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Porcine epidemic diarrhoea virus: a comprehensive review of molecular epidemiology, diagnosis, and vaccines

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Abstract The porcine epidemic diarrhoea virus (PEDV), a member of the Coronaviridae family, causes acute diarrhoea and dehydration in pigs. Although it was first identified in Europe, it has become increasingly problematic in many Asian countries, including Korea, China, Japan, the Philippines, and Thailand. The economic impacts of the PEDV are substantial, given that it results in significant morbidity and mortality in neonatal piglets and is associated with increased costs related to vaccination and disinfection. Recently, progress has been made in understanding the molecular epidemiology of PEDV, thereby leading to the development of new vaccines. In the current review, we first describe the molecular and genetic characteristics of the PEDV. Then we discuss its molecular epidemiology and diagnosis, what vaccines are available, and how PEDV can be treated.

Keywords Porcine epidemic diarrhoea virus. Review. Molecular epidemiology. Diagnosis. Vaccine

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Introduction

Porcine epidemic diarrhoea (PED), which was first observed among English feeder and fattening pigs in 1971 [1], is a devastating enteric disease that manifests as sporadic outbreaks during the winter, leading to damage on breeding farms. Characterised by watery diarrhoea, PED resembles transmissible gastroenteritis (TGE), but has less of an effect on suckling pigs (< 4-to 5-week old); this is what allowed PED to first be distinguished from the TGE virus and other recognized enteropathogenic agents. As it spread through Europe, the disease was named 'epidemic viral diarrhoea (EVD).' Unlikely what the disease used to outbreak in fattening pigs, different types of EVD caused acute diarrhoea in pigs of all ages in 1976. This type of EVD was classified as EVD type 2 [1], different from the previously recognized type 1 [2]. EVD type2 was turned out to be caused by a coronavirus-like agent in 1978 [3, 4] using experimentally designed CV777 which caused enteropathogenic infection in both piglets [3] and fattening swine. This was when the disease started to be called as 'Porcine Epidemic Diarrhoea (PED)' [4].

Both transmissible gastroenteritis virus (TGEV) and porcine epidemic diarrhoea virus (PEDV) are classified into group 1 of the genus Coronavirus. PEDV ranges in diameter from 95 to 1990 nm (mean diameter: 130 nm), including its projection. As in many particles with a tendency to a round shape, the PEDV contains a centrally located electron-opaque body; it also possesses widely spaced club-shaped projections measuring 18–23 nm in length. The internal structure of the virus remains unknown. The PEDV is sensitive to ether and chloroform and has a density in sucrose of 1.18 g/ml. The virus possesses a glycosylated peplomer (spike, S) protein, Poll (P1), envelope (E), glycosylated membrane (M) protein, and an unglycosylated RNA-binding nucleocapsid (N) protein [5]. Cell culture-adapted PEDV loses its infectivity when heated to C60°C for 30 min, but is moderately stable at 50°C; further, the virus is stable between pH 5.0 and 9.0 at 4°C and between pH 6.5 and 7.5 at 37°C[6]. PEDV shows no haemagglutinating activity [6].

The PEDV propagates by orally inoculating piglets, after which, during the early stages of diarrhoea, it collects in

the tissues and contents of the small intestine [3]. Vero (African green monkey kidney) cells support the serial propagation of PEDV and grow successfully in laboratory conditions; however, growth of the virus depends on the presence of trypsin in the cell culture medium. Cytopathic effects consist of vacuolation and formation of syncytia.

During the 1980s and 1990s, PED was prevalent throughout Europe, in countries such as Belgium, England, Germany, France, the Netherlands, and Switzerland (Table 1). PED is currently a source of concern in Asia, where outbreaks are often more acute and severe than those observed in Europe. In this respect, and in their high mortality rates, these resemble TGEV outbreaks. For example, Japanese outbreaks between September 1993 and June 1994 resulted in 14,000 deaths, with mortality ranging from 30 to 100% in suckling pigs. During these epidemics, adult pigs showed only temporary decreases in appetite and milk production [7]. Another PED epidemic occurred in the winter of 1996, during which 39,509 of 56,256 infant farrow-to-finish piglets died after experiencing diarrhoea. Between January 1992 and December 1993, 56.3% of viral enteric cases in infant pigs surveyed in Korea were attributable to PEDV, rather than TGEV. The vast majority of outbreaks (90%) involved piglets < 10-day-old [8]. The clinical lesions of PEDV in the small intestine of piglets were similar to those of TGEV. Lesions are confined to the small intestine, which is distended with yellow fluid (Fig. 1). PED outbreaks also occurred in Thailand from 2007 to 2008. Most of the affected farms reported that the disease first occurred in farrowing barns; 100% of newborn piglets were subsequently lost. Between August 1997 and July 1999, 50.4% of 1,258 enteric cases across 5 Korean provinces were diagnosed as PED [9]; further, a Korean abattoir serosurvey found PEDV seroprevalences of 17.6–79% (mean of 45%) in samples from 469 pigs from seven provinces. Cumulatively, these results suggest that the virus had become endemic in some areas [10] (Table 1). However, recent outbreaks seemed to be concentrated in certain countries where pork industry is prevalent, such as Philippines, South Korea and China.

Molecular and genetic characteristics of the PEDV

PEDV is an enveloped virus possessing an approximately 28 kb, positive-sense, single-stranded RNA genome with a 5' capand a 3' polyadenylated tail [11, 12]. The genome comprises a 5' untranslated region (UTR), a 3' UTR, and at least seven open reading frames (ORFs) that encode 4 structural proteins [spike (S), envelope (E), membrane (M), and nucleocapsid (N)] and three non-structural proteins (replicases 1a and 1b, and ORF3); these are arranged on the genome in the order 5'-replicase (1a/1b)–S-ORF3–E–M– N–3' (Fig. 2)[1, 5, 13–19].

The polymerase gene consists of 2 large ORFs, 1a and 1b, that cover the 5' two-third of the genome and encode the non-structural replicase polyproteins (replicases 1a and 1b). Genes for the major structural proteins S (150–220 kDa), E (7 kDa), M (20–3' kDa), and N (58 kDa) are located downstream of the polymerase gene [15, 18, 20]. The ORF3 gene, which is an accessory gene, is located between the structural genes. It encodes an accessory protein, the number and sequence of which varies among different coronaviruses [20].

The PEDV S protein is a type I glycoprotein composed of 1,383 amino acids (aa). It contains a signal peptide (1–18 aa), neutralising epitopes (499–638, 748–755, 764–771, and 1,368–1,374 aa), a transmembrane domain (1,334–1,356 aa), and a short cytoplasmic domain. The S protein can also be divided into S1 (1–789 aa) and S2 (790–1,383 aa) domains based on its homology with S proteins of other coronaviruses [21–26]. Like other coronavirus S proteins, the PEDV S protein is a glycoprotein peplomer (surface antigen) on the viral surface, where it plays a pivotal role in regulating interactions with specific host cell receptor glycoproteins to mediate viral entry, and stimulating induction of neutralising antibodies in the natural host [15, 21–23, 26, 27]. Moreover, it is associated with growth adaptation in vitro, and attenuation of virulence in vivo [28, 29]. Thus, the S glycoprotein would be a primary target for the development of effective vaccines against PEDV. Additional studies of this structure are essential for understanding the genetic relationships between, and diversity of, PEDV isolates, the epidemiological status of PEDV in the field, and the association between genetic mutations and viral function [29–33]. It was reported that aminopeptidase N is the receptor of TGEV, human coronavirus 229E (HcoV229E) and feline coronavirus (FeCoV) which all belong to group I coronavirus including PEDV [34].

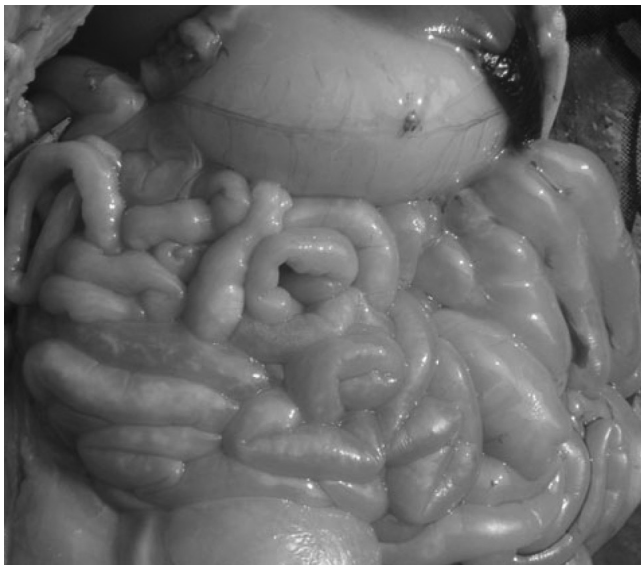
The PEDV M protein, the most abundant envelope component, is a triple-spanning structural membrane glycoprotein with a short amino-terminal domain on the outside of the virus and a long carboxy-terminal domain on the inside [35]. The M protein not only plays an important role in the viral assembly process [36, 37] but also induces antibodies that neutralise the virus in the presence of its complement [37, 38]. The M protein may play a role in α-interferon (α-IFN) induction [39]. Coexpression of M and E proteins allowed the formation of pseudoparticles, which exhibited interferogenic activity similar to that of complete virions [40]. Additional work on the M glycoprotein should increase our understanding of the genetic relationships between, and the diversity of PEDV isolates and the epidemic situation of PEDV in the field [30, 41–45].

The N protein, which binds to virion RNA and provides a structural basis for the helical nucleocapsid, is a basic

Table 1: Published cases of PEDV outbreaks from several countries (1978–2011)

Publication year	Country	Description	Reference
1978	England	Porcine epidemic diarrhoea type II was reproduced in experimental pigs of various ages by oral dosing with minced intestine from a naturally occurring case of the disease. Virus-like particles which probably represent an unidentified coronavirus were seen by EM the faeces and intestinal epithelium of infected animals.	Res Vet Sci. 1978 Sep; 25(2):255–256
1978	Belgium	Coronavirus-like particles were detected by EM in the intestinal contents of pigs during a diarrheal outbreak on 4 swine breeding farms in 1977. Diarrhoea was reproduced in experimental pigs with one of the isolates, designated CV777, which was found to be distinct from the 2 known porcine coronaviruses, transmissible gastroenteritis virus and hemagglutinating encephalomyelitis virus.	Arch Virol. 1978;58(3): 243–247
1993	The Czech Republic	PEDV was diagnosed in two swine herds. The causal agent was demonstrated in intestinal contents by EM and identified by immune EM.	Vet Med (Praha). 1993;38(6):333–341
1994	Belgium	Seven groups of pigs with acute diarrhoea during the months February to March 1992 were investigated. Seven of them had seroconverted to PEDV.	Vet Rec. 1994 Dec 17;135(25):594–597
1996	Hungary	When faecal samples of 92 live diarrhoeal weaned pigs (representing 19 farms) were tested, PEDV (5.5%) was detected.	Acta Vet Hung. 1996; 44(1):9–19
2000	Korea	Between August 1997 and July 1999, a total of 1258 cases from 639 pig farms were examined. Three hundred and four (47.6%) of 639 herds were diagnosed with PEDV infection.	Vet Rec. 2000 Nov 18;147(21):606–608
2005	China	PEDV LJB/03 was isolated from the faeces of piglets infected with PEDV on a pig farm, Heilongjiang province, China.	Virus Genes. 2005 Jan;30(1):69–73
2008	China	Six PEDVs were isolated from the faecal samples of piglets infected with PEDV in 2006 in China.	Virus Genes. 2008 Apr;36(2):355–364. Epub 2008 Jan 24
2008	Italy	There was an epidemic of diarrhoea affecting pigs of all ages in Italy between May 2005 and June 2006. In 63 herds the cause was confirmed as PEDV by EM, immune EM, PCR and serology.	Vet Rec. 2008 Mar 8;162(10):307–310
2010	China	Since early 2006, PEDV has been reemerging in immunized swine herds in China.	Arch Virol. 2010 Sep;155(9):1471–1476. Epub 2010 Jun 11
2010	Thailand	Since late 2007, several outbreaks of PEDV infection have emerged in Thailand.	Emerg Infect Dis. 2009 Jul;15(7):1112–1115
2010	Thailand	A PEDV outbreak was observed in March 2008 in a swine herd in Thailand. The disease was diagnosed by clinical symptoms, gross and histopathology and viral detection using RT-PCR.	AnimReprod Sci. 2010 Oct;122(1–2):42–51. Epub 2010 Jul 27
2011	China	PEDV CH/S strain occurred in a swine breeding farm in Shanghai in 1986 and was confirmed as PEDV by EM, direct IF testing, and serum neutralization testing.	J Virol. 2011 Nov;85(21):11538–11539

Figure 1: Photographic records of PEDV outbreaks. During a 2006 outbreak on a commercial farm in Kimpo. South Korean, piglets < 1 week of age died from severe watery diarrhoea after showing signs of dehydration. After the acute outbreak, piglets were anorectic, depressed, vomiting, and producing water faeces that did not contain any signs of blood. Necropsies of deceased piglets from the Kimpo outbreak uncovered gross lesions in the small intestines, which were typically fluidic, distended, and yellow, containing a mass of curdled, undigested milk. Atrophy of the villi caused the walls of the small intestines to become thin and almost transparent.



phosphoprotein associated with the genome [5, 16, 18, 46]. As such, it can be used as the target for the accurate and early diagnosis of PEDV infection. It has been suggested that N protein epitopes may be important for induction of cell-mediated immunity (CMI) [38].

Whereas the genes encoding the structural proteins have been thoroughly investigated for most coronaviruses, little is known about the functions of the accessory proteins, which are not generally required for virus replication in cultured cells [46–49]. On the contrary, their expression might lead to decreases of viral fitness in vitro, and mutants with inactivated accessory genes are easily selected during serial passage through cell cultures [50–53]. In general, accessory genes are maintained in field strains [50, 54], and their loss mainly results in attenuation in the natural host [55–57]. In the case of PEDV, the only accessory gene is ORF3, which is thought to influence virulence; cell culture adaptation has been used to alter the ORF3 gene in order to reduce virulence [52], as has been done for TGEV [53]. Differentiation of ORF3 genes between the highly cell-adapted viruses and field viruses could be a marker of adaptation to cell culture and attenuation of the virus [52, 58, 59]. Thus, measures of variation in ORF3 gene differentiation could be a valuable tool in molecular epidemiology studies of the PEDV [42, 45, 52, 59].

Molecular epidemiology of PEDV

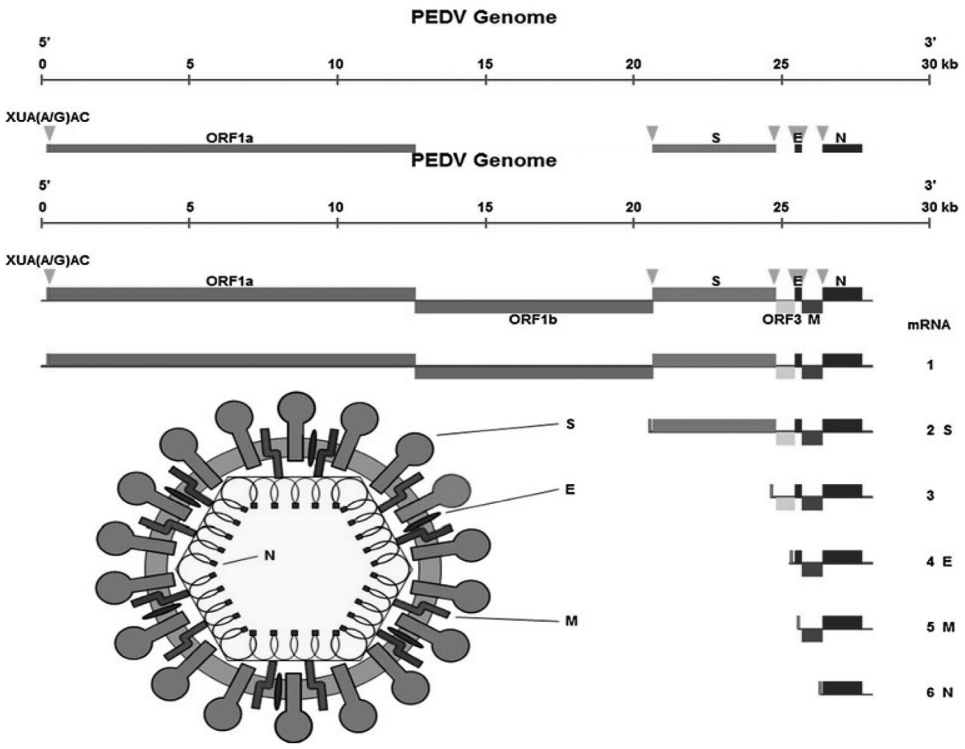
Genetic and phylogenetic analyses based on the S, M, and ORF3 genes have been used to determine the relatedness of PEDV isolates, both within Korea and among various countries in which PEDV has surfaced. Research on part of the S gene, and on the entire M gene, have suggested that PEDVs can be separated into three groups (G1, G2, G3), which have three subgroups (G1–1, G1–2, G1–3) [32]. According to analysis of the partial S genes, the G1 PEDVs had 95.1–100% nucleotide sequence similarities with each other, and they had 93.5–96.7 and 88.7–91.5% sequence identities with the G2 and G3 PEDVs, respectively. The G2 PEDVs had 96.7–99.8% similarities with each other, and they had 91.8–93.0% similarities with the G3 PEDVs [32]. These results reflect the existence of genetic diversity among the Korean PEDV isolates (Figure 3).

The majority of the Korean PEDV isolates are closely related to Chinese strains [32]. The Chinese PEDV clade also contains all strains isolated from several outbreaks of PEDV that have occurred in Thailand since late 2007. These classifications have been based on the phylogenetic relationship of the S genes, and support the results of Park et al. [32]. Recently, after analyzing the full S gene-based phylogenetic tree [31] reported that all PEDVs can be separated into 2 clusters, and that Korean field isolates are more closely related to each other.

In 2006, an analysis of the M gene of 6 PEDVs isolated from the faeces of Chinese piglets indicated that the isolates compose a separate cluster with Chinese strain JS-2004–02 [60]. These results demonstrated that there may be a new prevailing PEDV genotype in China [60]. Phylogenetic relationships of complete M gene nucleotide sequences indi-isolates have been found to be closely related to Chinese cate that recent Thai PEDV isolates are closely related to strains [45], and belong to the third of 3 PEDV groups con-isolates from China [30]. Likewise, most Korean PEDV taining all PEDV isolates [45].

Investigations of the ORF3 gene have revealed the reemergence of PEDV in immunised swine herds since early 2006 [42]. ORF3 genes have been used to divide Chinese field strains and PEDV reference strains into 3 groups; further, Chinese field strains appear to be closely related to Korean strains, but genetically different from PEDV vaccine strains. Another report revealed that PEDV has caused enteric disease with devastating impact since the first identification of PEDV in 1992 in Korea, and recent, prevalent Korean PEDV field isolates are closely related to Chinese field strains but differ genetically from European strains and vaccine strains [45].

Figure 2: Schematic representation of the PEDV genome based on the CV777 (GenBank accession No. AF353511) strain



Diagnosis

A diagnosis of PED cannot be made on the basis of clinical signs and histopathological lesions [61–64]. Due to the similarities in causative agents of diarrhoea, differential diagnosis is necessary to identify the PEDV in the laboratory [64, 65]. Many techniques have been used for the detection of PEDV, including immunofluorescence (IF) tests, immunohistochemical techniques, direct electron microscopy, and enzyme-linked immunosorbent assays (ELISA). However, these techniques are time-consuming and are low in sensitivity and specificity [66]. Kim et al. [67] compared three techniques (RT-PCR, immunohistochemistry and in situ hybridization) for the detection of PEDV. They concluded that although RT-PCR identified the presence of PEDV more frequently than the other methods, when only formalin-fixed tissues are submitted, immunohistochemistry and in situ hybridization would be useful methods for the detection of PEDV Ag and nucleic acid. The PEDV leader sequence was used to develop a reverse transcriptase polymerase chain reaction (RT-PCR) diagnostic technique [68, 69] that has successfully been used to detect both laboratory and field isolates [70, 71]. M gene-derived primers can be used in an RT-PCR system to obtain PEDV-specific fragments [69], and duplex RT-PCR has been used to differentiate between TGEV and PEDV [66]. The past few years have seen several useful modifications of the basic RT-PCR method. For instance, it is possible to estimate the potential transmission of PEDV by comparing viral shedding load with a standard internal control DNA curve [72], as well as to perform multiplex RT-PCR to detect PEDV in the presence of various viruses [73] – a technique that is particularly useful for rapid, sensitive, and cost-effective diagnosis of acute swine viral gastroenteritis). The commercial dual priming oligonucleotide (DPO) system (Seegene, Seoul, Korea) was also developed for the rapid differential detection of PEDV. It employs a single tube 1-step multiplex RT-PCR with two separate primer segments to block a non-specific priming [74].

