 1) Control; 2) Control + 0.5% processed mineral; 3) Control + 2 ppm ZEA; and 4) Control + 2 ppm ZEA + 0.5% processed mineral. In EXP 2, 36 gilts with an average initial weight of 11.1 kg were fed four treatments. The treatments were 1) Control; 2) Control + 0.75 ppm ZEA; 3) Control + 1.5 ppm ZEA; and 4) Control + 1.5 ppm ZEA + 0.5% processed mineral. In both EXP gilts were fed individually for 21 d.

Pigs in both EXP were housed individually in 0.70 m^2 pens with ad libitum access to treatment diets and water. A corn-soybean meal basal diet was used in the studies. Cultured ZEA was provided by LAMIC, Santa Maria, Brazil and processed mineral (Calibrin®Z) by Amlan International, Chicago, USA. No mycotoxins were detectable in the basal diet. Feed intake, weight gain, and feed conversion ratio were measured and calculated weekly. Vulva size was determined by its length × width × height, and measured at 3 d intervals in EXP 1, and on d 0, 5, 10, 14, 18, and 21 in EXP 2. At the end of each EXP gilts were euthanized and reproductive tracts were removed for determination of reproductive tract weight and length. All measured and calculated variables were subjected to variance analysis (ANOVA). Differences between mean values were compared by Tukey's test and considered significant at $P < 0.05$.

Results

No treatment differences were found for animal growth performance in EXP 1 or 2. Adding 0.5% processed mineral did not decrease performance in any EXP. Vulva size increased with ZEA addition without processed mineral; but adding the processed mineral reduced this effect. Gilts fed ZEA without the processed mineral had increased reproductive tract weights and lengths compared to those fed the control diet. Adding 0.5% of the processed mineral to ZEA diets significantly, EXP 1, and numerically, EXP 2, reduced tract measurements. Thus, adding 0.5% clay enterosorbent to swine diets had no negative effects on growth performance but reduced the negative effects of ZEA.

Conclusions and Discussion

Early work showed that certain minerals prevent the effects of aflatoxin on livestock. If there was a way to process certain unique minerals to allow binding of non-polar mycotosins it could benefit producers. Previous reports showed that a processed mineral decreased availability of multiple toxins in vitro and the effects of fumonisin in growing swine. Adding the processed clay enterosorbent to the uncontaminated control feed in these experiments did not have any negative effects on growth performance of growing swine. Additionally, the processed mineral used in these experiments decreased the negative effects of zearalenone. The results of the current experiments combined with results of the previous research show that this mineral can ameliorate the negative effects of multiple toxins in swine.

Acknowledgments

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References

1. Bier, L., E. D. DeBore, S. L, Johnston, F. Chi. 2011. Enterotherapy of Calibrin-Z and modified Calibrin-Z against fumonisin effects in growing pigs. ASAS and AAPA Meeting, Mar de Plata, Argentina.
2. De Boer, E., G. Goss, S. Johnston, and F. Chi. 2012. In vitro adsorption of calcium montmorillonite, yeast glucomannan and enzyme based mycotoxin binding products on aflatoxin, fumonisin, ochratoxin, T-2 toxin and zearalenone. World Mycotoxin Forum, Rotterdam, Netherlands.

Effects of live yeast added to the weaning diet on health, immunity and gastrointestinal functionality, of susceptible weaning pigs orally challenged with *E. coli* F4ac

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Introduction

The use of antibiotic in livestock is a serious threat for the human health, thus EU aims to reduce it. The gut mucosa homeostasis around weaning is a prerequisite for optimal health of pig. Maintaining a good intestinal microbial balance by using probiotic helps to reduce pathogen invasion and may prevent the use of antimicrobials. *In vitro* tests show that certain live yeasts reduce the pathogenicity of *Escherichia coli* F4ac (ETEC) and *in vivo* effect on pigs highly susceptible to ETEC can be postulated. The ability of *Saccharomyces cerevisiae* (Sc) supplied at different patterns to counteract the infection of ETEC in weaned piglets was tested.

Materials and Methods

Seventy piglets with the sensitive alleles of the gene MUC4 that predict the presence of receptors for ETEC in the gut were selected. Pigs were weaned at 24 days and divided in 4 groups: control (CO), CO + colistin (AB), CO + $5x10^{10}$ CFU of Sc/kg feed, from d0 to d21 (PR), CO + $5x10^{10}$ CFU of Sc/kg feed from d7 (infection with ETEC) to d11 (CM). On d7 post-weaning, all the pigs were orally challenged with 10^8 CFU of ETEC. All the pigs were sampled for blood (d7; d8; d12; d21) and feces (d7; d10), for the total *E. coli* and ETEC plate counts. From 12h before the infection to 144h post-infection fecal consistency was determined by visual appraisal (1=hard, 5=watery feces). On d8, 5 piglets per groups PR; CM; CO and AB were sacrificed and sampled for jejunum, for immunohistochemistry, morphology and for *in vitro* testing of the ETEC specific receptors. On d21, remaining piglets were sacrificed and sampled for the same adhesion test.

Results

Based on the *in vitro* adhesion test, all pigs had the receptors for ETEC. The mortality in CO (7/10) group was higher than in AB (1/10), PR (3/10) and CM (4/10) groups ($P<0.001$; $P=0.01$ and $P<0.05$, respectively). Twenty-four hours after the infection (d8) the first pigs died in the CO group, while in PR and CM groups the first piglet died at d10. Before challenge, diarrhea score, *E. coli* excretion, total and ETEC-specific IgA concentration did not differ between the groups. Conversely, CO group showed a higher fecal score post-infection than AB (58% averagely) (from $P<0.05$ to $P<0.001$). Yeast administration tended to reduce the fecal score compared to CO diet 48h after the infection (19%) ($P=0.07$) without differences observed between the yeast groups. Post-infection, live yeast reduced the excretion of total *E. coli* and ETEC, compared with CO group ($P<0.05$ and $P=0.06$, respectively). Compared to CO group, colistin and yeast treatments reduced the ETEC-specific IgA concentration ($P<0.05$). One day post-infection, blood concentration of CRP and haptoglobin and the score for ZO-1 integrity on the villi did not differ between groups. In the jejunum, crypts were deeper in AB and yeast groups than in CO ($P=0.05$ and $P<0.05$, respectively). No differences were observed between the yeast treatments. Antibiotic treatment increased the number of mitotic cell in intestinal villi compared to the control group. Conversely, PR group tended to increase the mitotic cells in villus and crypt and tended to reduce the cells in apoptosis, compared with CM group.

Conclusions and Discussion

The protocol based on the infection with ETEC was effective. The CO group showed more days in diarrhea as previously described[1], while colistin protected the piglets against ETEC. The low mortality in the yeast-supplemented groups indicates that the tested dose was able to protect post-weaning pigs from the pathology. Moreover, the observed modification in the time course of pigs mortality, indicates that both the periods of yeast administration lighten the effect of ETEC on health. These data suggest a potential competitive effect of live yeast against this pathogen able to reduce the course of the disease. Additionally, data collected twenty four hours after infection show that a continuous supply of yeast was more efficient in maintaining the proliferation of enterocytes also after the infection.

Acknowledgments

Lesaffre Feed Additives, Marcq-en-Baroeul, FRANCE

References

1. Bosi P et al. 2004. J Recherche Porcine 36: 125-32.

Growth performance consequences of a low phosphorus intake: prevention of the deficiency by an inorganic source or a phytase and recovery after depletion

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Introduction

Reduced growth performance is often attributed to either the low availability of Phosphorus (P) in feedstuffs of plant origin, problems with diet supplements, or inappropriate use of Phytase (PHY). However, it also needs to be recognized that there is little information on the effects of both the severity and duration of a P deficiency on growth performance. This trial was conducted to assess the time course of a P deficiency imposed on young pigs, and to evaluate the prevention of the deficiency using graded levels of digestible P (dP) supplied either from an inorganic source or released by PHY activity. Also, body weight (BW) gain was monitored after the P deficiency was removed.

Materials and Methods

The study used 244 pigs and was carried out in 2 parts. Part 1 which used a RCBD (block = group of pigs), was from 33d of age (7.7±1.30 kg BW) for 42d, and compared 6 treatments: 1) Basal, a dP deficient diet (by 0.2% units); 2) Basal + 0.1% dP from KH_2PO_4 (iP); 3) Basal + 0.2% dP from iP; 4), 5), and 6), Basal + 500, 1000 and 2000, respectively, PHY units from Ronozyme HiPhos (DSM Nutritional Products México). Part 2 measured possible recovery in response to previous dietary Treatment (Trt) from d42 for 70d in 2 subgroups, either P depleted (Trt 1; n=40) or non-depleted pigs (Trt 2, 3, 4, 5, 6; n=204). In Part 1, pigs were housed in pens of 4-6 pigs, randomized to Trt from outcome groups formed on the basis of litter of origin, sex and BW. Two feeding phases were used with Basal dP levels of 0.16% (for 14d) and 0.08% (for 28d), respectively, at a fixed dietary Ca content, resulting in Ca:P ratios of either 1.6:1 (Basal) or 1.0:1 (at 0.2% units of dP from iP). Responses to iP and PHY were analyzed using PROC REG, and GLM (SAS, v9.2). For Part 2, pigs were sorted by weight and sex into finishing pens of 12 pigs (independent of previous Trt); all pigs received the same conventional diets in 3 feeding phases. These data were analyzed using the GLM, REG, and MIXED procedures of SAS (v9.2) and Statistical Process Control methods.

Results

The growth responses [average daily feed intake (ADF), daily weight gain (ADG) and feed efficiency (G:F)] to dietary Trt during Part 1 of the study (when the dP deficiency was created) is shown in Table 1. Pigs fed the Basal diet (Trt 1) had reduced performance than the other treatments which were similar in this respect (P>0.1). Diminished growth of pigs on Trt 1 was evident from d21 of the experiment with clinical signs of *rickets* being noted from d35, further reducing ADG and ADF. However, all signs of P deficiency were prevented by any of the iP and PHY addition levels, despite calculated dP levels of these diets being below published needs.

Table 1. Least squares means for growth performance of P-deficient pigs in response to graded levels of KH_2PO_4 or Phytase during Part 1 (42 d period).

Variable	Initial wt, kg	ADF, kg[a]	ADG, kg[a]	G:F, kg[a]
1) Basal [b]	7.62	0.64	0.31	0.49
2) + 0.1% iP	7.77	0.79	0.42	0.54
3) + 0.2% iP	7.63	0.84	0.52	0.62
4) + 500 PHY	8.01	0.79	0.44	0.56
5) + 1000 PHY	7.63	0.79	0.46	0.58
6) + 2000 PHY	7.68	0.88	0.52	0.59
SEM	0.470	0.034	0.021	0.013

[a] Linear effect (P<0.01) of iP and PHY.
[b] Basal had an iP deficiency equivalent to 25% of the requirement; pigs fed Basal had reduced (P<0.01) performance than the other Trt (2-6) which were similar (P>0.1).

In Part 2, once the deficient diet was withdrawn, the P depleted pigs grew at a lower rate than non-depleted during the 70d period (0.74±0.123 vs. 0.89±0.124 kg/d, respectively; P<0.01). However, is should be noted that 82% (33/40) of the depleted pigs were within the range of the BW gain observed for the non-depleted (=0.83±0.130 kg/d), thus, showing a recovery capacity.

Conclusions and Discussion

The severe P deficiency (25% of the requirement) reduced growth performance and impaired structural soundness, although it required a longer period for signs of the later problem to occur. Less than 20% of the pigs that had clinical P deficiency signs were unable to subsequently recover. These results also highlight that feeding a low level of PHY or a minimum iP addition to a severely deficient diet is sufficient to prevent any clinical manifestations. Issues like severity and duration of a P deficiency, as much as recovery capacity, must be considered in establishing minimum levels or suitability of the dietary dP levels.

Improved piglet performance with new probiotic *Bacillus subtilis*

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Introduction

Chr. Hansen aims to market a new probiotic *Bacillus subtilis* strain for piglets and as part of the documentation package for a registration the European Union the following controlled efficacy trial under commercial like conditions were conducted by independent scientists.

Materials and Methods

The efficacy of the probiotic feed product *Bacillus subtilis CH27273* in early weaned piglets was evaluated. A total of 216 crossbred piglets (Lithuanian White x Landrace) from a batch of health piglets were used and the *Bacillus subtilis CH27273* based product was tested in a trial with two dosages of test product: 4 and 8 x10^5 CFU/g feed respectively and a negative control. A wheat/barley/corn and soybean based diet was used and piglets allocated to treatment to form homogeneous replicates (with regard to sex and body weight) to 36 pens in 3 weaning rooms such that each treatment was tested on 72 piglets in 12 pens of 6 piglets.

Results

For the whole study period (from 28 to 70 days of age using prestarter and starter feeds respectively) piglets fed the *Bacillus subtilis CH27273* diets grew 8.3 - 9.4 % more ($p \leq 0.05$) and converted feed 5.7 - 6.2 % better ($p \leq 0.05$) than control pigs (Tabel 1). Administration of the *Bacillus subtilis CH27273* at both dosages from weaning 28 days of age to 70 days of age significantly reduce the diarrhea of piglets and significantly improve productivity performance: daily weight gain and feed conversion ratio. The diarrhea rate in the control (T1) group was more than three times ($p \leq 0.05$) as high compared with remaining two *Bacillus subtilis CH27273* groups. Sum of diarrhea duration days per pen in *Bacillus subtilis CH 27273* diet groups were more than four time less compare to the control diet (Tabel 2).

Table 1. Efficacy results obtained with *Bacillus subtilis CH27273* during 42 days from weaning

Treatment	ADG (g/day)	FCR (g feed /g gain)
Control	361 [a]	1.76 [b]
B.subtilis L	391 [b]	1.66 [a]
B.subtilis H	395 [b]	1.67 [a]

(a, b) Superscripts indicate statistically significant differences ($p \leq 0.05$) using a GLM procedure with initial body weight as covariate
L&H indicate the dosage 4 and 8 x10^5 CFU/g, respectively Low and High dosage

Tabel 2. Daily visual score on fecal consistency per pen in efficacy trial with *Bacillus subtilis CH27273*

Treatment	Days with 2 and 3 score[1]	Sum of diarrhea days per pen	Diarrhea outbreak (days)
Control	3.9 [b]	9.5 [b]	3.2 [b]
B.subtilis L	1.2 [a]	2.4 [a]	1.3 [a]
B.subtilis H	1.1 [a]	2.3 [a]	1.2 [a]

[1]Faeces consistence per pen daily according to the scale (0-3 scale: 0-normal consistence; 1-soft; 2-diarrheae/liquid; 3-serious diarrhea).
(a, b) Superscripts indicate statistically significant differences ($p \leq 0.05$) using a GLM procedure
L&H indicate the dosage 4 and 8 x10^5 CFU/g, respectively Low and High dosage

Conclusions and Discussion

Applying the new probiotic *Bacillus subtilis CH27273* strain to piglets in pelletized cereal based diets resulted in a clear reduction in the diarrhea frequency and severeness in addition to significantly improved production results without any dose-response seen with the narrow span of dosages of probiotic tested. The new probiotic product showed promising results that forms the basis for further scientific documentation.

PRRSV replication in porcine alveolar macrophages causes late and minor down-regulations of MHC class I and II molecules that are epitope presentation structures for CD8$^+$ CTL and CD4$^+$ helper T cells

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Introduction

PRRSV (Order *Nidovirale*, Family *Arteriviridae*, Genus *Arterivirus*) infects monocytes and macrophages in pigs using CD163 and CD169 as receptors. The replication of this virus causes immune modulations in the host, resulting in weak adaptive and innate immune responses as well as persistence of the virus in tissue macrophages. As an effort to define the mechanism of weak PRRSV-specific T cell responses and persistence of the virus in tissue macrophages, we tested whether PRRSV replication in macrophages down-regulates MHC class I and II molecules that are epitope presentation structures for CD8+ CTL and CD4+ helper T cells.

Materials and Methods

PRRSV SD23983 strain was used to study viral replication and resulting down-regulation of surface molecules on porcine alveolar macrophages (PAMs). PAMs were collected from the lungs of healthy PRRSV-free 14 day old piglets. To define the down-regulations of MHC class I, II and SWC on PAMs by PRRSV, the synchronized infection of PRRSV into PAMs was carried out using two types of magnetic nanoparticles (ViroMag^{TM} and ViroMag^{TM} R/L, OZ biosciences, France) and concentrated PRRSV SD23983. FACS surface staining and analysis were performed to measure expression level of molecules (CD3, CD4, CD8, SWC3, CD14, MHC class I and II) on PAMs. Intracellular staining (ICS) for PRRSV N protein was performed to determine the infectivity of PAMs at 0, 6, 12, 18 and 24 hours post-magnetofection (hpm).

Results

Using freshly isolated PAMs, we determined the efficiency of magnetofection and PRRSV replication kinetics. PAMs had 11.1% of intracellular PRRSV N staining (ICS)-positive cells at 0 hpm, 30.4% at 6 hpm, 47.0% at 12 hpm, and 27.1% at 18 hpm. In addition, we analyzed expression kinetics of MHC class I and II at various hpm. The down-regulations of MHC class I and II started at 18 hpm with minimal level. The down-regulation pattern was clear at 24 hpm, but relatively weaker (11.5–44.2%) than another surface molecule on PAM, SWC3, which had 67% down-regulation at 24 hpm.

Conclusions and Discussion

MHC class I and II molecules were moderately down-regulated after 24 hpm, but not before 18 hpm in PAM cells. This result suggests that PRRSV replication in PAMs may not be the mechanism to suppress T cell-mediated immunity including CTL and helper T cells. This finding will aid in the design of a vaccine that induces protective CMI responses against early and late presented PRRSV proteins.

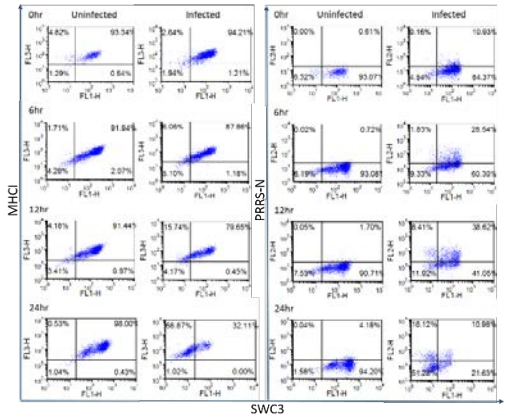

Figure 1. PRRSV replication in PAM down-regulates SWC3 significantly after 18 hour post-magnetofection, but not MHC class I molecule.

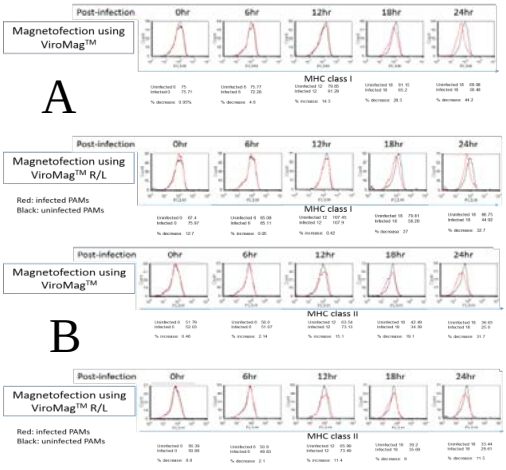

Figure 2. Late and minor down-regulations of MHC class I (A) and II (B) molecules on PAMs after PRRSV magnetofection

Acknowledgments

TJ Heiniger and Chandima B. Bandaranayaka-Mudiyanselage, VMRD Inc.

References

1. Sacha JB et al., 2007. J Immunol 178:2746-54.

Cell-mediated immunity following CIRCOVAC® vaccination in piglets: preliminary results on sustained antigen presentation in SPF vaccinated pigs

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Introduction

Type 2 Porcine Circovirus (PCV2) is now recognized as the causative agent of postweaning multisystemic wasting syndrome (PMWS) and other syndromes collectively referred to as PCV2 associated diseases (PCVD). The aim of the present study was to better understand the fine mechanisms leading to the efficacy of the immune response in piglets following CIRCOVAC vaccination.

Materials and Methods

Experimental designs:

- Study 1: Eleven 3-week-old SPF pigs were included in the study. Three piglets remained unvaccinated (NV) and eight were vaccinated (V) with 0.5 mL of CIRCOVAC at D0. Whole blood samples were collected in all piglets at D21 and D49.
- Study 2: Twenty-one 3-week-old SPF pigs were included and divided into 3 groups. The first one (V1) was vaccinated with 0.5 mL of CIRCOVAC (Inactivated PCV2 virus in Adjuvant at D0), the second (V2) with the same PCV2 inactivated antigen in an experimental adjuvant and the third (NV) remained unvaccinated. Whole blood samples were collected in all piglets every three weeks from D0 to D125.

Laboratory assays:

Peripheral blood mononuclear cells (PBMCs) were isolated and different cells fractions were obtained using a cell separation MACS® Technology indirect protocol with magnetic beads and specific anti-porcine CD5 and SWC3 monoclonal antibodies.

The phenotype of the different subpopulations was studied by flow cytometry. The functionality of the PCV2-specific responding cells were analyzed by IFN-γ ELIspot (1) and proliferation assays by flow cytometry. Tools for the monitoring of PCV2-specific memory responses were developed during those studies.

Results

In the first study, the IFN-γ producing cells obtained following PBMCs re-stimulation with PCV2 VLP were significantly higher ($p<0.05$) in vaccinated piglets at D21 compared to unvaccinated piglets. Likewise and only in CIRCOVAC vaccinated piglets IFN-γ responses could be detected in all tested conditions including restimulation with an irrelevant antigen or in absence of antigen only. This observation was further confirmed up to 41 days in the second study.

These observations prompted us to investigate whether some PCV2 loaded antigen presenting cells could persist for a prolonged period of time in CIRCOVAC vaccinated animals and explain the sustained IFN-γ responses observed in those vaccinated pigs independently of restimulation conditions.

Different cell fraction were prepared as followed:

- SwC3+ depleted PBMCs,
- Enriched CD5+ cell fraction,
- Purified SwC3+ cell fraction.

IFN-γ ELISPOT assays were carried out using these different cell fractions without PCV2 specific re-stimulation. Results are presented in Table 1.

Interestingly, only the combination of SwC3+ and CD5+ cell fractions from CIRCOVAC vaccinated animals allowed for IFN-γ production supporting our hypothesis that antigen presenting cells may be loaded with PCV2 antigen and capable of antigen presentation to T cells in a sustained manner.

Table 1. Study 1 - IFN-γ production monitoring by ELIspot of different cells fraction without restimulation in vaccinated (NV) and in unvaccinated (NV) piglets.

	IFN-γ production	
Cells fractions	**V**	**NV**
Total PBMCs	++	+
SwC3-depleted PBMCs	-	-
Purified $CD5^+$ cells	-	-
Purified SwC3+ cells	-	-
$CD5^+$ cells + $SwC3^+$ cells	++	-
$CD5^+$ cells + $SwC3^-$ cells	-	-

++: definite production; +: slight production; -: no production

Conclusion and Discussion

In CIRCOVAC vaccinated piglets, we demonstrated here that co-culture of CD5+ cells and SWC3+ cells purified from vaccinated animals could lead to IFN-γ production without any *ex vivo* antigenic stimulation.

Our results demonstrated that CIRCOVAC vaccination permitted a prolonged cell mediated response against PCV2 in vaccinated pigs. To further describe those preliminary observations, the immune response elicited by CIRCOVAC is being dissected to identify the humoral and cellular actors and being compared to the responses induced with an experimental adjuvant.

References

1. Piras et al., Viral Immunology, 2005;18(2):381-9

A novel oil adjuvant enhances the protection conferred by swine foot-and-mouth disease vaccines

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Introduction

Ready to use water-in-oil-in-water adjuvant technologies such as Montanide™ ISA 206 VG (ISA 206) have been used for over 20 years for foot and mouth disease (FMD) swine vaccination. These oil emulsion technologies have replaced aluminium hydroxide adjuvants. They induce a strong short-term and long-term immune response which is mainly mediated by neutralizing antibodies (1), with low viscosity and low side reactions.

A challenge for FMD vaccines is the existence of different serotypes of FMDV that are not cross-protective. As it has been shown recently that cellular CD8+ T-cell immune response could mediate cross-protection to different FMD serotypes (2), one option for cross-protection is to develop new adjuvants that stimulate more specifically a cellular immune response.

We have therefore selected a new adjuvant based on a specific enriched light mineral oil which stimulates both humoral and cell mediated immune responses. Montanide™ ISA 201 VG (ISA 201) has been developed specifically on the basis of the reference adjuvant Montanide™ ISA 206 VG. ISA 201 adjuvant has been shown previously in galenic and mice studies to preserve the injectability, stability and safety properties of double emulsion adjuvant ISA 206, while improving the antigen specific cell mediated immune response.

Here we show that this new adjuvant ISA 201 induces a higher humoral and cellular immune response against FMD than ISA 206 in swine and also increases protection against virulent challenge.

Materials and Methods

Fifty 2-month-old pigs, sero-negative for FMDV were randomly divided into 3 groups of 15 animals and one non-vaccinated control group of 5 animals. 15 pigs received inactivated FMDV type O vaccine based on ISA 206, 15 pigs received a vaccine based on ISA 201 and 15 pigs received a non-adjuvanted vaccine. All vaccines contained the same antigenic concentration. In each group, 5 pigs received a full dose (2ml), 5 pigs received 1/3 dose (0.67ml) and 5 pigs received 1/9 dose (0.22ml), in order to determine the PD50 of each vaccine.

To assess the safety of the vaccine, body temperatures of each pig was measured before vaccination and at 4 hours, 1, 2, 3 and 5 days post vaccination (dpv).

Blood samples were collected at 0, 3, 7, 14, 21 and 28 dpv to assay anti-FMDV antibody titers by ELISA analysis, IFNγ, IL-2, IL-4, IL-10 cytokines, and CD4+ and CD8+ specific T cells concentrations.

To demonstrate vaccine protective efficacy, all 50 pigs were challenged intramuscularly with 1000 PID_{50} (2 ml) of FMDV type O at 28 dpv. FMD symptoms were monitored for 10 days, and the PD_{50} of each vaccine was calculated using Karber's method.

Results

Both ISA 206 and ISA 201 based vaccines were safe in swine. For the full dose vaccines, hyperthermia post vaccination was ~1°C and disappeared after 2 days.

Antibody titers against FMDV were significantly higher in the ISA 201 group compared to the reference ISA 206 group for full dose, 1/3 dose and 1/9 dose vaccinated animals at 14dpv, 21dpv and 28dpv.

After full dose vaccination with ISA 201 vaccine, % of circulating CD4+ and CD8+ lymphocytes were enhanced compared to the ISA 206 group and the non adjuvanted vaccine group at 7dpv and 28dpv.

Finally, full doses or 1/3 doses of both ISA 206 and ISA 201 vaccines were fully protective against FMDV type O challenge at 28dpv (5/5 pigs without any clinical signs), whereas non adjuvanted vaccine failed to protect the animals (protection rate 2/5). All non vaccinated control animals showed clinical signs. However, when the vaccines were used at 1/9 dose, only the vaccine based on ISA 201 was fully protective, showing that ISA 201 adjuvant improves the PD_{50} of the FMD vaccine compared to reference adjuvant ISA 206 (PD_{50} of ISA 206 vaccine: 1/10 dose; PD_{50} of ISA 201 vaccine: 1/15.6 dose).

Conclusions and Discussion

We have shown in this study that a FMD vaccine based on the new double emulsion adjuvant Montanide™ ISA 201 VG are safe, and induce higher humoral and cellular immune response and protection against FMD than the reference vaccine based on Montanide™ ISA 206 VG.

Montanide™ adjuvants that increase cell mediated immune response could then extend the vaccinal protective shield against close variants such as local FMD virus strains while preserving the robustness, ease of injection and safety profile of FMD vaccines.

References

1. Barnard AL et al. 2005. Vaccine 23:1037-1047.
2. Guzman E et al. 2010. J Virology 84(23):12375-84.

The correlation between different acute phase proteins and average daily growth during a PRRSV field outbreak in nursery piglets

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Introduction

Acute phase proteins (APP) are suggested as sensitive biomarkers for the detection of clinical and subclinical infection in swine (1). To increase the sensitivity, several APP's can be combined, like Pig Major Acute Phase Protein (Pig-MAP), haptoglobin (Hp) or C-reactive protein (CRP) (2). During a field trial with the objective to evaluate the effect of a feed additive, we were confronted with an PRRSV outbreak which led to clinical streptococcal infections and subsequent increased mortality.

Materials and Methods

Four hundred piglets were allocated to 20 pens with 20 piglets per pen, divided over 5 rooms (4 pens per room). Piglets were weighed at weaning (at 21 days of age), at 14 days and at 32 days after weaning. Per pen 8 piglets were randomly selected and blood was collected at 14 and 32 days after weaning. Blood was centrifuged at 2000G and serum was stored in the freezer at -20˚C until analysis. Serum was analyzed for the acute phase proteins Pig-MAP, CRP and Hp using porcine specific Elisa kits. Linear regression was calculated using SPSS. APP concentrations were ln-converted to obtain a normal distribution of the data and statistically analyzed with ANOVA and Bonferroni post-hoc analysis.

Results

PRRS infection with an EU-strain was confirmed by the Dutch Animal Health Service (GD Deventer) by PCR. Average daily growth and mortality differed between rooms (Figure 1) as did Pig-MAP (Figure 2). Pig-MAP, CRP and Hp had a high significant linear regression at day 32 with the growth in period from day 14-32 after weaning (p<0,001). The R-square was differing with the highest value for Pig-MAP (0,36), followed by CRP (0,20) and Hp (0,19). Multiple linear regression of the three acute phase proteins together did not led to an increase in the R-sq and Pig-MAP was the only significant factor.

Conclusions and Discussion

The acute phase proteins Pig-MAP, CRP and Hp have a good correlation with growth. Of these proteins Pig-MAP has the best correlation and this is in accordance to previous results (2).As far as we know this is the first report describing the effect of a field outbreak of PRRS on the APP content in piglet blood serum.

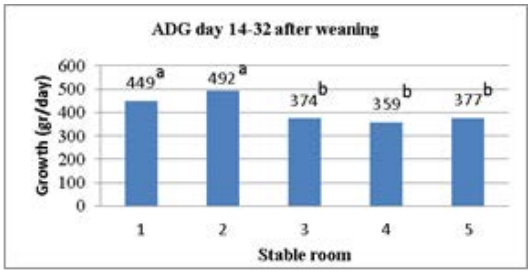

Figure 1. ADG for the different rooms. Results with differing superscript are significantly (p<0,005)

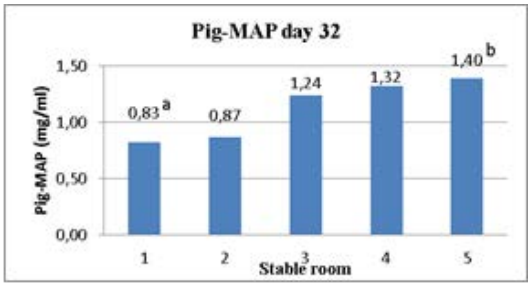

Figure 2. Pig-MAP 32 days after weaning of the different rooms. Differing superscripts are significant (p<0,05).

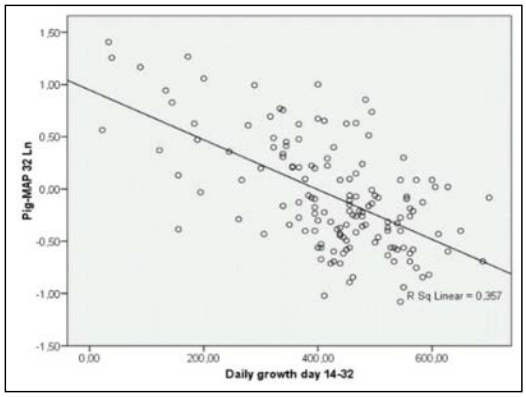

Figure 3. Ln converted Pig-MAP and daily growth. Linear regression was significant (P<0,001) and had a R-sq of 0,357

References

1 Peterson et al. 2004 Vet. Res. 35 pp163-187
2 Heegaard et al 2011 Vet. Res. 42:50

Tulathromycin promotes leukocyte apoptosis and inhibits pro-inflammatory CXCL-8 and leukotriene B_4 production in *A. pleuropneumoniae* and zymosan models of inflammation in the porcine lung

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Introduction

The induction of PMN apoptosis, their clearance by macrophages (MΦ; efferocytosis), and the reduction in synthesis of pro-inflammatory mediators, such as interleukin-8 (CXCL8) and Leukotriene B_4 (LTB_4), are critical events that mediate the resolution of inflammation following infection. Impairment of these processes in some diseases can have deleterious effects to host tissue. Porcine pleuropneumonia, caused by *Actinobacillus pleuropneumoniae* (APP) is characterized by excessive pulmonary inflammation and severe tissue damage. Lesioned areas of lungs infected with APP contain high numbers of active or necrotic neutrophils (PMNs). The superior clinical efficacy of some antibiotics used in the treatment of infectious inflammatory diseases has been associated with inherent immune-modulating effects. Tulathromycin was recently shown to have anti-inflammatory actions in a bovine model of pulmonary inflammation.[1] Given its growing use in swine practice, the objective of this study was to characterize the potential immuno-modulatory actions of tulathromycin (TUL) in a porcine model of pulmonary inflammation.

Materials and Methods

Freshly isolated porcine PMNs or monocyte-derived MΦ were treated with TUL (0.02-2.0 mg/mL) for 0.5-2h in the presence or absence of APP *in vitro*. *In vivo*, healthy piglets were treated with TUL (2.5 mg/kg) or vehicle and inoculated with APP ($1.5x10^7$ CFU) or sham. To observe the direct anti-inflammatory effects of TUL *in vivo* in the absence of bacteria, piglets were challenged with zymosan, (1 mg/kg). Bronchoalveolar lavage fluid was collected at 3h and 24h post-infection under sedation and general anesthesia, as previously described.[2] Necropsies were performed for gross morphological analysis and histology sampling following each time point. For all *in vitro* experiments, $n \geq 3$/group; for the *in vivo* studies, $n \geq 6$/group. Data were analyzed using ANOVA or t-tests, where appropriate.