Another useful reverse transcription-based diagnostic tool is RT loop-mediated isothermal amplification (RTLAMP). This assay, which uses 4–6 primers that recognize 6–8 regions of target DNA, is more sensitive than gel-based RT-PCR and ELISA, in large part because it produces a greater quantity of DNA [75]. Immunochromatographic assay kits can be used at farms in order to detect PEDV S proteins with 92% sensitivity and 98% specificity. This technique is less accurate than RT-PCR, but allows diagnosis within 10 min. Thus, it is particularly effective for quickly determining quarantine or slaughter policies in the field. Especially, endemic situation of PED infection brought the several commercialised PED virus

Figure 3: Relationships among PEDVs isolated from various countries based on the partial S gene including epitope region. The phylogenetic tree was constructed using the neighbor-joining method in MEGA version 5.05 with pairwise distances [99]. Bootstrap values (based on 1,000 replicates) for each node are given if > 60%. The scale bar indicates nucleotide substitutions per site. Asterisk represents PEDV isolate whose sequence available in GenBank database was shorter as compared to that of other reference strains. PEDVs isolated from various countries were marked with various colors: Europe (black), Korea (blue), China (red), Japan (olive green), Thailand (green) and Viet Nam (purple) (Color figure online)

detection systems using diagnosis techniques including conventional duplex RT-PCR (iNtRON Biotechnology, Inc, Korea), real time RT-PCR (kogenebiotech, Kore), DPO based multiplex RT-PCR (Seegene, Seoul, Korea), and immunochromatography (Bionote, Korea) in Korea.

Recently, a protein-based ELISA was developed to detect PEDV. In this technique, a polyclonal antibody is produced by immunising rabbits with purified PEDV M gene after its expression in *Escherichia coli*. IF analysis with anti-PEDV-M antibody is then able to detect PEDV-infected cells among other enteric viruses [76]. ELISA blocking and indirect IF have been used to detect PEDV antibodies at 7 and 10–13 days postinoculation, respectively [77]. For all tests, the second (convalescent) serum sample should be collected and examined no sooner than 2–3 weeks after the onset of diarrhoea. PEDV antibodies, detected by the ELISA-blocking and IF-blocking tests, have been found to persist for at least 1 year.

Due to the special features of the porcine mucosal immune system, the presence of serum antibodies against gastroenteric pathogens is not always correlated with protection; rather, detection of these antibodies only proves that individuals had contact with infectious microorganisms [78–80]. Additionally, Ha et al. [81] recently reported that colostrum IgA concentration is a better marker of protection from PEDV infection than serum neutralising (SN) titre from serum samples; however, SN titres may still be useful in determining herd infection status [81].

Vaccines

Until they are 4-to 13-day old, piglets are protected against PEDV by specific IgG antibodies from the colostrum and milk of immune sows [82]; the length of immunity depends on the titre of the mother. After antigenic sensitisation in the gut, IgA immunocytes migrate to the mammary gland, where they localise and secrete IgA antibodies into colostrum and milk. This 'gut-mammary' immunologic axis is an important concept in designing optimal vaccines to provide effective lactogenic immunity [83]. Pigs that regularly suckle the immune mother are constantly inoculating their lumens with milk-bound IgA antibodies, a process that confers passive immunity. IgG accounts for more than 60% of colostrum immunoglobulin content. However, IgA is more effective at neutralising orally infectious pathogens than either IgG or IgM because it is more resistant to proteolytic degradation in the intestinal tract and has a higher virus neutralising ability than IgG and IgM [84]. Therefore, only passive transfer of IgA from an immunised mother effectively induces immune responses in suckling piglets [85]. However, these antibodies do not protect against intestinal infection with PEDV.

Several PEDV vaccines, which differ in their genomic sequence, mode of delivery, and efficacy, have been developed. A cell culture adaptation of the CV777 strain had a strikingly different genomic sequence [18], was associated with much lower virulence in new born caesarean-derived piglets, and caused much less severe histopathological changes. However, in Europe, the disease caused by PEDV was not of sufficient economic importance to start the vaccine development. Therefore, the trial of vaccine development was mainly accomplished in Asian countries where the PEDV outbreaks have been so severe that the mortality of the new born piglets was increased. An alternative vaccine for suckling piglets may be an attenuated form of the virus derived from serial passage (passage level: 93) of the PEDV [86]. In Japan, a commercial attenuated virus vaccine of cell culture-adapted PEDV (P-5V) has been administered to sows since 1997. Although these vaccines were considered efficacious, not all sows developed solid lactogenic immunity [87].

Oral vaccination with attenuated PEDV DR13 (passage level: 100) has recently been proven to be more efficacious than injectable vaccine. Further, this vaccine candidate remained safe even after three back passages in piglets [88]. Piglet mortality can be reduced by orally inoculating pregnant sows with the DR13 strain. The viral strain was licensed, and used as an oral vaccine in South Korea from 2004 (patent No. 0502008). And the oral vaccine was registered and commercialised in Philippine at 2011. Despite the documented benefits of the DR13 vaccine, it does not significantly alter the duration of virus shedding – an indication of immune protection [79, 89] in challenged piglets. Shorter periods of virus shedding, as well as reduced severity and duration ofdiarrhoea in piglets, result from higher titres of serum antibodies; complete protection from PEDV infection prevents shedding after exposure to viral challenge [90]. Oral immunisation with highly attenuated PEDV confers partial protection against virulent challenge in conventional pigs, a result that is related to inoculation dose. At low doses of the attenuated PEDV, 25% of pigs are protected against PEDV challenge, but this proportion increased to 50% when pigs were inoculated with a dose 20 times stronger [91]. However, viral shedding may be difficult to measure accurately, as it is varies depending on viral strain and sensitivity of the detection tool [72].

Therefore, for the ideal and perfect development of vaccines, several criteria including the factors related the reduction of virus shedding in piglets, and the details of the mucosal immunity of PEDV should be focused in the course of development of next generation vaccines. Information on PEDV mucosal immunity has typically been limited. De Arriba et al. used the enzyme linked immunospot (ELISPOT) technique to characterise the isotype-specific antibody secreting cells in mucosal and

systemic-associated lymphoid tissues in pigs inoculated with PEDV. After infection with PEDV, levels of antibody secreting cell (ASC) in the gut were similar to those observed in response to TGEV and rotavirus infection; IgG ASCs were more prevalent than IgA ASCs. In PEDV-infected pigs, a limited number of IgM ASCs were detected at post infection day (PID) 4, and memory B cells appeared at PID 21 in mesenteric lymph nodes, spleen, and blood. Finally, the authors noted correlations between protection and both serum isotype-specific antibody and ASC response in gut-associated lymph tissues and blood on the challenge day [90–92].

There have also been reports of immune responses by transgenic plants and lactic acid bacteria that express the PEDV antigen [85, 93, 94]. The transgenic tobacco plants that express the S protein corresponding to the neutralising epitope of PEDV was tested whether feeding the plants induced the immune response in murine model. And the efficacy of orally administered antigen gene transgenic carrot and lettuce were tested after codon optimization and application of viral expression systems [85]. In mice, induced antibodies have neutralising activity against PEDV. No neutralising antibodies were detected in either mice or pigs given mucosal immunizations with recombinant *Lactobacillus casei* expressing PEDV N (nucleoprotein) on its surface. However, this treatment elicited high levels of mucosal IgA and circulation IgG immune responses against the PEDV N protein. Before this vaccine can be commercialised, further studies are needed; for instance, it will be necessary to understand discrepancies between test results of the first LAB scale vaccine and large-scale pilot vaccines.

Research into this and other potential vaccines should be made a priority, as PEDV-mediated diarrhoea causes significant economic losses in the swine industry. However, there is also a potential drawback to the use of live-attenuated vaccines. Recently, a survey conducted in China indicated close phylogenetic relationships between a Chinese PEDV field strain (CH/GSJIII/07) and two vaccine strains, suggesting that live vaccines can evolve into more infectious forms in the field [42].

Treatment

During the European outbreak of PEDV, pregnant sows were deliberately exposed to the intestinal contents of dead infected pigs, thus artificially stimulating lactogenic immunity and, hopefully, shortening the duration of outbreaks at farms [12]. However, several complications arose from this treatment. Because the intestinal contents did not have homogenous titres of PEDV, the induction of immunity – including solid lactogenic immunity – might not be expected. Diseases may be spread via contamination with viral agents, such as PRRSV and PCV2.

Immunoprophylactic agents may also be used to treat PEDV. For instance, anti-PEDV chicken egg yolk immunoglobulin (IgY) and colostrums from immunized cows have been found to increase survival rates of virally challenged piglets [95, 96]. Mouse monoclonal single chain variable fragment (scFv) antibodies to neutralised PEDV, which can be expressed in E. coli, are as potent as parental antibodies and block PEDV infection into target cells in vitro [97]. Thus, it is possible that recombinant E. coli cells expressing scFv can be used as prophylactic agents against PEDV infection. Epidermal growth factor (EGF), which stimulates the proliferation of intestinal crypt epithelial cells and promotes recovery from atrophic enteritis in PEDV-infected piglets [98], has also been proposed as a potential novel therapy to promote intestinal villous recovery in piglets with PEDV infections; it may also be useful in other species with viral atrophic enteritis. Drawbacks of this treatment include its high price and questionable safety.

References

1. E.N. Wood, Vet. Rec. 100, 243–244 (1977)

2. J. Oldham, Pig Farming (Oct suppl). 72–73 (1972)

3. P. Debouck, M. Pensaert, Am. J. Vet. Res. 41, 219–223 (1980)

4. M.B. Pensaert, P. Callebaut, P. de Bouck, Present knowledge. Int. Congr. Pig Vet. Soc. 52 (1982)

5. F.A. Murphy, Gibbs E.P., Horzinek M.C., Studdert M.J, in Veterinary virology, 3rd edn. (Academic Press, San Diego, 1999), pp. 496–501

6. P. Callebaut, Int. Congr. Virol, 420 (1981)

7. M. Sueyoshi, T. Tsuda, K. Yamazaki, K. Yoshida, M. Nakazawa, K. Sato, T. Minami, K. Iwashita, M. Watanabe, Y. Suzuki et al.,J. Comp. Pathol. 113, 59–67, (1995)

8. E.K. Hwang, K.J. Yoon, Y.H. Jean, Y.C. Bae, S.S.Yoon, C.K. Park, C.H. Kweon, Y.D. Yoon, M. Ackermann, RDA J. Agric. Sci. 36, 587–596 (1994)

9. C. Chae, O. Kim, C. Choi, K. Min, W.S. Cho, J. Kim, J.H. Tai, Vet. Rec. 147, 606–608 (2000)

10. C.H. Kweon, B.J. Kwon, Y.B. Kang, S.H. An, Korean J. Vet. Res. 34, 321–326, (1994)

11. M.B. Pensaert, P. de Bouck, Arch. Virol. 58, 243–247 (1978)

12. M.B. Pensaert, S.G. Yeo, in Disease of Swine, eds. by B.E. Straw, J.J. Zimmerman, S. D'Allaire, D.J. Taylor (Blackwell, Ames, 2006), pp. 367–372

13. A. Bridgen, M. Duarte, K. Tobler, H. Laude, M. Ackermann, J. Gen. Virol. 74(Pt 9), 1795–1804 (1993)

14. A. Bridgen, R. Kocherhans, K. Tobler, A. Carvajal, M. Ackermann, Adv. Exp. Med. Biol. 440, 781–786 (1998)

15. M. Duarte, H. Laude, J. Gen. Virol. 75(Pt 5), 1195–1200 (1994)

16. H.F. Egberink, J. Ederveen, P. Callebaut, M.C. Horzinek, Am. J. Vet. Res. 49, 1320–1324 (1988)

17. R. Kocherhans, A. Bridgen, M. Ackermann, K. Tobler, Virus Genes 23, 137–144 (2001)

18. C. Bernasconi, F. Guscetti, A. Utiger, K. Van Reeth, M. Ackermann, A. Pospischil, Clinical Histopathological and Immunohistochemical Findings, pp. 542–546, (1995)

19. A.A.F. de Vries, M.C. Horzinek, P.J.M. Rottier, R.J. de Groot, Semin. Virol. 8, 33–47 (1997)

20. K. Narayanan, S. Makino, in Nidoviruses ed. by Perlman, Snijder E.J. (ASM Press, Washington, DC, 2008), pp. 235–244

21. S.H. Chang, J.L. Bae, T.J. Kang, J. Kim, G.H. Chung, C.W. Lim, H. Laude, M.S. Yang, Y.S. Jang, Mol. Cells 14, 295–299 (2002)

22. D.J. Cruz, C.J. Kim, H.J. Shin, Virus Res. 132, 192–196 (2008)

23. M. Godet, J. Grosclaude, B. Delmas, H. Laude, J. Virol. 68, 8008–8016 (1994)

24. M.W. Jackwood, D.A. Hilt, S.A. Callison, C.W. Lee, H. Plaza, E. Wade, Avian Dis. 45, 366–372 (2001)

25. L.S. Sturman, K.V. Holmes, Adv. Exp. Med. Biol. 173, 25–35 (1984)

26. D. Sun, L. Feng, H. Shi, J. Chen, X. Cui, H. Chen, S. Liu, Y. Tong, Y. Wang, G. Tong, Vet. Microbiol. 131, 73–81 (2008)

27. B.J. Bosch, R. van der Zee, C.A. de Haan, P.J. Rottier, J. Virol. 77, 8801–8811 (2003)

28. S.J. Park, D.S. Song, G.W. Ha, B.K. Park, Virus Genes 35, 55–64 (2007)

29. T. Sato, N. Takeyama, A. Katsumata, K. Tuchiya, T. Kodama, K. Kusanagi, Virus Genes 43, 72–78 (2011)

30. S. Puranaveja, P. Poolperm, P. Lertwatcharasarakul, S. Kesdaengsakonwut, A. Boonsoongnern, K. Urairong, P. Kitikoon,

P. Choojai, R. Kedkovid, K. Teankum, R. Thanawongnuwech, Emerg. Infect. Dis. 15, 1112–1115 (2009)

31. D.K. Lee, C.K. Park, S.H. Kim, C. Lee, Virus Res. 149, 175–182 (2010)

32. S.J. Park, H.J. Moon, J.S. Yang, C.S. Lee, D.S. Song, B.K. Kang, B.K. Park, Virus Genes 35, 321–332 (2007)

33. W. Spaan, D. Cavanagh, M.C. Horzinek, J. Gen. Virol. 69(Pt 12), 2939–2952 (1988)

34. B.X. Li, J.W. Ge, Y.J. Li, Virology 365, 166–172 (2007)

35. A. Utiger, K. Tobler, A. Bridgen, M. Suter, M. Singh, M. Ackermann, Adv. Exp. Med. Biol. 380, 287–290 (1995)

36. V.P. Nguyen, B.G. Hogue, J. Virol. 71, 9278–9284 (1997)

37. P.J.M. Rottier, in The Coronaviridae ed. by S.G. Siddel (Plenum Press, New York, 1995) pp. 115–139

38. L.J. Saif, Coronavirus immunogens. Vet. Microbiol. 285–297 (1993)

39. H. Laude, J. Gelfi, L. Lavenant, B. Charley, J. Virol. 66, 743–749 (1992)

40. P. Baudoux, C. Carrat, L. Besnardeau, B. Charley, H. Laude, J. Virol. 72, 8636–8643 (1998)

41. G.S.V. Baquilod, Y.S. Yeo, Korean J. Vet. Res. 46, 355–361 (2006)

42. J. Chen, C. Wang, H. Shi, H. Qiu, S. Liu, X. Chen, Z. Zhang, L. Feng, Arch. Virol. 155, 1471–1476 (2010)

43. Y.Z. Chi, K.H., H.K. Jeong, J.H. Han, J. Vet. Res. 43, 219–230 (2003)

44. F. Jinghui, L. Yijing, Virus Genes 30, 69–73 (2005)

45. S.J. Park, H.K. Kim, D.S. Song, H.J. Moon, B.K. Park, Arch. Virol. 156, 577–585 (2011)

46. K.M. Curtis, B. Yount, R.S. Baric, J. Virol. 76, 1422–1434 (2002)

47. B. Schwarz, E. Routledge, S.G. Siddell, J. Virol. 64, 4784–4791 (1990)

48. S. Youn, J.L. Leibowitz, E.W. Collisson, Virology 332, 206–215 (2005)

49. B. Yount, R.S. Roberts, A.C. Sims, D. Deming, M.B. Frieman, J. Sparks, M.R. Denison, N. Davis, R.S. Baric, J. Virol. 79, 14909–14922 (2005)

50. A.A. Herrewegh, H. Vennema, M.C. Horzinek, P.J. Rottier, R.J. de Groot, Virology 212, 622–631 (1995)

51. A. Lissenberg, M.M. Vrolijk, A.L. van Vliet, M.A. Langereis, J.D. de Groot-Mijnes, P.J. Rottier, R.J. de Groot, J. Virol. 79, 15054–15063 (2005)

52. D.S. Song, J.S. Yang, J.S. Oh, J.H. Han, B.K. Park, Vaccine 21, 1833–1842 (2003)

53. Woods R.D., Can. J. Vet. Res. (Revue canadienne de recherche veterinaire) 65, 28–32 (2001)

54. R. Dijkman, M.F. Jebbink, B. Wilbrink, K. Pyrc, H.L. Zaaijer, P.D. Minor, S. Franklin, B. Berkhout, V. Thiel, L. van der Hoek, Virol. J. 3, 106 (2006)

55. C.A. de Haan, P.S. Masters, X. Shen, S. Weiss, P.J. Rottier, Virology 296, 177–189 (2002)

56. B.J. Haijema, H. Volders, P.J. Rottier, J. Virol. 78, 3863–3871 (2004)

57. J. Ortego, I. Sola, F. Almazan, J.E. Ceriani, C. Riquelme, M. Balasch, J. Plana, L. Enjuanes, Virology 308, 13–22 (2003)

58. J.H. Park, J.H. Han, H.M. Kwon, Virus Genes 36, 71–78 (2008)

59. S.J. Park, H.J. Moon, Y. Luo, H.K. Kim, E.M. Kim, J.S. Yang, D.S. Song, B.K. Kang, C.S. Lee, B.K. Park, Virus Genes 36, 95–104 (2008)

60. J.F. Chen, D.B. Sun, C.B. Wang, H.Y. Shi, X.C. Cui, S.W. Liu, H.J. Qiu, L. Feng, Virus Genes 36, 355–364 (2008)

61. E.N. Wood, Br. Vet. J. 135, 305–314 (1979)

62. D.C. Turgeon, M. Morin, J. Jolette, R. Higgins, G. Marsolais,

E. DiFranco, Can. Vet. J. (La revue veterinaire canadienne) 21, 100–xxiii (1980)

63. S. Dea, J. Vaillancourt, Y. Elazhary, G.P. Martineau, Can. Vet. J. (La revue veterinaire canadienne) 26, 108–111 (1985)

64. A. Pijpers, A.P. van Nieuwstadt, C. Terpstra, J.H. Verheijden, Vet. Rec. 132, 129–131 (1993)

65. K. Kusanagi, H. Kuwahara, T. Katoh, T. Nunoya, Y. Ishikawa, T. Samejima, M. Tajima, J. Vet. Med. Sci./Jpn. Soc. Vet. Sci. 54, 313–318 (1992)

66. S.Y. Kim, D.S. Song, B.K. Park, J. Vet. Diagn. Invest. 13, 516–520 (2001)

67. O. Kim, C. Chae, Can. J. Vet. Res. (Revue canadienne de recherche veterinaire) 66, 112–116 (2002)

68. K. Ishikawa, H. Sekiguchi, T. Ogino, S. Suzuki, J. Virol. Methods 69, 191–195 (1997)

69. C.H. Kweon, J.G. Lee, M.G. Han, Y.B. Kang, J. Vet. Med. Sci./ Jpn. Soc. Vet. Sci. 59, 231–232 (1997)

70. K. Tobler, M. Ackermann, Adv. Exp. Med. Biol. 380, 541–542 (1995)

71. K. Tobler, M. Ackermann, Schweiz. Arch. Tierheilkd. 138, 80–86 (1996)

72. D.S. Song, J.S. Oh, B.K. Kang, J.S. Yang, J.Y. Song, H.J. Moon, T.Y. Kim, H.S. Yoo, Y.S. Jang, B.K. Park, J. Swine Health Prod. 13, 269–272 (2005)

73. D.S. Song, B.K. Kang, J.S. Oh, G.W. Ha, J.S. Yang, H.J. Moon, Y.S. Jang, B.K. Park, J. Vet. Diagn. Invest. 18, 278–281 (2006)

74. C.S. Lee, B.K. Kang, D.H. Lee, S.H. Lyou, B.K. Park, S.K. Ann, K. Jung, D.S. Song, J. Virol. Methods 151, 30–34 (2008)

75. X. Ren, P. Li, Virus Genes 42, 229–235 (2011)

76. X. Ren, S. Suo, Y.S. Jang, Biotechnol. Lett. 33, 215–220 (2011)

77. A. Carvajal, I. Lanza, R. Diego, P. Rubio, P. Ca´rmenes, J. Vet. Diagn. Invest. 7, 60–64 (1995b)

78. L.J. Saif, R.D Wesley, in Disease of Swine, ed. by B.E. Straw, S. D'Allaire, W.L. Mengeling, D.J. Taylor (Iowa State University Press, Ames, Iowa, 1999), pp. 295–323

79. L. Yuan, S.Y. Kang, L.A. Ward, T.L. To, L.J. Saif, J. Virol. 72, 330–338 (1998)

80. T.L. To, L.A. Ward, L. Yuan, L.J. Saif, J. Gen. Vir. 79(Pt 11), 2661–2672 (1998)

81. G.W. Ha,B.K.Kang, S.J. Park,C.S.Lee, J.S. Oh,Y.G.Chai, B.K. Park, H.J. Moon, Korean J. Vet. Public Health. 34(1), 53–56 (2010)

82. M.L. De Arriba, A. Carvajal, I. Lanza, P. Rubio, P.C. Blanchard, Development of an ELISA for the detection fo antibody isotypes against porcine epidemic diarrhoea virus (PEDV) in sow's milk. Proc. 3rd Congr. ESVV, 222–225 (1995)

83. L.J. Saif, E.H. Bohl, R.K. Gupta, Infect. Immun. 6, 600–609 (1972)

84. P.A. Offit, H.F. Clark, J. Virol. 54, 58–64 (1985)

85. J.L. Bae, J.G. Lee, T.J. Kang, H.S. Jang, Y.S. Jang, M.S. Yang, Vaccine 21, 4052–4058 (2003)

86. C.H. Kweon, B.J. Kwon, J.G. Lee, G.O. Kwon, Y.B. Kang, Vaccine 17, 2546–2553 (1999)

87. Y. Usami, O. Yamaguchi, K. Kumanomido, Y. Matsumura, J. Jpn. Vet. Med. Assoc. 51, 652–655 (1998)

88. D.S. Song, J.S. Oh, B.K. Kang, J.S. Yang, H.J. Moon, H.S. Yoo, Y.S. Jang, B.K. Park, Res. Vet. Sci. 82, 134–140 (2007)

89. L.A. Ward, L. Yuan, B.I. Rosen, T.L. To, L.J. Saif, Clin. Diagn. Lab. Immunol. 3, 342–350 (1996)

90. M.L. de Arriba, A. Carvajal, J. Pozo, P. Rubio, J. Virol. Methods 105, 37–47 (2002)

91. M.L. de Arriba, A. Carvajal, J. Pozo, P. Rubio, Vet. Immunol. Immunopathol. 85, 85–97 (2002)

92. M.L. de Arriba, A. Carvajal, J. Pozo, P. Rubio, Vet. Immunol. Immunopathol. 84, 1–16 (2002)

93. T.J. Kang, J.E. Seo, D.H. Kim, T.G. Kim, Y.S. Jang, M.S. Yang, Protein Expr. Purif. 41, 378–383 (2005)

94. X.L. Hou, L.Y. Yu, J. Liu, G.H. Wang, Vaccine 26, 24–31 (2007)

95. C.H. Kweon, B.J. Kwon, S.R. Woo, J.M. Kim, G.H. Woo, D.H. Son, W. Hur, Y.S. Lee, J. Vet. Med. Sci./Jpn. Soc. Vet. Sci. 62, 961–964 (2000)

96. I. Shibata, M. Ono, M. Mori, J. Vet. Med. Sci./Jpn. Soc. Vet. Sci. 63, 655–658 (2001)

97. H.M. Pyo, I.J. Kim, S.H. Kim, H.S. Kim, S.D. Cho, I.S. Cho, B.H. Hyun, Vaccine 27, 2030–2036 (2009)

98. K. Jung, B.K. Kang, J.Y. Kim, K.S. Shin, C.S. Lee, D.S. Song, Vet. J. 177, 231–235 (2008)

99. K. Tamura, D. Peterson, N. Peterson, G. Stecher, M. Nei, S. Kumar, Mol. Biol. Evol. 28, 2731–2739 (2011)

Optimizing throughput and efficiency in production systems

Noel Williams

Paper not available at time of publication

Livestock associated MRSA: Tiger or Pussycat?

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"The larger the island of knowledge, the longer the shoreline of wonder (doubt)"

Ralph Sockman

The following comments are personal reflections on how the issue of "livestock associated methicillin resistant *Staphylococcus aureus*" (LA-MRSA) has evolved in the USA and globally. At the Leman conference, my presentation will emphasize details of recent research we have been conducting at the University of Minnesota to understand *S. aureus* ecology and the potential occupational risks for workers in the US swine industry. The comments below present a broader context of the epidemiology and sociology of LA-MRSA as a foundation for interpreting these recent observations.

Angst is born (again)!

It is approaching a decade since July 2004 when the phenomenon of LA-MRSA burst onto the scene in the Netherlands then echoed throughout the swine production world.[1] MRSA (deservedly) have high name recognition among the public as the flagship organism of a loosely defined fleet of "Superbugs" (or now more fashionably "nightmare bacteria"). Emergence of apparently novel pathogens rightly furrows the brows of infectious disease clinicians and epidemiologists. Will it be the 'big one' that wrecks an unprecedented toll on public and/or animal health and creates massive social and economic disruption? If there is a food animal reservoir, a few nanoseconds might expire before somebody asks what the industry has done wrong to deliver this evil upon society.

As each new ship sails in (BSE; *Salmonella* Typhimurium DT104; vancomycin resistant enterococci; Nipah virus; Hepatitis E virus, H5N1 and novel H1N1 influenza viruses; SARS and MERS; NDM-1, ESBL *E. coli*, carbapenem-resistant *Klebsiella*,…and LA-MRSA…) uncertainty is usually the prevailing sentiment among the medically and epidemiologically literate. It takes time, often many years, to get a reasonable handle on the magnitude of a novel microbial threat, with the public interest arguably best served when guarded consternation drives systematic research that is enhanced by thoughtful inference. Much of this appropriate response has occurred with the LA-MRSA issue, particularly in the Netherlands. The island of knowledge of LA-MRSA is now fairly extensive, predictably bringing new questions and uncertainties, but also some time to gauge the likely public health impact. Unfortunately it is rarely the guarded consternation of the prudent and epidemiologically informed that drives the public conversation.

Whipping up a frenzy!

While discovery of apparently emerging pathogens rightly troubles most people, these events are the lifeblood of those who make their living by peddling panic to the public. If food animals are implicated, even remotely, as having any role in the threat *du jour*, the tone from the 'science journalists' will usually degenerate towards apocalyptic finger pointing. History tells us that journalistic hyperbole often misses the mark by many orders of magnitude with respect to actual public health impact. BSE (one case where animal feeding practices were responsible for the propagation of the agent) illustrates the discrepancy between predictions of carnage purveyed by journalists (often cherry picking the upper confidence interval from the most pessimistic projection among highly uncertain models), and the actual toll on public health. Estimates aired in the media of predicted human deaths from variant CJD reached hundreds of thousands, but the ultimate (and still tragic) global toll over 16 years is around 230 – equivalent to about 2 days of road fatalities in the USA. SARS and H5N1 influenza are other examples where cumulative human deaths to date (respectively about 800 and 400 globally over about a decade) are of Lilliputian dimensions relative to the doomsday predictions, and are dwarfed by the public health burdens from daily risks like road deaths, and even lightning strike.[2] And just to place LA-MRSA in perspective, the global tally of fatal cases currently stands at ONE (more later), and in the USA the number of confirmed cases of clinical infection with LA-MRSA currently stands at ZERO. The ritual of 'crying wolf' benefits nobody but the scaremongering authors, contributes nothing to addressing any concerns and, like in the fable, is probably counterproductive for that rare future moment when the real wolf appears. I in no way want to trivialize the valid concerns that accompany all events of disease and pathogen emergence, but point out that those who hawk sensation for a living have little interest in the whole truth, or even reasonable perspective. Touting doomsday scenarios is a better business model.

I have written elsewhere of some myths and misinformation about LA-MRSA that was aired earlier in the course of the LA-MRSA 'panic-demic.[3] The romantic narrative of the swashbuckling journalists nobly fighting to inform a gullible public about the evils that are knowingly propagated by unscrupulous and money-grubbing food (and particularly meat) industries who hide their secrets from the hapless consumers has, for me personally, passed from tiresome to nauseating. My dismay about the quality of US journalism about LA-MRSA is not simply fatigue from listening to a 'broken record'. It is that the same refrain keeps repeating when they should be reading from a very different and more factual score. Recent exhibits of journalistic ineptitude on LA-MRSA[4] rival the stratospheric levels set in 2009,[5] when at least one could offer a defense of greater uncertainty. But as I have stated before, 'given the level of politicization of discussions about food animal production in general, and intensive livestock production in particular, it is now quaint if not delusional to dream of rational and factual public discourse on these issues.[3]

While the pedestrian standards of science journalism are dismaying, the media do not have a monopoly on hyperbole and overstatement. Science combines two basic processes: observation and inference. I contend that biased (or worse still deliberately hyperbolic) inference afflicts more than a small portion of the peer-reviewed literature published to date on LA-MRSA. Almost no studies refer to the fact that only one fatality in the world has been associated with LA-MRSA over 9 years and that the clinical burden of LA-MRSA to date has been very small. Another prevalent but misleading statement about the human health implications is that LA-MRSA represent a high proportion of 'cases' in countries such as the Netherlands, without pointing out that 'cases' in those countries include people who are colonized (i.e. culture positive nasal swabs) but do not have infections. A recent and well conducted Danish study concluded 'we therefore face an infectious occupational exposure of huge quantitative dimensions but of *unknown clinical importance*.[6] The statement *per se* is true, but the authors fail to point out that based even on their own data, not to mention the accumulated preceding studies, the real *unknown* is exactly how SMALL is the clinical impact of LA-MRSA. Current data are sufficient to conclude that the clinical importance of LA-MRSA (meaning those organisms now known to occur in livestock in many countries) is '*non-zero*' but very small. This is a mild example of insufficient context, but there are many more papers in which inferences drawn are questionable or even completely unjustified. The collective bias toward overstatement of the public health impact (real and projected) of LA-MRSA is pervasive, and the gatekeepers of quality (author responsibility and critical thinking, peer review and editorial standards) are inadequate. Two examples discussed below illustrate inferences that were so inappropriate (if not completely unjustified) that myself along with other colleagues submitted letters to the editor to document our concerns.

The first was a tragic report from France of a fatal case of ST398 MSSA (i.e. methicillin sensitive) infection of a girl with no known contact with livestock. The isolate involved had multiple attributes that were uncommon among ST398 MRSA isolates from pigs, yet the authors blithely inferred that 'spread of S. aureus ST398 among livestock is a matter of increasing concern because strains of this sequence type were able to acquire PVL genes and cause necrotizing pneumonia in a young immunocompetent patient.' Our response argued that apart from the different attributes of the organism (t571 MSSA) and the absence of any evidence of livestock involvement, there was substantial information supporting the possibility that the organism derived from a human rather than animal reservoir.[7] This has since been strongly endorsed by identification of distinct human and pig clades of that specific spa type (t571) of ST398 *S. aureus* that are likely epidemiologically independent.[8,9] Storm in an academic teacup, you say? But for the fact that the original paper immediately spawned considerable media and blogger interest, including a blog titled "News Break: PigMRSA involved in the death of a child" despite the fact that it was not MRSA, and there was no contact with pigs. The post remains alive and well at `http://www.superbugtheblog.com/2010/07/news-break-pig-mrsa-st398-involved-in.html`.

The second study published in late 2012 concluded that risk from LA-MRSA in livestock dense regions extended to the general community and not just to people with livestock contact.[10] We contend that this paper had serious flaws both in methodology and inference, and its final conclusion was unwarranted and contradicted by three much larger and methodologically superior studies (see below).[11] Unfortunately, a press statement issued when the paper was published ensured that the unjustified inference was echoed through scaremonger community. After all, the amplifiers merely relay what the credentialed scientists were stating in a 'peer reviewed journal'. In both cases, I believe that the authors of the papers we not sufficiently diligent (or perhaps just too busy) to consider alternative hypotheses before leaping to very questionable conclusions. Worse still is that the review process of the journal was patently inadequate for ensuring even rudimentary standards of inference. However, the most frustrating issue was the constipated process we encountered in publishing our concerned response to these papers. In both cases, publication of the letters (just 500 words) required almost a year, most of it spent trapped in the bowels of the journal while the babble that had been generated continued

to cycle around the Internet. Probably not a functional model for how science can inform public debate! Now that I have vented that frustration, let's get back to the Tiger and the Pussycat.

LA-MRSA: Where are the bodies?

I am emboldened to use this arguably tasteless subtitle by recalling an article I read as a graduate student over 30 years ago. During the 1960s when oral contraceptives were unleashed on the western world, there was a little research and much speculation that this might have grave consequences for cardiovascular health and mortality in young women. Unsurprisingly, much of the doomsday predictions were propagated by entities with philosophical objections to contraception. A couple of decades later, 'where are the bodies' was a rhetorical question pointing out that the wolf had not appeared. With LA-MRSA, where we confront concerns about an acute infectious process (rather than a chronic and metabolic process), a decade after the emergence of LA-MRSA is not too soon to pose this question.

In 2011, with support from the National Pork Board, I reviewed published studies of ST398 *S. aureus* to catalogue reports of human clinical disease. The charge was to identify all published information related to clinical infections with ST398 organisms [both MRSA and MSSA (i.e. susceptible to methicillin)]. Eighty three papers or reports were identified to contain information on ST398 associated with clinical cases in humans. Where available, information was recorded on country; numbers of isolates that were from screening swabs; numbers of isolates that were from clinical infections; clinical presentations (e.g., bacteremia; pneumonia; skin or soft tissue infection…); invasiveness (number of cases with invasive infections, i.e. not skin or soft tissue infections); history of animal contact; and number of fatalities. Using conservative criteria of invasiveness (i.e., likely overstating invasiveness by including sputum and urine cultures) there were 2,213 events of positive screening (isolates not linked to infection) and 495 isolates from humans with clinical infections. More than half of the specified presentations were skin or soft tissue infections, or infected wounds. There were 89 cases deemed invasive, and 5 fatalities (note 4 of these were MSSA and without association with livestock) documented globally over several years. Approximately 30% of the 89 invasive cases involved the t571 spa type that is relatively uncommon in pigs. For context, in the USA alone, in 2005 the CDC estimated 94,600 invasive MRSA infections and 18,650 deaths every year.[12] That is, if all the invasive and fatal cases of ST398 reported in the world from 2004 to 2011 had occurred in the USA in 2005, they would have represented 0.09% and 0.03% of those cases respectively.

As noted above, some European studies do not distinguish between events of colonization and clinical infection, and thus there is a lack of quantitative information about the actual clinical risks associated with livestock exposures and colonization with ST398 MRSA. The small number of reports of severe or fatal systemic infections with ST398 *S. aureus* have led to inferences that organisms of this lineage can be serious human pathogens. However, analysis of two years of data for a laboratory serving an estimated population of 800,000 people in a pig dense area in Holland identified 30 'clinical' ST398 isolates, of which 6 were pneumonia or systemic infection (1 blood, 3 sputum, 2 urine). These data suggest an annual risk of approximately 2 clinical infections (and 0.38 invasive infections) per 100,000 people.[13] A more recent report from the hog dense region of Jutland in Denmark (where all MRSA infections are reportable) recorded 3 clinical infections with ST398MRSA (all pig farmers) over 2 years in a population of around 580,000 people.[6] This implies an annual incidence around 0.25 clinical infections per 100,000 people per year. The infections were two cases of impetigo (superficial skin infection) and one case of tonsillitis (where isolation of the organism alone does not prove causation). For context, widely cited estimates for MRSA infection in the USA are 31.8 invasive cases and 6.3 fatalities per 100,000 people per year.[12]

To date, there have been 5 reported fatalities associated with ST398 *S. aureus*. In 4 of these cases, the organisms were t571 MSSA with no known livestock contact, and therefore are more likely related to the human than the pig clade of t571.[8] The other case was t011 ST398 MRSA (unlike t571, common in pigs in Europe) and the patient had indirect contact with pigs. This 85 year old man suffered from lung carcinoma and chronic obstructive pulmonary disease, and the organism was isolated from pleural fluid.[14] Other than infected bite wounds, reports of medically significant ST398 MRSA infections in healthy livestock workers are remarkably scarce and livestock contact is a notably inconsistent feature of invasive ST398 infections. Some authors default to the assumption that lack of livestock contact constitutes evidence of indirect spread (e.g. via food and the 'environment) putting the wider public at risk.[10] However, this is a very questionable assumption unless the organisms are known not be belong to the t571 human clade,[8] which itself may prove not to be unique (i.e., other spa-types deemed LA-MRSA may be found to have distinct human-adapted counterparts).

Information on human disease associated with ST398 MRSA is sparse in North America than Europe. A retrospective assessment of 3,687 MRSA clinical isolates in Canada identified only 5 cases with ST398 MRSA, 4 of which presented with skin or soft tissue infections.[15] The

CDC has examined over 12,000 US isolates and is yet to identify ST398 among human clinical isolates (Dr. Brandi Limbago, personal communication). Similarly, in the hog dense state of Minnesota, the MN Department of Health has tested over 7,000 clinical isolates of MRSA with *sma*1 PFGE (inability to type isolates with *sma*1 is a characteristic of ST398 lineage) and is yet to identify an 'untypable' isolate (Dr. Kirk Smith, personal communication). Given the known presence of ST398 in the North American swine industry, and the sporadic reports of clinical infections in swine workers in Europe, it is likely that some clinical infections may have occurred in occupationally exposed individuals in the USA. The absence of reported cases until now in the USA is likely attributable to both a low incidence and low severity of ST398 infections. On the other hand, t571 ST398 MSSA of the human clade has recently been identified as a relatively important source of human MSSA infection in New York, being identified in 5% of non-invasive MSSA cases and 2.5% of MSSA bacteremias.[9]. That is, the only documented cases of clinical ST398 infections in the USA to date involve the human clade of t571 that is considered 'animal independent'.[7]

The larger island of knowledge – do we know what LA-MRSA are?

In my experience around zoonotic pathogens over 20 years, simple phenomena rarely stay that way. The initial discovery of ST398 in Netherlands provided a fairly clear and simple narrative. A novel lineage of MRSA had been discovered in livestock (particularly pigs) and constituted a new threat to public health. Possession is nine-tenths of the law and the term 'livestock associated MRSA' or 'livestock associated *S. aureus'* understandably became synonymous with ST398-MRSA/*S. aureus*. However, this once reasonable terminology now causes more confusion than enlightenment. Firstly, we now know that at least one clade of ST398 is in not associated with livestock.[8,9] Secondly, ST398 *S. aureus* are not uniquely associated with pigs, a fact that is unsurprising both on the grounds of basic biology (assuming *S. aureus* is part of the normal flora of pigs) and from research conducted on isolates obtained before,[16] and after, the Dutch revelation. There is now accumulating evidence that *S. aureus* belonging to several MLST lineages (particularly ST9 and ST5, but also others included ST97 and ST1) may be equally 'pig associated'.[17-20] MRSA isolates belonging to these lineages (particularly ST9 in Asia) are being increasingly documented in swine. As research continues, a better understanding of the diversity of *S. aureus* in swine populations will be achieved, and it is clearly much broader than ST398.

'ST398' should no longer be seen as synonymous with 'livestock associated'. Its use as such should be eliminated henceforth, particularly in titles of peer reviewed literature. Though once having some utility, this terminology has been rendered inappropriate by recent research and contributes to misleading inferences[21,22] that can be carelessly cited as evidence of community spread of *S. aureus* from livestock sources, without any evidence that this was the case.[10] The use of the term 'livestock associated' with MSSA or MRSA should be reserved for isolates that have been directly obtained from or closely linked to livestock environments. Authors drawing inferences *indirectly* about livestock involvement in the epidemiology of human MRSA cases should be held to a higher level of accountability than has been the case to date.

Who are at risk of LA-MRSA exposure?

To date there are insufficient data to make any judgments about swine associated MRSA other than ST398. There has yet to be a community based outbreak of ST398 MRSA infection reported from any country, and two reports of hospital associated "outbreaks" were of small scale and predominantly involved asymptomatic carriage and contamination of superficial wounds. Evidence to date indicates the health risks associated with ST398 organisms *of livestock origin* are overwhelmingly restricted to people with direct animal contact and their immediate families.[23-25] Across these 3 studies, LA-MRSA prevalence (44%) was > 180 times higher in 352 occupationally exposed persons than in 2,094 rural residents without farm exposure (0.24%). Prevalence in family members of livestock workers was intermediate (5.2%). The studies collectively confirm that in livestock dense regions where exposure to ST398 MRSA is a common occupational risk for livestock workers, it is a lesser risk to their family members, and a negligible risk to persons without livestock or farm contact.[11]

MRSA myopia- what's in a gene?

MRSA are differentiated from MSSA based on the presence of a single gene (mecA). MRSA paranoia in the media creates the impression that MSSA have no importance as human pathogens, whereas MSSA contribute significantly to the burden of human *S. aureus* disease including fatalities across the world. It is remarkable that given the volume of research conducted on 'LA-MRSA', the biology and ecology of the parent organism (*S. aureus*) remains largely overlooked. Much of this focus was justified initially by the effort to understand the extent of the 'problem' in the early period of great uncertainty. However, as the conversation in some sectors drifts towards 'control and interventions' (albeit unwarranted and probably ineffective), it is essential to understand the basic ecology of *S. aureus* in pigs. Some very recent studies in pigs and veterinarians have broken this barrier,[26,27] and more are required if we are to obtain

any comprehensive understanding of the basic biology. At the University of Minnesota, we currently have ongoing studies of *S. aureus* epidemiology in pigs (funded by the National Pork Board) and swine veterinarians (funded by NIOSH through the Upper Midwest Agricultural Safety Center) in the USA, and some results will be presented at the Leman conference. This will include growing evidence that the prevalence of 'LA-MRSA' is lower in the USA than in many European countries, and that MSSA variants of most lineages of MRSA associated with pigs around the world are prevalent in the USA. Given that these organisms have likely been associated with pigs since well before the modern era of intensive swine production, the apparent lack of any incremental risk of *S. aureus* infections recognized swine farmers suggests that their pathogenic potential in humans is probably negligible. Further research into the virulence of livestock associated lineages will be required to explain the apparently minor risk of occupational *S. aureus* infections of swine farmers despite '*occupational exposure of huge quantitative dimensions*'.