Results

In vivo, bacterial burden of APP and pulmonary neutrophil influx were elevated in APP infected lungs relative to controls ($p<0.01$). Treatment with TUL did not alter recruitment of PMNs to the lungs. In both porcine models of pulmonary inflammation, as well as in culture, TUL induced PMN and MΦ apoptosis, but not necrosis, in a time- and concentration-dependent manner. This was determined using ELISA ($p<0.05$), TUNEL fluorescent staining ($p<0.05$) and Western blotting of cleaved (activated) caspase-3 ($p<0.05$). In addition, TUL-treatment enhanced PMN efferocytosis and phagocytosis by MΦ relative to control ($p<0.05$). In APP-challenged lungs, concentrations of pro-inflammatory CXCL8 were lower in the TUL-treated group ($p<0.05$). LTB_4, a potent neutrophil chemoattractant, was elevated in both APP- and zymosan-challenged lungs; in both cases, treatment with TUL was associated with a significant reduction in LTB_4 ($p<0.05$). Furthermore, TUL significantly attenuated disease pathology in APP-infected porcine lungs examined macroscopically and histologically.

Conclusions and Discussion

The excessive infiltration and activation of PMNs in response to a respiratory pathogen, such as APP, can lead to severe tissue damage. Consequently, therapeutics that target both the infectious agent and the overt host immune response are most beneficial for treating bacterial-induced inflammatory diseases, including pneumonia in swine. Findings from this study demonstrate that TUL has immune-modulating effects in porcine PMNs and MΦ *in vitro* and in the lungs of APP- and zymosan-challenged piglets. Indeed, TUL promotes PMN apoptosis, MΦ clearance of apoptotic cells, and lowers the production of pro-inflammatory mediators. Collectively, these data suggest that TUL, in addition to its anti-microbial properties, has inherent immune-modulating effects that deliver anti-inflammatory benefits in pigs.

Acknowledgments

Funding was provided by Zoetis, NSERC, and the Margaret Gunn Endowment for Animal Research.

References

1. Fischer CD et al. 2011. Antimicrob Agents Chemother 55(1):338-48.
2. Nerland EM et al. 2005. Am J Vet Res 66(1):100-7.

Evaluation and comparison of efficacy between killed and live vaccines against PEDV in field study

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Introduction

Porcine epidemic diarrhea virus (PEDV) is an infectious, highly contagious virus of swine, which belongs to the Coronaviridae family. PEDV was first reported in Belgium and the United Kingdom in 1978 and is an etiological agent of acute enteropathogenic diarrhea in swine. PEDV caused heavy losses to the swine industry in the Asia and currently in USA Newborn piglets are protected against PEDV until they are 4-13 days old by specific IgG antibodies from the colostrum and milk of immune sows. The length and efficiency of immunity in piglets is dependent upon the sows. Several PEDV vaccines, which differ in their genomic sequence, mode of delivery, and efficacy, have been developed. Prophylactic vaccines capable of preventing the initial stages of viral infection usually induce neutralizing antibodies against the viral pathogen. The ideal course of development of the next generation of vaccines for PEDV should focus on several criteria, including factors related the reduction of virus shedding in piglets and the details of the mucosal immunity to PEDV. In this study, we evaluated and compared vaccine efficacy between killed and live PEDV commercial vaccines.

Materials and Methods

Live and inactivated vaccine used in this study are registered vaccine in Korea In total, 20 sows were divided into four different groups with five sows per group. The four groups included the unvaccinated negative control group, the killed virus vaccination group with killed virus boosting (K/K), the live virus vaccinated group with live virus boosting (L/L) and the mixed group vaccinated with live virus and subsequently boosted with killed vaccine (L/K). Vaccination was conducted according to the standard vaccination program and guidelines for PEDV vaccines. Sows were vaccinated twice, at four week and two weeks prior to farrowing. Vaccination was done through intramuscularly. ELISA, Serum neutralization assay of sera and colostrums are did following standard method.

Results

In sow serum samples, the K/K group showed the highest IgG titer, which was about 2 times higher than the L/L or L/K groups, with no significance differences between IgA levels of all three groups. The IgA levels in colostrum samples were highest in the K/K group. The IgG titer in both the L/L and L/K groups were similar. The IgA titers were found to be similar in piglet sera from all three groups. The K/K group demonstrated the highest SN titer in all samples in comparison to the L/L and L/K groups (Fig. 1). In comparison to sow and piglet serum samples, the SN titer in colostrum was the highest with a dilution factor of 750 in the K/K group. In sow serum samples, the K/K group showed the highest SN titer with a dilution factorof up to 650. In piglet serum samples, the SN titer in the K/K group was also highest up to a dilution factor of 500, followed by 400 in the L/L group and 300 in the L/K group.

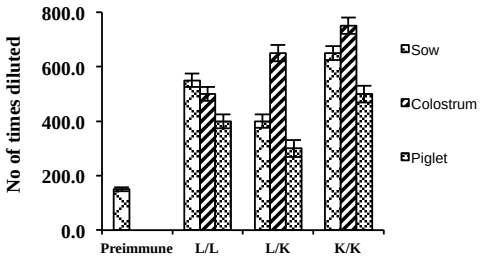

Figure 1. Serum neutralization titers. *To evaluate the potential protection provided by antibodies in the samples serum neutralization test of sow sera, colostrum and piglet seraof sow vaccinated with live (L/L), live-killed (L/K) or killed (K/K) vaccines was carried out.The SNT values are shown as the number of times the serum was diluted versus serum that gave 50% neutralization of PEDV.*:p <0.05 (highly significant) compared with NC, L/L and L/K.*

Conclusions and Discussion

The most critical factor for vaccine efficacy is serum neutralizing activity. The most effective neutralizing activity was found in the K/K group, particularly in colostrums, sow and in the piglets too. This finding could be very important because the vaccination strategy for PEDV mainly relies on passive immunity aimed at transferring protective antibodies from sows to piglets during uptake of colostrums.

In spite of prevailing controversies regarding the efficacy of PEDV vaccines, our current study demonstrated that vaccination and boosting with killed virus generated the highest levels of IgG and IgA antibodies. The K/K combination also provided the most effective neutralizing activity against PEDV in the piglets too. Consequently, the K/K vaccination strategy could be the best option for PEDV.

References

1. Park S.J. et al. Korea.Arch Virol 2011;156:577-85.
2. Kang T.J et al. Protein Expr Purif 2005;41:378-83.

Oral rice-based vaccine induces active and passive immunity against enterotoxigenic *E. coli*-mediated diarrhea in pigs

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Introduction

Enterotoxigenic *Escherichia coli* (ETEC) causes severe diarrhea in both neonatal and weaned pigs. Although the risk of mortality has not reported to be high by the single infection with ETEC, multiple infection with different pathogens exacerbates the diarrheic symptoms. The prevalence of ETEC in diarrheic piglets in Japan is second highest after rotavirus (1). Therefore, hygiene measure or vaccination program to reduce risks of ETEC invasion in neonatal and weaning piglets is requisite. We recently developed a rice-based cholera vaccine expressing CTB (MucoRice-CTB) (2, 3). This vaccine has the advantages of being suited to long-term storage without the need for a cold chain (>1.5 y), and delivery of the vaccine antigen is needle- and syringe-free. Cholera toxin B subunit (CTB) has 78% amino acid homology to B subunit of ETEC heat-labile toxin (LT), which is the major cause of diarrhea. Therefore, we selected MucoRice-CTB as an oral vaccine candidate.

Materials and Methods

One gram of MucoRice-CTB were mixed with feed and orally administered to 2-month-old minipigs or pregnant sows with for four times at 2-week intervals. Serum and intestinal wash fluids were collected from immunized minipigs and CTB/LTB specific IgG or IgA were measured by ELISA.

To measure the lactogenic immunity, colostrums and milk were also collected from sows until 2 weeks after fallowing.

Neutralizing effects of produced antibodies against LT were confirmed by intestinal loop assay. $4x10^6$ or $4x10^8$ CFU of enterotoxigenic *E.coli* strain S7 were injected into ileal loop of immunized minipigs and the accumulated fluid in the loop were measured after 16 Hrs of surgery.

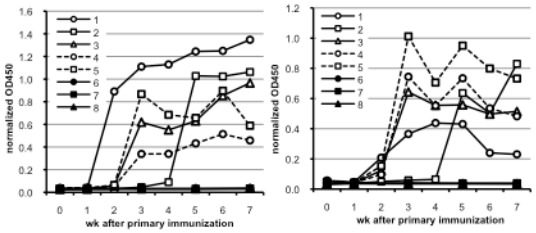
Results

All of vaccinated minipigs produced CTB-specific mucosal IgA as well as systemic IgG and IgA. The higher amount of CTB-specific IgG and IgA were also secreted to the colostrums and the secreted antibodies were passively transferred to sera of the progeny. All antibodies were also cross-reacted to LTB. Moreover, water volumes accumulated in the loops of vaccinated minipigs were significantly reduced than that in control minipigs, indicating that MucoRice-CTB can protect pigs from ETEC-mediated diarrhea.

Conclusions and Discussion

MucoRice-CTB, which generated by rice-protein expression system could induce both active and passive immunity against ETEC in pigs by oral route. LT of ETEC acts at mucosal surface. Therefore, toxin specific secretory IgA in the intestine or maternal milk would suit for neutralizing the toxin. Additionally, MucoRice-CTB could feed to animals simply by mixing with food, that it is easily-handed in farms and veterinary clinical site.

Figure 1. CTB specific IgG (left) and IgA (right) in



serum and milks of 2-week-old minipigs. Closed figures are of non-immunized controls.

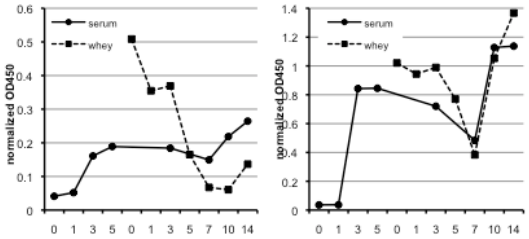


Figure 2. CTB specific IgG (left) and IgA (right) in serum and milks of the sow.

Acknowledgments

Ministry of Agriculture, Forestry and Fisheries of Japan

References

1. Katsuta K et al. 2006. J Vet Diagn Invest 18, 350-354.
2. Nochi T et al. 2007. Proc Natl Acad Sci U S A. 104, 10986-10991.
3. Tokuhara D et al. 2010. Proc Natl Acad Sci U S A. 107, 8794-8799.

Construction and immunogenecity of new PRRSV vaccine using attenuated *Salmonella* as live vector

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Introduction

The vaccine plays a very important role in control Porcine respiratory and reproductive syndrome (PRRS) worldwide. Inactivated vaccine is deficient in eliciting the cell-mediated immune response while live vaccine possesses the potential of recovering virulence and risk of recombination with PRRSV field or vaccine strain, yielding new virus. Thus, a novel vaccine which can elicit both humoral and cell-mediated vaccine is required in prevention of PRRS. PRRSV ORF5 and GP6 encoding protein, GP5 and GP6, are very strong immunogens in stimulating protective neutralization against PRRSV infection (1). Recently, attenuated *Salmonella* can be a good adjuvant in stimulating cell-mediated response and a live vector in engineering vaccine construction (2). In this report, the attenuated *Salmonella*–based new vaccine against PRRS was constructed and evaluated its immunogenecity.

Materials and Methods

The ORF5 and ORF6 genes were cloned using PRRSV WUH-3 strain (Accession No. HM853673)) and ORF5 gene was modified by insertion of specific sequence to expose the neutralization epitopes. Then, the amplified products were singular or fusion inserted into asd+ expressing plasmid, pYA3493, followed by electro-transformation with attenuated *Salmonella choleraesuises to* yield a recombinant *S.cholerasuises* expressing the target genes. The vaccine candidates were used to intramuscularly immunize the mice. C501-GP5m was used to vaccinate the piglets at dose of $10^{8.0}$CFU. The neutralization antibody (NA) and cell-mediated response were measured.

Results

The ORF5 and ORF6 were successfully amplified confirmed by sizes and sequence analyses. The individual original GP5, modified GP5 GP5/M and proteins were expressed with sizes of 27kD, 29kD and 43kD, respectively, in culture supernatant of recombinant *S.cholerasuises, designated as* C501-GP5), C501-GP5m and C501-GP5/M) by Western blot while individual M protein could not be expressed. The three recombinants *S.cholerasuises* have the same growth curves as the host *S.cholerasuises*.

In mice model after immunization with $10^{9.0}$CFU, in all tested vaccine candidates, C501-GP5m elicited highest NA titer. There were no difference in lymphocyte proliferative response at 4 weeks but C501-GP5/M elicited strongest response among three groups. The highest IFN-γ level was observed in C501-GP5m.

Based on the results in mice model, immunogenecity of C501-GP5m was further evaluated in piglet model. The average NA titers induced by singular and booster immunization with C501YA-GP5m) were 1:12.6 and 1:7.4, respectively.

Conclusions and Discussion

Three recombinant attenuated salmonella-based vaccine strains were constructed. The target proteins were expressed *in vitro*.Through mice experiments, the constructants could elicit NA and cell-mediated responses. The piglet model showed the good NA titer could be induced after immunization by C501-GP5m. Taken together, a novel vaccine candidate was constructed.

Currently, the concerns on possible virulence recovery and mutation in live PRRSV vaccine leads to the awkward siuation of PRRS control strategies. ORF5 encoding protein, GP5, is regarded as main immunigen in PRRSV but its neutralization epitopes are conformationally blocked, decreasing the immunogenecity. In this experiment, the epitopes were exposed effectively via insertion of the sequences. The animal tests showed the best immunogenecity among the other constructs.

The practical application of both whole PRRSV-based inactivated and live vaccine will of course interfere the epidemiological diagnosis and investigation of PPRSV due to the presence of antibody agaisnt ORF7 protein in immunized pigs when ORF7-protein was used as coating antigen. However, our new vaccine will not interfere with current antibody detection kit as no ORF7 protein will be synthesized by this construct.

Acknowledgments

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References

1. Qiu H J et al, 2005,Veterinary Immunology and Immunopathology, 106(3-4):309-319.
2. Kwon YM et al., 2007, Expert RevVaccine.6(2):147-52.

Expression of a PCV2 antigen and a molecular adjuvant, as an immune response enhancer, by a genetically engineered *Pichia pastoris* strain

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Introduction

The post-weaning multisystemic wasting syndrome (PMWS) is a viral disease with a high prevalence worldwide[1] affecting pigs between 6 and 15 weeks old[1, 2]. Nowadays, four vaccines are used to control Porcine Circovirus type 2 (PCV2) which has been described as the virus that trigger this wasting condition[3, 4, 5]. Although these commercial vaccines are able to control PCV2 infection they only induce humoral response in swine. Molecular adjuvants enhance not only humoral but also cellular immune response when co-administrated with a recombinant protein[6, 7]. The aim of this work was to obtain a molecular enhancer of immune response, produced in yeast culture, ready to be used within a PCV2 vaccine in swine.

Materials and Methods

A genetically engineered *Pichia pastoris* expressing both PCV2 antigen and a molecular enhancer, under the control of AOX1 promoter was developed. A single colony of *P. pastoris* transformed with the vector, grown on YPD agar plate, was inoculated into 10 mL of YPD medium and grown at 25°C for 16 h and was used as inoculum. All the shake flask experiments were carried out in 100 mL working volume. Methanol was added to the culture after every 12 h and induction was continued for the next 3 days. The cultures were centrifuged at 7.600 x g for 10 min and both pellet and supernatant were stored at -20°C. *Western blot* assay was used in order to analyze protein expression of PCV2 antigen and molecular enhancer. Protein purification was carried out by imidazole gradient using immobilized metal affinity chromatography (IMAC). Both proteins of interest were formulated as water-in-oil emulsion to immunize intramuscularly three weeks old pigs, followed by a booster, three weeks later.

Results

Methanol induction of recombinant *P. pastoris* resulted in the expression of both proteins PCV2-antigen (intracellular) and molecular enhancer (culture medium). The proteins were identified by *Western blot* as shown in figure 1, where only the recombinant clone Q1-1 expressed the adjuvant of interest which is not present within CAP controls. Both proteins were also semi-purified in order to prepare a water-in-oil formulation. Three weeks old pigs immunized with two doses of this preparation are under study to evaluate the increase in their PCV2-antibody titers, showing no adverse local reactions after two weeks post vaccination.

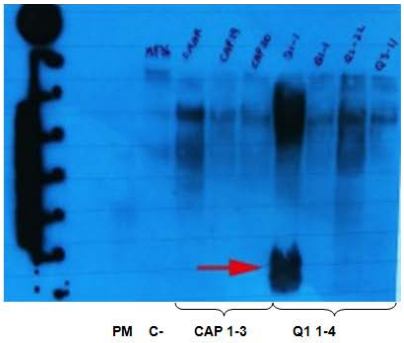

Figure 1. Western blot film after protein electrophoresis of culture medium after grown of *P. pastoris*. *PM: molecular weight, C-: negative control (untransformed yeast strain). CAP1-CAP3: clones transformed with PCV2 vector, Q1 1-4 clones transformed with vector containing the sequence of interest. The arrow shows the protein of interest expressed in clone Q1-1*.

Conclusions and Discussion

Genes encoding for both PCV2 antigen and molecular enhancer of immune response genes were successfully introduced into the genome of *P. pastoris* under the control of AOX1 promoter. The engineered yeast strain produced accumulation of both proteins when the gene expression is induced by methanol. The results have shown the potential of the innovative-engineered yeast strain as a source for production of proteins able to be used within PCV2 vaccine emulsions. Induction of PCV2 specific neutralizing antibody and T-cell mediated immune response in vaccinated pigs is under study.

References

1. Harding y Clark, 1997. *Swine Health and Prod.* 5: 201-203.
2. Alarcón *et al*., 2011. *Prev Vet Med*. 98: 19-28.
3. Nayar *et al*., 1997. *Can. Vet. J.* 38: 385–386.
4. Allan *et al*., 1998. *Vet. Rec*. 142: 467–468.
5. Ellis *et al*., 1998. *Mol Cell Biol.* 5: 1111-1121.
6. Le Bon *et al*., 2001. *Immunity.* 14: 461-470.
7. Cheng *et al*., 2007. *Vaccine*. 25: 5199-5208.

Testing of an inactivated subunit vaccine containing the ORF2 protein from the PCV2 in Mexico

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Introduction

Infection with Porcine Circovirus Type 2 is associated with Postweaning Multisystemic Wasting Syndrome (PMWS) and it is also related to the Porcine Dermatitis and Nephropathy Syndrome (PDNS). Circovirosis is distributed worldwide in all pig populations and in Mexico is prevalent in several states. As part of its control and prevention several vaccines have been used. To assess immunogenicity of the vaccines in pigs, physicochemical and biological tests should be performed in order to verify that it meets quality standards. Within the Mexican Official Standards PCV2 vaccines are not included, so the protocol for testing this biological was verified with the laboratory quality producer protocol and the standards set by the Code of Federal Regulations (CFR/USA). In this study it was carried out the general testing of an inactivated subunit vaccine containing the ORF2 protein from the Porcine Circovirus type 2 (PCV2).

Materials and Methods

The specifications of the vaccine under study were verified checking throughout physicochemical and biological tests according to the established by the NOM-063-ZOO-1999 and the CFR. Seventeen 4 week old hybrid gilts (Y x L) free of antibodies against PCV2 and two Guinea Pigs of 250 gr of the strain Hartley albino were used. Ten gilts were immunized in the neck muscles (2 ml) with a revaccination 15 days later and five were left as controls. The security test was carried out in parallel with two gilts, applying intramuscularly 2 doses in the table of the neck and 2 doses in the Guinea Pigs subcutaneously. Likewise the ELISA test was conducted to evaluate samples of sera obtained from days 0, 14 and 28, post-vaccination to assess seroconversion of antibodies using the kit SERELISA PCV2 Ab Mono Blocking SYNBIOTICS.

Results

The security test showed that the vaccine was safe and secure since it did not induced any negative reaction local or systemic in both vaccinated gilts and guinea pigs, the general physical condition and health were also monitored and remained stable throughout the test. The vaccine was found to have an average pH of 6.5, which is considered satisfactory. The sterility test showed that there were no contaminant organisms such as bacteria, fungi or micoplasmas. The vaccinated animals achieved an 80% seroconversion (Fig. 1), towards the viral antigen inoculated and 0% immune response was found in control pigs, based on the ELISA test performed on sera.

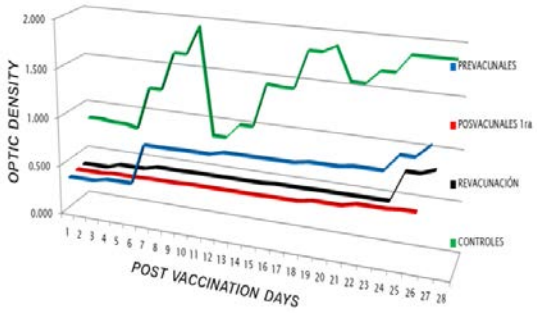

Figure 1. Seroconversion values found in vaccinated gilts by optic density after 28 days post inoculation.

Conclusions and Discussion

The requirements needed for their use in pigs were verified as satisfactory by the physical, biological and quality control tests carried out in this strudy and consequently this inactivated subunit vaccine against the CVP2 probed to be immunogenic and safe for pigs, and it may be useful for preventing infections of PCV2 or being used for the control of individual and group health disorders such as PMWS or PDNS associated diseases.

References

1. Clark, E. (1997). Procc. A.A.S.V. 28: 499-501.
2. Code Federal of Regulations (2006). Animals and Animal Products parts 9 to 133.33-38. National Archives and Records Administration. USA.
3. Petrini, S., Paniccia, M., Cagiola, M., *et al.* (2012). Procc. 22nd IPVS Congress/Satellite Merial Symposium, Jeju Island, South Korea.
4. Pradal-Roa, P. J. (2001). PDNS. Los Porcicultores y su Entorno. 4: 23.
5. Segales, J., Olvera, A., Grau-Roma, L., *et al.* (2008). Veterinary Record. 162: 867-868.
6. Taylor, D. J. (2013). In, Pig Diseases (Ninth edition). Wayment Print & Publishing Solutions Ltd., Hockley, Essex, UK. 72-78.
7. Ubeda, J. L., Menjón, R., Bollo, J.M., *et al.* (2010). Procc. 21st IPVS Congress, Vancouver, Canada. II: 398.

Efficacy of an anti-GnRF vaccine in suppressing estrus and estrus-related behavior in crossbred Iberian gilts

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Introduction

The Iberian pig is a native breed of Spain and Portugal, especially known for the production of dry-cured ham. It is typically kept in an extensive system, with a slaughter age ranging from 10 months to 2 years. To avoid welfare, sanitary and production issues linked to unwanted pregnancies caused by invading wild boar or co-housed males, gilts were traditionally spayed (1). However, EU Directive 2008/120/EC restricts this practice. Vaccination with an anti-GnRF vaccine (Improvac® / Vacsincel®, Zoetis) was tested for its ability to suppress estrus and estrus-related behavior in female pigs.

Materials and Methods

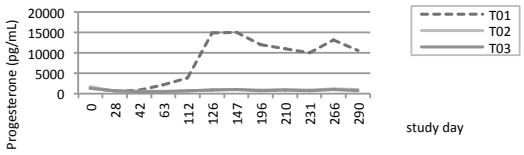
Sixty 18 week old crossbred (Iberian x Duroc) gilts were randomly allocated to one of three treatment groups within four preassigned pens:

	TREATMENT GROUP	DAYS OF INJECTION
T01	PBS 2 mL	0, 28, 112 and 196
T02	Improvac 2 mL	0, 28 and 112
T03	Improvac 2 mL	0, 28, 112 and 196

Animals were treated as indicated above. From day 1 until day 290 animals were tested for standing estrus in the presence of a boar, 3 times per week. Animals were regularly blood sampled, to assess serum titers of anti-GnRF antibodies by an in-house ELISA (Zoetis, Kalamazoo) and serum progesterone (using a commercially available enzyme immuno assay; Enzo Life Sciences). At slaughter (day 293-300), the weights of uteri and ovaries, the length of the uterus horn, and the presence of follicles on the ovaries were assessed.
The experimental unit was the individual animal. Treatment differences were assessed at a two-sided 5% significance level. Standing response was analyzed using the Cochran-Armitage test adjusting for block. All other variables were compared using linear mixed models.
This experiment was carried out in compliance with national legislation and subject to an ethical review.

Results

Standing estrus was observed on at least one occasion throughout the study in 17/20 gilts for T01 (85 %), in 3/20 gilts for T02 (15 %) and in 0/20 gilts for T03. The differences between T01 and T02, and T01 and T03, were significant (P<0.0001). The difference between T02 and T03 was not significant (P=0.3236). Whereas in group T01 most gilts showed standing estrus at multiple occasions throughout the study, the three gilts with standing estrus in T02 did so on a single occasion only. Two animals were judged to be in estrus around the time of the 2nd vaccination (day 29 and day 36), with a third animal detected at day 244 of the study.
Anti-GnRF antibodies were not significantly different between groups at day 0 of the study. Afterwards, both T02 and T03 had higher serum titers for all time points sampled (P<0.0001). The difference between T02 and T03 was not significant up to day 196 of the study. Afterwards, the titers were significantly higher for T03 (P<0.0001). Serum progesterone was not significantly different between treatment groups until day 63 of the study. From day 112 onwards, both T02 and T03 had significantly lower progesterone levels when compared to T01 (P<0.002).



At slaughter, average weight of the ovaries was 20.8 ± 2.1 grams for T01, 1.5 ± 0.4 grams for T02 and 1.2 ± 0.1 grams for T03. The difference between T01 and T02/T03 was significant (P<0.0001). In group T01, follicles were observed on all ovaries. In group T02 this was the case in 6 % of animals, whereas in T03 follicles were not observed on any ovary. Average uterus weight (grams) and length (meters, both horns combined) was 821 ± 94 g and 2.84 ± 0.27 m for T01, 47 ± 13 g and 0.80 ± 0.22 m for T02 and 34 ± 4 g and 0.79 ± 0.21 m for T03. Uteri for T02 and T03 were significantly smaller and lighter (P<0.0001) than those in T01. Between T02 and T03, weight and length were not significantly different (P=0.3498 and P=0.9737 respectively).

Conclusion

Both the three and four dose regimens of Improvac provide a prolonged reduction in estrus and estrus related behavior, and thus provide a promising alternative to surgical ovariectomy in Iberian gilts.

References

1. Hernández-García F.I. et al. 2013. *Acta agriculturae Slovenica*, Suppl 4, 123–126.

A. pleuropneumoniae serotype 2, vaccination with a vaccine based on the serotype

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Introduction

The prevalence of pleuritis recorded at Swedish abattoirs decreased from 8% in 1988 to 5% in 2002 due to a more prompt implementation of age segregated rearing systems [1]. However, during the last decade the incidence of outbreaks of actinobacillosis have increased and vaccination against *Actinobacillus pleuropneumoniae* (***App***) have had limited effect [2]. In 2012, pleuritis was recorded in 13% of the pigs slaughtered. *App* in Sweden has generally been associated with serotype 2 (***App2***) [3].

Materials and Methods

The trial was conducted in an age segregated system. A piglet producing herd with 900 sows and 43 farrowings each week delivered growers at the age of 11 weeks to a specialised fattening herd. Acute actinobacillosis caused by ***App2*** had occasionally been diagnosed, and the prevalence of pleuritis registered at slaughter had ranged between 20 and 40 % during the last years. Pigs in two consecutive batches were divided into two groups. One group was vaccinated with a vaccine based on ***App2*** (Hyobac App2; Salfarm, Kolding, Denmark) at the age of 6-7 and 10 weeks of age. The other group was kept as an unvaccinated control. Blood samples were collected every third week from the age of 6 weeks and onwards. Serum was analysed for the presence of antibodies to ***App2*** with an indirect ELISA [4].

Table 1. Productivity and recordings for pleuritis at slaughter

	Batch I		Batch II	
	Contr	Vacc	Contr	Vacc
Allocated, 11 w	172	182	316	252
Slaughtered	169	178	309	246
Mortality (%)	1.7	2.3	2.2	2.4
DWG (g/day)	893	875	793	813
F-conv (MJ/kg)	32.5	32.5	37.7	35.9
Pleuritis (%)	28.0	28.0	35.6	22.0

Results

No clear differences between the groups were recorded with respect to mortality and weight gain (Table 1).

The vaccinated pigs seroconverted to ***App2*** between 9 and 12 weeks of age in both batches. In contrast, the control pigs seroconverted between 15 and 18 weeks of age in the first batch and between 12 and 15 weeks of age in the second batch (figure 1). In the first batch, the prevalence of pleuritis recorded at slaughter was equal in both groups (28%). In the second batch, the prevalence of pleuritis recorded at slaughter was lower in the vaccinated group (22% vs 35.6%; χ^2 = 11.6, p<0.01; Table 1).

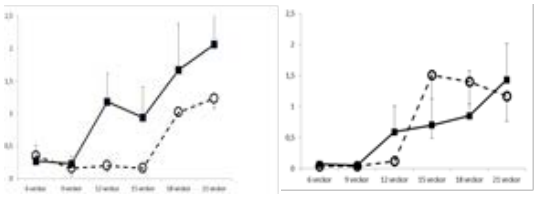

Figure 1. Serum concentrations of antibodies to ***App2*** in batch I (left) and batch II (right) in vaccinated (■) and non-vaccinated (○) pigs.

Conclusions and Discussion

A protective role of antibody-mediated immunity following ***App2***-infection has been demonstrated [5, 6], and the ***App2***-based vaccine apparently stimulated the immune system as indicated by the earlier seroconversion to ***App2*** in the vaccinated groups. Despite that, the prevalence of pleuritis at slaughter was equally high (28%) in both groups during the first batch. In the second batch, when the pathogen load was higher as indicated by a lower weight gain and an earlier seroconversion to ***App2*** in non-vaccinated pigs, the prevalence of pleuritis at slaughter was lower in the vaccinated group. That indicated a protective immunity induced by the vaccine, but only partially since pleuritis was recorded in 22% of the vaccinated pigs. Nor did the DWG differ significantly between the groups. This trial only included data from two batches, and a balance between infectious load and immunity maybe could arise with time. A longer evaluation period would therefore have been of interest.

References

1. Holmgren & Lundeheim, 2002. Sw Vet J. 54, 469-4.
2. Sjölund & Wallgren. 2010. Acta Vet Scand. 52:23
3. Wallgren et al., 2003. Sw Vet J. 55 (7) 25-28.
4. Wallgren & Persson, 2000. J Vet Med B. 47, 727-738
5. Krejci et al. 2005, J Vet Med B. 52, 190-196
6. Sjölund et al 20XX. Vet Rec. 10.1136/vrd2268.

Genetic selection for disease tolerance and robustness

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Introduction

Diseases like PRRS can adversely affect the productivity of the farm. In view of such diseases, pigs having inherent ability to resist or tolerate infections and keep up their productivity may be of great interest. In addition, especially in the context of global pork production, pigs also have to face other challenges due to heat stress, feed quality and other stressors. Hence, there is a growing need for pigs that are more robust to these stressors. Breeding for such robust pigs, however, requires a clear definition of robustness, estimation of the effects of these challenges, and estimation of available genetic variance to develop approaches for genetic selection. This report summarizes the results of a series of investigations and research findings including detection of disease outbreaks[1] and estimation of genetic parameters.

Materials and methods

Two sets of data were used to develop and validate measures of robustness to diseases and other stressors. The training dataset consisted of 57,135 reproduction records from 10,910 sows from a commercial farm in PRRS endemic area of Canada. The records included number of born alive (NBA), stillbirths (STB), mummified (MUM) and number of weaned piglets (NWP). The records on STB and MUM were summed together as number of lost piglets (NLP). These traits were combined in a disease load index (DLI) to predict disease outbreaks. Effectiveness of this DLI was compared to individual traits on a dataset from 20 farms in the Netherlands with known PRRS outbreaks. The DLI was then further validated using data from 431 farms from 8 countries in Europe and Americas.

Variances due to animal genetic effects, common environmental effects and service sire effects were estimated using animal models in ASReml[2]

Results

The results of validation using the proposed disease load index in the farms in Europe and America are given in Table 1. The majority of outbreaks detected were confirmed as PRRS (28 out of 41 outbreaks). The other 13 outbreaks were related with other infectious agents such as Leptospira or infectious diarrhea, and feed quality issues.

Table 1. Validation of outbreaks detected using disease load index

Country	Number of farms	Outbreaks detected	Outbreaks confirmed
Canada	13	2	2
Germany	47	1	1
Spain	38	5	5
Hungary	7	2	1
Italy	7	5	3
Netherlands	295	23	20
Portugal	11	4	4
Russia	14	6	5
Total	431	48	41

The proportions of animal genetic variance to the total phenotypic variance during disease outbreaks for NBA, NLP and NWP were 6.4, 8.3 and 5.3 percent, respectively. During healthy periods, these proportions were 7.2, 6.7 and 3.3 percent, respectively. The variances due to service sire effects during outbreaks were 4.2, 1.2 and 4.2 percent while those during healthy periods were 1.5, 0.8 and 1.1 percent, respectively.

Conclusions and Discussion

The results suggest that DLI is a suitable tool to detect outbreaks due to disease and disturbances due to other stressors. It can be a useful as an indicator for magnitude of production losses due to these disturbances. Analysis of variance components revealed that higher genetic variation is available in NLP and NWP during outbreaks for selection. There was also a higher variation due to the sires used for production of litters. This could be due to semen quality but mainly due to the interaction between genes of the sire and genes of the dam. These results reveal opportunities for genetic selection for improving robustness of pigs towards diseases and other challenges.

Acknowledgments

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References

1. Rashidi H et al. 2014. J. Anim. Sci. 92:95-105
2. Gilmour A R et al. 2009. ASReml user guide release 3.0 VSN International Ltd, UK.

Effect of genetic background and birthweight on the performance of growers and finishers

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Introduction

At Swine Innovation Centre Sterksel the effects of genetic background and birthweight on the performance and financial results of piglets, growing and finishing pigs were investigated. Genetic background (TPI; TOPIGS Profit Index) was expressed as the genetic index for daily gain, feed intake and lean meat percentage of the growing and finishing pigs.

Materials and Methods

In total 624 weaned piglets (48 pens x 13 piglets) and 576 growing and finishing pigs (48 pens x 12 growing and finishing pigs) (Tempo Boar x (Dutch Landrace x Dutch Large White) sow) were used in the experiment. Pigs were followed from birth until delivery to the slaughterhouse. There were four experimental treatments. See table 1.

Table 1.

Treatment	Genetic Index [1]	Birthweight Piglet [2]	Number of pens
1	High	High	12
2	High	Low	12
3	Low	High	12
4	Low	Low	12

[1] Sows with a high genetic index for daily gain, feed intake and lean meat percentage of the growing and finishing pigs were inseminated with a boar with a high genetic index; sows with a low genetic index were inseminated with a boar with a low genetic index.
2 High birth weight is higher than 1,340 gram; low is lower than 1,340 gram.