References

1. Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M. Methicillin-resistant Staphylococcus aureus in pig farming. *Emerg Infect Dis*. 2005;11(12):1965–1966.

2. Holle R. Annual rates of lightning fatalities by country. 10th International Lightning Detection Conference. 21–23 April 2008. Tucson, Arizona, USA 2008. http://www.vaisala.com/Vaisala%20Documents/Scientific%20papers/Annual_rates_of_lightning_fatalities_by_country.pdf

3. Davies PR. The misinformation game - does the villain have a voice. Proceedings, American Association of Swine Veterinarians,Omaha, pp.21–31. 2010.

4. Bittman M. Breeding bacteria on factory farms. *New York Times*. July 9, 2013. http://opinionator.blogs.nytimes.com/2013/07/09/breeding-bacteria-on-factory-farms/?_r=0

5. Kristof, N. Our pigs, our food, our health. *New York Times*. March 9 2009. http://www.nytimes.com/2009/03/12/opinion/12kristof.html

6. Omland O, Hoffmann L. Occupational acquisition of methicillin-resistant Staphylococcus aureus in humans—a description of MRSA carrier and infected cases from the region of north Jutland in Denmark. *Ann Agric Environ Med*. 2012;19(4):637–640.

7. Davies PR, Wagstrom EA, Bender JB. Lethal necrotizing pneumonia caused by an ST398 Staphylococcus aureus strain. *Emerg Infect Dis*. 2011;17(6):1152–1153.

8. McCarthy AJ, van Wamel W, Vandendriessche S, et al. Staphylococcus aureus CC398 clade associated with human-to-human transmission. *Appl Environ Microbiol*. 2012;78(24):8845–8848. doi: 10.1128/AEM.02398–12; 10.1128/AEM.02398–12.

9. Uhlemann AC, Porcella SF, Trivedi S, et al. Identification of a highly transmissible animal-independent Staphylococcus aureus ST398 clone with distinct genomic and cell adhesion properties. *MBio*. 2012;3(2):10.1128/mBio.00027–12. Print 2012. doi: 10.1128/mBio.00027–12; 10.1128/mBio.00027–12.

10. Feingold BJ, Silbergeld EK, Curriero FC, van Cleef BA, Heck ME, Kluytmans JA. Livestock density as risk factor for livestock-associated methicillin-resistant Staphylococcus aureus, the netherlands. *Emerg Infect Dis*. 2012;18(11):1841–1849. doi: 10.3201/eid1811.111850; 10.3201/eid1811.111850.

11. Davies PR *et al*. Livestock-associated methicillin-resistant Staphylococcus aureus in humans, theNetherlands. *Emerg Infect Dis*. 2013 (in press)

12. Klevens RM, Morrison MA, Nadle J, et al. Invasive methicillin-resistant Staphylococcus aureus infections in the united states. *JAMA*. 2007;298(15):1763–1771. doi: 10.1001/jama.298.15.1763.

13. Wulf MW, Verduin CM, van Nes A, Huijsdens X, Voss A. Infection and colonization with methicillin resistant Staphylococcus aureus ST398 versus other MRSA in an area with a high density of pig farms. *Eur J Clin Microbiol Infect Dis*. 2011. doi: 10.1007/s10096–011–1269-z.

14. Lozano C, Aspiroz C, Ezpeleta AI, Gomez-Sanz E, Zarazaga M, Torres C. Empyema caused by MRSA ST398 with atypical resistance profile, spain. *Emerg Infect Dis*. 2011;17(1):138–140.

15. Golding GR, Bryden L, Levett PN, et al. Livestock-associated methicillin-resistant Staphylococcus aureus sequence type 398 in humans, canada. *Emerg Infect Dis*. 2010;16(4):587–594.

16. Armand-Lefevre L, Ruimy R, Andremont A. Clonal comparison of Staphylococcus aureus isolates from healthy pig farmers, human controls, and pigs. *Emerg Infect Dis*. 2005;11(5):711–714.

17. Frana TS, Beahm AR, Hanson BM, et al. Isolation and characterization of methicillin-resistant Staphylococcus aureus from pork farms and visiting veterinary students. *PLoS One*. 2013;8(1):e53738. doi: 10.1371/journal.pone.0053738; 10.1371/journal.pone.0053738.

18. Cui S, Li J, Hu C, et al. Isolation and characterization of methicillin-resistant Staphylococcus aureus from swine and workers in china. *J Antimicrob Chemother*. 2009;64(4):680–683. doi: 10.1093/jac/dkp275.

19. Guardabassi L, O'Donoghue M, Moodley A, Ho J, Boost M. Novel lineage of methicillin-resistant Staphylococcus aureus, hong kong. *Emerg Infect Dis*. 2009;15(12):1998–2000.

20. Khanna T, Friendship R, Dewey C, Weese JS. Methicillin resistant Staphylococcus aureus colonization in pigs and pig farmers. *Vet Microbiol*. 2008;128(3–4):298–303. doi: 10.1016/j.vetmic.2007.10.006.

21. Rasigade JP, Laurent F, Hubert P, Vandenesch F, Etienne J. Lethal necrotizing pneumonia caused by an ST398 Staphylococcus aureus strain. *Emerg Infect Dis*. 2011;17(6):1153.

22. Moritz ED, Smith TC. Livestock-associated Staphylococcus aureus in childcare worker. *Emerg Infect Dis*. 2011;17(4):742–744. doi: 10.3201/eid1704.101852; 10.3201/eid1704.101852.

23. van Cleef BA, Verkade EJ, Wulf MW, et al. Prevalence of livestock-associated MRSA in communities with high pig-densities in the netherlands. *PLoS One*. 2010;5(2):e9385. doi: 10.1371/journal.pone.0009385.

24. Bisdorff B, Scholholter JL, Claussen K, Pulz M, Nowak D, Radon K. MRSA-ST398 in livestock farmers and neighbouring residents in a rural area in germany. *Epidemiol Infect*. 2011:1–9. doi: 10.1017/S0950268811002378.

25. Cuny C, Nathaus R, Layer F, Strommenger B, Altmann D, Witte W. Nasal colonization of humans with methicillin-resistant Staphylococcus aureus (MRSA) CC398 with and without exposure to pigs. *PLoS One*. 2009;4(8):e6800. doi: 10.1371/journal.pone.0006800.

26. Oppliger A, Moreillon P, Charriere N, Giddey M, Morisset D, Sakwinska O. Antimicrobial resistance of Staphylococcus aureus strains acquired by pig farmers from pigs. *Appl Environ Microbiol*. 2012;78(22):8010–8014. doi: 10.1128/AEM.01902–12; 10.1128/AEM.01902–12.

27. Verkade E, van Benthem B, den Bergh MK, et al. Dynamics and determinants of Staphylococcus aureus carriage in livestock veterinarians: A prospective cohort study. *Clin Infect Dis*. 2013;57(2):e11–7. doi: 10.1093/cid/cit228; 10.1093/cid/cit228.

Increase the value of your diagnostics and your value as a diagnostician

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Introduction

During lean financial times, all veterinary expenses incurred by the pork producer come under scrutiny. Therefore, it is essential to properly invest time and effort into all diagnostic submissions to get meaningful answers worthy of the diagnostic costs. But what if, despite your best efforts, the results you receive from the diagnostic laboratory just do not make sense or seem worthless? This paper discusses the issues of proper sampling technique, submission and interpretation of results that will increase the value and benefit received from diagnostic work ups for your clients.

Pig selection

The selection of pigs for testing, whether for serology or necropsy, is the most important step in obtaining an accurate and timely diagnosis. The majority of the veterinarian's time should be spent observing pigs, taking temperatures, and obtaining complete histories prior to collecting samples or performing euthanasia.

Sampling pigs that have been treated for days or weeks with antibiotics will be unrewarding if your goal is to isolate pathogenic bacteria. Likewise, sampling only pigs that are chronically ill will skew the diagnostic picture, resulting in isolation only of common secondary or opportunistic pathogens and finding histopathological lesions masked by scarring or regeneration rather than the inciting lesion.

In some situations, sample selection is straightforward – such as in outbreaks of PEDV, TGEV or ileitis in naïve herds. In other, more complex situations such as porcine respiratory disease, many agents may be involved. Diagnostic sample selection may involve testing pigs at recurring intervals in order to define all the agents involved. Sampling carefully to include acutely affected pigs will be the greatest help to the producer, the pathologist, and the veterinarian seeking treatment or prevention strategies.

Consultation

For common disease situations, as mentioned above, your sample selection will be straightforward and you can rely on your experience and best judgment to collect samples. For more complex situations or emerging diseases, consultation with a trained diagnostic pathologist may bring immediate benefit to the disease investigation.[1] The discussion should be followed by careful animal observation free of time constraints and other distractions. By doing this, you should be able to determine what is actually occurring with the pigs, not what the producer says is occurring with the pigs. An excellent example of what can be gained by evaluating pigs for clinical signs personally occurs in situations of central nervous system disease. CNS disease can present in many forms and the clinical signs displayed can sometimes help localize the lesion to a particular area of the central nervous system. While it is true that discrete lesions of the central nervous system resulting in well-defined neurological signs are not as common in pigs as they are for dogs and cats, careful observation and recording of clinical signs can be very helpful. For example, pigs that are down or ataxic but alert are likely suffering from lesions in the spinal cord or perhaps musculoskeletal system. Pigs that are unaware of their surroundings or near comatose are likely suffering from lesions in the cerebral cortex, such as those due to bacterial meningoencephalitis and viral encephalitis, in cases of primary neurological disease.[2]

Once the clinical observations are made, the clinical information should be documented and submitted in detail because it is needed by the pathologist to generate a report that is correct and meaningful. At present, laboratory submission forms are sometimes completed by a lay person and are either useless or contain incorrect information. Important or relevant information is sometimes omitted or is indecipherable and occasionally no information is provided at all. Where possible, historical data and production records should be submitted electronically therby enabling analysis using high speed computers to uncover biological information hidden in the mass of laboratory and epidemiologic data. Great care should be taken to avoid searching for a narrow list of infectious agents when faced with an unusual disease. It is precisely the wrong approach and sometimes leads to overconclusion and erroneous diagnostic endeavors. Instead, subroutines used for diagnostic investigations should be expanded to include

a complete physical examination and complete necropsy. Several animals displaying typical signs at various stages of disease should be necropsied and a full set of tissues collected for histopathology and other laboratory testes.

Evaluation of diagnostic laboratory results

Laboratory results should be evaluated with an "open mind" through a process of inductive and deductive reasoning. Diagnostic hypotheses should be developed, investigated, and continually refined while separating what you know from what you think you know. Avoid disbelieving or dismissing laboratory data if the results cannot be related to a specific disease entity because pre and post analytical errors are far more common than analytical errors. By studying a similar pattern of laboratory data in a large series of cases, confidence in the existence of a new disease or syndrome will emerge and will increase as similar findings are reported by others. When the findings have been replicated several times, a disease or syndrome will assume and increasing degree of believability.

Errors in laboratory medicine

While it may be tempting to dismiss some findings as error, be cautious before doing so. An explanation on the types of errors that exist in laboratory medicine may help you determine why results seem to make no sense. So, to understand laboratory errors, it is important to think of the diagnostic investigation as a series of events. The interval between the time a patient's sample is collected until the result is reported to the veterinarian can be divided into 3 phases: the pre-analytical phase, the analytical phase, and the post-analytical phase.

Pre-analytical phase errors

The pre-analytical phase occurs in the clinic after visiting the farm. The analytical phase occurs in the laboratory. The post-analytical phase is a shared responsibility and occurs both in the client and laboratory's environments. There are several errors that can occur in the pre-analytical phase. Errors regarding the patient status and information sharing include inaccuracies such as incorrect age listed, no age listed, or only a number listed as age and no units. A diagnostic investigation regarding diarrhea in a pig listed only with an age of "3" can result in several different test strategies on the fecal sample submitted. A 3 day old pig fecal sample will be tested for rotavirus, TGEV, *Salmonella sp., E. coli,* anaerobic bacteria such as *Clostridium perfringens*, and *Clostridium difficile* toxin. A 3 week old pig fecal sample will be tested for rotavirus, TGEV, aerobic bacteria, and protozoan parasites. A 3 year old pig fecal sample will be tested for TGEV, *Salmonella sp., Brachyspira sp.*, *Lawsonia intracellularis, Yersinia enterocolitica,* and metazoan parasites. You can see that a pathogen may be missed or tested for extraneously if the age of the pig is not shared with the laboratory. Errors in specimen collection may also occur. For example, attempting to detect the red blood cell surface pathogen *Mycoplasma haemosuis (*formerly *Eperythrozooan suis)* in pale piglets by PCR requires the submission of whole blood in EDTA. If only serum is submitted, the PCR test cannot be performed and the serology test for *M. haemosuis* antibodies in piglets is uninterpretable as they may represent maternal antibodies or may be false negative as antibody production to *M. haemosuis* occurs in waves.[3] Even when the correct sample is collected, errors in specimen processing can occur. Centrifugation at high speeds for prolonged times and in the presence of high temperatures can destroy viral RNA making it undetectable by polymerase chain reaction assays. Specimen transport occur especially in places with extremes in seasonal temperatures. Accidental freezing of formalin-fixed tissues can result in freeze-thaw artifacts that destroy tissue integrity rendering samples useless for histopathology examinations. Improper insulation and refrigeration of tissues or serum can result in high temperatures that destroy pathogen RNA or DNA or promote decomposition.

Analytical phase errors

All the steps involved in the actual analysis of the sample in the laboratory can be considered analytical phase errors. These "lab errors" include such technical mistakes such as mis-pipetting, fibrin interference (from clotting), hemolysis (from clotting), equipment errors, tube numbering or numerical ordering errors, and transcription errors. Such mistakes can occur if there is a lack of sufficient quality assurance and quality control measures in place. A laboratory with an insufficient quality assurance and quality control program may have at their disposal a lack of validated assays. Additionally, the laboratory may have chosen the inappropriate method for the sample and situation if a specific method was not available or not clearly requested. The American Association of Veterinary Laboratory Diagnosticians has in place an accreditation system that requires all accredited laboratories to have in place quality assurance programs, training records, calibration records, and validation of all tests used in each laboratory. The accreditation process is lengthy and taken seriously and repeated every 3 to 5 years. The accreditation process is in place to reduce the number of analytical phase errors and increase client satisfaction.

Post-analytical phase errors

Reviewing results for discordance, interpretation and utilization of results, and appropriateness of reference intervals

are tasks shared by the laboratory and the client. Basically, the laboratory has a responsibility to report results that have been checked for accuracy and repeatability using only validated tests. The laboratory has the responsibility of providing reference ranges and test interpretations where appropriate. Regarding test interpretations provided by the laboratory, these include cut-off points for positives and negatives (such as the IDEXX Herd Check PRRSV ELISA test has a cut-off of 0.40 s/p ratio for positive) that come from the validation information. In some cases, interpretations are not possible from the laboratory either due to insufficient numbers of test values to analyze or running a new test on a different species. Some reference ranges are age specific. Liver iron levels in pigs very greatly by age and by supplementation or diet status. Since information on age, supplementation, and diet status is often not provided with the liver samples, an interpretation on adequate, low or toxic levels of iron often cannot be made with certainty. A post-analytical phase that is primarily the responsibility of the laboratory is post analysis sample storage. Keep in mind sample storage is limited by space, time and money. Therefore, any precious, rare, or valuable samples should be archived by the submitter or special arrangements made with the laboratory.

Which errors occur with the greatest frequency?

According to a 2002 publication, "Errors in Laboratory Medicine" by Bonini, et al. from the Journal of Clinical Chemistry in 2002, a large percentage of laboratory errors occur in the pre-and post-analytical phases, with fewer mistakes (13-32%) occurring during the analytical phase.[4] They determined this by evaluating 8 years of literature (Medline and hand searches) and searching for "mistakes, blunders, errors, problem or defect associated with laboratory or medical laboratory." They found that even when different study designs, patient numbers, and discovery techniques were used, the distribution of errors across the different phases of the entire testing process was very similar, and usually occurring in the pre-and post-analytical phases. Another study conducted in 1997 analyzed the results of 40,490 "stat" results in a human clinical laboratory.[5] This is especially important to consider in our world of ever increasing needs for just-in-time testing and "RUSH" result reporting for such diseases of porcine veterinary importance like PRRSV. In this 1997 study, 189 errors were found for an error rate of 0.47%. Of those 189 errors found, 68% were pre-analytical, 13% were analytical, and 18% were post-analytical.

Reducing laboratory errors – a shared responsibility.

It is important to remember that the client and laboratory share in the responsibility of reducing laboratory errors at the pre-analytical and post-analytical levels. To prevent pre-analytical errors, the submitter and the laboratory need to be conscious of sample labeling, tube type, tube fill level, pre-centrifugation intervals, post-centrifugation intervals, storage time and temperatures, transport time and temperatures, and the condition of the samples received at the laboratory. The laboratory should serve as a reference for the client, providing the necessary submission information for optimal results.

Conclusion

The contemporary swine diagnostician must have the ability to carefully observe and describe diseases; correlate history, physical examination, clinical signs, and production data; must know when, how, what type and to what extent of laboratory investigations to undertake; must understand and interpret the significance of the laboratory results obtained; must use knowledge of pathological principles to formulate possible diagnoses; must develop hypotheses about underlying mechanisms, and must be able to explain the basic mechanisms underlying the disease processes to the producer. Having the ability to incorporate these skills will make it easier to deal with results that don't seem to make sense and increase the knowledge of all those involved in finding the correct answers.

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References

1. Collins JE. Pathogens as their role evolves: a view from the diagnostic laboratory. Proceedings of the Allen D. Leman Swine Conference, St. Paul, MN 2006; 33: 33–39.

2. Radostits OM, Gay CC, Blood DC, Hinchcliff KW. Diseases of the Nervous System, pp. 501–504. *In* Veterinary Medicine – A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats, and Horses, 9th Ed. WB Saunders, London, UK. 2000.

3. Thacker EL. Mycoplasma Diseases, pp. 710–714. *In* Straw BE, et. al., Diseases of Swine, 9th Ed. Blackwell Publishing, Ames, IA. 2006.

4. Bonini P, et al. Errors in Laboratory Medicine:. Clin Chem 48:5, 2002.

5. Plebani M et al. Mistakes in a stat laboratory: Types and frequency. Clin Chem. 43, 1997.

Haemophilus parasuis: Infection, immunity and antibiotics

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Introduction

H. parasuis is considered one of the most important bacterial pathogens affecting pigs. Vaccines and other management strategies have not always been successful in controlling the losses associated to *H. parasuis*. This bacterium frequently colonizes the mucosal of the swine upper respiratory tract. Oliveira et al., (2004) showed that exposure of young pigs to a low dose of virulent *H. parasuis* strain (controlled exposure) reduced mortality due to Glasser's disease and could induce the development of protective immunity. Under field conditions, factors that may disrupt colonization patterns of *H. parasuis* at weaning, such as antibiotic treatment, might result in delay of disease to late nursery phases, due to lack of immune response priming.

H. parasuis, as a gram-negative bacterium, interacts with the immune system at different levels, including the innate and the specific immune system. The literature offers a vast characterization of antibody-mediated immune responses, whereas little information is currently available for innate immune and cell-mediated responses.[2] A recent study showed that *H. parasuis* susceptibility to phagocytosis by porcine alveolar macrophages (PAMs) correlates with the clinical origin of the strain.[3] *H. parasuis* strains isolated from systemic lesions (virulent) were resistant to phagocytosis, while nasal strains (non-virulent) were efficiently phagocytosed by PAMs in vitro, followed by subsequent bacterial death within the macrophage. Similarly, *H. parasuis* was able to stimulate the production of IL-1 expression in lung of pigs undergoing severe disease following experimental infection, whereas IL-4, IL-10, tumor necrosis factor-alpha (TNF-α), and (IFN-γ) were expressed in significantly higher levels in spleen, pharyngeal lymph nodes, lung and brain of survivors, which suggests that these cytokines might contribute to protection against *H. parasuis*.[4] Specific increases in the relative proportions of T and B lymphocytes isolated from peripheral blood mononuclear cells (PBMC) were also found in colostrum-deprived pigs after challenge with virulent *H. parasuis* strain, but not after immunizations.[5,6]

Most frequently, pigs exposed to *H. parasuis* live cultures or vaccinated with killed bacterins generate progressively increasing serum IgG antibody response. Pigs with high homologous titers are protected against challenge,[7] while Glasser's disease has been associated with absence or low titers of serum antibodies.[8,9] Likewise, pigs lacking maternal immunity were susceptible to Glasser's disease upon inoculation with a virulent *H. parasuis* strain, whereas pigs that received maternal immunity were protected.[10] Cerda-Cuellar et al. (2010) further demonstrated that piglets from vaccinated sows had significantly higher levels of antibodies earlier after birth and were colonized later and to a lower degree than piglets from non-vaccinated sows. Furthermore, the increase in colonization rate was associated with a decrease in *H. parasuis* serum antibodies in piglets, which indicates that the level of maternal antibodies in piglets might be able to modulate the timing and level of colonization by *H. parasuis*.

According to Oliveira et al., (2004), the success of *H. parasuis* controlled exposure in preventing mortality during the nursery stage suggests that *H. parasuis* might interact with the mucosal of the upper respiratory tract, possibly resulting in colonization, priming of the immune response and protection from disease. Therefore, the development of a protective immune response against *H. parasuis* might be delayed by factors that interfere with bacterial colonization. Recent studies have shown that early antibiotic treatment not only prevented the development of an effective immune response against *Chlamydia trachomatis* and *Salmonella* sp., but also rendered the animals susceptible to challenge infection.[12,13] In contrast, however, a recent study demonstrated that early antibiotic treatment with enrofloxacin against *Salmonella typhimurium* infection primed specific antibody response, which protected against secondary challenge.[14] Enrofloxacin, the primary compound of Baytril (Bayer Animal Health), is considered efficacious against *H. parasuis*, but limited information is currently available on the effects of enrofloxacin on *H. parasuis* colonization and immunity. Therefore, a better understanding of *H. parasuis* colonization over time and the effect of antibiotics on colonization and the development of an effective immune response requires further investigation in order to prevent and control disease.

The objective of our study was to evaluate the effect of enrofloxacin (Baytril 100) in *H. parasuis* colonization in

weaned pigs. Additionally, in order to recreate *H. parasuis* colonization under conditions representative of what occurs in the field, a new experimental inoculation model was developed in conventional pigs to better understand dynamics of colonization and immune responses to virulent *H. parasuis*.

Material and methods

Study 1: Effect of enrofloxacin on *H. parasuis* colonization

Forty five pigs were identified in a commercial herd and screened for *H. parasuis* using gel-based PCR.[15] Twenty-four of the weaned pigs that tested positive for *H. parasuis* were selected and moved to the University of Minnesota research isolation facility. Pigs were randomly divided into one treatment group of 12 pigs and one control group of 11 pigs and housed in two separated rooms (one pig in the control group died shortly after arrival). On arrival at the research facility, blood samples and nasal and tonsil swabs were collected from all the pigs. Pigs in the treatment group were treated with a single dose of injectable enrofloxacin (0.034ml/lb / 7.5 mg/Kg Baytril) at 24 h post arrival. Pigs in the control group received saline solution intramuscularly but not antibiotics. Pigs were monitored for 15 days. Throughout the study pigs were sampled by tonsillar and nasal swabs and selected pigs were necropsied at 3, 8 and 14 days post-treatment (DPT). At necropsy, swabs from the nasal cavity, tonsil, trachea, lung, and peritoneal and pleural serosas were collected for diagnostic investigation. Nasal swabs were processed for qPCR testing. DNA from swabs were extracted using DNeasy Blood & Tissue Kit Qiagen kit and then tested individually by quantitative PCR (qPCR),[16] with some modifications. Differences between the proportions of *H. parasuis* positive pigs in treated vs control groups at each sampling time point were calculated using Fisher's Exact Probability Test. The Bonferroni correction was used to address multiple comparisons ($\alpha = 0.003$).

Study 2: Colonization model

Sixteen conventional weaned pigs were divided into 3 groups and placed into 3 different rooms at the University of Minnesota research isolation facility. At day 0 of the study, groups 1 and 2 (n = 6 each) and 3 (n = 4) received 10^6 or 10^4 CFU/ml of highly virulent *H. parasuis*, strain Nagasaki, or saline, intranasally, respectively. Clinical evaluation and nasal swabs (for bacterial culture) were collected before and every day after inoculation (dpi) during 7 days. Blood samples were collected on 1, 3 and 4 dpi for bacterial isolation. At 2 time points (4 and 7 dpi), half of the pigs were euthanized and assayed for the presence of *H. parasuis* in the respiratory tract and systemic sites. *H. parasuis* isolates obtained were genotyped by ERIC-PCR[17] in order to differentiate strain Nagasaki from any other *H. parasuis* strains that the pigs may carry.

Results

Study 1: Effect of enrofloxacin on *H. parasuis* colonization

Pigs tested positive by gel-based PCR[15] at the herd of origin and on the day of arrival. Results from qPCR showed that twenty two out of twenty three pigs (95.7%) tested positive from tonsil swabs on arrival, while only 11 pigs (48%) tested positive from nasal swabs. All treated pigs tested *H. parasuis* negative by qPCR at 1 day post-treatment (DPT). Moreover, treatment effect persisted partially until 12 DPT. The control pigs tested positive throughout most of the days of the study. Differences between the proportion of positives for control and treated pigs were statistically significant on days 1, 2, 3, 4, 5, 6 and 7 post-treatment for nasal swabs and on days 2, 4 and 5 post-treatment for tonsil swabs (P-value < 0.003). At necropsy, nine out of eleven control pigs were positive for *H. parasuis* in at least one of the five samples tested. One sample from the serosas was cultured positive and two isolates were recovered from the nasal cavity. In contrast, only 4 out of 12 pigs in the treatment group tested positive at necropsy. Three pigs tested positive in the tonsil, 3 in the trachea, and one in the nasal cavity. None of the serosas were cultured positive and only one bacterial isolate could be cultured from the nasal cavity at necropsy. Interestingly, all isolates were recovered at 15 DPT. No clinical signs or lesions of any kind were observed during the experiment.

Study 2: Colonization model

ERIC-PCR genotyping demonstrated that the *H. parasuis* strains isolated before inoculation were identical, and frequently isolated from the nose of all the pigs throughout the study. The Nagasaki strain, also identified by ERIC-PCR, was recovered from the nose of 5 pigs after inoculation. Moreover, tracheal swabs collected at necropsy yielded only Nagasaki isolates in 10 pigs. Overall the Nagasaki strain was isolated at least once from all 12 inoculated pigs, but was never recovered from systemic sites of experimentally inoculated pigs, control pigs or before inoculation. There were no differences in isolation of the Nagasaki strain based on inoculation doses. The absence of fever, clinical signs, lesions and bacteremia demonstrates that there was no systemic infection, even though the Nagasaki strain can be highly virulent. Trachea represents a less competitive niche for *H. parasuis* colonization, which may explain why tracheal swabs yielded higher number of Nagasaki isolates compared to nasal swabs.

Conclusions

Under the conditions of this study, enrofloxacin treatment significantly reduced the number of pigs colonized with *H. parasuis* and this effect was mostly seen during the first week post treatment. In addition, enrofloxacin also reduced the presence of *H. parasuis* on the nasal cavity and the tonsils of naturally colonized pigs, but was unable to completely eliminate the organism. Nevertheless, the results showed that the effect of enrofloxacin was rapid and active for relatively long time against *H. parasuis* harbored on the nasal cavity and tonsils of weaned pigs, since all treated pigs tested negative at 1 DPT and continued to test negative during most of the study.

Reduction of *H. parasuis* in the nasal cavity and tonsils may help to control the disease during susceptible stages such as the weaning period, but further research is needed to evaluate the lasting effect of enrofloxacin in colonization patterns and immune response. We intend to investigate these findings further by using the *H. parasuis* colonization model that we have established in conventional pigs to study the early events of *H. parasuis* infection and immune response in conventional pigs.

References

1. Oliveira, S. et al. 2004. Evaluation of *Haemophilus parasuis* control in the nursery using vaccination and controlled exposure. *J Swine Health and Production* 2: 123–128.

2. Aragon, V. et al. 2012. Glasser's Disease. In: Zimmerman, J.J. et al. Diseases of Swine. 10th Ed. Wiley-Blackwell, Ames, IA, USA, p.760–769.

3. Olvera, A. et al. 2009. Differences in phagocytosis susceptibility in *Haemophilus parasuis* strains. Vet. Res. 40:24.

4. Martin de la Fuente, A.J. et al. 2009. Cytokine expression in colostrum-deprived pigs immunized and challenged with *Haemophilus parasuis*. Res. Vet. Sci. 87, 47–52.

5. Martin de la Fuente, A.J. et al. 2009. Blood cellular immune response in pigs immunized and challenged with *Haemophilus parasuis*. Res. Vet. Sci. 86, 230–234.

6. Frandoloso, R. et al. 2012. New insights in cellular immune response in colostrum-deprived pigs after immunization with subunit and commercial vaccines against Glasser's disease. Cell. Immunol. 277, 74–82.

7. Martin de la Fuente, A.J. et al. 2009. Systemic antibody response in colostrum-deprived pigs experimentally infected with *Haemophilus parasuis*. Res. Vet. Sci. 86, 248–253.

8. Riising, H.J. 1981. Prevention of Glasser's disease through immunity to *Haemophilus parasuis*. Zentralbl. Veterinarmed (B). 28, 630–638.

9. Rapp-Gabrielson, V.J. et al. 1997. *Haemophilus parasuis*: immunity in swine after vaccination. Nord. Vet. Med. 27, 20–25.

10. Blanco, I. et al. 2004. Comparison between *Haemophilus parasuis* infection in colostrums-deprived and sow-reared piglets, Vet. Microbiol. 103: 21–27.

11. Cerdà-Cuéllar, M. et al. 2010. Sow vaccination modulates the colonization of piglets by *Haemophilus parasuis*. Vet. Microbiol. 145, 315–320.

12. Su, H. et al. 1999. The effect of doxycycline treatment on the development of protective immunity in a murine model of chlamydial genital infection. J. Infect. Dis. 180,1252–1258.

13. Griffin, A., et al. 2009 Successful treatment of bacterial infection hinders development of acquired immunity. J. Immunol. 183, 1263–1270.

14. Johanns, T.M. et al. 2011. Early eradication of persistent *Salmonella* infection primes antibody-mediated protective immunity to recurrent infection. Microbes and Infection. 13: 322–330.

15. Oliveira, S., Galina, L., Pijoan, C. 2001. Development of a PCR test to diagnose *Haemophilus parasuis* infections. J Vet Diag Invest 13:495–501.

16. Turni, C., Pyke, M., Blackall, P.J. 2010. Validation of a real-time PCR for *Haemophilus parasuis*. *J Applied Microbiol* 108:1323–1331.

17. Rafiee, M. et al. 2000. Application of ERIC-PCR for the comparison of isolates of *Haemophilus parasuis*. Aust Vet J 78, 846–9.

Association of *Brachyspira* antibiograms with species and source

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Introduction

Brachyspira hyodysenteriae is the etiological agent of swine dysentery, a mucohemorrhagic diarrheal disease of grower-finisher pigs. Recently, a novel pathogenic species, "*B. hampsonii*", has been isolated from clinical cases of swine dysentery in North America. Other members of the genus *Brachyspira* cause varying degrees of clinical disease ranging from mild to mucoid diarrhea. Although these diseases continued to cause significant economic losses to swine rearing countries globally, they largely disappeared from the US in the 1990s. Recently however, *Brachyspira* species known to cause mucohemorragic, mucoid and mild diarrhea in grower-finisher pigs have been increasingly isolated in the US Limited information exists on the susceptibilities of pathogenic and commensal *Brachyspira* species towards commonly used antimicrobials. In addition, no routine diagnostic method currently exists in the US for testing *in vitro* antimicrobial susceptibilities of *Brachyspira* species. Therefore our aim was to identify the *in vitro* susceptibility patterns of various *Brachyspira* species towards commonly used antimicrobials in the US A second objective was to validate a broth microdilution method for routine diagnostic testing of *Brachyspira* species.[1]

Materials and methods

Bacterial isolates and antimicrobials: A sample set of 80 submissions made to the University of Minnesota Veterinary Diagnostic Laboratory (20 *B. hyodysenteriae*, 20 "*B. hampsonii*", 20 *B. pilosicoli* and 20 *B. murdochii*) originating from a variety of sites, companies and states across the US in 2009 to 2013 were tested. A set of 40 isolates (10 each of four different species) were evaluated by both the agar and the broth microdilution methods to test for comparability. An additional set of 40 isolates (10 each of four different species) were evaluated in duplicate by the broth microdilution method to test for reproducibility. Susceptibility of *Brachyspira* species towards commonly used antimicrobials including tiamulin, valnemulin, lincomycin, tylosin, tylvalosin and carbadox (agar dilution method only) was evaluated. The *B. hyodysenteriae* type strain B78^T (ATCC 27164™) was used as an experimental control.

Agar dilution method: Fresh sheep blood agar plates were prepared which contained two-fold serial dilutions of antimicrobials. A 5µl suspension of 1×10^8 *Brachyspira* per ml of BHI broth supplemented with 10% FBS was inoculated and a cut was made on the agar to observe the ring phenomenon. The plates were incubated anaerobically at 37°C for 4 days.[2]

Broth dilution method: A 500 µl suspension of 1×10^6 to 5×10^6 *Brachyspira* per ml of BHI broth supplemented with 10% FBS was inoculated into each well of the broth antimicrobial susceptibility panel (VetMIC™ Brachy panel, SVA Sweden). The panels were anaerobically incubated at 37°C for 4 days on a rotator. The internal growth controls for each isolate were checked microscopically to confirm purity.[3]

Analysis: The minimum inhibitory concentration (MIC) was identified as the lowest concentration of the tested antimicrobial that inhibited visible growth. The MIC_{90} and MIC_{50} values (in µg/ml) were calculated for each species evaluated. The association between isolates from sow/gilt sites (vs. finisher sites) and susceptibility towards tylosin and lincomycin was tested with an odds ratio and a z-test for two proportions.

Results

The concentration at which the growth of 50% (MIC_{50}) and 90% (MIC_{90}) of the isolates was inhibited is summarized in tables 1 and 2. Table 1 contains the comparative MIC_{50} and MIC_{90} results of the agar and broth microdilution methods categorized by species. In general, the results obtained by the broth dilution method were equal to or within two doubling dilutions of those obtained by the agar dilution method. In addition, the results of the broth dilution method showed good reproducibility. Table 2 contains the MIC_{50} and MIC_{90} results by species using only the broth microdilution method for tiamulin, valnemulin, lincomycin, tylosin and tylvalosin, and only the agar dilution method for carbadox. These results were interpreted by using epidemiological cut-off values proposed by Pringle et al.[4] In general, MICs for all species were high for lincomycin, tylosin and tylvalosin; and low for tiamulin, valnemulin and carbadox. MICs for *B. pilosicoli*

and *B. murdochii* were moderately higher for all tested antimicrobials than for other species. Several *B. pilosicoli* isolates with unexpectedly high MICs for tiamulin were identified. Table 3 shows the results of the odds ratio and the two-proportion z-test comparing high susceptibility to tylosin and lincomycin in sow/gilt sites as compared to finisher sites. The results indicate an approximately 13 and 10 times higher odds of high susceptibility towards tylosin and lincomycin respectively, for isolates originating from sow/gilt sites as compared to that of isolates originating from finisher sites. In addition, the proportion of isolates originating from sow/gilt sites with a high susceptibility to tylosin and lincomycin was found to be statistically different from that of isolates originating from finisher sites ($P < 0.0001$).

Table 1: Comparison of the MIC_{50} and MIC_{90} (in µg/ml) results by species using the agar and the broth microdilution methods

species	method	tiamulin		valnemulin		lincomycin		tylosin		tylvalosin	
		MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
B. hyodysenteriae	agar	≤ 0.063	0.25	≤ 0.031	0.063	32	64	128	> 128	8	32
	broth	0.125	0.125	≤ 0.031	0.125	32	64	> 128	> 128	2	8
"B. hampsonii"	agar	≤ 0.063	0.5	≤ 0.031	0.125	1	> 64	4	> 128	0.5	16
	broth	≤ 0.063	0.25	≤ 0.031	≤ 0.031	0.5	32	2	> 128	≤ 0.25	8
B. pilosicoli	agar	≤ 0.063	> 8	≤ 0.031	4	32	> 64	128	> 128	16	> 32
	broth	0.125	> 8	0.063	1	32	32	> 128	> 128	16	> 32
B. murdochii	agar	0.5	1	1	4	8	32	> 128	> 128	16	> 32
	broth	0.125	1	0.25	0.5	16	16	> 128	> 128	8	32

Table 2: MIC_{50} and MIC_{90} (in µg/ml) results by species using only the broth microdilution method (and only the agar dilution method for carbadox).

"*" indicates values with low susceptibility to an antimicrobial (4)		tiamulin	valnemulin	lincomycin	tylosin	tylvalosin	carbadox
MIC_{50}	*B. hyodysenteriae*	≤ 0.063	≤ 0.031	16*	> 128*	4*	0.0039
	"B. hampsonii"	≤ 0.063	≤ 0.031	≤ 0.5	4	0.5	0.0039
	B. pilosicoli	0.5*	0.5*	32*	> 128*	32*	0.0078
	B. murdochii	0.25	0.25	16*	> 128*	8*	0.0039
MIC_{90}	*B. hyodysenteriae*	0.5*	0.5*	32*	> 128*	16*	0.0078
	"B. hampsonii"	0.25	≤ 0.031	16*	> 128*	4*	0.0039
	B. pilosicoli	> 8*	2*	64*	> 128*	> 32*	0.0156
	B. murdochii	1*	0.5*	16*	> 128*	32*	0.0078

Table 3: Results of odds ratio comparing high susceptibility to tylosin and lincomycin from sow/gilt sites as compared to finisher sites

Antimicrobial	Odds ratio (95% CI)	Z – score (*P* – value)
Tylosin	12.68 (4.01 to 40.01)	4.7 ($P < 0.0001$)
Lincomycin	9.68 (3.2 to 29.3)	4.0 ($P < 0.0001$)

Discussion

While most *Brachyspira* species isolates showed a pattern of high susceptibility to tiamulin, valnemulin and carbadox, and low susceptibility to tylosin, lincomycin and tylvalosin; several isolates originating from sow/gilt sites with high susceptibility to all antimicrobials were detected. In addition, *B. pilosicoli* showed low susceptibility towards most antimicrobials tested. This study indicates that different MIC patterns were generally associated with the species of *Brachyspira* evaluated as well as with the source of animals (sow/gilt site vs. finisher site). In addition, this study validates the use of a broth microdilution method for routine diagnostic applications.

References

1. M. Karlsson et al., 2003. J.Clin.Microbiol., June 2003. pg 2596–2604.

2. J. Rohde et al, 2004. Vet Microbiol. 102, 2004. pg 25–32.

3. M. Karlsson et al., 2001. Vet. Microbiol. 84, 2002. pg 123–133.

4. M. Pringle et al., 2012. Acta Vet Scand., 2012, 54:54.

Antibiotics and their role in *Brachyspira* elimination programs

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Introduction

With any bacterial elimination program, sanitation coupled with discontinuing contamination of the environment is fundamental to eradicating the organism from a premise. Site depopulation is the easiest way to discontinue contamination of the environment and has led to all in all out pig flow or depopulation/repopulation strategies. The decision to depopulate involves a variety of considerations including feasibility, economics, and site biosecurity. In situations where the production economics of continuing to populate a facility with infected animals versus depopulation are advantageous, antibiotic interventions are employed to assist with discontinuing contamination of the environment. This strategy increases the risk of failure in the elimination program and increases cost through antibiotic usage but is calculated to be offset by continued production and the economics of the situation. An increase in the laboratory diagnosis of strong beta hemolytic *Brachyspira* and associated lesions since 2006[1] coupled with the recent identification of *B. hampsonii*[2] has reinvigorated the practitioners interest in this reemerging pathogen. Antibiotic selection and dosage play a key role in elimination of this pathogen.

Program

In large sow herds that are not depopulated, a structured and rigorous program with quality assurance checks must be employed to eliminate *Brachyspira*. Due to the labor requirements of the program, external labor and twenty four hour washing crews are often times warranted due to the considerable work load and scope of the project. This program requires excellent sanitation of the following areas:

1. Animal areas
   a. Presoak areas with a detergent
   b. Strict sanitation of flooring, side walls, ceiling, feed, and ventilation systems
   c. Inspection and certification by veterinarian or upper management
   d. Disinfection with quaternary/glutaraldehyde disinfectant with contact time of 10 minutes prior to moving animals[3]
   e. Removal of water nipples, cleaning and disinfection
   f. Prevention of backsplash – use of plastic and tarps during pressure washing between clean and dirty areas
2. Animals
   a. Washed prior to movement with particular attention to feet, legs and underline
   b. Animals disinfected with iodine
3. Fomites
   a. Danish changes between clean and dirty areas
   b. McREBEL during site sanitation. No use of hot boxes, processing carts, etc.
4. Water source
   a. Inspection of holding tank, ensure no rodent or bird exposure.
5. Rodent/pest control
   a. Bait stations every 50 feet around the perimeter, checked twice weekly.
   b. Bait stations installed in the barn attics, checked weekly.
   c. Grass and weeks mowed and sprayed around the barns. Intact rock barrier surrounds the perimeter of all barns.
   d. Implementation of The Rodent Index System (adapted from Penn State Cooperative Extension) to quantify rodent activity[4].
   e. Boric acid applied to all electric boxes and areas where cockroaches congregate. Creep dividers in farrowing house filled with foam spray to eliminate cockroach habitat. (Table 1)

Table 1: Example medication and sanitation schedule

Timeline	Stage	Inclusion rate	Medication start date	Medication end date	Washing startdate	Washing end date
Week 1-2	Gestation Lactation GDU	Denagard 200g/ton & 60 ppm H_20 Denagard 200 g/ton Mecadox 50 g/ton	June 20	July 3	June 27	July 3
Week 3-6	Gestation Lactation GDU	Denagard 35 g/ton Denagard 35 g/ton Mecadox 25 g/ton	July 4	July 31		
Week 7-8	Gestation Lactation GDU	Denagard 200g/ton & 60 ppm H_20 Denagard 200 g/ton Mecadox 50 g/ton	August 1	August 14	August 8	August 14

Antibiotics commonly used for treatment of *Brachyspira* include tiamulin, carbadox, tylosin, lincomycin, gentamicin, valnemulin, and salinomycin.[5,6] Of those tiamulin and carbadox are most commonly utilized in the United States. *Brachyspira* MIC values to tylosin are intermediate and high MIC values to lincomycin and gentamicin provide concern of resistance to these antibiotics.[5] Valnemulin is not approved for use in the United States and salinomycin is not labeled for use in swine.