At weaning piglets were grouped by genetic background, birth weight and gender. Five weeks after weaning, pigs were moved to the unit for growing and finishing pigs. Pigs out of the same weaning pen stayed together. The growing and finishing pigs were fed ad libitum. They received a starter diet for five weeks, a grower diet for four weeks and a finisher diet until delivery to the slaughter house.

Results

Genetic background

- Piglets from sows and boars with a high genetic index grew faster during the weaning period than piglets from sows and boars with a low genetic index. Feed intake and feed conversion ratio did not differ between these piglets.
- Growing and finishing pigs from high genetic sows and boars ate more, grew faster and had a better feed conversion ratio than growing and finishing pigs from low genetic sows and boars.

Birth weight

- Piglets with a high birth weight ate more and grew faster during both the weaning period and the growing and finishing period than piglets with a low birth weight. Feed conversion ratio during the weaning period and the growing and finishing period did not differ between high and low birth weight pigs.

Table 2. Performance of finishers per TPI and per category of birth weight

TPI	Low		High	
Birthweight	**Low**	**High**	**Low**	**High**
Nr animals	144	142	141	144
Nr pens	12	12	12	12
Startweight Kg	22.5	25.5	23.0	25.5
Endweight Kg	112.9	115.4	116.3	117.2
End-age	173.3^{a}	167.9^{b}	169.6^{b}	164.7^{c}
ADG (g/d)	818^{a}	851^{b}	874^{bc}	891^{c}
Feedintake (kg/d)	2.12^{a}	2.18^{b}	2.19^{b}	2.26^{c}
Feedconv.	2.60^{a}	2.56^{ab}	2.51^{b}	2.54^{ab}

[a,b,c] averages with a different letter within arrow are different ($p<0.05$)

Conclusions and Discussion

In conclusion, performance of growing and finishing can be improved by using sows and boars with a high genetic index for daily gain, feed intake and lean meat percentage. The genetic index can be used in the decision whether a sow should be replaced or not. Besides, the farmer can decide to use boars with a high genetic index. Pigs with a high birth weight have a better performance during both the weaning period and the growing and finishing period than low birth weight pigs. Birth weight is highly repeatable; in other words, sows that have heavy piglets will also have heavy piglets in the next litters. Thus, birth weight of the piglets can be used in the decision whether or not to replace a sow. High genetic growing and finishing pigs with a high birth weight are delivered 8.6 days earlier to the slaughterhouse than low genetic pigs with a low birth weight and are heavier at that age too. Therefore, it is financially interesting to keep these pigs in different compartments.

Acknowledgements

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Effect of pre-farrow administration of tulathromycin injectable solution on *M. hyopneumoniae* prevalence in suckling pigs at birth and weaning

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Introduction

M. hyopneumoniae(*M. hyopneumoniae*) is one of the most prevalent and economically significant respiratory pathogens in the swine industry. Vertical transmission of *M. hyopneumoniae* has been proposed as the most important means of transmission in segregated production systems. Additionally, *M. hyopneumoniae* prevalence at weaning has been shown to be an important factor in determining clinical presentation of disease downstream. The objective of this study was to determine if tulathromycin injectable solution administered pre-farrow to sows and gilts reduces the prevalence of *M. hyopneumoniae* in suckling pigs at birth and at weaning

Materials and Methods

Forty-eight females within one, multiple parity, 5000-sow farm were randomly assigned to either the treatment or control group. Treatment females received one intramuscular (IM) injection of tulathromycin injectable solution (2.5mg/kg) at day 112 of gestation. Nasal and tonsil swabs were collected from all females at day 112 of gestation, farrowing, and weaning. Treatment and control groups were housed in separate farrowing rooms. All viable piglets from treatment and control females were enrolled in the study. Piglets were individually weighed and nasal and tonsil swabs were collected at birth and weaning. Piglet swab samples were tested in pools of 5 by litter. All swab samples were submitted for *M. hyopneumoniae* detection by real-time PCR (VetMax™). Tonsil swabs were used to verify the status of "suspect" nasal swabs, when applicable. Proportions of positive samples were compared using Fisher's Exact test or McNemar's Chi Square test for paired samples using Statistix 9.0 software.

Results

The number of sows positive to *M. hyopneumoniae* in the two experimental groups at different samplings is shown in Table 1. The number of positive piglets (by pools) in the two experimental groups at birth and at weaning age is presented in Table 2. Sow treatment with tulathromycin was associated with significant reductions in *M. hyopneumoniae* prevalence in sows at farrowing (p=0.008) and at weaning (p=0.001) and in piglet pools at birth (p=0.01) and at weaning (p=0.042).

Table 1. *M. hyopneumoniae* PCR positive sows

	Day 112	Farrowing	Weaning
Control	4/23	10/23	8/23
Treatment	6/24	2/24	0/24

Table 2. *M. hyopneumoniae* PCR positive piglets (pools)

	Birth	Weaning
Control	6/54	8/49
Treatment	0/58	2/56

Conclusions and Discussion

Results from this study indicate that it is possible to reduce the *M. hyopneumoniae* shedding in sows and gilts by administering tulathromycin injectable solution at day 112 of gestation. Subsequently, this reduced shedding in sows and gilts led to a reduced number of *M. hyopneumoniae* positive piglets at birth and at weaning. As prevalence at weaning has been associated with disease severity in downstream pigs, further studies are indicated to define the potential benefits of this approach in reducing respiratory disease and improving performance in growing pigs.

References

1. Sibila M, Calsamiglia M, Vidal D, et al. Dynamics of Mycoplasma hyopneumoniae infection in 12 farms with different production systems. Can J Vet Res. 2004;68:12–18.
2. Fano, E., C. Pijoan, and S. Dee. 2007. Effect of Mycoplasma hyopneumoniae colonization at weaning on disease severity in growing pigs. Can. J. Vet. Res. 71:195-200

Assessment of safety and efficacy of Hyogen® - a single dose *M. hyopneumoniae* bacterin vaccine in commercial pigs under field conditions

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Introduction

A randomized, blinded, placebo controlled field trial was performed to evaluate the safety and efficacy of Hyogen® a single dose *M. hyopneumoniae(M. hyo)* bacterin vaccine in pigs applied at 3 weeks of age.

Materials and Methods

The trial was carried out on a commercial farrow-to-finish farm of approximately 1200 sows with confirmed enzootic pneumonia in Hungary. A total number of 522 piglets at 21±3 days of age were either vaccinated with Hyogen® or phosphate buffered saline (PBS) injected in a ratio of 1:1 (D0h0: day of vaccination). The piglets were randomized at inclusion (D-1) within each litter by their bodyweight. Fifty-six (56) piglets per group were selected for individual safety observation. Body temperature of these animals were recorded at D-1, D0h0, D0h4, D1 and D2. General and local reactions were also checked at the same time and on D7 and D14. Lung lesions typical of enzootic pneumonia were observed at slaughter. Mortality, culling, individual antibiotic treatment data were collected during the whole observation period. All pigs were individually weighed at D-1, at the end of the nursery phase (approximately 13 weeks of age) and at the end of the fattening period (slaughter). Average daily weight gain (ADWG) was calculated. Blood samples were taken from 28 vaccinated and 28 control pigs at D-1, D21, 13 and 23 weeks of age to determine *M. hyo* antibody titres (IDEIA Mycoplasma hyopneumoniae, Oxoid, UK).

Results

Table 1. Safety and efficacy of Hyogen® vaccine in commercial pigs – summary of the field trial results

	Hyogen®	Placebo	*p*
Safety parameters			
Systemic reactions[1]	1/56 (1.8)[1]	0/56 (0)[1]	n.t.
iMIT[3]	2.0 °C	1.3 °C	n.t.
HMTI[4]	1.3±0.4[2] °C	0.3±0.3[2] °C	<0.01
Local reactions (LR)[1]	9/56 (16)[1]	2/56 (3.6)[1]	n.t
Duration of LR	< 7 days	<12 hours	n.t
Efficacy parameters			
Lung lesions[1]	61/169 (36.1)[1]	88/162 (54.3)[1]	<0.01
Mean LLS[5]	7.4±18.7[2a]	20.9±43.0[2a]	n.t
Median / Maximum LLS[5]	0 /164[a]	6 / 301[a]	<0.01
Pleurisy[1]	20/169 (11.8)[1]	36/162 (20.2)[1]	0.01
ADWG[6] fattening period	474±118[2] g	464±102[2] g	0.494
Mortality&culling rate	15/233 (6.4)[1]	13/233 (5.6)[1]	0.696
Antibiotic treatment rate	3/233 (1.3)[1]	8/233 (3.4)[1]	0.044

[1]№ incidence/№ observed (% in brackets); [2]Mean±standard deviation
[3] individual Maximum Increased Temperature; [4] Highest Mean Temperature Increase; [5]lung lesion score; [6]average daily weight gain
[a]according to Ph.Eur.Monograph 2448 scoring

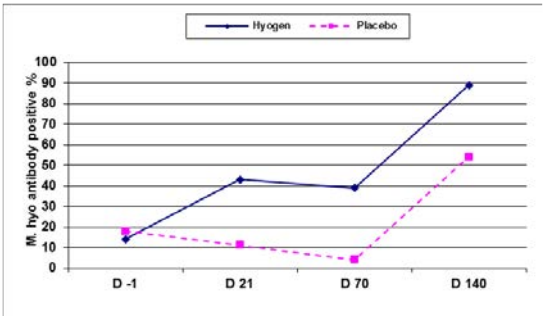

Figure 1. Proportion of *M. hyopneumoniae* antibody positive sera from vaccinated and saline injected pigs

Conclusions and Discussion

Safety General health was not affected by the vaccination. A small temperature increase in both groups was observed 4 hours after vaccination, which persisted for less than 12 hours only (Table 1). Moderate incidence, a maximum 16% of mild local reactions (i.e. slight, <5 cm swelling at the injection site) were observed 4 hours and 2 days post vaccination.

Efficacy Incidence and severity of lung lesions observed after natural farm challenge at the end of the fattening period were significantly reduced in the Hyogen® vaccinated group compared to the Control (Wilcoxon rank sum test p<0.01). ADWG was not significantly different between the treatment groups. Mortality and morbidity rate was generally low, however, significantly lower number of pigs needed antibiotic treatment in the Hyogen® group (chi-square test p=0.01). Antibody response confirmed both the vaccine take (D21 and D70 post vaccination) and the field challenge of *M. hyo* during the fattening period (Figure 1).

A single dose of Hyogen® vaccine proved to be safe in 3 week old piglets and as efficacious to cover the whole fattening period (until commercial slaughter).

Hyogen® is a brand name of Ceva Santé Animale

References

1. European Pharmacopoeia Ed.8th Monograph 2448.
2. Dawson, A., Harvey, R.E., Thevasagayam, S.J., Sherington, J., Peters, A.R. 2002. Vet. Rec. 151, 535–538.

C.003

In vitro study to update on antimicrobial susceptibility of *M. hyorhinis* in Thailand

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Introduction

Mycoplasma hyorhinis is a systemic pathogen causing pneumonia, arthritis-synovitis and lameness in pigs. Its occurrence in Thai pig farms in nursery to slaughtered pigs are observed frequently. Recent reports indicated higher resistance of *M. hyorhinis* to antimicrobials than other porcine mycoplasmas such as *M. hyosynoviae* and *M. hyopneumoniae* (3,4,5). This study aims to follow up on the susceptibility status of various strains of *M. hyorhinis* derived from field cases and slaughtered lung lesions. The antimicrobials chosen for MIC tests are based on the medicines used in farms such as doxycycline (DOXY), lincomycin (LINCO), tylosin (TYL), tilmicosin (TILM), tylvalosin (TYLV), tiamulin (TIA), and valnemulin (VAL).

Materials and Methods

Thirty-nine strains of *M. hyorhinis* derived from pigs with respiratory signs, lameness in six farms and the affected lungs in slaughtered pigs of two farms during 2012 to 2013 were selected. Mycoplasma isolates were subcultured until it could grow well within 3-4 days. Each of Mycoplasma isolate at the stationary phase was kept in -80 °C until use for susceptibility testing. BTS-7 was used as the referent strain.

The MICs were conducted by using agar dilution method (2). The seven antimicrobials were prepared for the test by making serial dilutions (10 times of the test concentration) ranging from 320 to 0.08 µg/ml. 2 ml of each dilution was mixed with 18 ml of modified Hayflick's agar and pouring plate. Therefore, the test concentration of each antimicrobial ranged from 32 to 0.008 µg/ml. The agar was left for solidification and ready for the test.

M. hyorhinis isolate was diluted to 10^8 CFU/ml in 0.5 ml Hayflick's broth and transferred into the well of the replicator seed block. One µl of each isolate was inoculated on the agar plate using the replicator so that there would be 10^5 of the isolate per spot. The inoculated plates were incubated at 37 °C in a humidified incubator with 5% CO_2 for 5 days. The lowest concentration of antimicrobial agent that inhibited the growth of mycoplasma was interpreted as the MIC value of each isolate. MIC range, MIC50 and MIC90 were determined. Resistant breakpoint of each antimicrobial was compared with the MIC to identify resistant isolates (1,2,6).

Results

Antimicrobial susceptibility of *M. hyorhinis* and its distribution were illustrated in Table 1 and Table 2.

MIC µg/ml	DOXY	LINCO	TYL	TILM	TYLV	TIA	VAL
BTS-7	0.256	4	1	8	0.128	0.512	0.016
MIC Range	0.256 -4	1 - >32	0.512 - >32	0.512 - >32	0.064 -16	0.256 -1	0.008 - 0.032
MIC 50	1	>32	>32	>32	16	1	0.032
MIC 90	2	>32	>32	>32	16	1	0.032
Break-point	≥16	>8	≥4	≥32	≥4	≥16	≥16

Table 1. MIC of *M. hyorhinis* (N=39)

Table 2. Distribution of *M. hyorhinis* MIC and % resistance (%R) to each antimicrobials.

MIC	Antimicrobials						
(µg/ml)	DOXY	LINCO	TYL	TILM	TYLV	TIA	VAL
0.008							6
0.016							7
0.032							26
0.064					1		
0.128					12		
0.256	1				4	3	
0.512	1		2	2		7	
1	24	7	15	3		28	
2	11	4				1	
4	2	7					
8							
16					22		
32				10			
>32		21	22	24			
%R	0	53.8	56.4	87.2	56.4	0	0

Conclusions and Discussion

M. hyorhinis field strains from Thailand tend to be resistant to all generations of macrolides (tylosin, tilmicosin, and tylvalosin) and to lincomycin at the resistance percentage from 53.8% to 87.2%. As a result of cross resistance among antimicrobials, the prophylactic application of macrolides and lincosamides is considered as one reason of resistance development. The prudent use of these groups is important (1,2,4,6). Nevertheless, in case of endemic infection, the antimicrobials causing non- or less resistance are the better alternative. In this study, doxycycline and pleuromutilins (tiamulin and valnemulin) can be the drugs of choice for the prophylaxis as well as treatment in field cases of *M. hyorhinis* infection.

References

1. EMA 2009. Scientific discussion, p.17.
2. Hannan, P.C.T., 2000, Vet. Res. 31: 371-395.
3. Ishii et al. 2013. Proc. of 6th APVS, PO19.
4. Makhanon, M., 2012, Doc. Thesis. Chulalongkorn University.
5. Makhanon et al. 2013. Proc. of 6th APVS, OR1.
6. NCCLS 2004. M31-S1, p.14.

C.004

Differences in susceptibility to PCV2 challenge between pure and crossbred Landrace pigs

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Introduction

In the literature, there are several reports suggesting that the genetic background of the animals may influence the outcome of natural (1) or experimental (2,3) porcine circovirus type 2 (PCV2) infection. Results of these studies indicate that Landrace pigs might be more susceptible to develop PCV2-associated lesions than other breeds (2,3). However, it is not known if this susceptibility is increased in pure Landrace pigs compared to crossbred ones. Therefore, the objective of this study was to assess the differences in susceptibility to PCV2 challenge between pure and crossbred Landrace pigs.

Materials and Methods

This experiment was carried out in compliance with national legislation and subject to an ethical review. Twenty pure Landrace (L) and 20 Landrace per Large White (L x LW) pigs, matched by age and weight, were included in this study. Groups were housed separately, but in identical rooms and management conditions. At 5-7 weeks of age, all animals were challenged with 3 ml of $10^{4.5}$ TCID/ml (2 ml intranasal + 1 ml intramuscular) PCV2b strain. Blood, nasal and faecal swabs were collected at 0, 7, 14, and 21 days post inoculation (dpi). Animals were necropsied at 21 dpi. Average daily weight gain (ADWG) was calculated from the body weight (BW) recorded at challenge and at necropsy. Clinical signs were daily registered. At necropsy, PCV2 associated gross lesions were registered and lung and lymphoid tissues were taken and immersed in formalin 10% for further histopathological studies. PCV2-associated microscopic lesions (lymphocyte depletion [LD] and histiocytic infiltration [HI]) were scored (from 0 to 3) in the following lymphoid tissues: tonsil (T), superficial inguinal lymph node (SILN), mesenteric lymph node (MLN) and spleen (S). *In situ* hybridization (ISH) was performed and scored (from 0 to 3) in the abovementioned lymphoid tissues (4). PCV2 antibody titres in serum were tested by means of an immunoperoxidase monolayer assay (IPMA) (4). Detection and quantification of PCV2 in serum, nasal and faecal swabs was done using a quantitative PCR (5).

Results

PCV2 associated clinical signs (poor body condition and rough hair) were observed only in L pigs (5 out of the 20 [25%]). One of these 5 L animals died at 18 dpi. Interstitial pneumonia was observed in 9 (45%) and 6 (30%) L and LxLW pigs, respectively, ($p>0.05$). Presence of craneo-ventral pulmonary consolidation was observed in 9 (45%) and 6 (30%) L and LxLW pigs, respectively, ($p>0.05$). Results of PCV2 ISH and microscopic lesion scoring are detailed in table 1. An increase in PCV2 IPMA titres from challenge onwards was observed in both genetic lines. However, LxLW pigs had significantly higher IPMA titres at 7 and 14 dpi than L pigs. From challenge onwards, L pigs showed a progressive increase of PCV2 viral load in serum, nasal and faecal swabs. On the contrary, in LxLW group, PCV2 viral load increased up to 14 dpi when started to decrease. Statistically significant differences in PCV2 viral load at 14 and 21 dpi in all three samples (serum, nasal secretions and faeces) between both genetic lines were observed.

Table 1. Percentage of animals with an ISH, LD and/or HI score ≥2

Variable	Breed	T	ILN	MLN	S
ISH	**L**	30^{a}	30^{a}	50^{a}	10^{a}
	LxLW	$11{,}1^{a}$	5^{b}	10^{b}	0^{a}
LD	**L**	35^{a}	35^{a}	45^{a}	15^{a}
	LxLW	0^{b}	5^{b}	5^{b}	0^{a}
HI	**L**	35^{a}	35^{a}	45^{a}	15^{a}
	LxLW	0^{b}	5^{b}	5^{b}	0^{a}

Different letters in superscripts means $p<0.05$ between genetic lines

Conclusions and Discussion

In this study, PCV2 associated clinical signs were only observed in pure L pigs. Moreover, L pigs showed more severe PCV2-associated microscopic lesions in lymphoid tissue and had lower PCV2 IPMA titres at the moment of highest PCV2 viral loads than crossbred L pigs. These results suggest that pure L pigs are more prone to suffer from PCV2 associated lesions than LxLW pigs. However, further studies are needed to elucidate if these differences are due to the breed, genetic line or individual susceptibilities.

References

1. López-Soria S et al., 2011. Vet Microbiol 149(3-4):352-7.
2. Opriessnig T et al. 2006. Vet Pathol 43(3): 281-293
3. Opriessnig T et al. 2009. J Anim Sci 87(5): 1582-1590
4. Fort M et al., 2009. Vaccine. 27: 4031-4037

C.005

PCV2 preferentially infects fetal thymus

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Introduction

PCV2 is widespread in the pig population and it is connected with several costly pig diseases referred to as porcine circovirus diseases (PCVD's) including postweaning multisystemic wasting syndrome (PMWS). However, most infections are subclinically. PCV2 is also a fetal pathogen linked to late term abortions and stillbirths. Nevertheless, these losses seem to be infrequent under field conditions (5). Many intrauterine infections are subclinically as Shen et al. (6) and Chery et al. (1) found in serum of healthy pre-suckling piglets a 39.9% and 63% PCV2 genome prevalence, respectively.
Thymus atrophy is a common lesion in PMWS (3) and infections of the thymus with PCV2 in diseased weaned pigs, as well as in fetuses, are documented (3). Nevertheless, these PCV2 infections were mostly evaluated by the presence of high concentrations of PCV2 antigen content detected by immunohistochemistry (IHC) or immunofluorescence respectively. (3). Moreover, in fetal PCV2 pathogenesis other fetal organs than the thymus and other cells attracted more attention including cardiomyocytes, lymph nodes, spleen, hepatocytes and macrophages (4).

Materials and Methods

135 IHC-PCV2 negative fetuses older than 55 days (d) (6 from 55-70 d, 37 from 70 - 110 d, 73 at normal birth time and 10 post-term fetuses) from 66 sows of 38 farms with anamnestical increased reproductive failures were from a previous prevalence study (7) conducted before PCV2 vaccination became common in Switzerland. Fetal organs were investigated with fluorescence *in situ* hybridization (FISH) for presence of PCV2-DNA. At necropsy, tissue from thymus, spleen, mesenteric lymph node, tonsil, heart, liver and placenta were collected and routinely processed (formaldehyde fixed and paraffin embedded) for histology with hematoxylin-eosin (HE) and PVC2 specific IHC using mAB F217 and FISH with specific oligonucleotides described in Khaiseb et al. (2) and a newly designed oligonucleotide P2O-O1r 5'GCA TGT TGCT GCT GAG GTG CTG CCG 3' with 5' and 3' Dyomics 630 labeled. FISH signals in the organs were interpreted semi-quantitatively and scored from negative (-) to ++++. From 45 randomly selected tissue blocks of the fetuses older than 55 days conventional PCR (9) and from additional 4 fetuses about 30 days old without histologically identifiable thymus, nested PCR (first primer set 5' GGA GGA TTA CTT CCT TGG TAT TTT GG 3' and second primer set (2) 5'GGT GCT GCC GAG GTG CTG 3') was performed.

Results

PCV2 cap protein was absent in the investigated fetal organs and no myocardial or lymphatic tissue lesions were seen. Of the 45 fetuses older than 55 days, investigated by conventional PCR (9) 43 (96%), were PCR positive and contained PCV2b genotypes. The 4 fetuses about 30 days of age were in nested PCR also positive and contained PCV2b genotypes too.
In FISH virtually 100% of the fetuses were PCV2 infected with highest prevalence and most infected cells in thymus. Other organs were significantly less infected (descending order: mesenteric lymph node, liver and spleen, heart and placenta).

Conclusions and Discussion

Proverbially 100% of the thymi of fetuses from a field investigation of losses from sows contained PCV2 genomes. PCV2 antigen was not detected in these fetuses and thus, the losses could hardly be attributed to the PCV2 infection. Whereas, PCV2 related losses are associated with high amounts of virus within the myocardium and necrotizing to fibrosing myocarditis (4), our piglets in which only PCV2 DNA could be found, showed even the lowest PCV2 DNA content in the myocard compared to the other organs. These piglets had also no histological lesions in the myocardium.
Most studies, experimental infections and field studies, imply fetal myocardial cells and in second rank liver and secondary lymphatic organs as mainly infected due to detection of PCV2 cap protein. However, in subclinical infections with predominantly PCV2 genome presence the tissue tropism seems to be different than in the viral overflow.
We suggest that the PCV2 infections are latent and already in fetal pigs present. The virus is probably early vertically introduced into the embryos. This and a certain thymus tropism were also noted in Chicken Infectious Anemia Virus (CIAV) infections, the only other well-known *Circoviridae* (8).

References

1. Cheryl MT et al. Vet. Microbiol. 2013; 166, 365-74.
2. Khaiseb S et al. J Virol. 2011;85:11111-20.
3. Saha D et al. 2010, Vet. Microbiol. 145:62-8.
4. Sanches RE Jr et al. Vet Microbiol. 2003; 95:15-25.
5. **S**egales J et al. In: Diseases of swine 9th edition. 2006, p 299-307.
6. Shen H et al. Prev Vet Med. 2010;97:228-36.
7. Sydler T et al. 2012. IPVS Korea, 863.
8. Vaziry A et al. Avian Pathol. 2011; 40:377-85.
9. Wiederkehr D et al. Vet. Microbiol. 2009;136:27-35.

C.006

Humoral immune response to PCV2 in vaccinated and non-vaccinated animals

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Introduction

Porcine Circovirus 2 (PCV2) is the causative agent of the post-weaning multisystemic wasting syndrome (PMWS), an economically devastating disease affecting six to twelve week-old pigs, resulting in poor production rates in affected animals (1). PCV2 vaccines have dramatically diminished the prevalence of PMWS in the field by reducing viral load in the animals, and inducing neutralizing antibodies (NAs) against PCV2 that correlate with protection under experimental conditions (2). Another subset of antibodies against a linear epitope of PCV2, denominated "decoy" epitope, has been described as non-protective and rather predisposing to PMWS in the animals (3). Our objective was to bring insight into the humoral response of animals against PCV2 in different stages of pig production.

Materials and Methods

A hundred and sixty samples were collected from thirteen randomly selected farms in AB, Canada. The samples were initially classified as vaccinated (V, n=80) and non-vaccinated animals (NV, n=80) and further divided into four age groups: 1(0-21 days), 2 (21-84 days), 3 (84-180 days) and 4 (>180 days).

We developed a high-throughput virus neutralizing assay as well as a peptide ELISA to measure the response towards the "decoy" epitope. Viral load in the serum was assessed using a sensitive probe-based qPCR.

The variance between V and NV animals, as well as between age groups was analyzed (SPSS® version 17.0, USA). Normally distributed data was analyzed using one-way ANOVAs with Bonferonni tests for *post-hoc* comparisons. If data was not normally distributed, a Kruskal-Wallis test was used with Mann-Whitney test for *post-hoc* comparisons. A $p<0.05$ was considered significant.

Results

A significant difference of NA titers between V and NV was observed in age group 2 (Figure 1). The highest NA titers were observed in V and NV of age groups 3 and 4.

"Decoy" ELISA demonstrated higher S/P ratios in NV animals compared to V animals in age groups 3 and 4. No significant difference was observed in the viral load of V and NV animals.

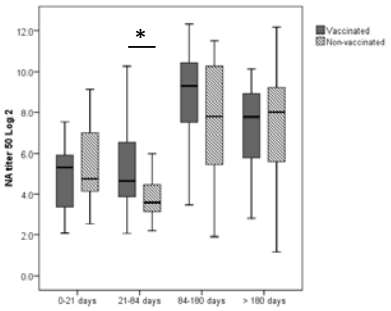

Figure 3. NA titer 50 Log 2 of V and NV animals from four age groups. Significance is shown as a star.

Conclusions and discussion

V animals presented significantly higher titers of NAs in the main age group in risk of developing PMWS, demonstrating a positive effect of vaccination on protection against the disease. We also observed a delayed induction of high titers of NAs compared to previous experimental studies. This difference might be due to PCV2 subclinical infection in the animals. However, in the same age group, we did not observe a difference in the antibody titers against the "decoy" epitope; the clinical importance of these antibodies needs to be further analyzed. PCV2 load in serum was not significantly lower in V animals, demonstrating no vaccine effect on lowering serum PCV2 levels.

Our study demonstrates a diverse humoral response against PCV2 and a possible impact of subclinical infection in the correct development of the adaptive immune response.

Acknowledgements

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References

1. Gillespie, J et al. 2009. J Vet Intern Med. 23: 1151-63.
2. Fort, M., et al. 2007. Vet Microbiol. 125: 244-55.
3. Trible, B. et al. 2011. Clin Vaccine Immunol. 18: 749-57.

In vitro activity of twelve antimicrobials against an oral attenuated live *L. intracellularis* vaccine isolate

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Introduction

The use of the oral attenuated live *L. intracellularis* (LI) vaccine Enterisol Ileitis requires an antibiotic free period of three days prior the day of and three days after the day of vaccination to ensure its efficacy. On some farms the vaccination against LI and necessary antibiotic treatments may coincide. So antibiotic substances which do not interfere with LI could be helpful and the concurrent use with the vaccine might be possible.
In this study the *in vitro* minimal inhibitory concentrations (MIC) of 12 antibiotic substances were determined for extracellular LI and for LI propagating in a cell culture.

Materials and Methods

Previously reported (1) test systems were modified:
A deep frozen stock of live attenuated LI vaccine (Enterisol Ileitis) was inoculated (100 µl) on fresh McCoy cells in a concentration of 56 $TCID_{50}$ units/ml (Tissue Culture Infectious Dose; determined prior to this study in order to get a stable and repeatable infection of susceptible cells in the intra- and extracellular test setup).
The MIC was defined as the lowest concentration of an antibiotic substance in the microdilution test which does inhibit the growth of LI tested in 3 replicas each. Each antibiotic substance was tested separately in different dilutions for extracellular and intracellular effects on LI propagation. To ensure comparability of the results, the same working dilutions were used for both tests simultaneously. As the stock solutions of some antibiotics have to be prepared in Ethanol, sodium hydroxide solution or citrate buffer, the effect of these substances on LI growth were tested separately without any detectable effect.

Results

	MIC BP	(lit source)	extracellular		intracellular	
Bacitracin			>1024	**R**	>1024	**R**
Neomycin	8	(2)	>256	**R**	>512	**R**
Virginiamycin	8	(3)	32	**R**	32	**R**
Bambermycin	8	(4)	8	**R**	64	**R**
Ceftiofur	8	(2)	256	**R**	64	**R**
Colistinsulfate			>256	**R**	>512	**R**
Florfenicol	8	(2)	2	s	0.5	s
Tilmicosin	32	(2)	2	s	1	s
Sulfonamide	512	(2)	4	s	8	s
Trimetoprim/ Sulfonamide	4/76	(2)	1/19	s	0.5/9.5	s
Ampicillin	32	(2)	64	R	2	s
Tulathromycin	64	(5)	16		8	
Penicillin (BP only for gram+)			16		4	

Table 1 shows the MIC values for tested substances compared to breakpoints (BP) defined in literature (R= resistant, s= sensitive). A clearly positive result in infected wells was considered if at least five cells showed a minimum of five fluorescent LI inside or around it, detected by the use of a specific monoclonal antibody against LI and an FITC-Labelled detection antibody.

Conclusions and Discussion

A highly standardized test setup was established to detect the MIC of antibiotic substances for an oral attenuated live LI vaccine strain.
Bacitracin, Neomycin, Virginiamycin and Bambermycin did not influence the propagation of LI in this in vitro study. The same applies to Ceftiofur and Colistinsulfate which confirms former in vivo challenge trials in Enterisol Ileitis vaccinated pigs when the respectively highest approved US dosage in pigs was used for IM injection (Ceftiofur: 5mg/kg bwt (6), and Colistin-sulphate: 8mg/kg bwt (7)).
Florfenicol, Tilmicosin, Sulfonamides and the combination of Trimetoprim and Sulfonamide did exhibit a substantial inhibitory effect on LI growth.
In the extracellular tests Ampicillin inhibited the growth of LI only at concentrations above BP whereas the intracellular MIC value was below it, indicating an effective inhibition of LI propagation.
Tulathromycin inhibited LI growth which is in contrast to a previous *in vivo* study (6) where a dosage of 2.5mg/kg bwt in pigs did not interfere with the concurrent administration of Enterisol Ileitis indicating that the levels of Tulathomycin were not sufficient to have an effect on the vaccine at the site of its action – the enterocytes.

References

1. Wattanaphansak et al (2008) Vet Microbiol 134(3-4), 305-310
2. NCCLS guidelines (2002) Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard - 2nd ed. M31-A2 22:6
3. McDermott et al (2005) Appl Environ Microbiol 71(9), 4986-4991
4. Hayes et al (2003) Appl Environ Microbiol 69(12), 7153-7160
5. Godinho (2008) Vet Microbiol 129, 426-432
6. Husa et al (2009) Proc 36th Allen D. Leman Conf Rec Res Rep p188
7. Grosse Liesner et al (2012) Proc 22nd IPVS Congr p663

C.008

Fostera™ PRRS vaccine efficacy against a Canadian heterologous field strain of PRRSV

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) represents the most economically important viral disease of the swine industry in North America. Vaccination is one of the options used to control PRRSV infection. Up to now many PRRSV vaccines have been developed, including products that contain modified live virus derived from cell culture attenuation of virulent field isolates. These vaccines have been widely used and have shown some efficacy in reducing clinical disease and severity, as well as the duration of viremia and virus shedding but have failed to provide complete sterilizing immunity[1]. Efficacy of modified live virus vaccines is greater for the homologous strain and in some instances declines dramatically when facing genetically unrelated heterologous PRRSV strains[2]. The objective of this study was to evaluate the efficacy of a modified live PRRSV vaccine, Fostera™ PRRS, to protect against respiratory diseases and specific lung lesions induced by an experimental challenge with a heterologous field strain widely circulating in eastern Canadian swine herds.

Materials and Methods

Forty-six, 16 day-old conventional piglets were included in this study. Animals were randomly divided into four groups (non-vaccinated/non-challenged: n=7; non-vaccinated/challenged: n=15; vaccinated/challenged: n=15 and vaccinated/non-challenged: n=9). After one week of acclimation, 23 day-old animals were vaccinated IM with Fostera™ PRRS. Three weeks following vaccination, challenged animals were infected IM and IN with a heterologous field strain (FMV12-1425619). Pigs were monitored daily for temperature, weight and clinical signs for 28 days. Blood samples were collected on day -1, 3, 7, 10, 13, 21, and 28 post-challenge (pc) and tested for PRRSV RNA by RT-qPCR and for virus specific antibodies. Animals were sacrificed on day 14 or 28 pc. At necropsy, lung macroscopic lesions were assessed and sections of lung and assorted tracheobronchial lymph nodes were collected for microscopic examination and PRRSV viral load evaluation.