MIC data for the pleuromutalin drugs, valnemulin and tiamulin, suggest that antibiotic resistance patterns in US isolates of *Brachyspira* are not developing as quickly as what has been described in other countries. Carbadox and salinomycin MIC levels are at the low end of the range tested and expected to be effective.[5] The MIC_{90} of *Brachyspira hyodysenteriae* and *spp* is 0.03 and 0.015 respectfully.[5] Administration of carbadox at 50 g/ton provides colonic activity at 4 mg/g (Dwain Guggenbiller. personal communication. June 2012).

When a herd is infected with *Brachyspira hampsonii*, MIC patterns are generally elevated compared to *Brachyspira hyodysenteriae*. Susceptibility testing in 2011, examined MIC patterns of 11 North American sourced *Brachyspira spp.* isolates. 91% (9/11) of these isolates exhibited strong beta hemolysis. After the study was published, these isolates were sequenced and mostly identified to be *B. hampsonii* (Eric Burrough, personal communication, June 2012). The median MIC values from *B. hampsonii* are 8 time higher and the MIC_{90} 4 times higher than isolates of *B. hyodysenteriae* tested in this study (n = 24).[3] Tiamulin colonic contents activity is outlined in Figure C with the MIC_{90} setting a target of 2 for *B. hampsonii*. Ad libitum feed intake in lactation results in a calculated daily drug dose of 2.4 mg/lb (500 lb sow with 12 lb per day intake – 200 g/ton tiamulin). This is slightly less than the published daily dosage of 3.0 mg/lb which supplies activity in colon contents at 2.84 mg/g[7] but is estimated to supply tiamulin activity of > 2 mg/g (Keith Erlandson, personal communication, June 2012). Restricted feed intake in gestation results in a calculated daily drug dose of 0.9 mg/lb (500 lb sow with 5 lb per day intake – 200 g/ton tiamulin) which results in colonic content concentrations below the limit of quantification (<1.62 mg/g)[7]. Since this is less than our target MIC value of 2, supplemental tiamulin must be administered via the drinking water. Additional dosage of tiamulin at 60 ppm provides a calculated daily drug dose of 2.27 mg/lb[7] (500 lb sow with 5 gal per day intake). Total combined dosage is estimated to be ~3.27 mg/lb which is estimated to provide tiamulin activity of >2 mg/g in the colon contents (Keith Erlandson, personal communication, June 2012). (Tables 2 and 3)

References

1. Burrough E. Brachyspira-Associated Colitis. ISU Swine Disease Conference proceedings. 2012. pp 43–47.

2. Chander Y, Primus A, Oliveira S, et al. Phenotypic and molecular characterization of a novel strongly hemolytic Brachyspira species, provisionally designated "Brachyspira hampsonii". JVetDiagn Invest 2012;24:903–910.

3. Gebhart C. Efficacy of Synergize against *Brachyspira hyodysenteriae*. Preserve International report. Received by email June 2012.

4. Martin G. The Rodent Index System. Penn State Extension – poultry. www.personal.psu.edu/gpm10/Rodent_Index11.pdf. Accessed on July 4, 2012.

5. Clothier K, Kinyon J, Frana T, Naberhaus N, Bower L, Strait E, Schwartz K. Species characterization and minimum inhibitory concentration patterns of *Brachyspira* species isolates from swine with clinical disease. 2011. *J VET Diagn Invest*. 23: 1140.

6.Straw B, Zimmerman J, D'Allaire S, Taylor D. Diseases of Swine; 9th Edition. Swine Dysentery pp. 785 – 805. Blackwell Publishing, 2006.

Table 2: MIC for *Brachyspira hyodysenteriae* and *spp*.

		Tiamulin MIC (µg/ml)	Carbadox MIC (µg/ml)
B. hyodysenteriae (n = 24)	Median	0.125	0.015
	Mode	0.125	0.015
	MIC_{90}	0.5	0.03
	Range	0.125 - 4	0.008 - 0.06
B. spp. (n=11)	Median	1	0.008
	Mode	1	0.015
	MIC_{90}	2	0.015
	Range	0.125 - 4	0.004 - 0.03

Clothier K. 2011. *J VET Diagn Invest*. 23: 1140.

Table 3: Tiamulin activity

Treatment group	Calculated daily drug dose, mg/lb	Tiamulin activity, mg/g			
		Lung	Tonsil	Colon mucosa	Colon contents
I. Feed medication (THF)					
35 g/ton	.9	a	a	a	a
105 g/ton	3.0	1.46 (1)	a	a	2.84 (4)
200 g/ton	6.2	1.99 (3)	a	1.57 (1)	8.05 (5)
II. Water medication (THF)					
60 mg/L	2.8	1.11 (1)	a	a	2.16 (2)
120 mg/L	6.0	4.26 (5)	a	1.56 (3)	5.59 (5)
180 mg/L	9.5	8.50 (5)	2.50 (1)	3.39 (4)	18.58 (4)
Limit of quantitation (LOQ)		**0.94**	**2.31**	**0.92**	**1.62**

a Below the Limit of Quantitation. Anderson, M.D. 1994. AASP Annual Meeting. p. 115-117.

7. Anderson, M.D.; Stroh, S.L.; Roger, S. 1994. Tiamulin ("Denagard®") in certain swine tissues following oral and intramuscular administration. Proc. Ann. Mtg. American Association of Swine Practitioners, Chicago, Ill. USA p. 115–117.

Methodology to investigating CNS disease in pigs post-weaning

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Introduction

Central nervous system signs in nursery pigs are not uncommon and it doesn't take long for someone new to the hog industry to experience their first "paddling pig" when frequenting young pigs. Often times this scenario is described as a 'Strep' pig and treatment with an antibiotic such as penicillin is successful. However, this scenario may not always be quite so simple and may require additional intervention. What steps should be taken when investigating central nervous system signs in nursery pigs? What differentials should be considered? The discussion below outlines a practitioner's methodology to diagnosing central nervous system disease in young growing pigs.

Interviewing the eyewitness

The caretaker involved with the day to day care of the pigs can provide a great deal of insight when investigating disease events in a population of pigs. When a veterinarian arrives on a sick call it is a point in time observation that may or may not be an accurate portrayal of what has been going on. Involving them in the investigation is key to understanding the big picture and will enhance the ability of the veterinarian to arrive at the correct diagnosis.

Gathering evidence

The next step in the investigation is to review pertinent records that may help to further narrow the scope of differentials. Looking at mortality records and noting how many animals have died in the past 7 days is useful. Also it is helpful to know whether or not these pigs are sudden death pigs or chronic pigs. Records should also be available for treatments that have been occurring and should indicate how many pigs have been treated per day, with what, and how much.

Observing the scene

Quietly opening the door and observing the pigs in their environment will give clues as to whether or the pigs are comfortable in their environment. Next, physically entering the pens and walking through the pigs is essential to get a true picture of the condition of the pigs. Pigs displaying neurological signs should be observed closely for ataxia, head tilt and circling. Get all of the pigs up and observe them for tear staining, nasal secretions, skin lesions, lameness and activity level. Completely evaluate the entire population before coming to a conclusion on the diagnosis. Make a mental note of how many pigs are visually ill and estimate the percent of the population that is affected. Begin forming a list of differentials.

Necropsy any dead pigs that are present and note gross lesions. If the diagnosis is still unclear euthanize 2-3 acute pigs for submission to the diagnostic lab.

Developing a key suspect list

Differentials vary depending on the distribution of disease. Primary differentials for diagnosing neurological signs affecting populations of pigs should include:

- Infectious causes
  - Bacterial meningitis-commonly caused by *Strep. suis*
  - Edema disease caused by various types of *E.coli*
  - Meningoencephalitis caused by *Haemophilus parasuis*
- Non infectious causes:
  - Salt poisoning (water deprivation)
  - Toxicities (i.e. organophosphates, ionophores)
  - Nutritional deficiencies (i.e. vitamin A)

Other differentials exist but should be considered after ruling out the primary infectious causes.

Gathering samples

Necropsy of recent mortalities may be helpful when evaluating gross lesions but are not ideal for tissue submission due to level of autolysis that has likely taken place. When choosing pigs to sample it is important to choose at least 2-3 acutely affected pigs that are representative of the population. Euthanasia method should be taken into consideration and if possible done in a way that preserves

the brain from damage. A set of fresh and fixed tissues should be taken and should include a full sampling of both thoracic and abdominal tissues (lung, heart, lymph nodes, liver, spleen, kidney, small intestine, colon, and brain). Tissues should be placed on ice and fixed with 10% formalin as soon as possible to prevent further autolysis. Swabbing the lateral cerebral hemispheres and other parts of the brain may also be warranted in cases where bacterial meningitis is suspected.

Treatments

Treatment for central nervous system disease in pigs should not only involve antibiotic use but should also include management related interventions such as reviewing ventilation and stocking density of the barn. Overcrowding and poor ventilation including high humidity, can lead to increased incidence of bacterial meningitis.[1]

In infectious cases, antibiotic choices should include those antibiotics that are capable of crossing the blood brain barrier and may include an anti-inflammatory drug such as Predef. Common injectables, specifically for treatment of *Strep.* meningitis in post-weaning pigs, should include penicillin or cephalosporins. Oral antibiotic choices include a combination of chlorotetracycline and tiamulin.. Successful outcomes using potassium penicillin G via the water to treat *Strep. suis* have also been documented.[2] However, an effort should be made to obtain a sensitivity specific to the isolate obtained from the farm of origin.

Case 1

A nursery located in MN was placing approximately 2000 PRRS positive pigs every 9 weeks at a rate of 500 pigs per week. The pigs ranged in age from 17-21 days and were placed by delivery in 4 rooms housing 500 head each.

At 10-14 days post-placement the grower would start to notice pigs displaying signs of ataxia with some progressing to lateral recumbency and paddling movements of the front and rear limbs. Pigs in the affected room would rapidly deteriorate and sometimes as many as 25% of the pigs would be showing the clinical signs described including head pressing and chomping at the mouth. Mortality rates were as high as 25% on a per room basis.

Diagnostics revealed a highly resistant pathogenic hemolytic *E.coli*. Genotyping revealed the presence of the StX2e toxin and F18 pilus. A combination of injectable and oral antibiotics was attempted with marginal response. This disease process was highly predictable and occurred at various severity levels for over a year despite numerous and extensive interventions. Interventions included ventilation adjustments, gating modifications, well treatments, downtime, oral inoculants etc. however clinical signs continued. The final intervention was to seal the concrete floors with two coats of a concrete sealer after which the *E.coli* related death loss stopped.

Case 2

2400 PRRS negative pigs were being placed into a continuous flow nursery site in MN every other week. Around 4-7 days post-placement the caretaker reported finding several good pigs found dead and numerous others down and paddling. Death loss quickly approached 1% within 3 days. Oral antibiotic intervention using a combination of chlorotetracycline and tiamulin was implemented to address the current disease issue and tissues were sent off. Diagnostics confirmed meningitis and septicemia due to *Strep. suis* serotype 1.

The following groups placed on feed also showed similar clinical signs so a decision was made to implement an autogenous bacterin on the pigs at the sow farm as well as to treat the pigs with ceftiofur upon weaning.

References

1. Straw et. al. *Disease of Swine 9th Edition*. 2006. Blackwell Publishing, Ames, IA.

2. Byra C, Gadbois P, Cox WR, Gottschalk M, Farzan V, Bauer SA, Wilson JB. Decreased mortality of weaned pigs with Streptococcus suis with the use of in-water potassium penicillin G. *Can Vet J.* 2011 Mar; 52(3):272–6.

CNS disease diagnostics

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Introduction

Central nervous system (CNS) disease or like diseases can be frustrating circumstances in growing pigs, even if there are only a few animals affected. The challenges we as swine veterinarians face is accurately diagnosing these issues, mitigating, and then preventing future problems. The intent of presentation/proceedings to provide guidance, understanding, and sample collection tips for CNS disease scenarios or disease presentations that may arise in nursery aged pigs.

Disease diagnosis is different for each of us. It basically comes down to how each of us thinks followed by incorporating previous experiences. What you see, determine, or link together with a thorough investigation in the clinical setting will hone in your diagnostics skills when it comes to particular pathogens or syndromes, but this will also guide proper sample submissions when CNS disease problems are not so clear cut from the normal.

Bear in mind that not every condition with perceived neurological signs is infectious or is even related to the central nervous system. Too often we as veterinarians make broad assumptions on the cause of disease. There is nothing wrong with this. Experience usually lends our differentials to be true. However, not understanding potential differential will hinder finding the actually etiologic cause of the clinical syndrome.

Oh, where to begin? Well, how about on a complete clinical examine of those pigs displaying signs of disease. Neurology was maybe not your favorite during veterinary school, but that does not mean that we should forget about proper procedure. An exam can localize the issue or possibly disprove CNS involvement all together. Some clinical examples would include proprioceptive defects, varied coordinated movement, posture and mentation. A second key aspect of an investigation is gathering a detailed history including recent procedures such as vaccinations, feed changes, or mechanical issue within the facility affecting air, feed or water.

Before we start listing conditions, a mini review of basic neurology may help. The cerebrum is responsible for behavior, voluntary movement and consciousness; it is the hub for normal thought. "Down paddling" and nystagmus is a loss of voluntary movement of the limbs and ocular muscles and therefore would indicate cerebrum involvement. This is most commonly in the form a bacterial meningitis, but viruses and toxins (i.e. salt), can also result in this clinical presentation. Seizures, tremors, and hyperesthesia are other clinical signs that would indicate the cerebrum is involved. If normal or perceived normal mention is apparent, the cerebrum is likely not affected.

The cerebellum is responsible for coordinated movement, balance, and posture. Ataxia, proprioceptive deficits, and incoordination are typically present with defects in the cerebellum. However, spinal cord issues can also present with similar clinical problems as seen in with cerebellar disease, and can also include muscle rigidity or flaccidity. Table 1 summarizes clinical signs that can be seen with different segments of the CNS.

I hope that you are not confused yet because we also have to rule out cardiovascular, muscle, bone and joint lesions that may appear as "CNS signs." This is where your "routine" necropsy is important; don't just drill down on CNS tissue for sample submission! Pain associated with trauma, broken or weak bones, or inflammation within the axial skeleton can cause incoordination, proprioceptive deficits, or recumbency. However, the pig's awareness/mentation is typically not altered. Cardiovascular problems resulting in tissue hypoxia may also mimic CNS disease syndromes in certain scenarios.

CNS differentials

Swine medicine is population medicine at its finest. With that said, we are typically dealing with infectious pathogens when CNS disease issues within the nursery phase of production occur. This does not exclude metabolic conditions, toxin issues, or traumatic problems as the cause of clinical CNS disease. Below is a list of common differentials for CNS like diseases in nursery aged pigs. This list is not complete, but captures the vast majority of cases that are typically seen at diagnostic laboratories.

1. Bacterial septicemia: *Streptococcus suis*, *Haemophilus parasuis* and *Mycoplasma hyorhinis*
   - Lesion: Meningitis, but otitis is possible
   - Clinical sign: Nystagmus, "down paddling," head tilt, and circling

Table 1: Central nervous system clinical signs and insult location

Location	Clinical signs
Cerebrum	Altered mention, decreased awareness
	Loss of consciousness
	Loss of voluntary movement (nystagmus)
	Seizures
	Tremors
Cerebellum	Ataxia
	Proprioceptive deficits
	Incoordination
Spinal cord	Ataxia
	Proprioceptive deficits, knuckling
	Incoordination
	Rigidity
	Flaccidity

2. Viral diseases: Porcine Reproductive and respiratory syndrome virus, porcine circovirus type 2, and enteroviruses (Teschoviruses)
   - Lesion: Encephalitis or meningoencephalitis, typically perivascular. Lesions associated with teschoviruses are in the brainstem
   - Clinical sign: Dulled mentation, seizures, incoordination, ataxia, recumbency and proprioceptive deficits. Knuckling-over and/or posterior paresis more common with Teschovirus infection.
3. Toxin: Stx2e toxin (edema disease)
   - Lesion: Perivascular edema and neuronal necrosis. Lesions typically start within the brainstem, but cerebellar inflammation and edema is can also be present. Gastric and mesocolonic edema is sometimes viewed.
   - Clinical sign: Sudden death, eyelid edema, and diarrhea. Diarrhea is not a prerequisite for edema disease
4. Nutritional: Water deprivation (salt toxicosis), Vitamin A toxicity or deficiency, Vitamin D and/or calcium deficiency, and tremorgenic mycotoxins
   - Lesion: Water deprivation is associated with eosinophilic perivascular encephalitis. Lesions associated with other nutritional issues are often not apparent.
   - Clinical sign: Dulled mentation, seizures, incoordination, ataxia, recumbency and proprioceptive deficits
5. Trauma: Improper vaccination technique and broken bones
   - Lesion: Dependent on the type of insult
   - Clinical sign: Ataxia, paresis and prolonged recumbency are typical

Diagnostic sampling

The old adage is collect samples from "acutely affected and non-treated" animals exhibiting clinical signs that predominate within the population. This is still true in CNS disease scenarios. It also advised to submit animals in the subacute stage as some lesions, both gross and microscopic, are not always apparent in the acute cases. Your routine, complete, and clean necropsy technique, examining all organ systems will assure a proper diagnosis. Don't just focus on animal tissues is another important piece of advice, feed could be the issue as well.

The below list is a guide to proper sampling when working up CNS cases.

- Serum and whole blood from affected and non-affected pigs
  - ▸ Reason: Serum can be useful for analyte evaluation (sodium, calcium, etc), agent and antibody detection. Whole blood may be useful to determine if the process is infectious (white blood cell count) or if toxins are a concern.
- Brain: Fresh and formalin fixed cerebrum, cerebellum, and brainstem (PLEASE collect all, not just cerebrum)

- ▸ Advice: Please try not to use captive bolt or blunt force trauma euthanasia methods in pigs being evaluated for CNS disease.
- ▸ Advice: Swab the brainstem prior to brain extraction for a "clean" bacteriology sample. Sending a fresh chilled intact head is better than not submitting brain at all
- ▸ Advice: Split brain longitudinally, putting the "best half" in formalin. Don't cut into little pieces. "Bread-loafing" is encouraged
- ▸ Advice: Collect proximal cervical cord as possible

- Spinal cord: Fresh and formalin fixed 1" segments of cervical, thoracic, and lumbar cord
  - ▸ Reason: Pieces of spinal cord can be invaluable for diagnosis and it doesn't need to be hard. Remove with a Barnes dehorner, cleaver, or saw. Sending intact vertebrae with the spinal cord inside is better than no spinal cord at all
- Muscle and Heart: fresh and fixed
  - ▸ Reason: To rule out toxins or other diseases such as mulberry heart disease that may mimic CNS clinical signs.
- Bone and joints
  - ▸ Advice: Routinely break ribs for bone strength; vitamin D, calcium or phosphorus issues. If weak or questionable, Collect rib bone which includes the costochondral junction.
  - ▸ Advice: Sample joint fluid, then open them (versus contaminating them first) by removing the overlying skin and using a syringe and needle
  - ▸ Advice: Open multiple joints, examine, and collect synovium. Submitting intact, chilled joints is an alternative.
- A complete set of tissue; other organs
  - ▸ Reason: Add strength to the diagnosis and rule in/or out other issues
  - ▸ Reason: Need intestine to confirm *Escherichia coli* isolation and genotyping (edema disease)
- Feed and water
  - ▸ Reason: You are there, why not collect? It doesn't cost anything to collect

Diagnostic testing

Diagnostic testing can be expensive with CNS like disease scenarios. The reasoning being there are numerous differentials to potentially rule out and there is not always a "PCR" for what we are trying to diagnose! Some testing modalities, especially when investing nutritional reasons, are not always clear cut with a yes or no answer either. An important aspect to bear in mind is that sample submission will drive testing; meaning that if the appropriate sample is not received, then the test will not be selected or run. Teschovirus testing is a prime example of this potential issue. If the brainstem or cervical spinal cord is not received, the likelihood of diagnosing this pathogen as the etiologic agent involved in the clinical picture is almost none.

At diagnostic laboratories, testing is highly driven off the clinical history. Yes, that means what you write on the submission form is important! This helps the diagnostician! Please be more specific that "CNS signs". Take a moment and write something smart on the paper; if that is not an option, call.

"Routine" testing of CNS disease in the nursery pig generally involves bacterial culture of the brain, small intestine, and other major organs, and combining these finding with microscopic lesions that would be compatible with bacterial septicemia or edema disease. Microscopic evaluation would then potentially lend to further testing based on the findings. Examples include Teschovirus testing in the case of brainstem changes or vitamin D if bone lesions are viewed at the costochondral junction.

On occasion there are cases that do not align; meaning testing may be negative but there are microscopic lesions indicating a particular etiology or vice versa. In these events, further submission is recommended, but ultimately the "diagnosis" is made by you incorporating all pieces of the puzzle.