Results

Temperature and clinical signs were significantly lower in the vaccinated/challenged group compared to the non-vaccinated/challenged group, starting on day 11 pc through day 21. On day 13 and 21 pc, vaccinated/challenged animals had a significantly lower viremia compared to the non-vaccinated/challenged animals (Fig. 1). Lung lesions scores tended (P=0.071) to be higher in the non-vaccinated group on day 14 pc (Fig. 2). Lung viral load was significantly higher on days 14 and 28 pc in non-vaccinated/challenged animals compared to vaccinated/challenged animals, indicating that vaccination had a positive impact on the lung viral load and virus clearance.

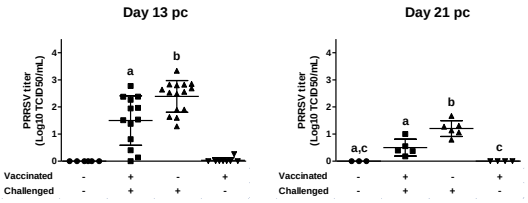

Figure 1. PRRSV viremia on day 13 and 21 pc

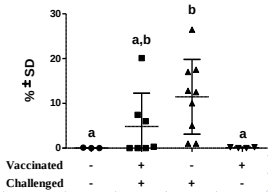

Figure 2. Macroscopic lung lesions scores (%) on day 14 pc

Conclusions and Discussion

Overall, the FosteraPRRS vaccine had a positive impact on several parameters such as clinical signs, body temperature, and viremia. A significant difference between non-vaccinated and vaccinated animals was detected for some parameters from 11-13 days pc which suggested that cell mediated immune response or other delayed response could play a more important role than the pre-existing PRRSV antibodies in vaccinated animals within the context of heterologous vaccine protection.

Acknowledgments

CS and CP were recipients of CSHB postdoctoral fellowships. CS and FA were recipients of FRQNT postdoctoral and FPPQ graduate student fellowships, respectively. This research was supported by Zoetis Canada.

References

1. Kimman TG et al. 2009. Vaccine 27, 3704-3718.
2. Murtaugh MP and Genzow M. 2011.Vaccine 29, 8192-8204.

C.009

Modified live PRRSV vaccination is efficacious following challenge with eight genetically diverse PRRSV isolates

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Introduction

Modified-live vaccine (MLV) can significantly reduce lung lesions following a heterologous PRRSV challenge.[1] It is necessary for PRRS MLV vaccines to be effective against newly emerging PRRSV field isolates. The objective of this report is to summarize experimental data on PRRSV MLV efficacy following challenge with eight genetically diverse PRRSV isolates.

Materials and Methods

Field isolates 1 (1-18-2) and 2 (1-4-4) were isolated in 2008 and 2011, respectively, from herds in the upper Midwest. Field isolate 3 had a RFLP type of 1-5-2. All strains were associated with reproductive problems and high mortality in growing pigs. Conventional pigs were vaccinated at approximately 3 weeks of age and challenged at either 21 or 28 days post vaccination. Pigs were challenged with a 2ml intranasal dose of 5.92 log $TCID_{50}$/ml, 5.13 log $TCID_{50}$/ml, or 4.78 log $TCID_{50}$/ml viral stock, respectively for Isolate 1, 2 and 3. All pigs were humanely euthanized and necropsied 14 days following challenge. Lungs were collected and scored for percent pneumonia associated with PRRSV exposure. Studies involving the remaining challenge isolates [NADC, VR2332, SDSU 73, 1-8-4 and 1-4-2] have been previously described.[1] Table 1 provides a summary of the genetic diversity of the challenge isolates.

Table 1. Summary of the percent nucleotide similarity of challenge isolates to Ingelvac PRRS® MLV & ATP based on ORF 5 sequence

Challenge isolate	Lineage†	Ingelvac ATP	Ingelvac MLV
U66394 (NADC)	5.1	91%	94%
AF535152 (VR2332)	5.1	90%	100%
AY656993 (SDSU73)	8	90%	89%
Field isolate 1 (1-18-2)	1	87%	87%
Field isolate 2 (1-4-4)	1	86%	86%
Field isolate 3 (1-5-2)	NC*	87%	88%
17198-6 (1-4-2)	9	92%	90%
EF484031 (1-8-4)	1	86%	87%

*NC= not classified based on the analysis
†Lineage classification based on Shi, M. et al. 2010. J Virol 84(17):8700

Results

Table 2 provides a summary of lung lesions (percentage) by treatment for multiple studies using various challenge isolates. Differences between vaccinated and challenge control animals for all studies are statistically significant ($p<0.05$)

Table 2. Lung lesions (%) in vaccinated and non-vaccinated pigs challenged with PRRSV

	Lung lesions, %		
Study	Vaccinated	Non-Vaccinated	Challenge isolate
1*	1.0	31.6	NADC
2*	10.0	37.5	VR 2332
3†	17.8	70.1	SDSU 73
4†	37.1	82.0	SDSU 73
5†	5.8	23.3	VR 2332
6*	0.7	26.0	17198-6 (1-4-2)
7*	0.3	37.7	SDSU 73
8*	0.6	14.8	17198-6 (1-4-2)
9*	12.0	39.9	MN 1-8-4
10*	10.4	36.2	MN 1-8-4
11†	8.7	62.5	MN 1-8-4
12*	8.0	47.0	SDSU 73
13*	15.9	52.7	SDSU 73
14*	29.8	36.0	MN 1-8-4
15†	27.4	46.6	SDSU 73
16*	4.5	32.9	MN 1-8-4
17†	13.8	58.1	FI 1 1-18-2
18*	1.4	18.0	FI 3 (1-5-2)
19†	0.7	18.0	FI 3 (1-5-2)
20*	37.4	52.9	FI 2 (1-4-4)
21*	2.4	17.6	MN 1-8-4

***** Ingelvac PRRS® MLV, †Ingelvac PRRS® ATP , FI=Field isolate

Conclusions and Discussion

Use of a modified-live PRRSV vaccine significantly reduced lung lesions in multiple challenge models which used genetically diverse heterologous PRRSV isolates.

References

1. Roof, M. B. et al. 2007. International symposium of emerging diseases.

Comparison over a twelve month period of type I and type II PRRS vaccine in commercial farm infected with type II PRRSV

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Introduction

Porcine Reproductive and Respiratory Syndrome is an economically important disease for the swine industry (1). To control PRRS in Thailand, it is important to coincide virus strain, vaccine strain and farm management (1). We have found 6 main clusters of PRRS genetic diversification in Thailand (2 for Type I and 4 for Type II) (2). Both Type I and II PRRS virus were used as vaccine in Thailand. The aim of this study was to compare pig performance in a farm which used Type I and Type II PRRS vaccine strain in the same farm but in different unit.

Material and Method

This study was done in a 2,400 sow farrow to Nursery farm, which was mainly infected with Type II PRRS virus strain. This farm organized their operation in 3 similar units. The laborers were rotated between units during the years. Between the end of 2012 to beginning of 2013, the farm experienced a minor PRRS outbreak. In Feb 2013, a new PRRS vaccination protocol was implemented. In **Unit 1,** all sows and Piglets were vaccinated with Type I PRRS vaccine and **Units 2**, **3** were vaccinated with Type II PRRS vaccine. The Performances of Sow herd and Nurseries were monitored from Jan -Dec of 2013.

Results

Table 1. Performance in Sows Unit

	U1	U2	U3
Sow no.	2 091	2 217	1 966
Wean pig	10.0±2	10.0±2.1	9.3±1.1
Wean weight	6.5±0.3	6.6±0.3	6.7±0.27
% loss	9.5	12.3	12.7

During the 12 month observation period, no serious PRRS outbreak was reported even though a farm in the area experienced an outbreak in Q3. The performance of Sow and Nursery unit are summarized in Tables 1 and 2. Trend of % loss in nursery decreased after implementing new vaccination protocol (Figure 1), with exception of U3 where some seasonal increase occurred.

Table 2. Performance in Nursery Unit

	U1	U2	U3
Piglet no.	17 926	18 310	16 591
ADG	394±28	381±30	382±17
FCR	1.37±0.1	1.42±0.11	1.44±0.05
FCG	28.22±2.4	30.66±3.4	30.08±1.6
% loss	4.36	5.63	5.59

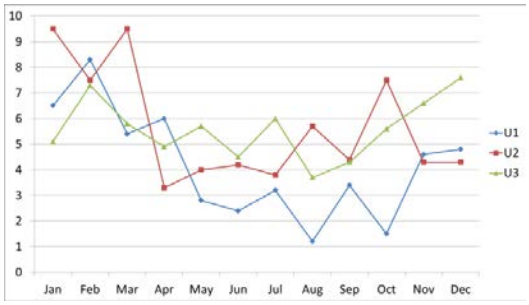

Figure 1. % loss of Nursery in each period

Conclusion

Both Type of vaccine strains improved farm performance when combined with biosecurity measures, proper vaccine and pig flow management. The date supports that PRRSv type of vaccine isolate was not a good predictor of efficacy, i.e. a Type I PRRS vaccine can also be efficacious in farms infected with type II PRRS virus.

References

1. Urairong K.2012. Viral Disease of Swine in practice1:68-97.
2. Nilubol D et al. 2013. Acrh Virol 158, 943-953

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

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Introduction

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

Material and Methods

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

Result

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

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

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

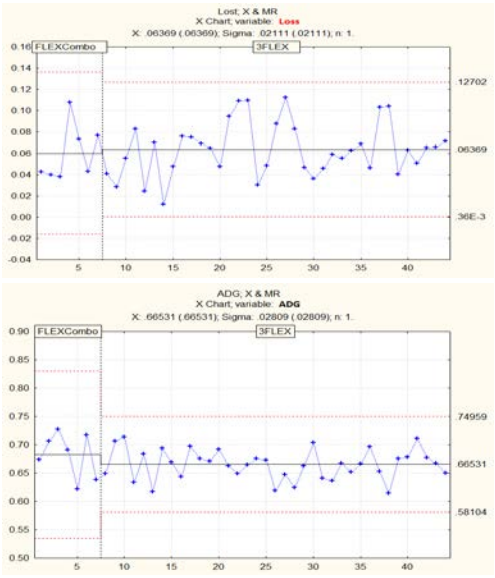

Figure 2. SPC chart of Percentage Loss and ADG comparing 2 periods

Discussion

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

C.012

PRRS oral fluid PCR cycle threshold (Ct) values analysis in sequencing success in growing pigs

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Introduction

PRRS sequencing is a tool that helps for a better epidemiological understanding. Oral Fluid (OF) samples provide advantages over serum but its sequencing success rate is low.[1] Objectives of this paper are to analyze the sequencing success rate (able or unable to sequence) from nursery and finisher pigs in OF, analyze Ct values distribution, and determine if there is a relationship between Ct values and production phase with the ability to obtain a sequence from OF samples.

Materials and Methods

Samples included in the analysis were collected during a large study conducted in a production system located in the USA. The sampling period was from November 2010 to May 2011. Sampling protocol was in nursery (8-9 weeks of age) and in finishing (15-16 weeks of age). A total of 452 oral fluid samples were analyzed (279 Nursery and 173 Finishing). All samples were analyzed for sequencing at the Iowa State University Veterinary Diagnostic Laboratory (ISUVDL) in Ames, Iowa. Proportion of Able and Unable to sequence was generated from total samples and by production phase. Descriptive statistics were run on all Ct values from OF PCR along with a comparison of able/unable Ct means using 2-samples t-test. A distribution of Ct values from able and unable to sequence were plotted in a histogram and a Binary logistic regression was applied taking sequencing success as a response variable and included Ct values and production phase in the model. Additionally, prediction values of sequencing success and Ct values from nursery and finishing pigs were calculated (Minitab 16.2.3 College PA USA).

Results

Table 1 shows the able/unable to sequence proportion of total samples, and divided into production phases.

Table1. Proportions of able and unable to sequence

Total Samples	**452**	
Unable to Seq	**266**	**58.8%**
Able to Seq	**186**	**41.2%**
Nursery	**279**	
Able to Seq	133	**48%**
Unable to Seq	146	**52%**
Finisher	**173**	
Able to Seq	53	**31%**
Unable to Seq	120	**69%**

The mean Ct values from OF in which a successful sequence was obtained were significantly lower (P value <0.001**)** than those were unable to be sequenced (31.28 vs 32.24). Logistic regression showed a significant association (P value (<0.010) of Ct values and production phase and the ability to get a sequence from OF.

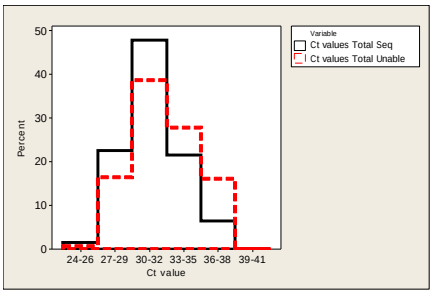

Figure 1. Oral fluid PRRS PCR ct value histogram from able and unable to sequence.

Fig 2 shows a binary regression prediction plot for new observations on ct values from nursery and finisher pigs.

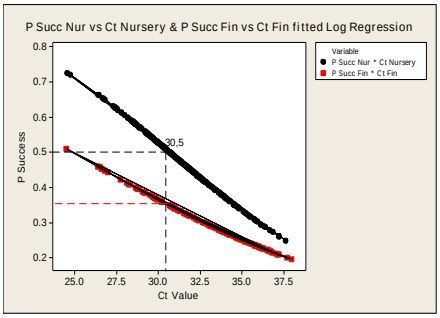

Figure 2. Probability of sequencing success vs ct values

Conclusions and Discussion

The data analysis shows a low proportion of able to sequence in OF samples. Ct values are statistically associated to sequencing success, but other variables like cold chain, sample handling, enzyme inhibitors, time after sampling, etc. are needed to include in future regression models in order to understand why samples with the same Ct value sometimes are able or unable to sequence as it is shown in figure 1. From a practical stand point and using this data set, a Ct value of 30.5 would provide a 50% chance to get a sequence in nursery pigs, but only a 35% chance in finisher pigs (Fig 2). This kind of analysis could help practitioners in the decision to invest or not in sequencing from OF samples. More importantly, this analysis re-confirms the great need to investigate further to enhance the ability for successfully sequencing OF.

References

1. Main R et al. AASV Full circle sequencing. 2013:5-8

C.013

Sow reproductive performance using Triptorelin Gel and fixed-time AI in commercial swine farms

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Introduction

Triptorelin Gel (OvuGel®) is a gel formulation containing the GnRH analog, triptorelin. A single dose of Triptorelin Gel deposited intravaginally 96 hours after weaning induces ovulation 40 to 48 hours later, thereby facilitating a single fixed-time insemination 24 hours following treatment without regard to estrus. The objective of this study was to further demonstrate the use of Triptorelin Gel under a variety of management conditions in commercial swine farms.

Materials and Methods

The study was conducted at six farms in the United States. Conventional artificial insemination (AI) was used on one farm, while others used post-cervical AI. Breeding eligible sows (BES) were blocked by parity and body condition score at weaning and randomly assigned to either Triptorelin Gel or Contemporary (Control) treatments. Control BES (n=1479) were inseminated following normal farm SOP on the day detected in estrus, and 24 hours later if still in estrus. Triptorelin Gel BES (n=1475) were treated 96 hours post-weaning and inseminated once 22+/-2 hours later. Pregnancy was determined by ultrasonography approximately 30 days after AI. Data were subjected to analysis of variance using the PROC MIX procedure of SAS (version 9.2) to determine main effect of treatment, replicate, and treatment by replicate interactions. Differences between treatment means were tested on least squares means estimates using the T test at $P<0.05$.

Results

Reproductive performance data are presented in Table 1. There was no difference among Triptorelin Gel sows bred once and Control sows bred an average of 1.9 times in weaned sow farrowing rate/weaned sow utilization (no. farrowed/no. weaned, $P=0.41$), born alive ($P=0.66$) and total born ($P=0.60$). This resulted in live pigs per 100 sows of 1100 for Triptorelin Gel sows and 1081 for Control sows. Fewer semen doses were required for the single fixed-time insemination, therefore, total born per semen dose was greater ($P<0.01$) for Triptorelin Gel sows than for Control sows (11.9 vs. 7.5, respectively).

Table 1. Reproductive Performance at Six Farms[1]

	Control	Triptorelin Gel	*P*-Value
Breeding Eligible Sows	1479	1475	.
Inseminated by 7 Days Post-Weaning	1363	1475	.
Number Semen Doses	1.9	1.0	0.0001
Sows Pregnant	1257	1261	.
Pregnancy Rate (no. pregnant/no. weaned)	85.0	85.5	0.68
Sows Farrowed	1224	1239	.
Farrowing Rate (no. farrowed/no. bred)	89.8	84.0	.
Weaned Sow Farrowing Rate (farrowed/weaned)	82.8	84.0	0.41
Total Born	14.2	14.2	0.60
Born Alive	13.1	13.1	0.66
Total Born/Semen Dose	7.5	11.9	0.0001
Live Pigs per 100 Sows	1081	1100	.

[1] The data presented in this table are raw means.
Sows farrowed spring and summer of 2013.

Discussion and Conclusions

Because only Control sows which expressed estrus were inseminated, while all Triptorelin Gel-treated sows were inseminated once, comparing conventional farrowing rates (no. farrowed/no. bred) is not appropriate. Thus, we suggest that weaned sow farrowing rate or weaned sow utilization rate (no. farrowed/no. weaned) and live pigs per 100 weaned sows are the most appropriate economic measures to compare farrowing performance. In this study, sows treated with Triptorelin Gel and inseminated once had both a weaned sow farrowing rate and a litter size similar to Control sows inseminated multiple times during estrus. By breeding all weaned sows on a single day, utilization rates of weaned sow inventory increased and improved throughput. Our data show that breeding all weaned sows on a single day allow sows to be induced to farrow with no increase in number of stillborn pigs. Furthermore, 92% of the induced sows farrowed on the same day. This decreased the number of days spent on assisted farrowing and Day 1 pig care. Also, 90% of the piglets were the same age (20 days) at weaning. After Triptorelin Gel implementation in the spring of 2013, average pregnancy rate of weaned sows at three farms increased from 82.3% (7289 pregnant/8857 weaned sows) to 85.8% (7187 pregnant/8377 weaned sows) compared to the same time period in the previous year, indicating improvement in weaned sow utilization. Conventional AI and post-cervical AI produced similar results. These data demonstrate that Triptorelin Gel effectively synchronizes time of ovulation in weaned sows, thereby facilitating a single fixed-time AI without regard to estrus. As a result, the cost of labor and semen is reduced, while a high level of reproductive performance is maintained.

C.014

A period of growth inhibition in newly born piglets

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Introduction

Colostrum intake is essential to assert maternal antibodies and energy for the new born piglet. This has been investigated by measuring immunoglobulin in piglet blood 12 and 24 hours after farrowing (1), or by calculating colostrum intake on the basis of piglet weight gain between birth and 24 hours (2). An investigation (3) found in 3 piglets per litter in 23 litters, that piglets stop growing 15 hours after birth of the first piglet (BFP), and that growth was impeded until 33 hours after BFP. In this investigation this growth inhibition is analyzed in details.

Materials and Methods

In one farm 11 parity 1 and two parity 2 Landrace/Large White sows of DanAvl genetics were included at start of farrowing. A total of 223 live born piglets (17.2 live born piglets per litter) were ear tagged at birth. Time of birth and weight was registered. All piglets were weighed every two hours for 40 hours. To reduce litter size to 14 or less piglets per litter, some piglets were removed from the litter after eight to 24 hours after BFP. The final data set included 165 piglets that were still present with their own mother sow at 40 hours (12.8 piglets per litter). Sow rectal temperature was measured every 6 hours after BFP. To investigate the effect of piglet size at birth, piglets were divided into small (< 1250 g, 48 piglets, av. 850 g), medium (57 piglets, av. 1270 g) and large (> 1550 g, 54 piglets, av. 1720 g).

Results

The average birth weight of the 165 piglets in the trial was 1410 g per piglet. At 40 hours, these piglets weighed 1570 g per piglet, after growing an average of 160 g during 40 hours.

The average growth curve of the piglets did resemble the curve presented by (3), but the interval with inhibited piglet growth was reduced into lasting from 16 until 24 hours after BFP. Small, medium and large piglets at birth all had the same shape of the growth curve, including the same period of the growth stop, when corrected for birth weight (Figure 1). Before the growth stop the piglets grew by 7 g per hour. After the growth stop the piglets grew 6 g per hour.

Dividing piglets into piglets born during the first hour (n=35) and piglets born after four hours after birth of the first piglet (n=70) indicated, that the growth stop happened at the same time after birth of the first piglet, and was thus not mediated by the time of birth of the specific piglet.

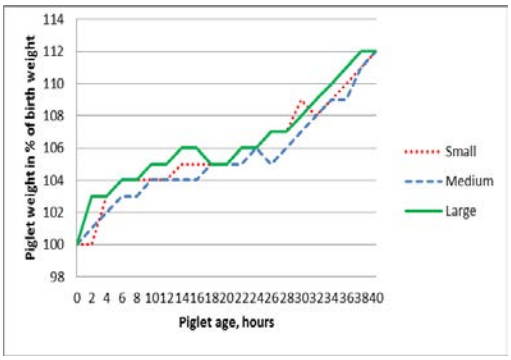

Figure 1. Average piglet weight in percent of birth weight by age of the specific piglet for small, medium and large piglets.

Conclusion and Discussion

The first days after birth, piglet growth is similar in small, medium and large piglets, when accounting for birth weight.

Piglets did not grow from app. 16 to 24 hours after birth of the first piglet in the litter. The cause of this is not clear, but may be a result of the transition from colostrum to sows milk in the sow udder.

If this is a general feature, this knowledge is important, when piglet growth is based on two observations. Weighing the first born piglet at birth and at 16 hours will indicate a growth rate of 7 gram per hour. weighing the same piglet at 10 hours after birth and 16 hours later will indicate a growth rate of just 3,4 g/hour ((6x7 g + 8x0+2x6 g)/16).

References

1. Thorup F. et al. 2004. Proceedings 18th IPVS congres. P. 478. Germany.
2. Devillers N. et al. 2004. J. Anim. Sci. 78, 305-313.
3. Krogh U. et al. 2012. European Ass. For Anim. Prod. 63rd ann. Meeting. P 43.

C.015

Improved piglet quality and sow reproductive performance following treatment with a GnRH analogue (peforelin) on a high productive sow herd

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Introduction

Optimal reproductive performance is crucial for economic success in commercial pig herds. Different management strategies (1), such as optimized feeding strategies, batch farrowing systems and extended photoperiod during the post-weaning phase (2), are applied in order to meet the high performance expectations of modern sow farmers (3,4). Pharmaceuticals, such as progesterone-analogues and gonadotropins, are used in practice to control reproduction with the aim to increase the reproductive performance of gilts and sows. Estrus synchronization is an important tool in reproductive management to group the onset of estrus of gilts or sows in one to two days, thus resulting in grouped inseminations and batch farrowings (4). The use of gonadotropins post-weaning in sows or following altrenogest treatment in gilts, both in order to stimulate the follicular development, can achieve an even better synchronization effect (5,6). In order to decrease the number of non-productive days by a reduction in the weaning-to-estrus interval (WEI), exogenous administration of gonadotropins is a common practice on most high productive sows herds, although other fundamental reproductive problems also have to be solved simultaneously.

The present study investigated the effect of a GnRH analogue (peforelin, Maprelin®) administration in gilts, primiparous and pluriparous sows on sow reproductive performance and piglet quality at birth in a high productive sow herd under Belgian conditions.

Materials and Methods

In a high productive sow herd with 450 sows, sows were stratified in gilts, primiparous and pluriparous sows and randomly allocated to 2 groups: peforelin treated (Maprelin® = M-group) or no treatment (control = C-group). Gilts were injected 48h after the last altrenogest treatment and sows 24h after post weaning (PW). Weaning-to-estrus interval (WEI), insemination rate (IR), farrowing rate (FR), farrowing efficiency index (FEI), number of total (TBP), life and stillborn piglets, mummies and life piglet index (LPI) were calculated and compared between treatment groups. To assess piglet quality at birth, 6033 piglets from 426 litters were weighed individually within 24 h after birth.

Results

IR improved significantly (P = 0.0119) in the M-group compared to C-group with similar WEI for both groups for sows in estrus until day 7. FR was not impacted resulting in a significantly better FEI (= farrowings per 100 treatments) (P = 0.0078) for M-group (79.2%) as compared to the C-group (70.2%). All parameters concerning number of piglets born were similar between both groups, resulting in a significant effect on the LPI (C-group, LPI = 1032; M-group, LPI = 1151; P = 0.0078). Overall, no effect of peforelin treatment on piglet birth weight could be observed, although specific subcategories (1st parity and older (5+ parity) sows) did have a significant positive impact of treatment on birth weight. During late summer (August-September) all treated sows profited from peforelin treatment, although the weight improvement was not statistically significant.

Table 1. Comparison of reproductive parameters between control (C-group) and Maprelin-treated (M-group) animals

	C-group	M-group	Significance level
WEI ≤ 7 d	5.08 ± 0.26	5.10 ± 0.34	P > 0.05
pw			
IR	87.2%	93.2%	P = 0.0119
FR	81.3%	85.7%	P > 0.05
FEI	70.9%	79.9%	P = 0.0129
LPI	1032	1151	P = 0.0078

Conclusions

In conclusion, peforelin treatment had a significant positive effect on several to the crucial sow performance parameters (IR, FEI and LPI) without a negative impact on piglet birth weight.

References

1. Peltoniemi et al., 1999
2. Claus and Weiler, 1985
3. Kim et al., 2013
4. Vangroenweghe et al., 2012
5. Brüssow et al., 2010
6. Martinat-Botté et al., 2010

C.016

Use of incubator and milk replacer to raise delayed six-day-old piglets

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Introduction

Late adoption of piglets is a technique of management that is commonly used to rescue pig neonates that are losing body weight in their first week of life. However, the survival and growth of piglets will be less if the adoption takes a long (1). In addition, in cross adoptions piglets have more injuries by fights, greater number of vocalizations and less effective feeding (2) so the use of nurse sows is still a controversial topic. An alternative to avoid uses nurse sows as well as its consequences is the use of incubators which provide security, a comfort area for the neonate and a continuous source of milk supply (3).

Material and methods

The aim of this study was to evaluate if the use of an incubator and milk replacer may to rescue piglets with delayed in its body weight in the first week of life and improve its productive performance in the weaning area. Fourteen piglets of 6 days old with delayed in its growth and with an average body weight of 1.30±0.05kg were selected to this study. The average of body weight of the piglets under study was lesser than the average at birth of the piglets from the farm where the study took place, 1.32±0.05kg. The piglets were kept in an incubator with nipple drinkers, natural ventilation, heat lamps and a continuous source of milk substitute during the 24 h of day. The milk replacer was offered by 7 days, after this period of time, it was offered a pre-starter pelleted diet, SEW, in the form of porridge which was modified during the follows 4 days according to the follows ratio (milk replacer: SEW) 80:20, 60:40, 40:60 and 20:80. After this period of time SEW was offered as only food for the last three days, moment in which the piglets were weaned at 21 days of age. In other hand, 9 piglets of 7 days old with an average weight of 1.70±0.06kg were used as control group and were kept with a nurse sow. The piglets received SEW from the 10 day of life until the weaning. At weaning the pigs from the incubator and the pig from the nurse sow were moved to the weaning area and were monitored for 16 days and they were fed with SEW. Records of mortality and the average daily gain (ADG), was evaluated during the time that last the study. The data were analyzed with ANOVA. Initial body weight and initial age were used as covariates and means were compared with Tukey test. Mortality was analyzed by Xi^2.

Results

In the period of 6-37 days, the piglets mortality in the incubator was of 14% compared with those piglets raised with the nurse sow which was of 11%, however, there were not significant differences (P = 0.82). In addition, there were no significant differences (P>0.05) in the ADG between the piglets from incubator 140±0.10g versus the piglets from nurse sow, 180±0.20g. Similar performance was observed in weaning area, where the ADG was slightly higher for piglets coming from the incubator, 200±020g than those ADG from piglets raised by a nurse sow, 190±040g, however, there was no significant differences (P=0.90).

Conclusions and Discussions

The results showed in this study indicate that piglets with a delay in its growth has the similar opportunities of survive than piglets raised by a nurse sow; however, it is important to consider that in this study the piglets used as a control were 28% heavier at beginning of the study than the piglets raised in the incubator, which suggests that the incubator and the milk replacer were able to rescue piglets, which in most of the case die if they stay with its mother or in some case with a nurse sow. Finally, ADG in both groups was similar and there were no significant differences (P> 0.05). Table1

Table1: ADG and mortality in piglets from incubator vs. piglet under nurse sow

Treatment	Mortality (%)	ADG 6-37d (g)[1]	ADG 21-37d (g)[1]
Incubator	14%	140±10	200±20
Nurse sow	11%	180±20	190±40

[1]Values are shown in media ± SE.

In summary, the use of incubators with a continuous supply of milk replacer may rescue animals delayed its growth during the first week of life and allows to take them to weaning with higher productive potential to the next phase of development.

Acknowledgments

Dr. John Vignes from Ralco Nutrition for his invaluable cooperation.

References

1. Straw B et al. 1998. Prev. Vet. Med. 33:83-89
2. Martineau G P 2001. J Anim Sci 79:88-93
3. Cabrera R et al. 2003. Carolina Swine Nutrition Conf.

An effective worming schedule for sows using flubendazole in the prevention of pre-weaning *Ascaris suum* infestation of their offspring

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Introduction

The prevalence of *Ascaris suum* in modern pig husbandry remains high and its damage is described mainly in fattening pigs (1, 2, 3). Insights on the impact of migrating *Ascaris* larvae on the immune response against *Mycoplasma hyopneumoniae* (5), both after vaccination and infection challenge, have increased the awareness about controlling early *Ascaris* infestations. Correct worming of sows can prevent *Ascaris* egg contamination of the farrowing houses. A previous study with fenbendazole, even at very high dose (9 mg/kg spread over 3 days), did not stop egg shedding completely (4).

The objective of this study was to investigate the use of flubendazole to completely stop *Ascaris* egg excretion in sows before moving them to the farrowing house.

Materials and Methods

Individual fresh feces samples were collected from sows in late gestation within a week before treatment. The positive sows were randomized into a treated group (T) (n=11) and a non-treated control group (C) (n=9). The treated sows received 1 mg/kg/day flubendazole (Solubenol®, Elanco Animal Health) for 5 consecutive days, starting on day 18 before expected farrowing date (EFD). Solubenol® was diluted in drinking water and drenched directly into the mouth of the sow to ensure full uptake of the dose. The control sows did not receive any treatment. Fresh feces samples were collected daily from the first day of treatment until the day before movement to the farrowing house (=day 6 before EFD), on day 5 before EFD, on farrowing and weaning day. Fecal egg counts were performed blinded and were expressed as Eggs Per Gram (EPG) using the McMasters technique. The individual fecal egg counts were listed together with the descriptive statistics and a frequency table (positive/negative samples) over time for each group. The mean values were used to make graphical presentations over time for each group. On the individual area under the curve values, the two-by-two Wilcoxon Mann-Whitney U test was used to do the statistical comparisons between groups. To test the evolution over time (before and after treatment), a paired statistical test, Wilcoxon Signed Rank, was performed between the individual area under the curve values.

Results

For C, there was no statistical difference between the fecal egg counts before/during the treatment period and the post-treatment period (day 13 - 6). For T, there was a significant drop in fecal egg counts between the treatment period (day 18-14) and post-treatment period (day 13 - 6) ($p = 0.0078$). There was no difference in fecal egg counts between C and T during the treatment period ($p = 0.3514$), but during the post-treatment period the fecal egg counts in T were significantly lower than the ones of C ($p = 0.0070$). The third day after the last treatment day all sows from T were completely negative and remained negative until weaning. The results are shown in Figures 1 and 2.

Figure 1 Average faecal egg counts (EPG) over time

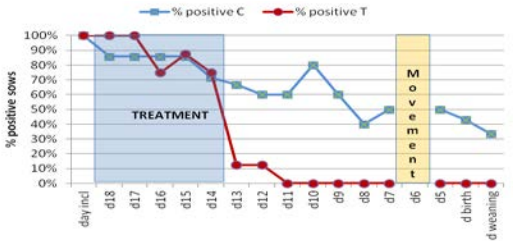

Figure 2. Percentage of positive sows over time

Conclusions and Discussion

The use of Solubenol® in sows prevented the contamination with *Ascaris suum* of the piglets and farrowing house via the sows during the lactation period. All sows became completely negative already at the third day after the end of the treatment. Therefore, we recommend starting the treatment at least 8 days before the sows are moved to the farrowing house.

References

1. Kanora A et al. 2004. Proc. 18th IPVS vol 1: 241
2. Knecht D et al. 2011. Vet. Parasitol., doi:10.1016/j
3. Marchand D et al. 2012. Proc. 22th IPVS, 230
4. Pittman JS 2012. Proc. 4th ESPHM, 100
5. Steenhard N et al. 2009. Vaccine 27, 5161–5169

Gilts batch synchronization using oral Altrenogest, by use of automatic dosing through Electronic Sow Feeding Stations (ESFS)

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Introduction

The use of heat synchronization by oral manual administration of Altrenogest is a well documented management tool to group the estrus presentation of batches of gilts[1]. However, in large farms, manually administrating the drug for 18 consecutive days, in order to group a batch of gilts, is labor demanding.

This paper evaluates the result and practicality of an automatically controlled dosing Altrenogest system, associated to a modified Electronic Sow Feeding System (ESFS) used during the training faze of the gilts, in commercial pig farms in Spain.

Materials and Methods

Standard oral Altrenogest Spray 540 ml (Altresyn® CEVA) were used for the dosing. Compident® Junior Electronic Sow Feeding Stations (ESFS) (Schauer, Agrotronic GmbH.) are designed for gilt individual feeding in the training faze prior to the service and group gestation. Gilt feeding training usually begins when gilts surpass 100 kg of Body Weight and usually lasts 4 to 6 weeks. Feed training takes place until they are moved to crates previous to service. During this training faze, first heat was detected (non served) after which, the Altrenogest treatment was implemented, as a means to induced heat grouping.

The ESFS was modified to automatically administer the 5 ml dose of Altrenogest into the sows feed trough. The administration system was programmed for a daily dosing of 5 ml. for 18 consecutive days, to all gilts of the group previously selected for service. A double can system was adjusted to avoid running out of product during treatment. The ESFS was programmed to switch automatically to the second can, once the first can is empty. Staff was trained to manage the can replacement.

All gilts that came on heat after the treatment were moved to individual crates to be served on the next heat. This system was used to start up 3 large farms in Catalonia, Spain, between January 2011 and August 2013. Twenty consecutive gilt batches on each farm were successfully dosed. A total of 11.340 gilts were synchronized in weekly groups to come on heat.

Results

Of the 11340, gilts treated, 204 gilts were culled before service, for various reasons (1,79%).

10.445 gilts (93,79%) of gilts automatically fed Altrenogest came into estrus during the 21day heat check period after the withdrawal of Altrenogest. All of them had come on heat 5 days after the withdrawal of Altrenogest. Average conception rate of all 3farms at 28 to 35 days (Ecography), for the gilts was 89,97 %.

Conclusions and Discussion

The proportions of “in heat” gilts that received Altrenogest automatically, was not significantly different from those reported for manual dosing of. (94%).

In a few cases, gilts had to be manually dosed for safety; frequently at the end of the can, or in gilts that did not eat for one or two days. Although the number of cases was not registered, staff reported them as to be of very low incidence.

Although automatic dosing of Altrenogest requires daily checks, it was considered by the staff in charge as; a comfortable, reliable, easy to manage tool that also reduced human error at dosing.

An additional advantage observed was that it reinforced sow training, facilitating management of sows in all other sections of the farm.

The automatic Altrenogest dosing was implemented in large Site 1 farms that are run by staff in a proportion of 230 to 250 sows per person. Automatic dosing resulted in a very valuable management tool under these conditions.

The veterinarian practitioners involved in the start up of these farms considers this technique very useful for the efficient introduction of gilts to new large scale farm with ESFS.

Acknowledgments

Albesa-Ramadera farm staff. Lerida, Spain.

References

1. C. M. Wood, E. T. Kornegay, and C. F. Shipley[2]
2. Efficacy of Altrenogest in Synchronizing Estrus
3. in Two Swine Breeding Programs and Effects
4. on Subsequenct' Reproductive Performance of Sows'. J Anim Sci. 1992 May;70(5):1357-64
5. J. Patterson1, N. Williams2, G. Spronk and
6. G.Foxcroft. Efficacy of a 7 vs 14 day Regu-Mate Protocol in Cyclic Gilts. Advances in Pork Production (2008) Volume 19, Abstract #25
7. Martinat-Botte y cols. Synchronization of oestrus in gilts with Altrenogest. Effect on ovultation and foetal survival (1995) Animal Reproduction Science,vol.39, 267-274

C.019

Effect of individual pig, pen-wise and room-wise treatment on faecal shedding of *L. intracellularis*

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Introduction

The intracellular, gram negative bacterium *Lawsonia intracellularis* (LI) is a common cause of enteritis in pigs[1]. Enteritis in the nursery unit accounts for the major part of antimicrobials consumed in Danish pig production[2]. As antimicrobial consumption is of great public concern, we wanted to study different treatment strategies, in order to evaluate whether antimicrobial reducing treatment strategies could ensure an effective therapy measured as reduction in faecal shedding of LI.

Materials and Methods

A clinical field trial was conducted from October 2011 until April 2013 in three Danish pig herds with a history of LI induced diarrhoea in the nursery unit. One of five treatment strategies with oxytetracycline administrated orally or by injection for five days was randomly selected for treatment in a room with nursery pigs when a diarrhoea outbreak occurred (Table 1).

Table 1. Treatment strategies with oxytetracycline for five days used in nursery rooms

STRATEGY	DOSE	ADMINISTRATION
High dose	20 mg/kg	Oral medication in drinking water of all pigs in room
Normal dose	10 mg/kg	
Low dose	5 mg/kg	
Pen	10 mg/kg	Oral medication of all pigs in affected pens
Individual	10 mg/kg	Intramuscular injection of affected pigs only
No treatment	0 mg/kg	No medication, rooms with no diarrhoea

Each treatment strategy was repeated in three rooms in each herd. Faecal samples were obtained from 15 randomly selected pigs in each room two days after completion of treatment. LI was determined by qPCR[3] at the National Veterinary Institute, Denmark. For statistical analysis a non-parametric Kruskal Wallis rank sum test was performed using R-software[4].

Results

LI qPCR results were obtained from 708 pigs. The distribution of LI qPCR data is presented as boxplots in Figure 1 and as median and inner quartile in Table 2.
The overall difference between treatment strategies was significant ($p < 2.2e\text{-}16$). High and normal dose of oxytetracycline used for room-wise oral treatment resulted in more than 75 % of the pigs being below the LI detection limit two days after termination of treatment. Pen-wise oral treatment and individual pig injection with oxytetracycline resulted in a higher number of pigs shedding LI after medication.

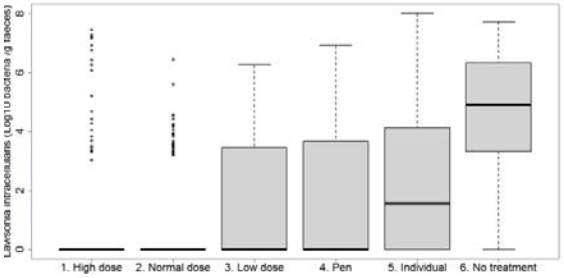

Figure 1. Boxplot of LI levels in faecal samples for six treatment strategies two days after end of treatment

Table 2. Medicated fraction and LI range in faecal samples for six treatment strategies two days after end of treatment

STRATEGY	n pigs	Medicated fraction	LI Log10/ g Median [25Q ; 75Q]
High dose	147	100 %	0.00 [0.00 ; 0.00]
Normal dose	129	100 %	0.00 [0.00 ; 0.00]
Low dose	131	100 %	0.00 [0.00 ; 3.45]
Pen	146	78 %	0.00 [0.00 ; 3.67]
Individual	125	54 %	1.55 [0.00 ; 4.12]
No treatment	30	0 %	3.33 [4.91 ; 6.27]

Conclusions and Discussion

Medication of all pigs in a room with high (20 mg/kg bdw) or normal dose (10 mg/kg bdw) of oxytetracycline provided the most effective reduction in feacal shedding of LI. Although pen-wise and individual treatment included a high number of medicated pigs (78% and 54% respectively) the feacal shedding of LI in these rooms was higher than observed for room-wise treatment. In conclusion treatment strategies that did not involve all pigs in a room were less effective in reducing feacal shedding of LI.

References

17. Stege H et al. 2000. Prev. Vet. Med. 46, 279–292.
18. DANMAP 2012. ISSN 1600-2032.
19. Ståhl M et al. 2011. Vet. Micr. 151 (2011) 307-314.
20. R Development Core Team 2011. www.R-project.org

C.020

Evaluation of loop-mediated isothermal amplification for the detection of *L. intracellularis* in feces from experimentally and naturally infected pigs

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Introduction

Lawsonia intracellularis, the causative agent of proliferative enteropathy, is an obligate intracellular bacterium. Fecal shedding of this organism is routinely detected by polymerase chain reaction (PCR).[1] Loop-mediated isothermal amplification (LAMP) is a gene amplification method which is carried out at a constant temperature using a single enzyme and can be evaluated visually.[2] The LAMP test has the potential to be used pen-side as it requires minimal expertise, cost and equipment to perform as compared to PCR-based assays. We compared the LAMP test to conventional PCR for the detection of *L. intracellularis* in fecal samples from both experimentally and naturally infected pigs.

Materials and Methods

Fecal samples from 111 pigs which had been experimentally infected with *L. intracellularis* (n=55) or sham infected (n=56) were collected for evaluation by both PCR and the LAMP test. A further 254 fecal samples were longitudinally collected from numerous sites from each of two production systems that had histories of proliferative enteropathy at biweekly intervals and tested by both conventional PCR and LAMP. A LAMP protocol was optimized using known *L. intracellularis* positive and negative samples from both feces. DNA from all fecal samples was extracted using a Qiagen stool kit. Analytical sensitivity of the LAMP test was evaluated and compared to the conventional PCR on samples spiked with 10-fold dilutions of *L. intracellularis.* Analytical specificity was determined by testing swine enteric organisms, normal fecal flora, and *L. intracellularis*' closest genetic relative (*Bilophila wadsworthia*). Diagnostic sensitivity, specificity and accuracy of the LAMP test was calculated as compared to the conventional PCR for feces from both the experimentally infected and naturally infected pigs.

Results

Analytical sensitivity (detection limit) of the conventional PCR and the LAMP test for detecting *L. intracellularis* in spiked samples was 10^2 and 10^3 organisms/ml, respectively. No cross reactivity was detected with DNA from a panel of swine pathogens, normal fecal flora, or *Bilophila wadsworthia*. Using the conventional PCR test as the gold standard, the diagnostic sensitivities and specificities of the LAMP test were 71% and 100%, respectively, for feces from experimentally infected pigs and were 35% and 100%, respectively, for feces from naturally infected pigs. The overall agreement between conventional PCR and LAMP for fecal samples from experimentally infected pigs was 79%, and from naturally infected pigs was 76%.

Conclusions and Discussion

This study evaluated the efficacy of the LAMP test for detection of *L.intracellularis* in fecal samples. Though previous reports have demonstrated that the analytical sensitivity of LAMP is higher than that of conventional PCR, we found the LAMP to be 10-fold less sensitive than PCR. However, LAMP test's specificity was comparable to PCR. The lower sensitivity of the LAMP test for naturally infected pig feces was likely due to the likelihood that that sample set had lower concentrations of *L. intracellularis* per gram of feces as compared to the experimentally infected pig feces. There was a very good overall agreement between the conventional PCR and the LAMP test for feces from either sample set, likely due to the high specificity of the test compared to the PCR (100%). The LAMP does show potential for use in pen-side or field conditions, or in resource-limited laboratories. However, evaluations of new primer and enzyme combinations are warranted to increase the sensitivity for the test in the field.

Acknowledgment

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References

1. Guedes, R.M., C.J. Gebhart, et al., 2002. Can. J. Vet. Res. 66:99-107.
2. Notomi, T., H. Okayama, et.al., 2000. Nucleic Acids Res 28:E63.

C.021

Effect of specific mix of monoglycerides and diglycerides of short and medium chain fatty acids in fattening pigs diets to control swine dysentery

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Introduction

Swine dysentery (SD) is an important disease in growing pigs with high economic impact (1). *Brachyspira hyodysenteriae* is the most important species, very resistant environment pathogen (2) even if new species are widely diffused (3). SD is controlled by use of lincosamides, pleutomutilins and macrolides (4), but the risk of occurrence of antibiotic resistance and the ban of grow promoters in EU make difficult the control of the disease nowadays. Recent studies have considered the effectiveness of new substances for the SD control(4). In this study, we evaluated the effect of a specific mix of monoglycerides and diglycerides of short and medium chain fatty acids (WO2010/106488A2) supplemented in fattening pigs diet in order to control SD.

Materials and Methods

A fattening site, naturally *B.hyodysenteriae* infected was selected and divided in two units for the study. Two previous fattening batches reported clinical signs of SD and *B.hyodysenteriae* was isolated from single pigs. 1022 pigs were randomly selected and included in control group and 974 in treatment group. The control group (CG) was fed with a commercial diet, the treatment group (TG) was fed with the same commercial diet added with 0,4 ml of LB/ kg lw, from day 0 to day 15 and from day 99 to day 112. CG was divided: 508 pigs in unit 1 and 514 in unit 2. The same for TG: 471 pigs in unit 1 and 503 in unit 2. Forty sentinels were ear tag identified in CG and forty in TG (20 pigs in each of the four units) to singularly monitor performances and microbiological status. Faeces were collected individually from sentinels on day 0 (T0), day 60 (T1) and caecal content was collected at slaughterhouse. Environmental faecal pools were sampled at day 0 (T0), day 60 (T1) and day 180 (T2). *B. hyodysenteriae* isolation and PCR were performed in individual faeces, environmental faecal pool and caecal content. The following zootechnical measures were recorded: total mass of pigs entering and exiting the fattening unit, weights of sentinels at day 0 (T0), 71 (T1), 120 (T2), 203 (T3), and feed consumption. Parameters included are Total Feed Intake (TFI), Average Daily Feed Intake (ADFI), Average Daily Gain (ADG), Feed Conversion Rate (FCR), Gain/Feed ratio (G:F). Necropsy and general post-mortem investigations were performed for dead animals.

Results

B. hyodysenteriae microbiological results in individual faeces and environmental pool samples are reported in Table 1. SD specific anatomo-pathological lesion (colitis) and microbiology results in caecal contens in necropsied pigs are shown in table 2. In Table 3 and Table 4 are reported microbiological results in caecal content of slaughtered pigs and zootechnical parameter data.

Table 1. *B. hyodysenteriae* isolated in individual faeces (sentinels) and environmental pool samples (positive/total)

	Control	Treatment
Fecal samples		
T0	0/40	0/40
T1	0/40	0/40
Enviromental samples		
T0	0/8	0/8
T1	2/8	1/8
T2	4/8	2/8

Table 2. Anatomo-pathological lesions and microbiology results in necropsied pigs (positive /total)

	Control	Treatment
Colitis	16/51	12/55
B. hyodysenteriae isolation	6/42	1/41

Table 3. Microbiology results in caecal content in slaughtered sentinel pigs (positive/total)

	Control	Treatment
B. hyodysenteriae isolation	5/79	2/80

Table 4. Zootechnical records

	Control	Treatment	Unit
TFI	492,34	479,88	Kg TM
ADFI	2,36	2,30	Kg TM
ADG	0,668	0,687	Kg /Day
FCR	3,53	3,35	
G:F	28,37	29,85	

Conclusions and Discussion

B. hyodysenteriae isolated in environmental and pig samples confirmed that the groups acquired infection. Infection rate. was higher in CG than in TG concerning environmental faecal pools (6/16 vs 3/16), caecal contents of necropsied (6/42 vs 1/41) and slaughtered pigs (5/79 vs 2/80). ADG and G:F were higher in TG than CG while FCR was lower in TG than CG. The fattening pigs diet supplemented with specific mix of Monoglycerides and Diglycerides of short and medium chain fatty acids showed to have a reduced risk to acquire *B.hyodysenteriae*, increased the feed intake and improved the performance. Further studies are needed to investigate the effects against other enteric pathogens and in farms with severe clinical form of SD.

References

1. Alvarez A et al. 2013. Int J Env Res Pub Health 10:1927-1947.
2. Schwartz T. et al. 2012. AASV An Meeting 85-90.
3. Osorio J et al. 2013. Res Vet Sci,
4. Pringle M et al. 2012. Acta Vet Scand: 54:54.

C.022

Decision model development for application of PRRS mitigation strategies post-weaning

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Introduction

Pork production systems routinely capture large amounts of real-time data. Decision tools can be developed to effectively utilize this data in strategic decision making. For example, Porcine Reproductive and Respiratory Syndrome (PRRS) creates economic losses in the period from weaning to market (WTM).[1] Several tactics can be employed to mitigate these losses in WTM batches. However, understanding the mean cost of PRRS infection in a batch of pigs and the ability to predict which batches are most likely to suffer those losses are critical in designing mitigation strategies that optimize economic returns. This paper describes an economic decision model for PRRS mitigation strategies in WTM pigs that was constructed and applied within a large production system.

Materials and Methods

A two stage decision model was constructed. The first stage estimates the Marginal Economic Cost (MEC) of PRRSv infection in the WTM period based on the performance differences of infected (p) and non-infected (n) groups.

*MEC=[[(weight gain/nADG)-(weight gain/pADG)] x housing cost/day]+ [[(Weight gain*nFCR)-(weight gain*pFCR)] x feed cost] +[[nMortality-pMortality] x value of a dead pig]*.

Values used in the model are determined with a multivariate approach (ANOVA) that estimates the mean performance differences between n and p batches and accounts for other sources of variation including: Source Farm, Area Pig Farm Density, Site level biosecurity, Time of year at Placement and Timing of PRRS Infection.

The second stage predicts which batches are more likely to suffer losses associated with PRRSv infection through application of multivariate logistic regression using the same variables in a backwards stepwise fashion.

A production system provided a data set containing 503 individual batch records. Of the 503, 53 batches included all of the data points necessary for this analysis. Site level bio-security was estimated with PADRAP[2] and an Internal Biosecurity audit. Area density was estimated based on the number of farms per square mile in the site's county based on 2007 USDA Census of Ag. Serial Oral Fluid PRRS PCR tests that were typically, but not consistently collected at 4 week intervals were used as a way to estimate timing of infection. Twenty seven positive batches (having at least one PCR positive) and 26 negative batches comprised the data set.

Results

PRRSv infection post-weaning increased mortality, impaired feed conversion and reduced growth rate, resulting in an additional 2.8 (1.4, 4.3) days on feed and 4.77 (2.9, 6.6) kg of feed per head.

Table 1. Summary of Biological performance (Least square means, 95% CI of the difference)

	Difference (Neg-Pos)	P-value
Mortality (%)	-1.88 (-2.43,-1.33)	<0.001
ADG (g/day)	13.6 (4.5,22.7)	0.03
FCR (kg/kg)	-0.04 (-0.06,-0.02)	0.03

Using cost assumptions (Mortality= $50/hd, yardage=$0.12/day and feed=$0.33/kg), the MEC of PRRS infection during WTM was estimated to be $2.84 ($1.78, $3.92). The risk of losses due to PRRSv infection in a batch of pigs could not be predicted by placement date, source, area pig density or either measure of site biosecurity assessed by this system.

Conclusions and Discussion

With a PRRS infection cost of $2.84 in roughly 50% of batches and the inability to predict which batches will suffer losses, the system should implement a cost conscious mitigation strategy in a system-wide manner rather than attempt a targeted approach. The ability to measure the biological and systematic sources of variation is critical to make informed decisions. Although in this case just 10.5% of batches had a complete data set; small, accurate, representative data sets can be useful in models to inform decision making and aid in achieving optimal long term performance in production systems.

References

1. Holtkamp, et al. *JSHAP* 2013;21(2):72-84.
2. www.padrap.org

C.023

Implementation of air filtration for PRRSV prevention in USA

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Introduction

Over eight years, air filtration was implemented in 93 client swine herds. The farms implementing filtration are: 61 sow farms, 23 boar studs, 6 finishing sites, and 3 research sites. 54 of the farms are filtered 100% of the time. 34 have partial filtration, in which there is no filtration in the summer months when the ventilation rates are high. Five of the farms started with partial filtration and later converted to 100% filtration. Other published work has shown a reduction in the rate of infection with PRRSv after the implementation of an air filtration system.

Materials and Methods

Upon implementation of a filtration program, data was collected to determine the number of new PRRS virus introductions into the herd in the 5 years prior to the filters being installed, or as long as the farm has been in existence if it is less than 5 years old. Data was collected after filtration and any new virus introductions recorded, regardless of source of the virus. This includes new virus introductions in the summer for the partial filtered farms, even though the air is not going through the filters at that time. The filters used included Camfil L6 (up to 4 years of age), Camfil L9 (up to 5 years of age), and Clarcor VPP (up to 2 years of age) filters.

Results

Before filtration, the 93 farms were infected with a new strain of PRRS an average of 52.5% per year. After filtration, the break rate per year has been 11.3%. The average number of years between PRRS breaks before filtration was 1.9 and the average after filtration was 8.9 years (P<.0001). 58 of the 93 farms are considered "high break rate" farms, having had a history of PRRS introductions of at least 2 in the previous 5 years and being in hog dense areas. The break rate on these farms was 70.9% previous to filtration and 14.9% after filtration (P<.0001). 90 of the 93 farms have had fewer breaks after filtration than before, while 3 of the 93 farms have had more PRRS introductions after filtration than before. The reason for PRRS introductions is not taken into consideration, nor is it considered whether the farm breaks when filters are on or off for the partial filtered farms. 18% of the filtered farms that have been infected with PRRS virus have been infected within one year of putting the filters in. Often this is when other parts of the project are being completed such as shutter walls, loadouts, etc.

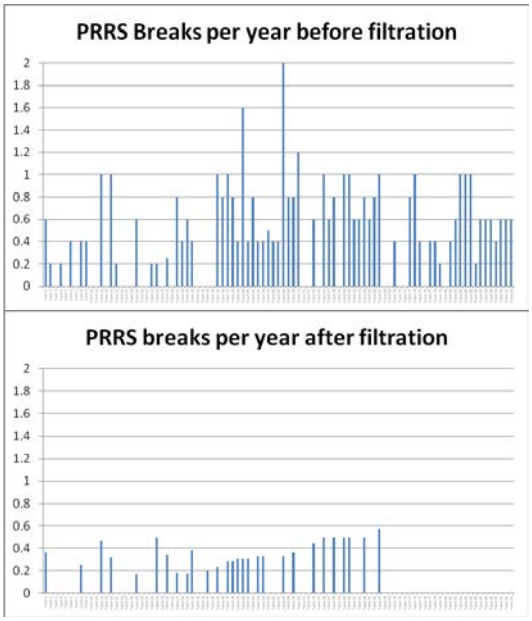

Figure 1. Break rate of each of 93 farms prior to and after the implementation of an air filtration system.

Conclusions and Discussion

Overall, there is a clear reduction in the number PRRS virus introductions in farms that have implemented air filtration. The farms have not changed biosecurity practices before or after filtration. The exception is that the majority of the sow farms have built a new weaned pig load out area or made modifications to what they had to allow for a double door system. Over the last two years, many farms have implemented shutter walls to further remove backdrafted air from the facilities. The break rate per year on the filtered farms is on an improving trend since that time. Positive pressure for entry and exit points to the facility also seem to correlate with less PRRS virus introductions. This seems to further the argument that aerosol transmission is a major source of new PRRS virus introduction in farms in the USA. The implementation of an air filtration system can be an effective way to reduce the rate of PRRS virus infection.

References

1. Dee S et al. 2010. Vet Record. 2010. 167:976-977.
2. Dee S et al. 2012. Viruses 2012, 4, 543-662.

C.024

An evaluation of the effectiveness of sanitation procedures using an accelerated hydrogen peroxide (Accel) disinfectant to reduce virus transmission via livestock transport vehicles

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Introduction

The modern pork production industry is characterized by frequent movement of swine. Varying health statuses of transported swine and the necessity to transport multiple groups in a single day or over the course of a week make transportation vehicles a risk for spreading infectious diseases (1). The purpose of this study was to compare the efficacy of Accel disinfectant (Virox Technologies Inc., Oakville, Ontario, Canada) and Synergize disinfectant (Preserve International; Reno, NV) against porcine reproductive and respiratory syndrome virus (PRRSV) and transmissible gastroenteritis virus (TGEV) in model livestock trailers under conditions similar to those experienced in commercial pork production.

Materials and Methods

An incomplete block design with treatment groups of Accel (n=8), Synergize (n=8), a positive control (n=9), and a negative control (n=4) was completed. Model livestock trailers were contaminated with a slurry mixture containing PRRSV ($3x10^5$ $TCID_{50}$ per ml) and TGEV ($10^{5.25}$ $TCID_{50}$ per ml) and allowed to sit in a 4°C cooler for 60 minutes. After removal, the contamination procedure was completed a second time. A limited washing procedure was then done, followed by the disinfection treatment. The positive control group was sham-disinfected with tap water and the negative control was not disinfected.

Four samples were collected from each replicate at five separate time points: 1) Immediately after the second contamination procedure, 2) Immediately following the washing process, 3) 15 minutes post-disinfection, 4) 30 minutes post-disinfection, and 5) 60 minutes post-disinfection. Samples were pooled by time point and replicate and were tested by PRRSV qPCR and TGEV PCR.

A bioassay was also completed. Six different bioassay groups were tested using the previously collected environmental samples (Table 1). Pigs were inoculated by oral gavage and intramuscular (IM) injection. On day 7 post-inoculation bioassay pig serum was tested for PRRSV by qPCR and fecal swabs were tested for TGEV by PCR. On day 14 serum was tested for PRRSV by qPCR and PRRSV and TGEV antibodies by ELISA Fecal samples were tested for TGEV by PCR. TGEV IHC was performed on intestinal tissue samples.

Table 1. Treatment groups for bioassay

Group	Treatment	Collection Time
Accel15	Accel	15 minutes
Accel60	Accel	15 minutes
Synergize15	Synergize	15 minutes
Synergize60	Synergize	60 minutes
Pos15	Tap water	15 minutes
Neg15	None	15 minutes

Results

PCR PRRSV positive replicates in the environmental sampling phase were found at all time points for the Accel, Synergize, and positive control treatment groups. No positive replicates were identified for the negative control group. Bioassay pigs in the Pos15 group were identified PRRSV positive on qPCR testing on days 7 and 14 post-inoculation and PRRS ELISA positive on day 14. No other bioassay groups were found PRRSV qPCR or ELISA positive.

Environmental sampling for the presence of TGEV was inconclusive due to the inability to maintain positive control groups throughout the course of the study. No bioassay pigs tested positive on TGEV PCR, ELISA, or IHC testing.

Conclusion and Discussion

After the washing procedure was completed, model trailers still contained puddles of water and a noticeable amount of organic debris. A limited washing procedure paired with Accel and Synergize disinfectants were effective at inactivating PRRSV in the environment of these model transport trailers with a short amount of contact time. The washing procedure itself was not effective at eliminating virulent PRRSV.

This study shows that washing processes similar to those found at commercial truck washes in the United States may be effective if the proper disinfectant and contact time are used.

Acknowledgements

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References

1. Dee S et al. 2004. Can J Vet Res. 68:128-133.

C.025

Field observation on the effect of PCV2 vaccination on the production parameters of selected swine herds

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Introduction

PCV2 is the essential infectious agent of Porcine Circovirus Associated Diseases (PCVAD), which are considered to have a severe economic impact on swine production (4). It has been proven that PCV2 vaccination of sows has helped in improving reproductive performance. PCV2 vaccination in piglets is now a routine practice worldwide to reduce the negative impact of infection on pig productivity from weaning onward (2, 3). In Poland less than 30% of piglets are vaccinated against PCVAD, whereas the average in EU is 80% and in some countries, e.g. in Germany and the Netherlands it accounts for 90% (1). The aim of this paper is to check effect of piglets PCV2 vaccination in Polish middle and large swine herds.

Materials and Methods

The field observation was performed in 2 farrow to finish pig herds. Herd A has 50 sows and herd B has 500 sows. In 2011 both herds were negative to PRRSV, but production parameters were unsatisfactory, despite of high management standards. Both herds used own gilts for sows replacement. Production parameters were monitored on the both farms from Jan 2010 to Dec 2010 (before period) and from Jan 2013 to Dec 2013 (after period), to exclude seasonal influences. PCV2 vaccination was implemented in herd A in Sep 2011. Piglets received single dose of commercial PCV2 vaccine Circovac (Merial) at 5 week of age. In herd B Porcilis PCV (MSD Animal Health) was used since Nov 2011, piglets were vaccinated at 3 week of age. In order to demonstrate the effect of PCV2 vaccination, selected production parameters before and after introducing of PCV2 vaccination were compared.

Results

Mean production parameters for herd A and B before and after vaccination are shown in Table 1. and Table 2., respectively. After implementation of vaccination program with a PCV2 vaccine in piglets, the number of pigs weaned/litter increased, in both herds. Furthermore, pre-weaning mortality decreased in both herds. Also average daily gain was higher after PCV2 vaccination.

Table 1. Herd A, production parameters before and after PCV2 vaccination.

Parameter	Before	After	Diff.
Live born/litter	11	12,5	+1,5
Weaned piglets/litter	10,5	11,3	+0,8
Pre-wean mortality (%)	15,4	9,2	-6,2
Post-wean mortality (%)	2,3	2,5	+0,2
Average daily gain (g)	636	663	+27

Table 2. Herd B, production parameters before and after PCV2 vaccination.

Parameter	Before	After	Diff.
Live born/litter	11,5	11,3	-0,2
Weaned piglets/litter	10,3	10,6	+0,3
Pre-wean mortality (%)	13,2	9,2	-4
Post-wean mortality (%)	8,7	7,3	-1
Average daily gain (g)	602	698	+96

Conclusions and Discussion

The use of PCV2 vaccines, regardless of manufacturer, time of vaccination and the herd size, resulted in all cases in improvement of growth performance as well as decrease in pre-weaning morality rate. Positive effects were also seen with regard to the number of piglets weaned per litter.

References

1. Baekbo P et al. 2012. Transbound Emerg Dis. 59:60-67.
2. Grimbeek P. 2010. IPVS. P. 372
3. Pejsak Z et al. 2012. Pol J Vet Sci. 15:37-42.
4. Segalés J et al. 2005. Animal Health Res Rev. 6:119-42.

C.026

Influence of Circumvent® PCV M vaccination timing on nursery performance

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Introduction

Vaccination for controlling porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* is a common practice in many swine operations and is typically performed in young pigs less than 6-7 weeks of age. The impact of vaccination on nursery performance has been investigated by several researchers in laboratory and field settings and the results have been variable.[1,2] In most field situations, the benefits of vaccination from controlling disease outweigh the negative impacts of vaccination. The objective of this study was to determine if altering the timing of vaccination with Circumvent® PCV M would impact nursery growth performance.

Materials and Methods

The study was conducted at the JBS United Burton-Russell Research Farm. Pigs from 4 consecutive farrowing groups (every 4 weeks) were used. Pigs were allocated to treatment group at 1 week of age (WOA). At weaning, the pigs were penned by treatment group to enable collection of feed intake data. Each group contained 19 pens with each pen holding 23-27 pigs. The pigs were weighed individually at 1 WOA (4-5 days, at processing) and pens were weighed and feed intake determined weekly from 3-9 WOA. Three treatment groups, as directed by the producer and herd veterinarian, were evaluated: 1) A - vaccination at processing (1 WOA) and weaning (3 WOA); 2) B - vaccination at processing and at 6 WOA; and 3) C - vaccination at weaning and 6 WOA (per label directions).

Results

Body weight data is presented in Table 1. Groups A and B were lighter than Group C at processing and at weaning. At 6 WOA, Group A was lighter than Groups B and C. By 9 WOA, there were no differences in body weight among the three groups.

Table 1. Average weight (lb) by age

Group (Vacc. Wk)	1 wk	3 wk	6 wk	9 wk
A (1/3)	4.69[b]	14.25[b]	25.97[b]	50.15
B (1/6)	4.70[b]	14.27[b]	26.77[a]	50.73
C (3/6)	4.78[a]	14.68[a]	26.99[a]	50.26

Daily gain, daily feed intake and feed conversion ratio data is presented in Table 2. The data is organized in to three periods: 3-6, 6-9 and 3-9 WOA. For daily gain, Group A grew slower than Groups B and C from 3-6 WOA. From 6-9 WOA, Group C grew slower than Group A and Group B grew similar to Groups A and C. For daily feed intake, the only differences occurred from 3-6 WOA where Group A ate less than Groups B and C. For feed conversion, there was no difference from 3-6 WOA but from 6-9 and 3-9 WOA, Group A was more efficient than Groups B and C.

Table 2. Results by age period

Group (Vacc. Wk)	Para-meter	3 to 6 wks	6 to 9 wks	3 to 9 wks
A (1/3)	Daily Gain	0.529[b]	1.150[a]	0.832
B (1/6)		0.565[a]	1.133[ab]	0.843
C (3/6)		0.558[a]	1.101[b]	0.815
A (1/3)	Daily Feed Intake	0.702[b]	1.635	1.156
B (1/6)		0.741[a]	1.651	1.187
C (3/6)		0.736[a]	1.597	1.145
A (1/3)	Feed Conv. Ratio	1.329	1.422[a]	1.389[a]
B (1/6)		1.313	1.458[b]	1.408[b]
C (3/6)		1.314	1.451[b]	1.405[b]

Conclusions and Discussion

Overall, the data indicate that vaccination at either 1, 3 or 6 weeks of age has some impact on performance but by 9 WOA, the impact is no longer apparent based on body weights. Differences in daily gain are apparently due to reduction in feed intake as demonstrated by Group A during 3-6 WOA. In contrast, the feed conversion ratio for Group A was significantly better than Groups B and C during 6-9 and 3-9 WOA. In summary, all three regimens resulted in similar nursery performance and no clear advantage with a particular regimen was evident.

Acknowledgments

Thank you to the farm staff that assisted with ths study.

References

1. Dritz, S. In: Proc. 51st George A. Young Swine Conference. South Sioux City, NE, 2011, pp. 24-34.
2. Thacker, B, et al. In: Proc. 43nd Annual Meeting of the American Association of Swine Veterinarians, Denver, Colorado, 2012, pp. 127-129.

C.027

Virginiamycin: Lack of interference with *Lawsonia* immunization, and improvement in feed efficiency

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Introduction

Porcine proliferative enteropathy (PPE), a highly prevalent and costly swine disease (1) caused by *Lawsonia intracellularis* (*Li*) (2), can be controlled by vaccination with an avirulent live oral *Li* vaccine. In-feed antimicrobials are often used in weaned/growing pigs for treatment, control or prevention of various diseases. The objective of this study was to determine if concurrent in-feed medication with virginiamycin (VM) at 11 g per tonne interfered with effective immunization with an avirulent live oral *Li* vaccine, as measured by differences in *Lawsonia*-specific lesion scores following oral experimental challenge with virulent *Li*.

Materials and Methods

Eighty-two 4-week-old castrated male pigs were used in this study. Ten pigs were used as non-challenged controls (NCC), divided into two pens of five pigs. The remaining pigs were divided into four pens of six pigs for each of three treatment groups; a challenged control group (CC), a vaccinated control group (VC), and a vaccinated medicated group (VM).

The VM treatment group received 11 g per tonne virginiamycin in their feed from Day 0 through Day 27. Vaccinated groups (VC, VM) were individually orally drenched once with the label-approved dose of an avirulent live oral *Li* vaccine on Day 1. Sixty mL of gut homogenate inoculum containing 10^8 virulent *Li* cells was administered via intragastric gavage to pigs in challenged groups (CC, VC, VM) on Day 31. Due to lab handling capabilities, pigs were euthanized over a two day period, on Days 51 and 52. All pigs were necropsied, and ileal immunohistochemistry (IHC) scores were assigned based on percent of ileal crypts colonized with *Li*.

This study consisted of two phases: a vaccination phase from Day 0 through Day 27 and a challenge phase from Day 28 through Day 51. Pen body weights were measured before and after each trial phase. Leftover feed was vacuumed out of the feeders and quantified at the end of each phase. This enabled determination of ADG and F:G for each phase of the trial.

Performance data were analyzed using ANOVA, treating the pen as the statistical unit. If the ANOVA *P* value for treatment effects was significant ($P < .05$), Tukey's HSD was used to discern differences among groups. *Lawsonia*-specific lesion score data were analyzed using the Kruskal-Wallis rank sum test, using the individual pig as the statistical unit, since pigs were vaccinated and challenged individually.