Take home points

1. Diagnosis of "CNS signs" is multifaceted as differentials can be vast for nursery-aged pigs and begins with a complete clinical examination. Clinical examination will aid in localizing a potentially insults or rule in/out other organ system involvement.
2. CNS disease syndromes or like diseases require submission of multiple tissues including cerebrum, cerebellum, brainstem, and spinal cord to arrive at an accurate diagnosis. Sample collection methods and cleanliness add strength to the diagnostic findings.
3. Diagnostic testing is driven by sample submission and reported clinical signs; Arriving to the correct etiology is based upon testing the appropriate tissue or sample and combing these with the clinical findings.

Blood-brain barrier basics: A selective passageway for bacterial pathogens and antimicrobial drugs

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Meningitis is a serious complication of *Streptococcus suis* bacteremia in young piglets. This microorganism is also an emerging human pathogen that has been recently implicated in a case of meningitis in a swine handler (Wertheim et al., 2009; Fowler et al., 2013). In order to access and infect the brain meninges, blood-borne *S. suis* must penetrate the blood-brain barrier of affected animals or humans. The purpose of this article is to briefly review some key aspects of the locations and protective functions of this important physical barrier in swine and other animals and summarize our current understanding of how *S. suis* breaches this barrier.

There is more than one "blood-brain barrier"

The blood-brain barrier plays several key roles in central nervous system (CNS) homeostasis in that it hinders the entry of microorganisms and potentially neuroactive or toxic substances into the brain, maintains concentrations of proteins and ions at levels optimal for neural activity, and orchestrates immune responses that incur a minimum of inflammation and cellular damage (that would disrupt barrier function, see below). There are in reality, three major barriers within the CNS, which include (1) the endothelial cells lining continuous capillaries supplying the brain parenchyma, which encompasses the largest surface area for barrier function; (2) a less extensive blood-cerebrospinal fluid (CSF) barrier consisting of CSF-secreting choroid plexus epithelial cells lying in close proximity to fenestrated capillaries; and (3) an arachnoid epithelium within the meninges (Saunders et al., 2012; Abbott, 2013). The microvascular endothelial cells of brain capillaries interact with closely apposed pericytes, and adjacent neurons, astrocytes and microglia (the "macrophages of the brain") to form a so-called neurovascular unit (Iadecola, 2004). These cells act in a concerted manner to maintain barrier function. Compared with these three regions, the circumventricular organs possess a "leakier" barrier composed of ventricular ependymal cells. This more permeant cellular structure permits brain chemosensors to monitor and respond appropriately to changes in serum osmolality or the presence of blood-borne toxicants.

Tight junction and adherens junction proteins are the main contributors to blood-brain barrier integrity

Brain microvascular endothelial cells (BMECs) and epithelial cells of the choroid plexus and meninges are held together by a complex array of specialized membrane proteins to form tight junctions. Claudin-5, occludin, junctional adherens molecules (JAMs) and zona occludens adapter proteins are proteins in tight junctional complexes that are commonly expressed in BMECs and choroid plexus epithelial cells (CPECs) from pigs (Malina et al., 2009; Schroten et al., 2012). These tight junctions physically exclude proteins and other macromolecules as well as microbes present in the brain blood supply. Even blood-borne molecules that are essential for brain function are generally excluded by these intercellular junctions and normally cannot cross the barrier through a paracellular pathway. Instead, these substances move across the blood-brain interface by means of selective carrier proteins (e.g. ion and water channels, glucose and amino acid transporters, transferrin receptors, efflux pumps). Many inflammatory, immune and neuroactive substances can regulate blood-brain barrier permeability (Liu et al., 2012). For example tumor necrosis factor-α, a pro-inflammatory cytokine, has been reported to increase paracellular permeability to both small and large molecules in neonatal pigs (Megyeri et al., 1992; Abraham et al., 1996). On the other hand, transforming growth factor-β, which is secreted by astrocytes and pericytes present in the neurovascular unit, decreases blood-brain barrier permeability by increasing tight junction protein gene expression in BMECs (Takeshita and Ransohoff, 2012).

Streptococcus suis breaches blood-brain and blood-CSF barriers

Streptococcus suis appears to migrate across the blood-brain and blood-CSF barriers of piglets after adhering to and invading BMECs and CPECs respectively. This process appears to involve several *Streptococcal* proteins, including adhesins, invasins and cell wall components as well as bacterial interactions with serum components such

as fibronectin (Fittipaldi et al., 2012). The *Streptococcal* exotoxin suilysin may disrupt barrier function through cytotoxic effects on endothelial and epithelial cells, but it is not required for invasion. Capsular polysaccharide, which is otherwise considered a major virulence factor for this bacterium, may actually reduce *S. suis* invasion in BMECs and CPECs by covering up cell wall adhesins (Vanier et al., 2007, 2009a).

Interactions between *Streptococcus suis* serotype 2 and cultured porcine CPEC monolayers in a Transwell system have been the subject of recent investigations (Tenenbaum et al., 2009; Schroten et al., 2012). These have revealed that *S. suis* shuttles across the choroid plexus epithelium and enters the CSF in a polarized fashion. Bacteria cross the basolateral membranes of epithelial cells, are enclosed in endocytic vacuoles and trafficked to the apical membrane, and the cocci are subsequently released intact into the CSF via exocytosis. This process, which involves virulence factors and direct bacterial-epithelial contacts, disrupts blood-CSF barrier function and enhances the entry of additional bacteria and white blood cells into the CSF (Fittipaldi et al., 2012). Administration of an anti-inflammatory glucocorticoid (dexamethasone) inhibits *S. suis*-induced alterations in tight junction re-organization and breakdown in CPECs (Tenenbaum et al., 2008). Although the mechanistic aspects of *S. suis* migration across BMECs are less well-defined, it is known that this organism can reside within porcine BMECs for up to 7 hours (Vanier et al., 2004).

Host cells residing in the blood-brain barrier can detect and respond to pathogen attack

Cells comprising the neurovascular unit that participate in first line defense against pathogenic microbes must have the ability to recognize conserved, pathogen-associated molecular patterns, such as the presence of lipopolysaccharide or lipopeptides on bacterial surfaces. BMECs and neighboring cells express a number of pathogen recognition receptors, including Toll- and NOD-like receptors, and virus-detecting RIG helicases (Kristensson, 2011; van Sorge and Doran, 2012; Lampron et al., 2013). Streptococcal cell wall components can activate these receptors in BMECs and CPECs to produce pro-inflammatory responses, which can potentially degrade barrier function if unchecked (Schwerk et al., 2011; Fittipaldi et al. 2012). Inflammatory mediators, including cytokines, chemokines and arachidonic acid metabolites, facilitate the attachment of leukocytes to the basement membranes of BMECs and, by loosening tight junctions, allow the paracellular transmigration of these immunocytes into the CNS (Lampron et al., 2013). Neutrophils appear to move directly through porcine CPECs in response to inflammatory mediators formed during *S. suis* infection (Wewer et al., 2011). *Streptococci* can degrade the chemokine interleukin 8 generated by porcine BMECs and thereby slow the appearance of neutrophils at sites of infection (Vanier et al., 2009b). On the other hand, exposure of porcine CPECs to the inflammatory cytokine interferon-γ increases the activity of epithelial indolamine dioxygenase, an enzyme that degrades tryptophan necessary for *S. suis* growth. CPECs can thus have a bacteriostatic effect on this organism (Adam et al., 2004).

Drugs, including antimicrobials, and even large proteins can cross the blood-CSF barrier, especially in young piglets

Although the blood-brain barrier function of BMECs and the overall neurovascular unit appears does not appear to mature with age, the blood-CSF barrier of neonates is generally more permeable than that of adults (Saunders et al., 2012). Relatively large proteins such as IgG from colostrum or bilirubin can enter the CSF in suckling piglets (Lee et al., 1995; Harada et al., 2002). This barrier may be particularly vulnerable to *S. suis* infection in neonatal swine. However, it may likewise be more permeable to antimicrobial drugs, such as β-lactam antibiotics that are routinely used to treat *Streptococcal* infections in swine and humans and to which microbial resistance is currently low (Nau et al., 2010; Varela et al., 2013). Meningitis is associated with increased permeability to hydrophilic drugs (e.g. penicillin) that normally penetrate the blood-brain barrier poorly (Lutsar et al., 1998). Drug accumulation in the CSF to concentrations effective in arresting *Streptococcal* meningeal infections may even be enhanced, based on studies in neonatal rodents reporting a reduced expression of drug efflux pumps such as P-glycoprotein (Tsai et al., 2002).

Summary

Work with porcine experimental models, including BMEC and CPEC cell lines from swine, has provided significant insights into the mechanisms by which *Streptococci* interact with cells of the blood-brain and blood-CSF barriers to produce meningitis in piglets. Many questions remain to be addressed, however. These include the following. What is the role of the blood-arachnoid barrier in *Streptococcal* meningitis? What factors affect the achievement of effective antibacterial drug concentrations in the CSF of piglets? Can effective *S. suis* vaccine be produced? As *S. suis* has recently been recognized to be an emerging human pathogen, it is expected that this avenue of research with swine-based models will continue to yield new knowledge of benefit to both piglets and people.

References

1. Abbott NJ. Blood-brain barrier structure and function and the challenges for CNS drug delivery. J. Inherit. Metab. Dis 36:437–449, 2013.

2. Abraham CS, Deli MA, Joo F, Megyeri P, Torpier G. Intracarotid tumor necrosis factor-alpha administration increases the blood-brain barrier permeability in cerebral cortex of the newborn pig: quantitative aspects of double-labelling studies and confocal laser scanning analysis. Neurosci Lett 208:85–88, 1996.

3. Adam RA, Tenenbaum T, Valentin-Weigand P, Laryea M, Schwahn B, Angelow S, Galla HJ, Däubener W, Schroten H. Porcine choroid plexus epithelial cells induce *Streptococcus suis* bacteriostasis in vitro. Infect Immun 72:3084–3087, 2004.

4. Fittipaldi N, Segura M, Grenier D, Gottschalk M. Virulence factors involved in the pathogenesis of the infection caused by the swine pathogen and zoonotic agent *Streptococcus suis*. Future Microbiol 7:259–279, 2012.

5. Fowler HN, Brown P, Rovira A, Shade B, Klammer K, Smith K, Scheftel J. *Streptococcus suis* meningitis in swine worker, Minnesota, USA. Emerg Infect Dis 19:330–331, 2013.

6. Harada E, Araki Y, Furumura E, Takeuchi T, Sitizyo K, Yajima T, Kuwata T. Characteristic transfer of colostrum-derived biologically active substances into cerebrospinal fluid via blood in natural suckling neonatal pigs. J Vet Med A Physiol Pathol Clin Med 49:358–364, 2002.

7. Iadecola C. Neurovascular regulation in the normal brain and in Alzheimer's disease. Nat Rev Neurosci 5:347–360, 2004.

8. Kristensson K. Microbes' roadmap to neurons. Nat Rev Neurosci 12:345–357, 2011.

9. Lampron A, Elali A, Rivest S. Innate immunity in the CNS: redefining the relationship between the CNS and its environment. Neuron 78:214–232, 2013.

10. Lee C, Stonestreet BS, Oh W, Outerbridge EW, Cashore WJ. Postnatal maturation of the blood-brain barrier for unbound bilirubin in newborn piglets. Brain Res 689:233–238, 1995.

11. Liu WY, Wang ZB, Zhang LC, Wei X, Li L. Tight junction in blood-brain barrier: an overview of structure, regulation, and regulator substances. CNS Neurosci Ther 18:609–615, 2012.

12. Lutsar I, McCracken GH Jr, Friedland IR. Antibiotic pharmacodynamics in cerebrospinal fluid. Clin Infect Dis 27:1117–1127, 1998.

13. Malina K C-K, Cooper I, Teichberg V. Closing the gap between the in vivo and in vitro blood-brain barrier tightness. Brain Res 1284:12–21, 2009.

14. Megyeri P, Abrahám CS, Temesvári P, Kovács J, Vas T, Speer CP. Recombinant human tumor necrosis factor alpha constricts pial arterioles and increases blood-brain barrier permeability in newborn piglets. Neurosci Lett 148:137–140, 1992.

15. Nau R, Sörgel F, Eiffert H. Penetration of drugs through the blood-cerebrospinal fluid/blood-brain barrier for treatment of central nervous system infections. Clin Microbiol Rev 23:858–883, 2010.

16. Saunders NR, Liddelow SA, Dziegielewska KM. Barrier mechanisms in the developing brain. Front Pharmacol 3:46, 2012.

17. Schroten M, Hanisch FG, Quednau N, Stump C, Riebe R, Lenk M, Wolburg H, Tenenbaum T, Schwerk C. A novel porcine in vitro model of the blood-cerebrospinal fluid barrier with strong barrier function. PLoS One 7:e39835, 2012.

18. Schwerk C, Adam R, Borkowski J, Schneider H, Klenk M, Zink S, Quednau N, Schmidt N, Stump C, Sagar A, Spellerberg B, Tenenbaum T, Koczan D, Klein-Hitpass L, Schroten H. In vitro transcriptome analysis of porcine choroid plexus epithelial cells in response to *Streptococcus suis*: release of pro-inflammatory cytokines and chemokines. Microbes Infect 13:953–962, 2011.

19. Takeshita Y, Ransohoff RM. Inflammatory cell trafficking across the blood-brain barrier: chemokine regulation and in vitro models. Immunol Rev 248:228–239, 2012.

20. Tenenbaum T, Matalon D, Adam R, Seibt A, Wewer C, Schwerk C, Galla HJ, Schroten H. Dexamethasone prevents alteration of tight junction-associated proteins and barrier function in porcine choroid plexus epithelial cells after infection with *Streptococcus suis* in vitro. Brain Res 1229:1–17, 2008.

21. Tenenbaum T, Papandreou T, Gellrich D, Friedrichs U, Seibt A, Adam R, Wewer C, Galla HJ, Schwerk C, Schroten H. Polar bacterial invasion and translocation of *Streptococcus suis* across the blood-cerebrospinal fluid barrier in vitro. Cell Microbiol 11:323–336, 2009.

22. Tsai CE, Daood MJ, Lane RH, Hansen TW, Gruetzmacher EM, Watchko JF. P-glycoprotein expression in mouse brain increases with maturation. Biol Neonate 81:58–64, 2002.

23. van Sorge NM, Doran KS. Defense at the border: the blood-brain barrier versus bacterial foreigners. Future Microbiol 7:383–394, 2012.

24. Vanier G, Segura M, Friedl P, Lacouture S, Gottschalk M. Invasion of porcine brain microvascular endothelial cells by *Streptococcus suis* serotype 2. Infect Immun 72:1441–1449, 2004.

25. Vanier G, Segura M, Gottschalk M. Characterization of the invasion of porcine endothelial cells by *Streptococcus suis* serotype 2. Can J Vet Res 71:81–89, 2007.

26. Vanier G, Fittipaldi N, Slater JD, Dominguez-Punaro M de la C, Rycroft AN, Segura M, Maskell DJ, Gottschalk M. New putative virulence factors of *Streptococcus suis* involved in invasion of porcine brain microvascular endothelial cells. Microb Pathog 46:13–20, 2009a.

27. Vanier G, Segura M, Lecours MP, Grenier D, Gottschalk M. Porcine brain microvascular endothelial cell-derived interleukin-8 is first induced and then degraded by *Streptococcus suis*. Microb Pathog 46:135–143, 2009b.

28. Varela NP, Gadbois P, Thibault C, Gottschalk M, Dick P, Wilson J. Antimicrobial resistance and prudent drug use for *Streptococcus suis*. Anim Health Res Rev 14:68–77, 2013.

29. Wertheim HF, Nghia HD, Taylor W, Schultz C. *Streptococcus suis*: an emerging human pathogen. Clin Infect Dis 48:617–625, 2009.

30. Wewer C, Seibt A, Wolburg H, Greune L, Schmidt MA, Berger J, Galla HJ, Quitsch U, Schwerk C, Schroten H, Tenenbaum T. Transcellular migration of neutrophil granulocytes through the blood-cerebrospinal fluid barrier after infection with *Streptococcus suis*. J Neuroinflammation 8:51, 2011.

Diagnostic considerations

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How much should we spend on diagnostic examinations? This is a question that veterinarians and producers continue to struggle with. There are a number of questions that must be considered when making this decision. The most common question from producers is how much is the testing going to cost. Obviously this will depend on what tests are going to be requested. This is often decided by the veterinarian with some input from the producer. Occasional submissions are left to the "discretion of the pathologist", but hopefully these are minimal. Testing "all tissues for everything" is not a sound financial diagnostic plan.

The decision to conduct diagnostics will vary depending on the type of producer:

Integrator: budgeted

Managed herd: budgeted

Large independent herd: as needed or budgeted. (May not be budgeted but it seems they know when they have spent too much)

Small independent herd: as needed. Veterinary discretion if it is needed and/or what tests are needed.

Backyard herds/ secondary income: as needed or "do it all". (Profit/cost is not considered)

Show pig: 4H vs. professional. (Cost may or may not be considered)

For the past year (June 1, 2012 to June 1, 2013), the 4 Star group submitted 308 tissue cases to the Iowa State University Veterinary Diagnostic lab. The total cost was $56,020 or $182 per case. For the sake of discussion, we will use $182 as an average cost for tissue diagnostics for the rest of this article.

Diagnostic testing is divided into two broad categories: diagnosing the cause of sick pigs and disease surveillance.

Let's talk about diagnosing the cause of sick pigs first.

A vast majority of the tissue cases submitted by the 4 Star group were to diagnose the cause of sick pigs. Why? Because tissue submissions are not a cost effective plan for surveillance. It is not practical to submit tissues from enough pigs in a group or from a farm to make the findings significant.

It seems like an easy decision to spend money on diagnostics when pigs are sick. But even then, some producers are slow to make the call. As Dr. Tokach has pointed out some will call with the first sneeze and it's difficult to see the problem, while others will not call until 15% are dead and "maybe you should come out when you get a chance".

As farms have evolved, many submit their own tissues and the veterinarian does know until the lab report. Then veterinarians may not even know the history or clinical signs. This may be the only contact veterinarians has with small farms

It is nice to have a budget for diagnostics. It is difficult to know how much to set aside for diagnostics. Use previous year's expenses at a guide? And how do you budget for new agents that show up in the industry? For example:

In the late 80's, there were no tests for mystery swine disease. MSD turned into PRRS and diagnostic tests were developed. Now a large portion of diagnostic testing on any farm is for PRRS.

SIV is another example. Before 1998, there was only H1N1. Then H3N2 came into the picture. The next 10 years saw an increase in testing sorting the new strain and other new strains out. Recently pandemic H1N1 and variant H3N2 have become prevalent and now take up portions of the diagnostic budget.

Other examples include PCV2, Brachyospira and most recently PED. A few years ago these things were not tested for all. Now they are taking up a significant portion of the diagnostic budget.

If we discover that the "post weaning failure to thrive" syndrome has an infectious cause, you will likely see a spike in diagnostics for that particular agent.

Finally, FAD testing is not in anyone's budget. If a FAD shows up in the USA, testing will exceed most diagnostic budgets.

Surveillance testing is the other broad category for diagnostic testing.It is important to be looking for something or to be answering a question. "Monitoring for the sake of doing something does not justify itself in all but the highest health system."

For example, the testing a breeding herd pyramid is looking for positives vs. negatives. In this case it is vitally important to understand the sensitivity and specificity of the particular test you are conducting. What is the rate of false positives vs. negatives? Will the results create more questions than answers?

Looking back, the old SPF system looked at very few animals (10 sets of lungs per quarter) to determine *Mycoplasma hyopneumoniae* status of enrolled herds. This worked to a certain extent because most of these farms were small, one site, continuous flow farms. The theory was that if Mycoplasma was on the farm, at least one of these sets of lungs would show lesions. The big problem with this system was that if a herd failed, they would just continue to look at sets of 10 lungs until they all passed, then they kept their SPF status.

Most commercial herds will be looking for infection levels or percentage positive vs. negative for a specific agent. In other words, may be trying to determine stability of a herd or if a herd is shedding a particular agent. They may also be checking for exposure to an agent after an intentional exposure.

One thing that has come about with the PRRS area regional control projects is the concept of conducting surveillance across a wide geographical area instead of one farm. Is the area positive for PRRS? If yes, what strain? In the West Michigan ARC, we tested the entire area three times or every other year. We tracked results on a spreadsheet and created a dendogram for the area and even divided by year. We learned that the strains we had in our area did not change much over the six years (so far) of the project. With good communication, we would learn quickly if there was sick pigs in the area. We would then try to test in front of the outbreak and to warn producers downwind that something may be headed their way.

This is a good time to talk about the diagnostic laboratory's work. A few years ago, we were requesting IFA's, SN's, CF's, and virus isolation. Many of those tests lacked good specificity or sensitivity. Now we have Immuo-histochemistry, PCR's, and sequencing. PCR testing has become the most requested test at the ISU veterinary diagnostic lab. In the case of a new agent (PED for example), the first thing the laboratory network does is develop a PCR test. Producers can quickly determine if they have the agent or not, without waiting several days or weeks to see.

The most significant change in the field of diagnostics and surveillance is oral fluid testing. Oral fluid testing has changed how samples are taken on the farm. Oral fluid or rope testing has several advantages including the fact it is easy and fast to collect, more animals can be tested per sample than serum, the sample can be tested by PCR as well as for antibodies (the sample can be used for diagnostic and surveillance purposes), and one sample can be used for multiple agents. Multiple studies have shown that oral fluid testing is as accurate as serum testing and can accurately show if and when a barn is exposed to a particular agent. This is amazing technology and I can't wait to see what tests are developed for this technology in the future.