Results

Mean ileum IHC scores and F:G ratios are presented in Table 1 below. Mean ileum IHC scores in the VC and VM treatment groups were significantly lower than in the CC group. Mean ileum IHC scores in the VC and VM treatment groups did not differ. F:G was lowest in the VM treatment group compared to the other treatment groups during the vaccination phase (Days 0-27) when virginiamycin was offered, as well as during the trial overall (Days 0-51).

Table 1. Mean ileum IHC lesion scores and F:G ratios by treatment group

Response Variable	NCC[1]	CC	VC	VM	*P*
IHC[2]	0.00	1.78[a]	0.82[b]	0.83[b]	.02
Days 0-27 F:G	1.49	1.58[a]	1.46[b]	1.30[c]	<.001
Days 0-51 F:G	1.61	1.76[a]	1.62[b]	1.51[b]	.001

[1]Not included in the statistical analysis; raw means included for reference only
[2]Mean ileum IHC lesion score
(a, b, c) Values within rows with different superscripts are statistically different ($P < .05$)

Conclusions and Discussion

Both the VC and VM treatment groups had significantly lower lesion scores than the CC group, confirming immunization efficacy. Importantly, lesion scores were not significantly different between the VC and VM treatment groups, confirming the lack of interference between immunization and virginiamycin medication. F:G values were lowest in the VM treatment group compared to the other treatment groups during the vaccination phase (Days 0-27) when virginiamycin was offered, as well as during the trial overall (Days 0-51), confirming the feed efficiency benefits of virginiamycin administration.

References

1. McOrist S and Gebhart C. 2012. Diseases of Swine. 10th ed. pp. 811-820.
2. McOrist S et al. 1993. Infect Immun 61:4286-4292.

C.28

Acute salinomycin toxicosis in a swine herd

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Introduction

Toxicities in swine production are not frequently reported, however when appears could affect a large number of pigs with the consequent severe productive, economic as well as pig welfare impact (1,2). Salinomycin (SAL) is an ionophore licensed as coccidiostat in pigs in Argentina and growth promoter in other countries (3). It have a narrow range of safety that varies between from 30 to 60 ppm related with the age of pigs (1,2,3). This case report describes an acute toxicosis due to SAL associated an overdose in the feed formulation.

Materials and Methods

Case report: A one site 120-sow farrow-to-finish farm reported an outbreak of tremors, anorexia and lateral recumbence on the grower-fattening unit on October 18. The signs appeared 4 days after they start to eat *ad-libitum* in wet-dry feeders a new batch of on-farm produced feed. The population at risk was 516 pigs from 105 to 161 days old (55-112 kg/BW). Since August, the farmer has been used SAL as a growth-promoter (extra-label) at 60 ppm.

Case progression: Clinical signs appeared in 1 pig on October 15 and 3 new cases on 16 and 17. Clinical examination and rectal temperature were recorded and blood samples were taken for hematological and biochemical assay for aspartate transaminase (AST) and creatine kinase (CK) in 13 affected and 8 non-affected pigs. Necropsy was performed in 2 affected pigs and samples from several organs and tissues were taken for histopathological studies. Besides, representative samples of feed were taken for toxicological assay.

Results

A total of 15 pigs (2.7%) showed weakness, lateral recumbence, muscle tremors, vocalization and pain Signs related to central nervous signs, was not detected.

A pattern of hematological stress response was detected in all sampled pigs. In clinical affected pigs, median rate of CK and AST almost duplicate and fivefold increase respectively those not affected one (table 1).

Gross lesions were not observed. Microscopic lesions in skeletal muscle were characterized by hyaline necrosis and fragmentation of muscle fibers with proliferation of myoblast, satellite cells and macrophages. In cardiac muscles slight necrosis and fibrosis was seen. No lesions were observed in the kidney. A diagnosis of monophasic multifocal muscle necrosis compatible with ionophores toxicosis was informed. Thin-layer chromatography analysis detected 170 ppm of SAL on the feed samples.

Treatment and outcome: due to presuntive diagnosis of SAL toxicosis the feed bin was emptied and refilled with a new batch of feed without SAL and antibiotics. Thereafter, no new clinical cases were reported; however, all severely affected pigs were euthanized. The owner decided to sell most of the remained pigs before they reached the commercial weight (approximately 90 kg instead of 115 kg) due to the low group performance.

Table 1. Levels of CPK and AST in both analyzed groups, Units are expressed in UI/L.

	Affected		Non-affected	
	CPK	AST	CPK	AST
n	13		8	
median	15024	1662	7851	337
range	5105-19711	12-2677	873-14222	61-708

Reference levels: CPK <25 UI/L; AST 40-60 UI/L

Conclusions and Discussion

The clinical signs, biochemical findings and microscopic lesions leads to presumptive diagnosis of ionophore toxicosis. The detection of high levels of SAL in the feed confirm the diagnosis of SAL toxicosis. Event though all pigs eat the same feed, serum enzyme assays related with muscle damage such as AST and CK showed differences between clinical and subclinical affected pigs. Pathological differences with another reported case of SAL toxicosis in pigs might be related with the dose and the age of affected pigs (3). The origin of the overdosage was and misinterpretation of the feed formulation directives. This kind of mistake alerts us about the necessity of applied an Hazard Analysis Critical Control Points (HACCP) to minimize the risk of mistakes in feed formulation and ensure a safe produtcs to human consuption.

References

1. Carpenter J et al. 2005. J Swine Health Prod 13:333-336.
2. Croubels S et al. 2001. Vlaams Diergeneeskundig Tijdschrift 70:54-58.
3. Plumlee KH et al. 1995. J Vet Diag Invest 7:419-20

C.29

Utilizing a progesterone ELISA in gilts to augment the use of P.G. 600® and MATRIX™

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Introduction

Progesterone (P4) is an ovarian steroidal hormone produced by corpora lutea (CL) and is required for maintenance of pregnancy. Following ovulation, ovarian follicular tissue is rapidly luteinized to form CL and P4 production begins.[1] Pre-pubertal gilts are defined as those which have not yet had their first estrous cycle and therefore have low P4 levels due to the lack of CL. The estrous status of an individual gilt may be established by determining the P4 level and her history.[2] Sows that do not exhibit estrus within 7-10 days post-weaning and have low P4 levels are considered to be acyclic.[3] Animals with high P4 levels have CL on their ovaries and therefore are not considered to be pre-pubertal (gilts) or acyclic (gilts or sows).

Animals with normal estrous cycles have low P4 during their follicular phase and high P4 levels during their luteal phase. Animals with no history of detected estrus and high P4 levels either had a "silent" heat cycle or had a normal heat cycle that was missed by farm staff.

P.G. 600 is a combination of PMSG and HCG and is labeled in the US for the induction of estrus in pre-pubertal gilts and for the treatment of weaned sows experiencing delayed return to estrus. MATRIX (altrenogest 2.2%) is labeled in the US for estrus synchronization in gilts. MATRIX is fed orally for 14 days to gilts that have previously cycled; most will recycle within 4-9 days after the final dose. MATRIX is contraindicated in pre-pubertal animals. Conversely, P.G. 600 is contraindicated for post-pubertal gilts.

Herd A was the gilt developer for a 10,000-sow, parity segregated system experiencing an extended entry-to-service interval in gilts despite intensive boar exposure for at least 5 weeks. Herd B was a batch farrowing operation that needed to synchronize gilts to fit a specific breeding period. Due to labor and facility constraints, observed heat detection in gilts was not possible in Herd B. MATRIX was used to synchronize gilt groups, with variable success.

Materials and Methods

Herd A- Serum was collected from 29-week-old gilts that had not been detected in heat after 5 weeks of boar exposure. A total of 116 gilts were sampled over a period of 2 weeks.

Herd B- Serum was collected from 33 gilts one day prior to MATRIX use (day 0). Only gilts with high P4 levels (N=21) were placed on MATRIX. For those given MATRIX, P4 levels were also determined on day 14 of Matrix treatment.

All serum was assayed with a semi-quantitative ELISA to determine P4 levels (Ovucheck® Premate Porcine, Biovet, Inc., St Hyacinthe, Quebec, Canada).

Results

Herd A- Low P4 levels were detected in 113 of 116 gilts tested. Gilts with low P4 levels were injected with P.G. 600 within 24 hours of P4 testing; 107/113 gilts (94.7%) exhibited standing estrus within 8 days of P.G. 600 treatment.

Herd B- For the 21 gilts treated with MATRIX, 20/21 gilts (95.2%) had exhibited standing estrus within 8 days of the last treatment.

Conclusions and Discussion

Herd A- P4 testing indicated that most of the 29-week-old gilts with no history of observed estrus were pre-pubertal. The post-treatment estrus response to P.G. 600 was excellent.

Herd B- P4 testing was used as a proxy for pre-breeding estrus detection in developing gilts since facility and labor constraints prevented farm staff from gathering heat-no-service data. MATRIX response in gilts with high P4 levels was excellent. The historical subpar response rate was most likely due to its use in prepuberal gilts.

Timely onset of estrus in replacement gilts is crucial for all herds, especially those with non-continuous breeding programs and in parity segregated operations where gilts are the only source of animals available for meeting breeding targets. For herds without the ability to monitor estrus in developing gilts, P4 testing can help verify the estrous status of individual animals, aid in the selection of proper hormonal interventions and improve the outcome following hormonal therapies such as MATRIX or P.G. 600.

References

1. Dial G.D. and Britt J.H. 1986. The Clinical Endocrinology of Reproduction in the Pig. In: Current Therapy in Theriogenology, second edition. p 905-911. D.A. Morrow, ed. W.B. Saunders Co.
2. Lehman J.R. et. al. 2013. Optimizing PG 600® and MatrixTM usage with progesterone testing and real-time ultrasound evaluation. Proc AASV Annual Meeting. p 221-224.
3. Lucy, M.C., et. al. 2002. Ovarian follicular growth in sows. Reprod. Suppl. 58: 31-45

C.329

Influence of carbetocin and oxytocin on parturition and neonatal piglet vitality

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Introduction

The number of weaned piglets per sow is important for the profitability in modern pig farms. By an increase in the number of piglets in a litter the duration of parturition will be extended. Piglet mortality during parturition is around 8% and is correlated to the duration of parturition and is the highest in the last third of parturition. Oxytocin is used to accelerate the parturition in sows but can have a negative influence on the number of stillborn piglets and piglet vitality. It has been reported that carbetocin is also used to accelerates parturition in sows and has a more prolonged effect on the uterus longer with less side effects. A comparative study between the use of oxytocin and carbetocin on the farrowing duration and piglet vitality has not been done before. The aim of this research was to evaluate the use oxytocin and carbetocin in farrowing sows and its effect on the course of parturition, and the vitality of the neonatal piglets.

Materials and Methods

In this study 150 sows from 2 commercial farms (75 sows per farm) were involved. Cloprostenol induced parturition sows farrowing within 24 h and sows with less than 8 born piglets were excluded from this experiment. Per farm sows were randomly arranged into three groups. Oxytocin and carbetocin solutions were injected intramuscular after the birth of the fourth piglet. The carbetocin group (C) received 1 ml (0,07 mg) carbetocin (Longacton®, Dechra). The oxytocin group (O) received 1 ml (10 IU) oxytocin (Oxytocin®, Dechra). The control group (P) received no injection. The study was triple blinded, the observers nor the statistician knew the contents of the bottles. During the observations, sows and piglets were minimal assisted. Farrowing duration, expulsion interval, standing interval after birth and suckling interval of the neonatal piglets were measured. In addition other variables, head or breech presentation, umbilical cord rupture (broken, adhered different or adhered normal), sex and weight were also noted. Live born piglet vitality was scored by a modified Apgar score including the time interval between birth and first breath, the snout skin color, the skin staining with meconium and the time interval between birth and first standing. The data were statistically analysed by multivariable regression analysis in R. If P≤ 0.05, it was called significant.

Results

	Carbetocin	Oxytocin	control
Littersize	15.26	15.02	14.98
Stillborn type II	0.75	0.49	0.46
Total farowing duration ±sd	2.11 h ± 96	2.15 h ± 96	2.32 h ± 96
Mean int 1-4	25.8 ± 17.2	26.4 ± 19.1	24.8 ± 13.5
Mean int 5-8	12.5 ± 8.3	11.0 ± 7.0	13.3 ± 6.7
Mean int 9-12	11.0 ± 8.9	10.8 ± 7.5	14.5 ± 12.0
Mean int 13-	13.8 ± 10.8	18.0 ± 11.7	19.0 ± 26.9

Treatment did not have a significant influence on the

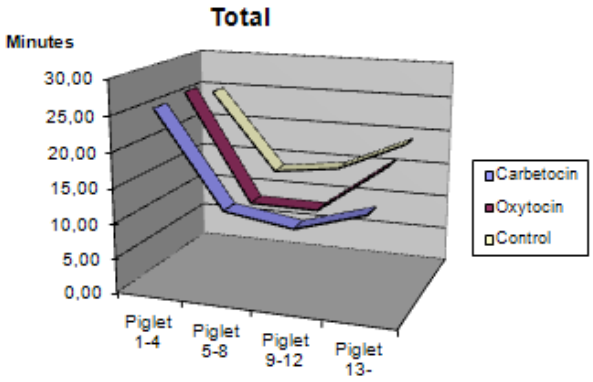

Figure1. Mean birth interval.

Treatment with carbetocin and oxytocin both had a lower odds ratio (0.63 and 0.53 respectively) with respect to piglet mortality within the first 48h of life in the control group, significantly different for the odds ratio of oxytocin (P<0.05). There was no impact on vitality at birth.

Conclusions and Discussion

In this study treatment had no significant effect on the duration of parturition, the expulsion interval and the piglet vitality score, but there was a significant effect between oxytocin treatment and piglet mortality within 48h of life. Oxytocin treated piglets were less likely to survive the first 48h of life compared to control piglets.

C.31

Improvement in the estimation of heritability for cured ham sensorial meat quality traits using high density 60K SNPs arrays

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Introduction

The production of cured ham in Spain represents a very important economic issue in the frame of pork production. The main focus of genetic selection up to the date was related to numeric production. Today, in a "genetic on demand" program the meat quality has increased its importance for costumers. So, in Spain the traits involved in cured ham quality and technologic characteristic of meat need to be investigated and included in such program. We present the preliminary results for genetic parameters related to cured ham in a Spanish Duroc genetic line.

Materials and Methods

Information from cured hams was available from 249 animals (all TOPIGS Duroc gilts). The software package ASREML was used to estimate genetic parameters for the different traits. Genetic correlations between traits were estimated within sub data sets.! The parameters studied were: Fat brightness (FTB), Intramuscular fat (IMF), Lean brightness (LNB), Uniformity of color (UNC) and Color intensity (INC), Cured smell intensity (CSI), Taste to raw meaty (RWM), Salty (SAL), Cured (CUR), Hardness (HDN), Chewiness (CWN), Mellowness (MLN), Juiciness (JUN), Total acceptance (ACP), Slice texture average (SLA) and Slice texture standard deviation (SLD). The phenotypic information related to cured ham sensorial meat quality traits was obtained by means of a sensorial analysis panel. Panel test consisting of 2 male and 6 female judges typical consumers of ham and it was done in accordance with the ISO 4121 (2003) regulations in a standardized room (UNE, 1979.On the other hand, DNA from 248 animals was isolated and every animal was genotyped using Illumina 60k chip at GeneSeek (USA).

Results

The results for heritability calculated by each method are shown in table 1. The heritability varied for several traits depending on the method used. Generally, the 60K calculations showed a much lower standard deviation as compared to the classical statistical method. Overall acceptance of cured ham quality (overall sensorial appreciation) was (phenotypically) correlated with cured taste (0.67), followed by mellowness (-0.56) and hardness (0.50).

Table 1. Results obtained for cured ham-related traits heredability by classical ASREML and 60K SNPs.

Group	Trait	h^2 (ASREML)	h^2 60K
Appearance	FTB	0.29±0.16	0.38 ± 0.09
	IMF	0.37±0.18	0.51 ± 0.09
	LNB	0.54±0.2	0.38 ± 0.12
	UNC	0.28±0.17	0.35 ± 0.13
	INC	0.11±0.16	0.28 ± 0.10
Smell	CSI		
Taste	RWM	0.08±0.15	0.16 ± 0.08
	SAL	0.43±0.18	0.35 ± 0.12
	CUR	0.02±0.13	0.17 ± 0.08
Texture	HDN	0.22±0.15	0.31 ± 0.09
	CWN	0.08±0.13	0.14 ± 0.08
	MLN	0.23±0.17	0.17 ± 0.09
	JUN	0.09±0.12	0.32 ± 0.11
Total	ACP		
Texture	SLA	0.72±0.23	0.63 ± 0.09
	SLD	0.03±0.12	

Conclusions and Discussion

Due to the low number of animals, the standard errors on the estimates are rather high, but comparable with other previously recorded for meat quality traits[1]. The heritabilities seem to be comparable with those published for meat quality traits[2] and higher than other previously reported for cured ham sensorial traits[3]. The use of high density 60K SNPs genotyping improves most of heritabilities values, in terms of standard deviations reduction. The traits related with cured ham should be included in any selection program develop in Spain for producers focused on transformation industry, especially those producing for cured ham.

Acknowledgments

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References

1. Russo et al. 2000. J Anim Breed Genet. 117 37–42
2. Suzuki et al. 2005. J Anim Sci . 83:2058-2065.
3. Guàrdia et al. 2012. Proc. ICQG Edimburgh. P258

C.32

Comparison of the carcass quality of Improvac® vaccinated entire male pigs with surgical castrates and female pigs

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Introduction

A key economic indicator of the efficiency of meat production is lean meat yield. This is often reflected in the "carcass grade" applied at slaughter. In Thailand, a measurement of the percentage of lean meat is used to simply evaluate carcass quality. It is a measure of the ratio of the thickness of the backfat and width of the loin muscle (2). Improvac (Zoetis, Madison, NJ) is a vaccine used for the immunological castration of male pigs. The 2nd dose of Improvac is timed to occur in the late finishing phase, and allows producers to maximize the production benefits of raising entire boars, and yet provide sufficient time for any boar taint compounds present in the body to be depleted. The aim of this trial was to compare the carcass grade assigned, by a commercial slaughterhouse, between Improvac vaccinated entire male pigs with the grade assigned to either surgically castrated pigs or female pigs.

Materials and Methods

This study was conducted on 2 farms. Farm A had a total of 494 pigs with an average weight of 112 kg at slaughter. There were 202 male pigs that were surgically castrated between 3-7 days after birth; 204 female pigs and 88 male pigs that were vaccinated with Improvac by subcutaneous injection at 15 and 19 weeks of age as per label instructions. Farm B had a total of 107 male pigs, 62 surgical castrates and 45 Improvac vaccinated males. On each farm all treatments were fed the same diets and kept under the same conditions. At 24 weeks of age the pigs were slaughtered in a commercial slaughterhouse. At slaughter, the warm carcasses were measured and the Lenden-Speck-Quotient (LSQ) index was calculated using the formulae of Sethakul et al (2) LSQ = (BF_3 + BF_4)/2b (Figure 1). A commercial grading matrix was used to convert the LSQ to a grade. Statistical differences in the grading distributions were tested using the Chi-square statistic.

Results

The carcass grading distribution for each group on each farm is shown in Figure 2. On both farms the Improvac vaccinated groups showed a statistically significant ($P<0.001$) shift to a higher percentage of A grade carcasses when compared to either the castrates or female carcasses. On farm A the female pigs also had a higher proportion of better graded carcasses compared to the castrates.

Figure 1. The positions for measuring backfat thickness (BF_3 and BF_4) and width of loin muscle (b) for use in calculating the LSQ (Sethakul et al. 2003).

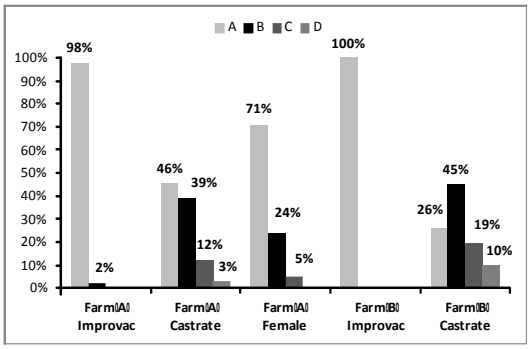

Figure 2. Distribution of the LSQ carcass grade assigned to Improvac vaccinated, surgically castrated or female pigs at slaughter.

Conclusions and Discussion

In the majority of carcass grading schemes the higher the grade the higher the lean meat yield and lower the fat content and hence the better the value. In the current study the Improvac pigs had the best carcass quality when compared with castrates or female pigs. The castrated pigs had the worst LSQ grading. The current findings of a shift in distribution to more high-value better grade carcasses in the Improvac vaccinated pigs confirms the findings from a 10 country, 13 study report by Allison et al, 2011. The higher carcass grade for Improvac vaccinates is reflected in a higher lean meat yield on dissection (2)

References

1. Allison, JRD et al. 2011. Proc 57th Int Cong Meat Sci & Tech, Belgium, pp 953-956.
2. Sethakul J et al. 2003. Thai J Agric Sci. 34: 3-6 (Supplement), pp 228-231.

C.034

Economic model to demonstrate benefit of improvements in sow farm to result in better productivity

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Introduction

One of the strongest predictors of sow farm productivity is productivity of parity 1 (P1) females (1). Thus, we propose a partial budget economic model to help demonstrating the benefit of improvements in the sow farm facilities to result in better productivity of females at P1 and beyond. By definition, partial budget models take into account changes (before and after implementation of a project) in cost and revenues (2).

Methods

We propose a 3 steps model:

1. Identify opportunities of improvement according to genetic companies recommendations.

1.1. List changes that require no investment.

1.2. List changes that do require investment in the production system, and estimate cost of each change (e.g. providing 4 types of feed to gilts at nursery instead of 3 will require 1 additional silo at cost of $2,000).

2. Define productivity goal according to genetic potential. As rule of thumb, F1 gilts farrow no less than 1 piglet (total born) compared to great grand parent female. Alternatively one could target to achieve the total born of the current top 25%.

3. Build economic viability project to compare costs of changes to expected project benefit.

Results

To demonstrate the model, we described a project to improve long term sow farm productivity by improving short term P1 productivity. The estimated benefit of the project was calculated considering the effects of improving sow farm productivity on a) the piglet production cost and on b) the profit margins (assuming farrow to finish operation) (Figure 1).

The cost to implement the project was the sum of the required adjustments on the facilities (Figure 2).

The overall benefit over costs analysis was performed assuming a 5-year return over investment analysis using a cost of capital of 8% (Figure 3).

Figure 1. Demonstration of project benefit:

Total opportunity of improving productivity at breeding herd

1- Impact on cost of production		
Impact of improvement on Piglets/Sow/Year (PSY) on production cost		Number of sows: 1,000
Cost of weaned pig	$	40.00
Actual PSY		29.00
Total cost per sow per year	$	1,160.00
Target (potential) PSY		30.00
(new) cost of weaned pig		38.67
Reduction on cost of production per piglet	$	1.33
Reduction of cost per year	$	40,000.00
2- Impact on profit margin		
Impact of PSY improvement on *margin over variable cost*		
Additional piglets weaned/year		1,000
Full value pigs sold/year (92%)		920
Acutal margin per head	$	76.00
Additional prof margin per year	$	69,920.00
3- Variable cost of producing additional piglets		
Variable cost of piglet production ($ 4.00/hd)		5,000
Total Benefit (1+2-3)	$	104,920.00

COMMENTS:
1- There will be reduction on production cost due to better production efficiency. That is, the cost of operating the breeding herd will be diluted by a larger amount of pigs weaned (this difference will be added on margin over variable cost).
2- Additional piglets result in additional profit.
3- Additional piglets have variable cost (vaccinations, injections, labor, others)

Required investments to allow proper gilt development from birth to farrowing

Adjustments on the Gilt Development Unit (GDU)		
Nursery - additional water nipples	$	1,280.00
Nursery - Mat under brooders	$	600.00
Growers - Additional silos to allow proper feeding	$	6,000.00
Growers - additional water nipples	$	1,500.00
Flushing - Water nipples in the crates	$	11,000.00
Adjustments in the Gestation area		
Proper semen storage unit	$	1,500.00
Water nipple in some crates (weaning and gilts)	$	25,000.00
Adjustments in Farrowing barn		
Adjustments in the farrowing crates	$	107,520.00
Replace heating source for piglets	$	12,000.00
TOTAL COST FOR ADJUSTMENTS	**$**	**166,400.00**

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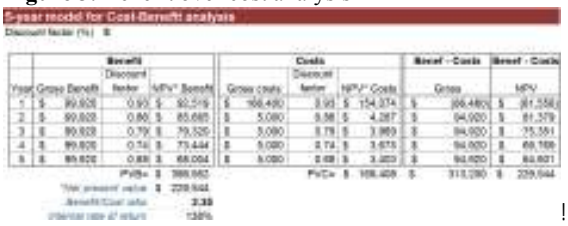
Figure 3. Benefit over cost analysis



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This case report showed that the amount of $166,400 required for adjustments on facilities had a benefit over cost ratio of 2.35 times, and an internal rate of return of 138%. The net present value of the project was $ 229,544.

Conclusions and Discussion

There are several key performance indicators (KPIs) used in the swine industry today. However, it is still not clear the economic value of many KPIs, and how much can producers invest on their facilities to improve productivity as measured by KPIs.

The economic model presented in this study was created to help veterinarians and producers to understand the economic value of throughput KPIs, allowing to pinpoint how much can be invested in adjustments on infrastructure to justify increases in productivity levels.

The spreadsheet used for this study is available by request to the author.

References

1. Pinilla J.C. et al. 2014. AASV Meeting 2014 [in press].
2. Abbitt & Townsend. 1979. Citrus & Veg Mag, 42 (12).

Data on stillborn piglets in nine Danish herds

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Introduction

Still born piglets are a major contributor to piglet mortality. In Denmark the average number of live born and still born piglets/litter is 15.1 and 1.7, respectively (1). The objective of this study was to analyse the association between parity, litter size, birth weight, and still born piglets in herds with large litter size.

Materials and Methods

Nine herds with an average number of 1.6 still born piglets per litter were included in the study. In each herd, 926 to1261 piglets from approximately 70 consecutive farrowings were ear tagged and weighed.

All still born piglets were autopsied either in the herd or stored in a freezer before shipment to the laboratory for pathological examination. The criteria of a piglet being still born were that lungs did sink when submerged into water. For all litters included in the study, parity of the sow and litter size (live born and still born) was recorded.

The probability for the individual piglet to be still born was analysed in a generalized linear mixed model with parity, litter size, and birth weight as explanatory variables while herd and sow was included as random effects.

The three explanatory variables were dichotomized. Parity 1 and 2 as low parity while higher parities were high parity. Birth weight < 1.29 kg was low birth weight while birth weight > 1.29 kg was high birth weight. Litter size < 19 total born was low litter while litter size > 18 total born piglets was high litter size.

Table 1. Data on material included in the 9 herds.

Herd	Sows	Live born per litter	Still born per litter	Total number born
1	60	16.6	1.2	1063
2	58	15.6	1.8	1017
3	62	16.5	1.8	1136
4	69	16.0	1.7	1225
5	67	14.1	1.1	1025
6	60	17.1	2.4	1166
7	72	15.4	2.1	1261
8	74	15.9	1.0	1250
9	56	14.5	1.9	926
All herds	578	15.8	1.6	10069

The three variables and their two level interactions were analysed in one model. The statistical analysis was performed with a binary distribution and a logit link function in Proc Glimmix in SAS (2).

Results

The number of sows, live born and still born piglets in each herd is shown in Table 1. Only one piglet was excluded from the analysis due to missing values. Thus a total of 10068 piglets from the nine herds were used in the analysis. Parity, litter size, and birth weight all had a statistically significant impact on still born piglets. There were no statistically significant two level interactions between parity, litter size, and birth weight. The results are presented in Table 2.

Table 2. Effect of parity, litter size, birth weight.

Parameter	Odds ratio	95% CI	P-value
Low parity[1]	0.41	0.31-0.54	<0.001
High parity	1	-	-
Low litter size[2]	0.76	0.58-0.98	0.04
High litter size	1	-	-
Low birth weight[3]	3.04	2.51-3.68	<0.001
High birth weight	1	-	-

[1]Parity<3 [2]Litter size <19 piglet [3]Birth weight<1.29 kg

Conclusions and Discussion

In this analysis piglets with a low birth weight had 3.04 higher odds for being still born than piglets with a high birth weight (> 1.29 kg). Piglets born by a low parity sows had a 0.41 lower odds for being still born than piglets born by a higher parity sow. Piglets born in litter sizes < 19 (low) had 0.76 lower odds for being still born than piglets born in larger litters.

Acknowledgments

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References

1. Vinther J. 2013.! http://vsp.lf.dk/Publikationer/Kilder/Notater/2013/1314.aspx
2. Base SAS® 9.3 Procedures Guide Copyright © 2011, SAS Institute Inc., Cary, NC, USA

C.334

Piglet cost determination

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Introduction

Small and medium scale pig production system, persist because they establishes a complex social and commercial network. According to different authors, pig production can be divided according technical and commerce goals. The emphasis of this work has been to determined benefits and cost production in piglet small-scale farms

Materials and Methods

A modified cost determination methods (1) were used to determine fixed and variables costs and final profit. 1249 litters were divided in strata and analyzed. Strata 1: from 5 to 10 sows (N=9); Strata 2= 11 to 49 sows (n=11); and Strata 3= 50 to 100 (n=5). Fixed costs formula was: F=L+S+Co+R+A+Fi+CO+Ot, where F=Fixed cost, L=Labor, S=supply, Co=Fuel and energy, R=maintenance, A=amortization, Fi=Financial cost, CO=Opportunity and OT Others cost. Variable cost formula was V=((SA+SF+BA+BF+PF+M+T+CO) /AS*W) PW where SA=sow amort. SF=sow feed, BA=boar amort. BF=boar feed, piglets feed, M=medicines, T=transport, CO=opportunities costs, AS=all sows, W=weighted factor, PW=piglets weaned. SA formula was: ((SA-(RP-(1-%SM))/((SB/BSY)-%SR)), where SA=sow price, RP= sow recovery price, %SM=5 sow mortality, SB=farm sow birth rate, BSY=birth sow year, %SR=% sow replacement. SB formula was: S(NSB*n)/AS NSB=number of sows depending birth number, n=number of birth. %SM formula was: %SM=BSY/SB. W formula was: w=BSY*PBA*(1-%PM)*(1-%TM) where PBA=piglets born alive, %PM=%piglet mortality, %TM=% transition mortality. Break-even point, net income and benefit/cost ratio were also calculated. Currency results are shown in USD.

Results

Table 1 resume the main results of Total costs for piglets production.

Table 1. Piglet cost production

	Strata 1	Strata 2	Strata 3
Fixed cost	16.03	9.40	7.78
Variable cost	29.00	24.90	25.55
Total cost	45.06	34.30	33.33

Strata 1 showed more fixed cost in relation to strata 2 and 3, but less variables costs compared with both, strata 2 and 3. Net income was $0.94, 11.70 and 12.67 USD/piglet weaned, for strata 1, 2 and 3 respectively. Break-even point was 105, 209 and 528 piglets weaned by farm, for strata 1, 2 and 3. The B/C ratio was 1.04, 1.35 and 1.39 for each strata.

Number of weaned piglets per year was 17.9±2.1, 22.5±1.2 and 21.5±1.3.

Conclusions and Discussion

Results showed a size effect in economic indicator. A lot as been discussed about farm size and economic scale size, but in this study, strata 1 showed the less profit and worst economic cost structure. This can be explained if you consider the opportunity cost of labor. Because, in Strata 1, producers don't hire labor. But the cost methodology needs to consider the opportunity cost of labor in order to establish the cost structure. For example, if we don't consider the labor cost of strata 1, $30.46, net income will be $15.81 USD, break-even point will be 11 weaned piglets and B/C Ratio will be 1.52. In this new scenario, Strata will be the Strata with best-cost equilibrium, and best profit and economic performance.

The main fixed cost was labor and the main variable cost was sows feed costs. The first one represented 31, 23 and 21% of fixed cost and the second one represented 51, 60 and 64% of variables costs, for strata 1, 2 and 3 respectively.

Data suggest that there is a size farm effect, but it is necessary to establish correctly the value or weight of each variable, especially in family labor cost. There is strong relationship between productivity and size cost structure.

The methodology allowed to analyze cost structure and establish the benefits of each strata. It also allowed to adjust the structure cost, depending on each strata characteristics, to know, the labor as economic concept. This economic concept is important to the strata 1 economic sustainability.

Acknowledgments

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References

1. Rouco YA et al. 2005.

C.37

In a co-infection model PRRSV type 1 aggravates virulence of a mild virulent *A. pleuropneumoniae* serotype 2 strain

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Introduction

PRRSV is endemic in most pig producing countries of the world and causes disease characterized by abortions and stillbirth, increased pre-weaning mortality and respiratory disorders in growing pigs. The genotype 1 strains of PRRSV are primary pathogens for reproductive failure and abortion. However, infections with this EU-type of PRRSV do not lead to clinical respiratory disease, but can induce a change in lung immune cell composition. In recent experiments we have been able to show, that the experimental infection had an effect on cellular immunity parameters and also induced mild, interstitial, inflammatory lung alterations, which can be predisposing for pneumonia. In the field situation, it has been shown that PRRSV predisposes for respiratory disease in pigs and pneumonia is correlated with co-infections with bacterial and viral pathogens. Fablet et al. (2011) have shown that PRRSV and *Actinobacillus pleuropneumoniae (A.pp)* serotype 2 are significantly associated with pneumonia and pleuritis (1). In experimental studies *A.pp* serotypes strongly differ in virulence depending on the expression of APX toxins, but although *A.pp* serotype 2 belongs to a mild virulent serotype, this serotype is currently the most diagnosed *A.pp* serotype in western European swine herds.

Materials and Methods

To be able to study predisposing and aggravating virulence mechanisms an experimental co-infection study with PRRSV genotype 1, subtype 1 (Lelystad strain) followed by a *A.pp* serotype 2 infection was performed. In this study, pigs were allocated to three groups (group 1:PRRSV/*A.pp* 2; group 2: PBS/*A.pp*2; group 3: PBS/PBS). Pigs of group 1 were infected with PRRSV by intranasal inoculation on day -7 and pigs of group 1 and 2 were exposed at day 0 to an aerosol during 20 minutes in an aerosol chamber. Aerosols were generated by an Aeroneb™Pro micropump nebulizer and 5 ml of the inoculum containing 1 x 10^9 cfu/ml was placed in the nebulizer vessel and dispersed by an airstream of oxygen. Pigs were followed clinically for 7 days after *A.pp* challenge and changes in haematology and leucocyte populations were addressed and pathological and microbiological examinations performed at the end of the study.

Results

In all PRRSV infected pigs highly febrile temperatures occurred ca. 4 hours after *A.pp* infection and increased body temperatures were observed in the following days.
60% of PRRSV/*A.pp* and 20% of PBS/*A.pp* infected pigs showed signs of fibrinous-necrotizing pleuropneumonia; *A. pp* was re-isolated from lungs of 6/10 PRRSV/*A.pp* pigs and 1/10 PBS/A.pp infected pigs.
PRRSV infection induced short lasting leucopenia, but no changes in phenotype profile of systemic lymphocytes were observed.

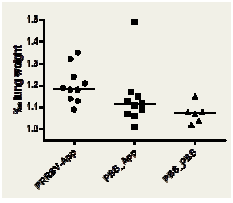

Figure 1. Lungs from pigs from PRRSV/A.pp infected group showed a significantly higher relative lung weight at necropsy

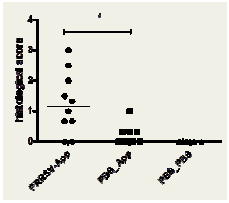

Figure 5. Histological assessment of 6 pre-defined lung areas resulted in a significant higher pneumonia score in PRRSV/A.pp group.

Conclusions and Discussion

From historical data it is known that under experimental conditions PRRSV type 1 alone does not induce clinical signs of pneumonia and that an experimental *A.pp* serotype 2 infection results in subclinical disease. In this study we showed that an infection with PRRSV type 1 (Lelystad strain) significantly aggravates the susceptibility to the mild virulent *A. pp* serotype 2 resulting in pathological signs of pleuropneumoniae. The high fever peak early after infection with gram negative *A.pp* is possibly related to an earlier described increased numbers of CD14 positive cells in lungs (2), which needs to be confirmed in BAL cells in this study. The results confirm that this animal model is suitable for future studies on pathogenesis, but especially to analyze new concepts for the prevention or intervention of porcine respiratory diseases after viral/bacterial co-infection.

References

1. Fablet C et al. 2012 Veterinary Microbiology 157:152-163
2. Van Gucht et al. 2005 Vet Immunol 18:116-126

Economic impact of *M. hyopneumoniae* eliminations

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Introduction

Mycoplasma hyopneumoniae continues to be an economically important respiratory infection in swine. There are a number of different ways to eradicate Mycoplasma from herds. Adaptations to the Swiss method have also been successful at eradication. These would include herd closures allowing the herds to continue to farrow during the process[1, 2]. Vertical transmission studies demonstrate that transmission occurs up to 8 months post infection[3]. Herd closures have typically been 8-9 months duration and may or may not have off site breeding projects incorporated. These procedures have included a whole herd medication program at the end of the closure.

Another method is whole herd medication without herd closure using a long acting antibiotic such as Draxxin® (10% Tulathromycin, Pfizer), treating all pigs on site with an injection and repeating in 2 weeks[4]. The advantage of this approach is that the herd returns to negative status faster.

This study looked at economic advantage of *Mycoplasma hyopneumoniae* elimination.

Materials and Methods

- Identified 39 herds that had been through a *Mycoplasma hyopneumoniae* elimination procedure.
  - 27 herds used a herd closure and whole herd medication program.
  - 12 herds did a whole herd injection of medication program and no closure.
- All farms had negative replacement breeding stock available.
- Herd's mycoplasma status where monitored using: pigs in the finishing flow and blood testing just prior to marketing.
  - Clinical signs of Mycoplasma (coughing) in sow herds or finishing pigs.
  - Serology of pigs at the end of the finishing phase.
    - Using IDEXX test if vaccinated
- Data from a production system that tracks health status in finishing was used to model the economic outcomes of the elimination process.

Results

Herd closure and whole herd medication was 89% and 67% successful overall respectively. The average "survivability" of herds in the study was 47 and 34 months respectively as shown in Table 1.

Table 1. *Mycoplasma hyopneumoniae* elimination success in 39 farms by type:

	Herd Closure	Medication	Total
Number of Sows	71000	23000	94050
Number of Herds	27	12	39
Percent Negative at 1 year	96%	75%	90%
Percent Negative to date	89%	67%	83%
Average Months negative	47	34	43

Table 2. Difference in performance between *M. hyopneumoniae* (+) and (-) pigs

Per 1000 sows	Myco (+)	Myco (-)	Difference
Finishing Mortality	3.6%	2.2%	-1.4%
Finishing Culls (Underweight MKT)	2.4%	1.4%	-1.0%
Total Pigs Sold	25614	26281	666
Cost of Treatments	$ 0.63	$ 0.37	$ (0.26)
Finish ADG	1.76	1.87	$ 0.11
F/G	2.73	2.65	$ (0.08)

Table 3. Economic impact of *Mycoplasma hyopneumoniae* elimination.

Grow Finish Performance Opportunity		Per Pig
Treatments savings / Total	$ 7,280	$ 0.28
Total Dead Pigs	386	
Cost of Mortality	$ 73,783	$ 2.81
Reduced number of culls (head)	256	
Cull opportunity $	$ 24,455	$ 0.93
Cost of Performance		
Cost ADG	$ 38,137	$ 1.45
Cost F/G	$ 48,155	$ 1.83
Total Finisher	$ 191,810	$ 7.30
Whole Herd Opportunity Cost Impact		
Total (Finisher)	$ 191,810	$ 7.30
Cost per Sow		$ 191.81

Table 4. Return on Investment of *Mycoplasma hyopneumoniae* elimination.

Myco Elimination per sow Closure		$ 7.50
ROI to 1		26
Months to break even		0.47
Myco Elimination per sow Medication		$ 30.00
ROI to 1		6
Months to break even		1.88

Discussion

Mycoplasma hyopneumoniae elimination programs have had a good success rate with 89% of closures and 67% of medication programs being successful. Not only does production improve but so does profitability as demonstrated in the economic model and a good return on investment. Although there is always risk of failure the reward is good and success rates have been reasonable.

References

1. Schneider P. 2006. Proc. Allen D Leman Swine Conference, 82-86
2. Yeske P. 2007. Proc. American Association of Swine Veterinarians 367- 370
3. Torremorell M. et al. 2007 Proc. American Association of Swine Veterinarians 9-10
4. Yeske P 2008 Proc. IPVS Durban, OR.02.25

Effect of tulathromycin given at weaning for the control of complex swine respiratory disease

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Introduction

Swine respiratory disease (SRD) is commonly found in weaned pigs and is attributable to both viral and bacterial pathogens. In the United States, Draxxin® (tulathromycin) is labeled for the control of SRD associated with *Pasteurella multocida*, *Actinobacillus pleuropneumoniae* and *M. hyopneumoniae(M.hyo)*. Previous studies have reported the benefits of using Draxxin for control of SRD when a portion of the herd is clinically ill and the remainder is at risk for developing disease [1,2]. The purpose of this study was to evaluate the derived production benefits of controlling complex SRD in piglets with one dose of Draxxin per label at weaning.

Materials and Methods

One thousand, one hundred weaned pigs from two commercial sow farms where clinical SRD due to *Pasteurella multocida* and PRRS had been diagnosed at weaning were blocked by gender and sow farm and 550 pigs were randomly enrolled to either Draxxin or saline control groups. The Draxxin group received tulathromycin at 2.5 mg/kg IM at weaning whereas the control group received a comparable dose volume of saline IM. The pigs were weighed at enrollment, study day 54 and study day 152. Starting from study day 1, if any pigs demonstrated a respiratory or depression score of ≥1 out of a 0-3 score range, as scored by an observer blinded to treatment, the pigs were treated with Excede® (ceftiofur) for Swine per label. The pigs ear tags were notched and the pig was not retreated for seven days. If the pigs continued to exhibit qualifying respiratory and depression scores after seven days, they were renotched and treated with enrofloxacin per label and another seven days were allowed to elapse. The enrofloxacin could then be repeated one more time, for a total of three retreatments. The pigs were maintained on unmedicated feed for the first 21 days of the study. Any pig that was pulled into a hospital pen was recorded and was kept on test. Any pigs that died or were euthanized were recorded. Initial weight was included as a covariate in the analyses of weight gain and ADG, which were analyzed by a linear Mixed Model approach. Percent retreatments, percent moved to hospital pen and percent mortality were defined as binary variables and analyzed using a Generalized Linear Mixed Model (GLMM). If the interaction of treatment-by-gender was significant at the 5% level for any parameter, a separate analysis was preformed for that parameter. (SAS® Cary, NC, USA).

Results

Pasteurella multocida, *Haemophilus parasuis* and *Streptococcus suis* were isolated from the pneumonic lung tissue samples of pigs that died or were euthanized. Piglets were PRRS positive at enrollment and at study day 33. Percent mortality, percent pulls to a hospital pen and ADG were significantly improved in the Draxxin treated pigs. The rate of death due to SRD in the control group was almost twice that of the Draxxin group.

Table 1. Results Summary

	Draxxin	Saline
ADG days 0-54 (kgs)	0.359[a]	0.341[b]
ADG days 0-152 (kgs)	0.655[a]	0.641[b]
% pulls to hospital pen	3.04[b]	7.18[a]
% retreatments gilts	40.5[b]	56.9[a]
%retreatments barrows	51.2	55.4
% mortality	16.8[b]	21.4[a]

Different superscripts within a row significantly different at p<0.05

Table 2. Cause of Death Summary

Cause of Death	# Saline	# Draxxin
CNS	1	2
CNS – SRD	21	22
GIT Torsion	5	2
LAME		2
Naval Ill		1
Rectal Prolapse		1
SRD	89	48
Septicema – SRD	1	2
Septicemia	3	11
Grand Total	**120**	**91**

Discussion

Due to the reduction in percent mortality, 29 more pigs were available to market in the Draxxin group. The improvement in ADG over the 152 day trial period made 2.07 more kilogramsper pig available at marketing. This study demonstrates that controlling SRD in pigs at weaning with Draxxin brings value by reducing mortality, morbidity and by increasing kilograms of pork available for market.

References

1. King et al. 2013.!Proceedings American Association of Swine Veterinarians 2013 poster 97
2. Garlaite et al. IPVS 2008 poster P05.036

C.40

Influenza prevalence within coordinated swine production systems

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Introduction

Influenza viruses are a common cause of respiratory disease in swine. Multiple serotypes of influenza A (IVA) are known to infect pig populations and often multiple-serotypes infect populations at the same time. We have demonstrated that it is routine to identify IVA in growing pigs without appreciable clinical disease[1]. Little is known about the reservoirs of IVA in pig populations; specifically what role does the breeding herd and replacement breeding stock play in maintaining IVA. We report the preliminary results from four closed, multi-site swine production systems that were sampled monthly across all phases of genetic multiplication and commercial weaned pig production.

Materials and Methods

Four production systems were selected that represented different geographic regions and local swine herd density (Table 1). Each production system consisted of six sites. One site dedicated to the breeding and farrowing of replacement breeding stock (MF), one site dedicated to growing replacement gilts (GDU), and four sites dedicated to breeding and farrowing commercial sows (BTW). In two of the systems replacement animals for the MF were raised on the MF site and in the other two systems they were raised at the GDU with the replacement females for the BTW farms.

Table 1. Site Locations and Local Swine Density

	System 1	System 2	System 3	System 4
MF	Illinois High	Illinois High	Nebraska High	Oklahoma Low
GDU	Illinois High	Illinois Moderate	Nebraska High	Oklahoma Low
BTW	Georgia Low	Illinois High	Nebraska High	Oklahoma Low

Thirty nasal swabs were collected each month from the following groups of animals: 7-8 month old gilts at MF (GMF), 21 day pigs at MF (WMF), 5-6 month old gilts at GDU (SEL), 7-8 month old gilts at BTW (GBTW), 21 day old pigs at BTW (WBTW). Results from samples collected between October 2012 and September 2013 are reported here. All samples were evaluated using qPCR. Positive samples were subjected to HA and NA typing and full genome sequencing.

Results

IVA was detected in every month of the study. Site level prevalence was 74% in the 212 samplings (>=1 swab positive). Sample set prevalence (>1 positive/30 swabs per age group per site) of IVA was 60% GMF, 68% WMF, 53% SEL, 53% GBTW and 83% WBTW. Multiple HA and NA types were detected in each of the systems across time and in specific sample sets. Across the four states in the study INFA was detected in all but one month of sampling (OK, Jul 13) with peak animal level prevalence never exceeding 25% (Figure 1). Over time all phases of each production system were infected with IVA. Pending sequencing will further elucidate the role of lateral and vertical transmission of IVA in modern production systems.

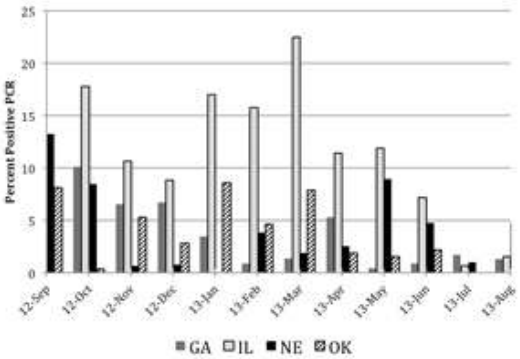

Figure 1. Sample level prevalence of IVA by farm location over time.

Conclusions and Discussion

These data are the first that describe the prevalence IVA in different phases of coordinated swine production systems over time. They suggest that the majority of farms are infected with IVA on a continuous basis, which agrees with our data from growing pig sites[1]. There appears to be multiple reservoirs of IVA in breeding herds and production systems based on nearly equal prevalence across multiple ages of pigs.

This project was funded by SJCRH-CEIRS.

References

1. Corzo CA, et al Active surveillance for influenza A virus among swine, midwestern United States, 2009–2011. Emerg Infect Dis [Internet]. 2013 Jun [Jan 20, 2014].

C.041

Genetic characterization of US PEDV and identification of a variant previously unrecognized in US swine

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Introduction

Porcine epidemic diarrhea virus (PEDV) was detected for the first time in US swine in April 2013[1] and has spread to 25 US states with 3310 cases confirmed as of February 16, 2014 (www.aasv.org/PEDV). However, little is known about the molecular epidemiology of PEDVs in US swine temporally and spatially. The purpose of this study was to characterize the genetic profile and diversity of PEDVs circulating in US swine since its emergence in April 2013.

Materials and methods

From October 2013 to February 2014, sequencing for PEDV S1 region (first 2.2 kb portion of the spike gene) were performed on 120 cases that tested positive by a PEDV specific real-time RT-PCR following the established procedures[2]. The full-length spike (S) gene sequences were determined on 5 cases using the Sanger method. The full-length genomic sequences were determined on 2 cases from different states using the next generation sequencing technology as described previously[2]. Sequence data were assembled and analyzed using the Lasergene 11 Core Suite (DNAStar). Sequences were compared to those of 10 additional US PEDV strains that were collected in May and June 2013 and 216 non-US strains with sequences available in GenBank. Phylogenetic analyses were performed using the MEGA5.2 software.

Results

Among 120 cases with PEDV S1 sequences determined, S1 sequences of 106 cases had 99.0-100% nucleotide (nt) identity to each other and to the PEDV strains identified earlier in US swine since April 2013 (hereafter designated as original US strain). In contrast, S1 sequences of the remaining 14 cases had only 92.4-93.8% nt identity to the the original US strains. They shared 99.6-100% nt identity to each other (hereafter designated as variant US strain). BLAST search showed that the S1 sequences of 14 PEDV variants had 99.4-99.6% nt identity to a Chinese PEDV strain CH/HBQX/10 (JX501318). Sequence alignment showed that these 14 PEDV variants had the same 129 nt changes within the first 1069 nt of the S1 region when compared to sequences of the same region of original US PEDV strains. In addition, the S1 region of these 14 PEDV variants had a total of 15-nt deletions (at 3 different locations with 1 nt, 11 nt and 3 nt deletions, respectively) and a 6-nt insertion in one location when compared to original US PEDVs. Interestingly, the majority of these 129 nt changes and the same deletion and insertion pattern observed among the 14 US variants were found in some PEDV strains previously identified in China, Korea and Europe.

Full-length S gene sequences of 5 selected variants had 99.7-100% nt identity to each other and had 96.1-96.5% nt identity to the original US strains. Whole genome sequencing demonstrated that 2 selected variants had 99.8% nt identity to each other and 98.8-99% nt identity to the original US strains.

Phylogenetic analyses using the partial or full-length S gene nucleotide sequences showed that the US variant strains clustered with some PEDV strains reported from China and were distantly related to the original US strains. When using the whole genomic sequences, the variant strains still formed a cluster distinct from the original US strains, but its relatedness to the cluster of the original strains was not as distant as that observed in S1 or S-gene based dendograms.

Conclusions and Discussion

Overall our data suggest that US PEDV is still at its early epidemic emergence and has not gone through significant genetic changes. Nonetheless, PEDV variants previously unrecognized in US swine was identified in this study. It is less likely that the PEDV variants originated from the PEDV strains previously identified in the US through a random mutation event. It seems more probable that more than one genotype of PEDV has been introduced into the US. To date, the PEDV variant had been detected in swine farms from 7 states and had been retrospectively detected in US swine at least from early October 2013. Additional retrospective testing is underway. Genetic relatedness and epidemiology of PEDV in US swine should be closely monitored.

Acknowledgements

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References

1. Stevenson et al. 2013. J Vet Diagn Invest 25:649-654.
2. Chen et al. 2014. J Clin Microbiol 52:234-243.

C.042

Effect of deoxynivalenol (DON; vomitoxin) intoxication on a PCV2 subclinical infection in piglets

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Introduction

Syndromes produced by consumption of high doses of mycotoxins are rarely reported today and scientific community is now attentive to the effects produced by intake of lower toxin-doses and their potential immunosuppressive effect[1]. Deoxynivalenol (DON) has strong immunomodulatory effects on swine and its ability to interfere in experimental immunization has been previously reported[2]. The aim of this work was to assess the effect of DON-exposure on the outcome of a porcine circovirus type 2 (PCV2) subclinical infection in swine.

Materials and Methods

Thirty-six two week-old piglets free from PCV2 infection and with low maternal anti-PCV2 Ab titers were distributed into four groups (n=9/group) according its weight and maternal anti-PCV2 Ab titers[3]. One week before challenge, half of the animals were fed with a DON-contaminated feed (4.3 ppm) whereas the others received a control one. On day 0, in each group, half of the animals were challenged with a PCV2b strain whereas the others were mock-infected (m.i.). Health status was daily checked and their weight was weekly recorded (average daily weight gain [ADWG] was subsequently calculated). Blood samples from all animals were weekly collected. PCV2 viral loads in serum were obtained by qPCR[3]. On day 28, all animals were sacrificed and both DON and PCV2 target-tissues were collected. Presence of PCV2 in tissues was assessed by *in situ* hybridization (ISH). In sera, both total IgA and IgG were measured by means of ELISA and anti-PCV2 antibody titers were assessed using IPMA[2, 3].

Results

Combined effects of DON and PCV2-infection are shown on tables 1 and 2. PCV2 was not detected by ISH in any of the tissues assessed (data not shown). No clinical signs or lesions compatible with PCV2-systemic disease were observed during the experiment as well as at necropsies.

Conclusions and Discussion

Obtained results indicated that a subclinical PCV2 infection was achieved in the PCV2-inoculated piglets. On the other hand, the ADWG underlined a deleterious combined effect of DON and virus. The measurement of the production of total IgA and IgG allowed confirming such combined effect, whereas no adverse effect was observed during the sole exposure to the contaminant or to the virus. The capability of DON to induce advancement in time of specific-Ab response (table 2) is in accordance with previous published works[2].

Table 1. Mean concentration of total IgA and IgG in sera samples at day 28 as well as evolution of AWDG between days 0 to 28.

Group	ADWG(±SD) (Kg)	IgA (±SD) (mg/ml)	IgG (±SD) (mg/ml)
DON/PCV2	0.18±0.02^{a}	0.65±0.10^{a}	8.21±1.21^{a}
control/PCV2	0.29±0.02^{b}	0.34±0.07^{b}	4.89±0.79^{b}
DON/m.i.	0.23±0.02^{a,b}	039±0.06^{b}	4.74±0.86^{b}
control/m.i.	0.25±0.02^{b}	0.34±0.03^{b}	3.92±0.49^{b}

Different letters in superscript within a column mean $p < 0.05$.

Table 2. Mean Log_2 PCV2 IPMA titers (±SD) and Log_{10} PCV2 viral load (PCV2 copies/ml of serum) through the study.

	Group	0	7	14	21
IPMA	**DON/PCV2**	3.4±1.9 ^{a}	1.9±2.3^{a}	6.5±1.6^{a}	9.2±1.8^{a}
	control/PCV2	3.4±1.9 ^{a}	0.5±1.9^{a}	6.1±1.9^{a}	8.8±1.3^{a}
	DON/m.i.	2.4±2.3 ^{a}	1.9±2.3^{a}	2.9±2.2^{b}	2.9±2.2^{b}
	control/m.i.	2.9±2.2 ^{a}	1.0±1.9^{a}	2.9±2.2^{b}	3.4±1.9^{b}
qPCR	**DON/PCV2**	ND*	5.5±2.1^{a}	6.4±0.7^{a}	5.5±0.5^{a}
	control/PCV2	ND	5.7±2.3^{a}	6.4±1.0^{a}	5.9±0.6^{a}
	DON/m.i.	ND	ND	ND	ND
	control/m.i.	ND	ND	ND	ND

Different letters in superscript within a column mean $p < 0.05$.
*ND= Not Detected.

Acknowledgments

This study was performed through a Transnational Access of the NADIR Project and was partially financed by the French ANR program DON&Co.

References

1. Oswald I et al. 2005. Food Add Contam 22:354–360.
2. Pinton P et al. 2008. Toxicol Lett 177:215–222.
3. Sibila M et al. 2012. J Comp Pathol 147:285-95.

C.043

Effect of pretreatment on detection of PRRSV in oral fluid by qRT-PCR assay

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Introduction

Collection and testing of oral fluid samples for disease surveillance is increasing in swine. However, it has been recognized that the oral fluid matrix presents a challenge to PCR detection, most often in the form of false negative results (1). The objective of this study was to evaluate the effect of specific factors on PRRSV qRT-PCR performance.

Materials and Methods

A total volume of 2.2 liters of oral fluid was collected from a group of ~250 5-week-old pigs that had been vaccinated 15 days prior with a modified-live PRRSV vaccine (Ingelvac PRRS® MLV, Boehringer Ingelheim Vetmedica, St. Joseph, MO). In the laboratory, the samples were pooled, stirred continuously while aliquoted into 25 ml volumes, and stored at -80°C.

Factors evaluated in the study included (Figure 1):

- Temperature 1 - samples were thawed at 4°C or 25°C for 24 hours
- Diluent - samples were diluted 1:2 with either Trizol® or nuclease-free water (total volume = 1.5 ml)
- Sonication - yes vs no. If "yes", samples were sonicated in a water bath sonicator for 10 minutes.
- Temperature 2 - temperature at which sonication was conducted (4°C vs 25°C). If sonication ="no", sample was held at either 4°C or 25°C for 10 minutes.
- Temperature 3 - temperature at which the sample was held (4°C vs 25°C) until the PRRSV RT-PCR assay was performed.

As demonstrated in Figure 1, the experiment allowed for direct comparisons of all the factors listed above. Overall, there were 32 treatments with 4 samples each, along with 1 negative control per treatment, i.e., 32 negative controls per replicate. This process was replicated 5 times and produced a total of 800 PRRSV RT-PCR results. Temperature was monitored throughout the process using NIST-certified thermometers that recorded the maximum and minimum temperatures.

Samples were tested at the Iowa State University Veterinary Diagnostic Laboratory. Viral RNA was extracted using the 5X Ambion® MagMAX™ Viral RNA kit. PCR was performed using the Tetracore EZ-PRRSV™ MPX 4.0 RT-PCR. Binary test outcomes were analyzed using logistic regression with SAS® Version 9.3. All factors and their interactions were considered in the model. Insignificant effects were excluded from the final model.

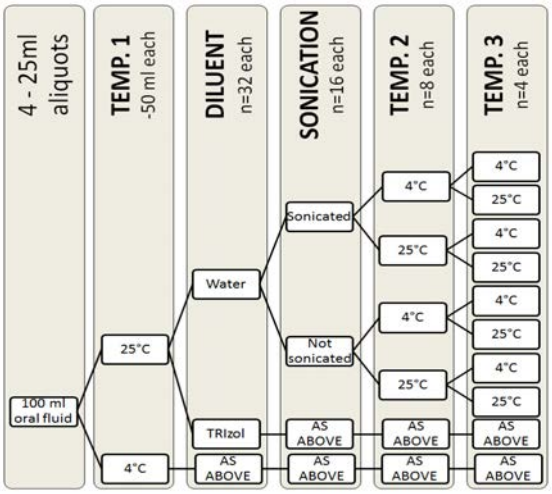

Figure 1. Flow chart summary of experimental design

Results

Testing of 640 known positive samples after treatments produced 85 false negatives (86.7% sensitivity). Testing of 160 negative controls produced 1 false positive (99.4% specificity). A summary of the statistical analysis on the qualitative (pos/neg) results is presented in Table 1. Odds ratio estimate for the effect of temperature 1 (25°C vs 4°C) was .265 (.145, .447), suggesting a false negative result is more likely when oral fluid is thawed at 25°C.

Table 1. Significant effects (outcome = positive PCR)

Effect	P-value
Temperature 1	<0.0001
Diluent	0.5953
Temperature 2	0.2583
Interaction (Diluent & Temp. 2)[1]	0.0428

[1]All 4 combinations of diluent (TRIzol or water) and temperature 2 (4°C vs 25°C) were found to have a significant effect on the outcome.

Conclusions and Discussion

This study identified that specific pre-extraction factors can significantly impact PRRSV qRT-PCR performance. Additional research is needed to continue the process of assay improvement.

Acknowledgments

This project was funded in part by USDA:NIFA:AFRI Animal Health and Disease

References

1. Chittick et al. 2011. J Vet Diagn Invest 23:248-253.

C.044

PRDC - Validation of a new diagnostic procedure for the diagnosis of PRDC in pigs

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Introduction

Respiratory disease is one of the most important diseases in pigs worldwide (1). Porcine Respiratory Disease Complex (PRDC) is the term for pneumonia caused by multiple pathogens. PRDC has been found to cause morbidity ranging from 30-70% and mortality ranging from 4-20% (1, 2, 3). *M.hyo*, PCV2, SIV and PRRSV are all pathogens that are often diagnosed as primary pathogen of PRDC in swine herds (5, 6).!

In Denmark the laboratory diagnosis of PRDC is routinely performed by a "Porcine Respiratory disease PCR package" which includes testing of 1-3 lung samples from each herd by single tube real-time PCR/reverse transcriptase-(RT-)PCRs specific for *Mycoplasma Hyopneumonia* (*M.hyo)*, Porcine Circovirus type 2 (PCV2), Swine influenza virus (SIV) and Porcine reproductive and Respiratory Syndrome virus (PRRSV). The aim of the present study was to compare the qualitative and quantitative results of the PCR package performed on lungs and on lung swabs.

Materials and Methods

The study compared the outcome of real-time PCR/RT-PCR performed on pieces of lung tissue samples in parallel with swabs obtained from the same lung lobe. The lungs included in the study were submitted from Danish herds for the diagnosis of PRDC. The standard sample consisted of lung tissue homogenized by bead-beating on TissuelyzerII (QIAGEN) prior to DNA and RNA purification. The other sample for parallel testing was generated by swabbing the same lung lobe with a standard cotton swab. The swab sample was collected in 2 mL of 0.9% saline with 0.1% peptone (peptone). DNA and RNA from both samples were purified using RNeasy Mini Kit and QIAamp DNA Mini kit, respectively (QIAGEN) and tested by real-time PCR/RT-PCR for *M.hyo*, PCV2, SIV and PRRSV. The use of peptone for collection swabs was validated prior to the study and no indication of PCR inhibition was observed. The results were analyzed qualitatively and quantitatively (comparison of Ct-values) for each sample and on herd level.

Results

A total of 99 paired lung and lung swab samples from pigs with respiratory symptoms were collected and tested. The samples originated from 44 herds. The qualitative results of the real-time PCRs/RT-PCRs of the diseased pigs are listed in table 1. A kappa analysis revealed results between 0.71 and 1.00 when comparing the two sampling methods. The quantitative results were graphically illustrated and analyzed by paired t-test (data not shown).

Table 4. The number of positive and negative samples and herds for each of the pathogens: *M.hyo*, PCV2, SIV, PRRSV Type 1 and PRRSV Type 2.

		Tissue		Swabs	
		+	-	+	-
M.hyo	Samples	28	71	25	74
	Herds	16	28	14	30
PCV2	Samples	28	71	34	65
	Herds	17	27	21	23
SIV	Samples	25	74	22	77
	Herds	13	31	11	33
PRRSV	Samples	11	88	9	90
Type 1	Herds	7	37	5	39
PRRSV	Samples	6	93	6	93
Type 2	Herds	3	41	3	41

Conclusions and Discussion

A good agreement was found between the qualitative results of test of the paired samples when compared by kappa analysis but the correlation was not perfect. The quantitative results revealed generally lower threshold cycles (CTs) for the lung tissue samples compared to the lung-swab sample by that indicating that lung tissue is more sensitive than swabs. Ongoing test on alternative swab material and adjusted swabbing technique may increase the sensitivity of this procedure which is cheaper and less resource demanding than tests on lung samples where a homogenization step is needed.

Acknowledgments

Technicians from *National Veterinary Institute* virology and diagnostic department and *Pig Research Centre*

References

1. Opriessnig T et al. 2011. Animal Health Research Reviews;12(02):133-48.
2. Harms PA et al. 2002. Journal of Swine Health and Production;10(1):27-32.
3. Kim J et al. 2003. The Veterinary Journal. 11;166(3):251-6.
4. Fachinger V et al. 2008. Vaccine;26(11):1488-99.
5. Fablet C et al. 2012. Vet Microbiol;157(1):152-63.
6. Hansen MS et al. 2010. J Comp Pathol; 143(2):120-31.

C.045

Influenza antibody detection in swine using the influenza A multispecies ELISA – experimental study

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Introduction

The continuously changing nature of influenza A in swine has caused subtype specific ELISAs to become less sensitive as new H1N1 or H3N2 swine influenzas evolve and become dominant (1,2). However the commercial AI multiS-Screen ELISA (AI-MS or NP ELISA) which recognizes a highly conserved influenza A nucleoprotein (NP) has been validated as a useful influenza antibody assay with swine sera, determined to be effective for the detection of antibodies for heterogeneous influenza viruses (3). The objective of this study was to further evaluate these commercially available serologic ELISA assays for influenza antibody detection in swine experimentally inoculated with a contemporary H1N1 or H3N2 influenza virus.

Materials and Methods

The study involved 82 piglets housed under BSL-2 conditions. A subset (n=28) was vaccinated twice with a multivalent IAV vaccine (FluSure XP™, Zoetis™). Thereafter, pigs were either intratracheally inoculated with A/Swine/OH/511445/ 2007 γ H1N1, or A/Swine/Illinois/02907/2009 Cluster IV H3N2, or served as negative controls. Blood was drawn from each animal by jugular venipuncture on DPI -42, -21,-7, 0, +7, +14, +21, +28, +35 and +42. All samples were aliquotted and frozen the day of collection, in a -20°C freezer. At study completion, one aliquot of all sera samples were randomized and submitted to the ISU Veterinary Diagnostic Laboratory, and the samples were assayed equivalently (same day, same technician) according to manufacturer's instructions, for influenza antibody detection by the (a) SIV H1N1 ELISA (IDEXX, Westbrook ME), (b) SIV H3N2 ELISA (IDEXX, Westbrook, ME), and (c) AIV MultiS-Screen ELISA (NP ELISA) (IDEXX, Westbrook, ME). Receiver operator characteristic analyses were performed by MedCalc® 11.3.5 using the ELISA assay results, where negative status was established by only unvaccinated unchallenged animals, and only challenged ≥ DPI +7 animals were used for influenza positive status.

Results

Using a total of 699 known status samples (279 negative, 420 positive), the AI multiS-Screen ELISA (NP ELISA) was superior to both the H1N1 and H3N2 commercial ELISAs in detecting influenza antibodies to both the H1N1 or H3N2 challenge viruses in vaccinated and unvaccinated animals. From MedCalc®, using a cut-off of S/N ≤ 0.60, the Se and Sp of the NP ELISA were 95.5% (95% CI: 92.2-96.7%) and 99.6% (95% CI: 98-100%) respectively. The performance of the H1N1 and H3N2 ELISAs, at the cut-off of S/P = 0.4 within the serotype specific treatment groups (n=210 positive, 279 negative, including challenged, vaccinated animals) were Se=28.57% and Sp=100% and Se=22.86% and Sp=99.64%, respectively.

Conclusions and Discussion

The NP ELISA is a sensitive and specific assay for the detection of contemporary swine influenza antibodies and should be considered the latest iteration of influenza A antibody ELISAs.

Acknowledgements

This study was supported by Pork Checkoff® funds through the National Pork Board (#09-193) and Zoetis™. Virus isolates were provided by Dr. Amy Vincent (USDA, NADC, Ames, IA) and Dr. Marie Gramer (University of Minnesota, St. Paul, MN).

References

1. Van Reeth et al., 2012. In: Zimmerman J et al., (eds). Diseases of Swine (10th ed). pp. 557-571.
2. Barbé F. et al., 2009.J Vet Diagn Invest 21:88-96.
3. Ciacci-Zanella et al., 2010. J Vet Diagn Invest 22:3-9.

C.046

Retrospective observation on efficacy of 3FLEX compare to conventional vaccination scheme in wean to finish period under Thai field conditions

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Introduction

PRRSv, PCV2 and *Mycoplasma hyopneumoniae* are the three pathogens which play major role in PRDC which is a major problem in swine industry worldwide. Although vaccination against 3 pathogens is commonly used in pig industry in Asia, conventional separate vaccination scheme faces the disadvantage in terms of labor effort, piglets stress and vaccination program design. The objective of this retrospective study was to evaluate the efficacy of 3FLEX vaccination scheme compared to conventional program in fattening period under Thai field conditions as demonstrated by other authors. [1,2]

Material and Methods

This retrospective study was conducted on a multi-site farm with 2,300 sows. Sow herd were PRRS stable through routinely mass vaccination using Ingelvac PRRS MLV four times a year. Piglets are weaned at 26 days old and moved to wean to finish facilities. The former vaccination program to control PRDC in piglets was: Ingelvac PRRS MLV at 14 days, Ingelvac M.hyo and Ingelvac CircoFLEX at weaning age. The current vaccination program is 3FLEX (vaccine with PCV2, Mhyo & PRRS in a single injection, Boehringer Ingelheim, St Joseph Missouri USA) at 14 days. Weaning pigs in each batch is around 400-450 pigs. Total of 8 batches applied with separate vaccination program and their performance were compared to 10 batches with 3FLEX program. The performance parameters used in these observations are mortality rate, ADG, FCR and slaughter weight in wean to finish period. Data was evaluated as before and after applying two sample t-test and also using standard statistical process control (SPC) method using Statistica version 8.0 for weight out.

Results

The results of production parameters are shown in Table 1. In both groups, the performance of wean to finish period have no statitiscal significant differences with the exception of ADG. This is most likley due to a genetic improvement implemented during the evaluation. The market weight peroformance is shown in figure 2.

Table 1. Production parameter comparison between Conventional and 3FLEX

	Conventional Mean (SD)	3FLEX Mean (SD)	p.value
Production batches	**8**	**10**	
Ending weight (Kgs)	**112.12 ± 2.975**	**113.48 ± 3.399**	**0.3687**
Days in house	**148.75 ± 4.676**	**145.20 ± 4.392**	**0.0995**
ADG (g/d)	**684.6 ± 0.019**	**703.8 ± 0.019**	**0.0426**
FCR	**2.42 ± 0.066**	**2.38 ± 0.042**	**0.1287**
% Loss	**5.30 ± 0.031**	**4.87 ± 0.029**	**0.7507**

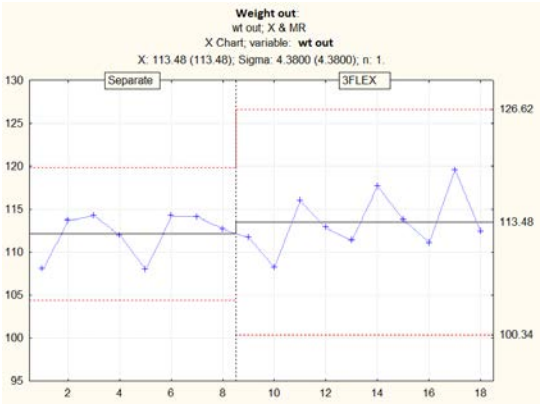

Figure 2. SPC chart of Market Weight of the 2 periods.

Conclusions and Discussion

The 3FLEX vaccination scheme can reduce labor effort and piglets stress with no negative effects in growing pig performance and leading to potentially better performance. 3FLEX fits to modern pig production management in today's pig production industry. In this case, the ADG improvement in the 3FLEX group could have also been influenced by genetic improvement.

Reference

1. Jose Angulo 2010,21st. IPVS congress O.236,p.268
2. Sopon Kongtes 2013,6th. APVS congress P.210

Farm case report: comparison of mortality rate in a farm that uses genotype II PRRS vaccine strain & genotype I PRRS vaccine strain at different period of time

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Introduction

For many years since its discovery PRRSV has become one of swine primary health concern in piggery farms causing great economic losses. Vaccination with PRRS MLV has become a necessity as vaccination can reduced feeder pigs mortality significantly (Guerzon et al 2008, Policarpio et al 2010). However, in farm with MLV PRRS vaccine program in placed where the performance is not satisfactory to bring the business at profitable level, thus, changing of vaccine is a common practice.

Materials and Methods

The farm is 500 sows, farrow to finish in conventional house farm operation located at Region 4A in Philippines. PRRSV vaccination using Genotype II vaccine strain is in place for many years prior to shifting to PRRSV Genotype I vaccine strain on June of 2012. Prior to shifting the farm experienced high feeder pig mortality. Similar vaccination program was applied in both vaccine strains for the breeders and feeder pigs, which is mass vaccination every 4 months and at 28 days old respectively. Prior to shifting, two laboratory tests were done at same time to confirm diagnosis of the disease and suspected co-infection; serology of serum samples cross-sectional tested for PRRSV Elisa (Civtest Suis PRRS E/S, Hipra Diagnos) and Lung Tissue samples tested for Haemophilus parasuis by PCR (Hipra Diagnos).

Results

PRRSV serology test result revealed un-stable breeder profile while high challenge was observed in feeder pigs (Figure 1). PCR test for Haemophilus parasuis was positive confirming the co-infection.

To compare the pre-weaning and post weaning mortality rate of the period the farm was vaccinating with Genotype II PRRS vaccine strain and after the farm shifted to Genotype I PRRSV vaccine strain (Amervac® PRRS, Hipra), the mortality rate dramatically improved by 119% in pre-weaning and 40% in post-weaning. In overall the mortality rate improved by an impressive 70% (Table 1).

Conclusions and Discussion

Though the overall picture of the farm is not yet at the ideal level as other production parameters has yet to yield desirable performance, the shifting to Genotype I PRRSV vaccine strain (Amervac® PRRS, Hipra) however, yielded dramatic improvement in the feeder pigs health situation. With the improvement in mortality rate the farm can expect better efficiency and would allow the farm management to give more attention to other aspect of operation to help further improve the farm overall performances.

Figure 1. PRRSV Serology result (Civtest suis PRRS E/S) April 2012

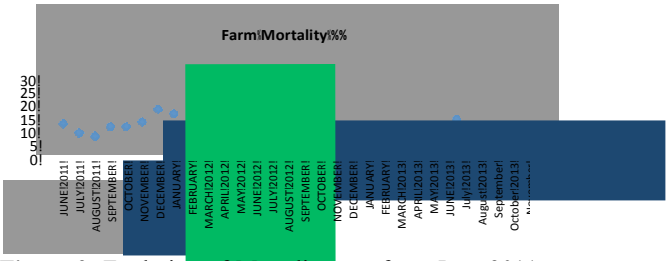

Figure 2. Evolution of Mortality rate from June 2011 to Nov 2013. Vertical broken line indicates timing of change in vaccine strain.

Table 1. Comparison of Mortality rate

	Pre-Wean Mort. %	Post-Wean Mort. %	Overall Mort. %
Genotype II Vaccine Strain (June'11 to June'12)	**7.18%**	**7.47%**	**14.65%**
Genotype I Vaccine Strain (July'12 to Nov'13)	**3.28%**	**5.32%**	**8.60%**
% Improvement	**119%**	**40%**	**70%**

Acknowledgments

BAPBI Farm & Hipra Diagnos (Hipra Philippines)

References

1. Guerzon et al, IPVS 2008
2. Policarpio et al, IPVS 2010

Glässer disease and governmental regulations: diagnostic problems and therapeutic restrictions

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Introduction

Glässer's disease caused by *Haemophilus parasuis* (HPS) occurs in all major swine-producing countries, leading to severe herd problems especially in 4 to 8 week old piglets [1]. Although there are no clinical signs specific to Glässer's disease, HPS can cause respiratory signs. In The Netherlands, HPS was cultured at necropsy most frequently from lungs of pigs diagnosed with Glässer's disease [2]. The diagnosis is traditionally made via a combination of clinical signs, lesions at necropsy and bacteriological culture. Due to the fastidious nature of HPS, culture results are often negative. Furthermore, serological diagnosis of Glässer's disease is inconsistent and inaccurate [3].

In The Netherlands, antimicrobial growth promoters (AMGP) have been banned and the prophylactic use of antibiotics is forbidden. Herd therapies with antibiotics are also restricted. Since then, veterinarians can be confronted with Glässer-like signs at herd level with negative bacteriology with exclusion of other possible infections and without the use of preventive antibiotic treatment as a therapeutic option.

This study describes a structured diagnostic and therapeutic approach to herd respiratory problems, suspected as being due to HPS infection, including two parameters to measure the effect of passive HPS immunization of piglets.

Materials and Methods

The procedure on a farm with coughing pigs and higher than national average mortality in piglets pre- and post-weaning:

1.A coughing index [4] was determined each week on batches of 3 to 8 week old pigs (Figure 1).

2.Curative antibiotic treatments were recorded. (Table 1)

3. The role of PRRSv, *Mycoplasma hyopneumoniae*, *Bordetella bronchiseptica*, *Pasteurella multocida* and Streptococci was excluded at necropsy and macroscopic lesions possibly caused by HPS infections but with negative bacteriological results were found.

4. Piglets were passively protected by vaccinating the sows with Porcilis Glässer®. The coughing index and antibiotic treatments were compared with previous scores in pigs which had been born to sows not vaccinated against Glässer's disease. (Table 1, Graph 1)

Results

Table 1: Coughing index and antibiotic treatments

	n. obs ervations	average coughing index	% units treated with antibiotics
not HPS protected piglets	24	1.19 (0.51 - 3.97)	54%
HPS protected piglets	11	0.36 (0.13 - 1.05)	0%

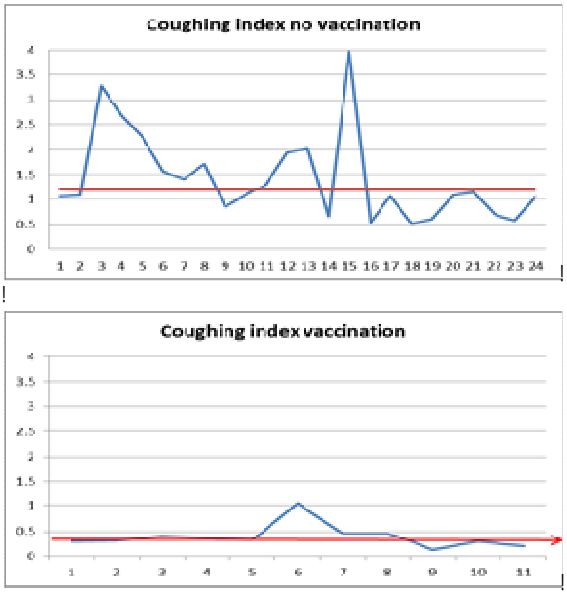

Figure 1. Coughing index per group (passive protection: N/Y) (Index per observation including the average score per the group)

Conclusions and Discussion

-Negative bacteriological examinations are often the reason for failing to fulfill all the diagnostic criteria for Glässer's disease. Prophylactic antibiotic treatments to prevent Glässer-like symptoms are not allowed. Nevertheless, the veterinarian must act within the Dutch regulations which order him to protect the well-being of piglets and prevent damage to health and economic loss.

-It is justifiable to institute a vaccination program even if not all the diagnostic criteria for Glässer's disease are fulfilled, and other possible causes are ruled out by necropsy and serology.

-In the case of HPS infections causing respiratory problems, the coughing index appears to be a suitable parameter by which to determine the effect of the passive HPS immunization of piglets. The coughing index on the farm in this study was reduced by 70% and antibiotic treatment reduced to nil when the sows were vaccinated with Porcilis Glässer®.

References

1. Aragon V. Disease of Swine 10th edition p.761-779
2. Esch E. 2009. GD Varken 1-2009
3. Oliveria S.: 2004. vet.microb. vol.99 p.1-12
4. Peter G.G.: Elsevier Handbook of Pig Medicine 200

C.049

The effect of supplying Lianol® Colostro to just born piglets: Mortality and medicine consumption

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Introduction

The intake of colostrum just after birth is essential for both health, growth and survivability: a reduced intake leads to an inadequate transfer of passive immunity and an increase in neonatal infections (1). Previous research has demonstrated that Lianol® Colostro, based on a highly digestible fermented potato protein, can increase the immunoglobulin G (IgG level in just born piglets (2). A better colostrum intake and hence a better passive immunity will results in a lower medicine use in the early stage of life.

The aim of the present study is to evaluate the effect of Lianol® Colostro on the mortality and medications use from birth to weaning in piglets.

Materials and Methods

The study involved 806 piglets; 71 litter. The piglets were divided over 2 groups based on sow parity; a control group (n=413 piglets) and a Lianol® group (n=393 piglets). The Lianol® group piglets received two doses of 1 ml of Lianol® Colostro into the mouth: the first immediately after birth, the second at the end of the farrowing day (8-12 hours later).

For each litter, the daily pre-weaning mortality was recorded. A distinction in the cause of death was made between crushing, starvation, diarrhea or other causes (1). Furthermore, medications use was monitored throughout the whole testing period, recording type of drug, number of administrations and volume used.

Data concerning mortality were analysed using a generalized estimating equation using PROC GENMOD with a Poisson model (SAS 9.2, SAS Institute Inc., Cary, NC). Data about medication use were analysed using a t-test considering the amount of active substance per head administered to piglets during the study.

Results

Mortality data are shown in Table 1. Starvation was the cause of death that shows a statistically significant difference between groups (P<0.0001). The overall pre-weaning mortality decreased numerically from 10.39% to 7.98% in the control and Lianol® group respectively. Furthermore, use of antibiotics and anti-inflammatory drugs was significantly reduced in Lianol® group (P=0.041 and 0.012 respectively, Figure 1).

Table 1. Relative Risk (RR) for piglets mortality from birth to weaning. CI: confidence interval

Reason of mortality	LS-MEANS (%) Lianol	Control	RR (95% CI)	*P* value
Crushing	3.33	3.62	1.09 (0.84-1.39)	0.512
Starvation	0.22	2.05	9.31 (4.43-19.6)	<.0001
Diarrhea	1.41	1.39	0.98 (0.66-1.46)	0.930
Other causes	3.02	3.33	1.10 (0.85-1.43)	0.457

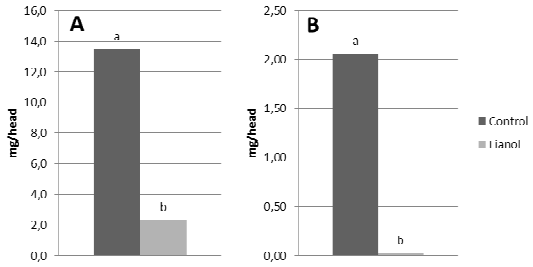

Figure 1. Mg of active substance per piglet from birth to weaning. A: Antibiotics; B: Anti-inflammatory.

Conclusions and Discussion

This research suggests an improved health status in piglets that received Lianol® Colostro at birth. This improvement is probably due to a greater colostrum intake and to a better transfer of passive immunity from the sow (2); previous work (2) demonstrated a significant effect of Lianol® Colostro on the IgG level in just born piglets. The current study finds that the administration of Lianol® Colostro can reduce pre-weaning mortality in piglets. Statistically differences were found in the mortality due to starvation (2.05% in the control compared to 0.22% in the treated group), considered one of the major causes of neonatal death (3). The present study confirms that starvation could be reduced by administrating Lianol® Colostro at birth. A significant benefit on the medicine use pre-weaning was also demonstrated.

References

1. Edwards SA: 2002Livest. Prod. Sci. 78 : 3–12.
2. Smulders D and Kanora A: 2012, Proceedings IPVS 2012, 555.
3. Kirkden RD et al.: 2013J. Anim. Sci. 91: 3361-3389.

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C.050

Evaluation of primary cut weights and cut yields of immunologically castrated pigs slaughtered 7.5 weeks after immunization under commercial conditions

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Introduction

The Mexican pig market is very diverse: about 44% of the hogs raised are sold as live pigs, 20% exported, 16% paid based on carcass quality and 20% processed in packing plants for the domestic pork demand. This situation requires producers to adapt farm management to fulfill the customer demand. For example, in Central Mexico the hog market demands barrows (physically castrated; PC) and gilts, and producers are typically paid according to *in vivo* weights. Immunological castration using Improvac® (Zoetis) is an alternative to control boar taint in commercial pig farms. Production of immunized boars (IB) has proven associated benefits in pig performance (e.g. improved feed conversion rate) and carcass characteristics (e.g. increased primal cut yields when compared to PC) [1,2]. The benefits in primary cut yields seen in experimental settings need to be confirmed under commercial conditions. The aim of this study was to investigate the effect of an extended Improvac® program (longer second dose to slaughter interval) on primary cut yields under field conditions.

Materials and Methods

A total of 436 IB and 397 PC were raised and finished under commercial production conditions. Boars received the first dose of Improvac® at 11 weeks of age and the second four weeks later at 15 weeks of age. All pigs were harvested at 22.5 weeks of age (7.5 wks after the 2nd dose). Prior to harvest, all pigs (IB and PC) were weighed and skin tattooed twice (loin and ham) for further follow up. Mean weight per group was calculated and sixty animals per group/sex were selected for individual follow up at the packing plant. The pigs selected were those whose final weight was the closest to the mean. Selected animals were shipped to a commercial slaughter plant located in Central Mexico and slaughtered according to the standardized approved procedures. To comply with standard practice of the farm and packing plant, pigs were slaughtered with 1 day difference between groups, and results were adjusted accordingly. After slaughter, individual hot carcass weight was recorded; carcasses were split at mid line, stored in the chilling room for 24h, and then transported to a commercial cutting room where the right half carcass was processed according to the packing plant's standard carcass cut out procedures[3]. Carcass skin was removed before the carcass cut out in shoulder, ham and belly; cuts were weighed individually.

Finally, primary cuts from each were obtained and individually weighed. Yields were expressed as hot carcass weight percentage (cold carcass weight not recorded). Data were analyzed using the Mixed Procedures of SAS, sex was set as a fixed effect and statistical differences were declared at a <0.05 P-value.

Results

Final farm weights of animals selected were 103.89 vs. 100.83 kg (SEM=1.492, P=0.15; IB vs. PC), whereas hot carcass weights were 85.47 vs. 82.45 kg (SEM=1.244, P=0.09; IB vs. PC). Due to market needs of the commercial packing plant, hams couldn't be further processed. Weights and carcass yields for the primary cuts are shown in Table 1.

Table 1. Carcass yields of Immunized boars (IB; n=60) *vs,* Physically Castrated (PC; n=60).

Item	IB	PC	SEM	P <
Shoulder, kg	5.66	5.40	0.130	0.16
Trimmed shoulder, kg	5.07	4.67	0.082	0.001
Trimmed shoulder yield, %**	11.87	11.34	0.104	0.001
Belly, kg	12.33	11.65	0.310	0.13
Trimmed Bone-In Belly, kg **	4.44	4.17	0.076	0.02
Trimmed Bone-In Belly yield, %**	10.40	10.12	0.102	0.06
Trimmed Bone-In Loin, kg	4.49	4.14	0.072	0.001
Trimmed Bone-In Loin yield, %**	10.50	10.06	0.095	0.002
Trimmed Collar, kg	2.31	2.13	0.040	0.002
Trimmed Collar yield, %	5.41	5.20	0.067	0.03
Ham§, kg	11.74	11.25	0.201	0.10
Ham§ yield, %	27.43	27.25	0.191	0.51

** Statistically significant at P<0.05; § Skin-on, Bone-In Ham

Conclusions and Discussion

Male pigs (IB) produced under an extended Improvac® program than common practice (slaughtered 7.5 weeks post-2nd dose, instead 3-4 weeks) had heavier live and carcass weights than PC. Under the commercial conditions of this study, carcass cut out characteristics were consistent to those reported in the literature. Cuts from the anterior carcass portion were heavier and had increased yield after trimming than PC. Improvac® is a flexible management tool that may help swine producers to improve the carcass quality of male pigs obtaining a significantly higher amount of meat.

References

1. Dunshea *et al.* 2013. Animal. 7:11, 1769-1778.
2. Boler *et al.* 2012. J. Anim. Sci. 90:334-344.
3. NMX-FF-081-SCFI-2003

C.051

The evaluation of narasin in grow-finish swine diets

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Introduction

Ionophores have been used in both cattle and poultry production; however, use of ionophores in swine diets in the US has not been approved until recently. Narasin has been approved in the US to be fed to grow-finish swine to improve feed conversion and/or average daily feed intake. However, little information is known about the effectiveness of such product. Therefore, the objective of the present study was to determine the impact of feeding narasin to grow-finish swine on feed conversion and feed intake.

Materials and Methods

A total of 1,180 pigs were placed on study at approximately 27.0 ± 2.6 kg in a wean-to-finish barn. Pigs were sorted by gender with the lower 10% being sorted off and the rest of the pigs were gate cut into a total of 48 test pens. Pens were randomly allocated to one of three treatments (control or narasin (13.6 g/ton (S1), Skycis®, Elanco, IN) or narasin 18.1 g/ton (S2). Treatments were randomly assigned within a block. Narasin was fed from d0 (27 kg average pig weight) to d105 (89 kg average pig weight). Diets consisted of 20% dried distiller's grain with solubles (DDGS) from d0-42, 10% DDGS from d42-84, and then 0% DDGS from d84-market. Diets met or exceeded NRC recommendations. Pens were fed with a Howema feed system to record feed intake. Pen weights and feed intake information were collected at each diet phase change. Pigs were vaccinated for PCV and given standard treatments for illness throughout the trial. Average daily gain and feed conversion was recorded up to the time when each pen of pigs is completely marketed. Data that was collected and analyzed included: wean-to-finish mortality (non-value pigs), full value pigs, non-full value pigs/culls (sub-standards at weaning and culls sold to secondary market), finishing ADG (pens), finishing feed intake and feed conversion (pens), and days to 198 lb. hot carcass weight. Data was analyzed as an incomplete block design using PROC Mix and starting average pig weight was used as a covariate (SAS® Enterprise Guide 4.3, Cary, NC, USA).

Results

The feeding of narasin resulted in no significant differences in ADG, ADFI, or G:F from d 0 to 21 of the study. However, feeding narasin significantly improved G:F from d 21 to 42 (0.431, 0.443 and 0.440, respectively; $P < 0.003$). From day 42 to 63, there were no significant differences in ADG, ADFI or G:F. However, narasin inclusion significantly improved G:F from d 0 to 63 (0.428, 0.435, 0.434; $P < 0.01$). Overall, the feeding of narasin from d 0 to 42 improved G:F when compared to control-fed pigs (0.466, 0.473, and 0.471, respectively; $P < 0.005$).

Conclusions and Discussion

In this study, pigs were followed after narasin was removed. It was noted that after narasin was removed, there were no significant differences between treatment groups. Based on this information, the feeding of narasin provides value on improving the utilization of nutrients while it is in the diets.

In addition, this study indicates that either the longer feeding of narasin or the reduction in DDGS resulted in a reduced response. The authors believe that the reduction in performance from days 42-63 is due to the removal of DDGS.

However, overall, there was a signficant improvement in feed conversion when feeding narasin in the grow-finish period.

Acknowledgments

Elanco Animal Health, Indianapolis, IN

C.052

Development of a slaughter house monitoring system as a tool for mycotoxins detection in swine

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Introduction

The most common mycotoxins in pigs around the world are: aflatoxin, fumonisin, ochratoxin, trichothecenes and Ergot (1). From a health point of view these mycotoxins are ingested gradually, the pigs are eating sublethal amounts of the toxin for a prolonged period of time. These affect significantly the health of the animals and this is manifested in a decline in their productive efficiency. An indispensable tool in the control of mycotoxins intoxication is a reliable diagnosis in target organs. The examination of lesions at slaughter houses is very useful, but more organs need to be included to look for these lesions (2). The aim of this work is to present a monitoring system (quantitative and qualitative) for those lesions related to mycotoxins in pigs.

Materials and Methods

The organs selected for evaluation were; digestive tract, spleens, lungs, livers, kidneys, and reproductive tracts.

It is important the extent or severity of the injury to give a proper rating. You must also take into account the consistency of the tissue, the color and size. The lesions evaluated in the organs were: Lung, edema and yellowish color; Liver: yellowish color, consistency (friable or presence of fibrous tissue), whitish or reddish spots; Kidney: yellowish color, increase in size, presence of cysts, whitish or reddish spots; Reproductive tract, presence of *corpora lutea*, hydrometra, and cysts; Spleen, whitish color, decrease in size; Digestive tract, ulcers, lymphoid reaction, and presence of undigested food.

The lesions of necrosis or friability in liver and kidney are detected by the fragility of the tissue when they are manipulated with your fingers.

All these lesions were included in a control sheet to be filled at the slaughter house.

Lungs, for the evaluation of edema, a value of 1 was given to low or moderate lesions and 2 for severe lesions (Edema between the lung lobes or the mediastinum). Yellowish lungs were given a value of 1

The Liver in pigs has 4 lobes, each one is evaluated and the value depends on the number of lobes affected: 0 = normal liver 1 = if a lobe is affected 2 = if two lobes are affected 3 = If three lobes are affected and 4 = when all lobes are affected. The lesions that are evaluated are: Fibrous tissue, friability, whitish spots, and reddish spots. In case of yellowish livers a value of 1 was given.

In kidneys, the values given were: Normal (no lesions observed) = 0, whitish spots or reddish spots, the value is given depending of the number of spots observed, 1= if there are 1 to 10 spots and a value of 2 = if there are >10. In the event of yellowish color a value 1 will be given. If the kidney is increased in size the value given will be =1

In addition to the organs examined quantitatively, some alterations were examined and described qualitatively in Spleens (decrease in size, whitish color, infarcts), digestive tracts (lymphoid reaction, irritation, undigested food, ulcers) and reproductive tract (cysts, Hydrometra, *corpora lutea, corpora albicans* and *corpora hemorrhagicum*). The evaluation also undertakes histopathological examinations of the most relevant lesions observed. This help to corroborate the presence of mycotoxins in the different organs examined.

Results

Figure 1 shows an example of the graphics included in the final report

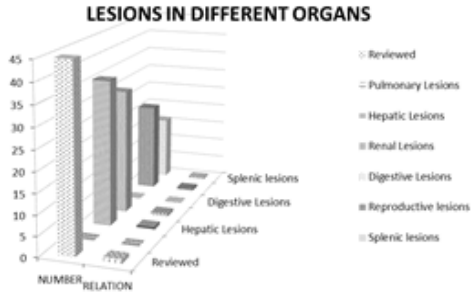

Figure 1. lesions in different organs of pigs examined at the slaughter house.

Conclusions and Discussion

The examination of organs at the slaughter house represents a diagnostic tool little used but very useful for the control of mycotoxin poisoning in different farms in Mexico. These monitoring along with histopathology lets us know the type of mycotoxin and the severity of the lesions found.

Acknowledgments

Slaughter houses in Mexico for allow us to carry out this examination in all the organs described in this paper.

References

1. Osweiler G.D. 1992: Diseases of Swine. Eds. Leman AD, Straw BE, Mengeling WL, D'Allaire S. and Taylor DJ 7 th Edition Iowa State Univ Press.
2. Pointon A.M., Davis P.R. and Bahnson PB 1999 Diseases of Swine 8th ED. Edited by Straw B., D'Allaire S. Mengeling W. and Taylor D. Iowa State Univ. Press Iowa

C.053

Field evaluation of immune response against PCV2 and *M. hyopneumoniae* vaccination through a needle-free injection device or conventional needle

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Introduction

Needle-free injection devices (NFIDs) have been available for humans since the 1930s. There use in swine farms has had a slow, but increased implementation supported by many benefits attributed to its use.[1] In Mexico, as well as many other countries their use is not a common practice. Due to this, a trial was conducted to evaluate the immune response of a vaccination program against Porcine Circovirus Type 2 (PCV2) and *Mycoplasma hyopneumoniae* (M hyo) administered with a Needle-Free injection system or conventional needle.!

Materials and Methods

In a commercial farm in Mexico, 60 pigs of 4 weeks of age were divided in two groups. Both groups received the same vaccination protocol of two doses of a PCV2 (4 and 10 weeks of age) and one dose of M hyo (4 weeks of age) commercial vaccines. Group 1 (G1) received vaccination with a commercial Needle-Free injection system (compressed gas powered), and Group 2 (G2) through conventional needle. Due to management, 15 pigs of each group were randomly selected and identified. Of these, 10 were randomly selected for blood collection at 4, 8, 12, 18, 20 and 22 weeks of age, and two pools of each sampling age per group were processed for PCV2 virus detection by real time RT-PCR. Commercial ELISA tests were performed for both agents. Serology S/P ratios were tested for normality using the Shapiro-Wilk test and therefore groups compared with the Mann-Whitney U test with significance level (α) set at 0.05 for each defined age with titers transformed to log10.

Results

As observed in Figure 1 (A), after vaccination for PCV2, median titers were higher at two ages for each group, 12 and 18 weeks for G1, and 20 and 22 weeks for G2. Higher mean titers were obtained at three ages, 8, 12 and 18 weeks for G1, and at two ages, 20 and 22 weeks for G2. After vaccination for M Hyo (Figure 1. (B)), median and mean titers were higher for G1 in all weeks with the exception of mean titers at 8 weeks, were the value was the same in both groups (3.57). The overall behavior of titers can be observed in Figure 1.

Although descriptive statistics show overall higher performance for the group vaccinated with the Needle-Free injection system, the Mann-Whitney U test did not detect a significant difference of titers at any week analyzed and p-values ranged from 0.540 to 0.921 for PCV2, and from 0.093 to 0.739 for M Hyo. A positive PCR for PCV2 with a viral load of 2310 particles/mL serum was detected from the animals from G2 at 18 weeks of age demonstrating field circulation of the virus during the trial.

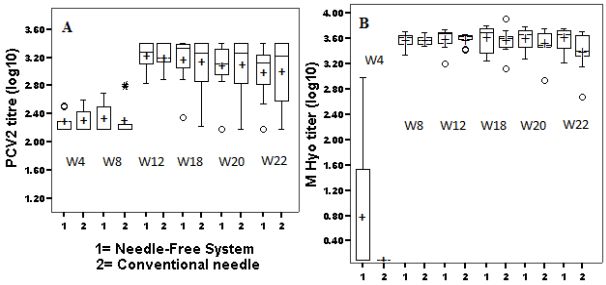

Figure 1. Means and box plot graphics for PCV2 (A) and M hyo (B) titers (log10) after vaccination with a Needle-Free system (1) or conventional needle (2).

Conclusions and Discussion

Although it has been quoted that immune response can be enhanced with the use of NFID´s due to the targeting of dendritic cells in skin and subcutaneous tissue[2], titers did not show a statistically significant difference in this field study but could be expected in other trials. On the other hand, it can be confirmed that producers should not doubt that an equivalent response can be obtained when using NFID´s in their production systems. Other aspects may also be considered for the use of NFID´s such as benefits for the vaccinator (lower injury risk, reduced vaccination time), the pigs (reduced stress and disease transmission)[3], and also for the companies (higher safety standards). Many international companies have adopted the use of these systems and their evaluation and implementation is a strategy when aiming for better vaccine efficacy and pork quality.

Acknowledgments

Lapisa® Diagnostic Laboratory; Michoacán, México

References

1. Christopher C et al. 2008. J Swine Health Prod. (16)5:254-261.
2. Knudssen N. 2006. PORK magazine. In: http://www.porkmag.com/printFriendly.asp?ed_id=4287
3. Daniels S.. 2010. High Plains Dairy Conference. pp. 25-36

Pharmacokinetics of APRAVET® 100 g/kg premix for medicated feeding stuff for pigs applied orally and its bioequivalence with apralan 100 granules

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Introduction

The aim of the study was to investigate the pharmacokinetics of an apramycin (APM) applied orally to pigs as Apravet® 100 g/kg premix for medicated feeding stuff for pigs (Huvepharma NV) and its bioequivalence to Apralan 100 granules (Eli Lilly Benelux S.A.).

Materials and Methods

A parallel experimental design study was performed with 16 pigs (Danube White), 9-10-week-old, equally male and female allocated into 2 groups of 8 animals each, weighing 11.5-13.5 kg, which were treated a single time orally with Apravet® 100 g/kg premix for medicated feeding stuff for pigs or Apralan 100 granules at a dose of APM 40 mg/kg b.w.[1,3] Heparinized blood samples were taken at predetermined intervals up to 24th h. APM plasma concentrations were determined by a validated HPLC method. Main pharmacokinetic (PK) parameters of both products were calculated by non-compartmental PK model.

Results

The PK parameters (mean±SEM) of APM following the administration of Apravet® 100 g/kg premix and Apralan 100 granules are presented in Table 1.

Table 1. PK parameters of APM in pigs

APM pharmacokinetic parameters (mean±SEM)		
	Apravet® 100 g/kg	Apralan 100
$t_{1/2\beta'}$	10.22±0.46 (10.15)^{DD}h	10.20±0.47 (10.12)^{OO}h
C_{max}	2.229±0.08 µg/mL	2.185±0.12 µg/mL
T_{max}	1.50±0.00 h	1.50±0.00 h
$AUC_{0\rightarrow tlast}$	14.38±0.58 µg.h/mL	13.95±0.75 µg.h/mL

^{O} – harmonic mean time

Plasma concentrations of APM higher than 50 ηg/mL for the two preparations were found in all treated animals until the 12th h (Fig. 1).

No statistically significant differences between Apravet® 100 g/kg premix and Apralan 100 granules were established in the plasma concentrations of APM as well as in the respective PK parameters.

The 95% confidence limits of the ratios between the mean values of AUC, C_{max} and T_{max} respectively for Apravet® 100 g/kg premix (as a test product) and for Apralan 100 granules (as a reference product) were within the bioequivalence interval for AUC and C_{max} 0.80 – 1.25, and for T_{max} 0.80 – 1.20.

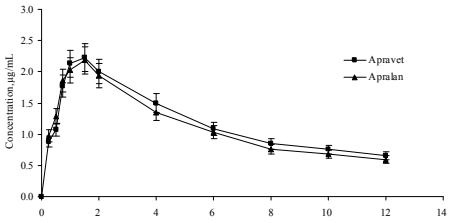

Figure 1. Mean serum concentrations of APM in pigs following single oral administration

Conclusions and Discussion

The PK parameters of APM determined in the present study are considered typical of aminoglycoside antibiotics after orally application to pigs. Our results correspond with the reports on the PK of APM in pigs and demonstrate that APM is poorly absorbed and tissue distribution is limited after orally administration.[2,4] Following oral administration of Apravet® 100 g/kg premix or Apralan 100 granules at a dose of APM 40 mg/kg b.w., similar results for serum concentrations and PK parameters were established with the two products. The analyses performed on the basis of the parameters AUC, C_{max} and T_{max} show that there is a bioequivalence between Apravet® 100 g/kg premix for medicated feeding stuff for pigs and Apralan 100 granules.

Therefore, the results are helpful for understanding the PK characteristics and correct application of APM to pigs.

References

1. Anonymous (2001), EMEA/CVMP/016/00-corr-Final;
2. Shang, R et al. (2004), Ch. J. of Vet. Med., 40-03;
3. Martinez, M. N. et al. (1998), Developm. and Form. of Vet. Dosage Forms, 2-nd edn., 429-468.
4. Veterinary Pharmacology and Therapeutics, Jim E. Riviere and Mark G. Papich (Editors), 9th Ed., Wiley-Blackwell, 2009, 915-944.

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