As our industry continues to evolve, we undoubtedly will continue to conduct diagnostics for sick pigs and surveillance to monitor exposure and disease levels. The tests we conduct will change as the older and better controlled diseases fade away and new agents show up. I trust our diagnostic labs will develop new testing methods and fine tune existing tests. The goal will be to use more sophisticated surveillance tests to head off sick pigs and the need to true diagnostic tests.

References

1. Hanson, J. 2009. Large system approach to increasing health and production. 2009 Proc. AASV Annual Meeting, pp 11–15.

2. Conner, J. 2009. Learning from the best. Proc. 2009 Proc. AASV annual meeting, pp 557–562.

3. Tokach, L. 2010. Pre-weaning Mortality. 2010 AASV Preconference Seminar, Pre-weaning mortality, pp13–16.

4. Davies, P. 2006. Principles of diagnostic testing. 2006 Proc. AASV Annual Meeting, pp 345–350.

5. Davies, P. 2009. New approaches to swine disease surveillance. 2009 Proc. Lemen conf, pp 28–33.

6. Prickett, JR, J. Zimmerman, J Johnson. 2010. Detection of Viral Pathogens of Swine using oral fluid samples. 2010 Iowa Disease Conference, pp 129–131.

7. Pelger, G. 2002. Serologic monitoring: Uses and implications. 2002 Lemen conf, pp 102–105.

8. Defresne, L, 2011. Field application of oral fluid sampling and testing in swine. 2011 AASV preconference seminar, Diagnostics. pp 5–8.

9. Olsen,C et al. 2012. Swine oral fluids diagnostic update. 2012 Iowa Disease Conference, pp 89–93.

Mycoplasma infection costs in a naïve swine population

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Introduction

Mycoplasma hyopneumoniae (M hyo) remains a ubiquitous pathogen in the worldwide swine population; especially in countries with a major swine industry.[1] This paper estimates the economic effects of reduced performance due to M hyo infection in a naïve commercial grow/finish flow in the United States.

Materials and methods

A 2200 sow, M hyo naïve farrow to finish operation located in the eastern corn belt of the United States was diagnosed with M hyo in March 2010. Individual PCR's performed on the nasal swabs for *Mycoplasma hyopneumoniae*; PRRSV and SIV were used to determine positive results for M hyo and negative results for SIV and PRRS virus.

Production records were evaluated comparing performance prior to and after the M hyo break. Parameters included average daily gain (ADG) and feed efficiency (FE), and the economic value for extra days on feed (overhead cost).

Economic analysis was made with the following evaluation assumptions: average feed cost of USD $239.42/ MT; average market animal revenue of USD $130.00; overhead cost of days on feed of USD $0.10 per day and a maintenance cost for feed due to more days on feed of USD $2.49 kg per day. The average initial weight at placement was 22.7 kg and average market weight was 115.7 kg. For the three parameters measured, the data were normally distributed. Student's T-test was used for the analysis and results considered significant if $P = 0.05$.

Results

The M hyo outbreak significantly impacted ADG ($P = 0.021$). Average daily feed intake ($P = 0.74$) and FE ($P = 0.07$) was not numerically improved, but failed to reach significance based on the a set. (Table 1).

The economic analysis revealed a combined loss of USD$7.92 per market animal in the outbreak phase. The majority of the cost impact was due to slower gains (7.2 days longer to market weight). Treatment costs, mortality rate and culls were not included in this study.

Table 1: Mean performance parameters pre and post M hyo outbreak.

	ADG	FCR	ADFI
Naive period	1.99[a]	2.58	5.11
Post-infection	1.86[b]	2.71	5.05

[ab] Values in columns with different letters are significantly different.

Conclusions and discussion

M hyo and the pneumonia associated with an infection remains a concern to the swine industry throughout the world. The clinical signs associated with a naïve population infected with M hyo are expressed as a respiratory challenge. The clinical signs include but are not limited to coughing, gaunt appearance, fever, labored breathing and an increase in mortality if timely treatments are not preformed.[1,2] Co-infections with other primary and secondary pathogens will complicate the diagnosis and increase costs associated with treatments.

In this M hyo and PRRS virus naïve production system, an acute outbreak occurred in March 2010 and was clinically expressed as respiratory disease in the finishing age animals with reduced growth performance. Even though antibiotic therapies (using both individual animal and mass treatments) were utilized to control mortality and reduced under-valued animals at market time there still was a significant decrease in growth. This paper illustrates what a worldwide distributed pathogen costs when an infection occurs in a naïve swine population. Biosecurity programs can use this economic data to show why the effort is needed to limit entry of a major pathogen like M hyo. Calculations of how costs were evaluated will be discussed and illustrated during the presentation.

References

1. Thacker, EL. Mycoplasmal Diseases. In: Straw, BE, Zimmerman JJ, D'Allaire, S, Taylor, DJ. *Diseases of Swine*. 9th ed. Ames, Iowa: Iowa State University Press; 2006:701–717.

2. Desrosiers, R.: 2001, *Journal of Swine Health Production*.2001; 9(5): 233–237.

Valuing an increase in litter size

John Kolb

Paper not available at time of publication

What have we learned using load close expose to produce PRRSv-negative pigs from positive breeding herds?

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Herd closure is a strategy widely used in the US swine industry to control and eliminate PRRSv from infected breeding herds. As part of the herd closure program some veterinarians expose the breeding herd with PRRSv in an effort to increase herd immunity (Desrosiers and Boutin, 2002; Voglmayr et al., 2006). In conjunction with deliberate exposure, the program often includes filling the facility with extra replacement pigs and then temporarily closing the breeding herd to any replacement pig introduction. Hence, the herd closure program for PRRSv is commonly thought of as having "load, close and expose" components. There are different immunogens that can be used for whole-herd exposure including the resident live virus and attenuated virus vaccine. Recently, our group conducted a series of studies to compare effectiveness of LCE using live virus inoculation (LVI) to that of using modified-live virus vaccine (MLV). Full details of the studies are available in the Ph.D thesis of Daniel Linhares at the University of Minnesota's College of Veterinary Medicine.

One study compared the LCE programs using LVI or MLV in regards to the time it takes to reach PRRSv stability (TTS) from infected breeding herds. Results showed that despite the great variability of TTS between enrolled herds, there were some variables associated with shorter TTS. Specifically, herds that used LVI as the method of whole-herd exposure reached TTS earlier than herds that used MLV vaccines. Moreover, herds with history of PRRSv infection in the 3 years prior to our study reached TTS sooner than herds without history of PRRSv in those same 3 years. Furthermore, herds assisted by a specific veterinary clinic reached TTS sooner than all other herds suggesting that there might be management practices associated with shorter TTS. Our results also showed that PRRSv-monitoring must be done repeatedly over time to increase confidence of PRRSv-negative status of weaned piglets. Altogether, those findings represent prove of concept that herds can reach PRRSv stability as soon as 84 days from establishment of LCE.

Another study evaluated the effect of attenuated PRRSv vaccine inoculation compared to the use of live-virulent virus inoculation on production performance in breeding herds. It was shown that herds that used MLV vaccine as part of load-close-expose herd closure programs recovered production levels and had a less severe production impact than herds that used LVI. Also, herds that reported previous PRRSv infection reached time to baseline production (TTBP) sooner than herds with no history of PRRSv infection in the previous 3 years. Interestingly, herds assisted by a specific veterinary clinic recovered production faster than other herds, raising the hypothesis that specific management practices could be associated with herd closure effectiveness.

Because results of TTS and TTBP studies pointed to different directions in regards to advantage of MLV compared to that of LVI, we built economic models to assist veterinarians to make informed decisions between LVI and MLV as part of LCE program to eliminate PRRSv. Taking in consideration the future prices for market hogs and pig feed from January to December 2012, and $13.52 loss per pig that is PRRSv-positive, the results suggested that MLV would be a better economical choice.

Furthermore, the final study investigated herd-closure practices associated with successful PRRSv-elimination from breeding herds. It showed that the success rate of LCE programs was 76% and 92% for LVI and MLV herds, when failures associated with unrelated PRRSv were excluded from the analysis although these differences were not statistically significant. Moreover, one out of six herds that achieved 90 days of failure to detect PRRSv in due to wean piglets failed to achieve AASV category III, which indicates that more strict monitoring programs should be adopted in herds undergoing PRRSv elimination. Interestingly, herds assisted by different veterinary clinics had different set of recommendations of management practices to be followed during LCE, indicating that there was no agreement between veterinarians on what is the relative importance of each management practice evaluated. The variables associated with failure to reach AASV category III were a) being infected with a PRRSv of RFLP pattern 1-4-4 and b) holding back pigs at weaning for quality.

Results from these studies provided science based information on LCE with live PRRSv as a method to produce PRRSv-negative pigs from infected breeding herds. Further studies are needed to compare herd closure without the "exposure" component on the c) effectiveness of

reaching TTS, b) total loss followed by the infection and intervention, and c) chance of program success. Also, further studies are needed to better understand specific factors associated with reaching short TTS and minimized total loss, such as farm layout, pig flow and actual implementation of specific management practices during the period of herd closure.

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PRRS control and eradication options for breed to wean farms

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Introduction

The swine veterinarian is charged with delivering optimal health management in a socially and economically sustainable manner. This expectation is complicated by the lack of available evidence for disease prevention, control and treatment forcing the swine veterinarian to regularly make decisions with less than ideal evidence. Never is this complication more evident than in the face of epidemic disease on a Breed to Wean (BTW) farm. A novel porcine reproductive and respiratory syndrome virus (PRRSV) introduction on a BTW farm results in dramatic performance losses that with or without intervention threaten farm and producer viability. In the face of this challenge the swine veterinarian must craft a health management plan which optimizes the client's biological and economic performance. To maximize BTW farm efficiency in the face of a novel PRRSV infection, the swine veterinarian must apply the best available evidence to their decision making process, and ultimately to their health management recommendations. This paper will communicate how The Maschhoffs health team has used best available evidence to control and eradicate PRRSV from BTW farms in our system, and specifically how we have applied learning's from the Time to Negative Pig Study conducted by the University of Minnesota. PRRS control and eradication options presented below will focus on a Load, Close and Expose (LCE) model of PRRS management, with particular consideration given to two key decision points; the final goal of the PRRS management plan (PRRS Control vs. PRRS Eradication), and the method of BTW herd exposure (Live Virus Inoculation vs. Modified Live Virus Vaccination).

System PRRS management background

PRRS impact on a BTW farm is most noticeable during the acute outbreak, and our system has historically modeled biologic and economic performance relative to growing pig losses, specifically post-weaning mortality. Accordingly, the health team's recommendation for PRRS management following a novel PRRSV introduction has been based on PRRS elimination to most rapidly achieve PRRSV negative piglet production, described in the Time to Negative Pig Study as Time to PRRSV Stability (TTS). Herds achieve "TTS status" when there is a failure to detect PRRSV RNA in serum of pre-weaning pigs by PCR over a 90-day period, following the AASV PRRSV-status criteria for a category IIB herd. The value of prior herd immunity (defined as decreased total pre-weaning piglet losses and decreased TTS) has been harder to quantify and therefore not valued in previous PRRS control bio-economic models. The Time to Negative Pig Study provides quantified estimates of Time to Baseline Production (TTBP) and TTS associated with various PRRS management strategies as well as previous herd immunity to PRRSV. Adding this information to our model and therefore to our decision making process allows valuation of the PRRSV negative weaned pig in relation to total sow farm losses assuming the BTW herd has either previous immunity or no immunity to PRRSV.

Updated PRRSV impact estimates

Time to Negative Pig Study updates provided in November, 2012 supported the quantification of two critical decision making points in PRRS management, the method of exposure (Live Virus Inoculation vs. Modified Live Virus Vaccination) as well as the value of prior immunity (PRRSV antibody positive vs. PRRSV antibody negative) in the face of subsequent novel PRRSV infections. TTS and TTBP were quantified for each option using median results of reported data. The resulting model outputs have truly revolutionized our approach to PRRS management. It is important to note that like all studies, the Time to Negative Pig Study has limitations. Specifically, participant herds were not randomly allocated to treatment, therefore the comparison relies on observational analysis and association does not infer causation. There is high variability and low control of loading, exposure and sampling methods. The TTBP estimates make major assumptions and do not consider the impact of other infectious agents present within a BTW herd. Finally, genetics, nutrition, environmental factors and numerous other variables are not controlled. Despite these challenges, our team has identified this to be the "best available information" and in the spirit of evidence based medicine, we will utilize this resource until superior evidence is presented.

Our updated impact estimates can be most easily explained relative to biologic performance. When reviewing the associations with TTS, our group noted that consistent with The Maschhoffs experiences, tremendous variability in TTS existed throughout the industry. Regardless of the variability in TTS, two significant associations could be made:

- BTW herds implementing Live Virus Inoculation (LVI) for exposure achieved TTS significantly faster than comparable herds achieving stabilization through Modified Live Virus (MLV).
- BTW herds with prior immunity (defined as PRRSV antibody positive at the time of novel PRRSV introduction) had achieved TTS significantly faster than comparable herds without prior immunity (defined as PRRSV antibody negative at the time of novel PRRSV introduction).

When reviewing the associations with TTBP (defined as 21 week performance of the affected farm prior to novel PRRSV outbreak), we noted several critical findings:

- TTS was poorly correlated with TTBP.
- TTBP was highly correlated with total pre-weaning production losses.
- Prior herd immunity resulted in earlier TTBP, with a drastic increase in total weaned piglet production relative to PRRSV naïve herds and an apparent dose impact with herds exposed multiple times reaching TTBP sooner than herds exposed once.
- BTW herds using MLV reached TTBP significantly faster than herds using LVI.

The following tables (Table 1 and Table 2) reflect our updated assumptions used to evaluate the biologic and economic performance of BTW farms engaging in an LCE PRRS management strategy utilizing LVI or MLV as their exposure method. These models assume that the same minimum criteria defined for inclusion in the Time to Negative Pig Study are in place and that the BTW farm currently experiencing a novel PRRSV infection flawlessly implements MCREBEL practices throughout herd closure.

These assumptions and associated economic impacts have resulted in two dramatic changes in PRRS management on BTW farms. We first changed our method of exposure from LVI to MLV on all BTW farms implementing an LCE PRRS management program. While our assumption that LVI would result in faster TTS was correct, the value of the increased PRRSV naïve weaned piglets was less than the value of faster TTBP and the associated increased weaned piglet production during herd closure. We continue to challenge the model as the value of a weaned pig changes, but the model is insensitive to weaned pig values, resulting in consistent PRRS management recommendations regardless of the current weaned pig price. Our second dramatic change was a transition from exclusive use of an "Eradication" approach to PRRS management to a decision making process that uses historical PRRS "outbreak frequency" to determine whether PRRS management attempts will result in Control (PRRSV vaccinated gilt introductions) or Eradication (PRRSV negative gilt introductions). Given that BTW herds regularly challenged by novel PRRSV introductions have inherently more value in being PRRSV antibody positive, we found the point of inflection in our model at which the benefits of decreased performance losses associated with each novel PRRSV introduction outweigh the cost of maintaining PRRS immunity in the BTW herd. For the purposes of our very basic model, this break frequency is approximately 1 novel PRRSV introduction every three years. Farms with a historic outbreak frequency of less than one outbreak every three years create more value by Eradicating PRRSV and farms with a historic outbreak frequency of more than one outbreak every three years create more value by Controlling PRRSV. This assumes that the historic outbreak frequency is an accurate predictor of future break frequency, an assumption that should be challenged regularly by the veterinarian working with the impacted herd.

Historical vs. current performance

While caution should be taken in comparing biological performance between historical and current PRRS management strategies, it is good practice to evaluate actual results in relation to model assumptions. Such information provides a quantitative "response to therapy" that can assist continuous improvement of PRRS management decision making and at a minimum, can point to specific model assumptions which need further validation. The performance data listed below represents the impact of novel PRRSV infection on a Maschhoffs BTW farm using an LCE PRRS management strategy utilizing LVI (Historical) vs. MLV (Current) as our exposure method. Results of biologic performance are presented specific to one BTW herd and include the herds TTS, TTBP and Post-Weaning Mortality. Definitions for these performance metrics are listed below:

> TTS – Number of weeks from herd closure (date of exposure followed by no live animal introductions) until the achievement of PRRSV status category IIB. Category IIB represents the time at which pre-weaning piglet testing for PRRSV has provided evidence of decreased PRRSV transmission to allow for negative (Eradication) or vaccinated (Control) gilt entry and subsequent sentinel testing.

Table 1: Value of prior immunity – per 1000 sows

	No immunity	Prior immunity
Cost of an outbreak		
Weaned pig value	$40.00	$40.00
Lost weaned pigs	3,575	645
Value lost	$143,000.00	$25,800.00
Cost of prior immunity		
PSY drag from immunity	0	1
Cost of immunity per year	$0.00	$40,000.00
Outbreak frequency to justify prior immunity		
Value of immunity in an outbreak	$0.00	$117,200.00
Outbreak frequency (years between outbreaks) to justify prior immunity	2.93	

Table 2: MLV vs. LVI exposure – per 1000 sows

Weaned pig value	$40.00
Improvement in pigs weaned from MLV	1,300
Value of increased weaned pigs	$52,000.00
Pre infection pigs per week	519
Post infection pigs per week with MLV	487
Reduced value of pigs weaned with PRRSV (per pig weaned)	$7.00
MLV TTS (weeks)	36
LVI TTS (weeks)	30
MLV PRRSV positive pigs weaned	17,532
LVI PRRSV positive pigs weaned	13,310
Lost value of MLV due to PRRSV positive weaned pigs	$122,724.00
Lost value of LVI due to PRRSV positive weaned pigs	$93,170.00
Increased cost of weaning PRRS positive pigs with MLV	$29,554.00
MLV cost ($1.00 per dose, two doses per sow during exposure)	$2,000.00
Net value of MLV for exposure	$20,446.00

TTBP – Number of weeks from herd closure until weekly PSY has returned to baseline performance (defined as the average herd PSY during the 12 months prior to novel PRRSV introduction).

Post-Weaning Mortality Increase– Average weekly mortality increase for all pigs weaned in the first six months immediately following herd closure when compared to the average weekly mortality during the six months immediately prior to novel PRRSV diagnosis.

Given our recent adoption of PRRSV Control on farms with high historic PRRSV break frequencies, we do not yet have data available on actual impact of prior immunity. We recognize the value in this data, and will monitor performance of PRRSV Control farms in the face of novel PRRSV introductions to challenge our model in a similar manner as our LCE-MLV vs. LCE-LVI approach.

Conclusions

- While ideal evidence to support PRRS management decision making on a BTW farm does not exist, there is an ever growing body of information which should be consulted regularly by the swine veterinarian prior to implementing a PRRS management plan.
- The Time to Negative Pig Study provides performance impact estimates of TTS and TTBP using either LVI or MLV for exposure.

Table 3: Historical performance of BTW farms utilizing LCE-LVI for PRRS management

	Diagnosis date	Closure date	TTS (weeks)	TTBP (weeks)	Post-weaning mortality increase (per week)
Farm 1	2/7/2011	2/15/2011	36	24	0.02%
Farm 2	12/5/2011	12/12/2011	33	33	0.23%
Farm 3	12/19/2011	1/9/2012	32	28	0.38%
Farm 4	12/19/2011	1/9/2012	26	25	0.11%
Farm 5	3/19/2012	5/2/2012	43	22	0.01%
Average	N/A	N/A	34	26	0.15%

Table 4: Current performance of BTW farms utilizing LCE-MLV for PRRS management

	Diagnosis date	Closure date	TTS (weeks)	TTBP (weeks)	Post-weaning mortality increase (per week)
Farm 1	11/6/2012	1/9/2013	23	20	0.23%
Farm 2	11/26/2012	1/2/2013	26	26	0.11%
Farm 3	11/26/2012	1/2/2013	25	26	0.09%
Farm 4	11/26/2012	1/9/2013	23	25	0.06%
Average	N/A	N/A	24	24	0.12%

- The Time to Negative Pig Study provides performance impact estimates of TTS and TTBP based on the presence of prior immunity in a BTW herd in the face of a novel PRRSV introduction.
- TTS and TTBP estimates can be used to create biological and economic performance models to improve the quality of decision making prior to implementing a PRRS management plan.
- Using TTS and TTBP estimates, homogenizing a BTW herd through MLV results in more value generation than exposure through LVI in spite of the fact that LVI results in a shorter TTS.
- Using TTS and TTBP estimates, maintaining a PRRSV antibody positive status results in more value generation than eradication of PRRSV on a BTW farm that experiences a novel PRRSV introduction more frequently than once every three years.

Coordinated herd closures for PRRSv control and elimination

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PRRS virus infections in breed to wean farms has negative consequences for animal health, farm productivity, and financial performance. Elimination of endemic PRRS virus from infected breeding herds has been practiced with success. Through analysis of both successful and unsuccessful PRRS elimination projects, Pipestone Veterinary Clinic (PVC) has developed a list of best practices. These best practices have been incorporated into a standardized protocol for PRRSv elimination projects implemented in the Pipestone System.

Summary of PVC philosophy: Elimination of field virus (wild type virus) will be the goal for every farm following a new PRRS virus introduction. Principle of elimination is based on

1. Exposure of all resident breeding animals to new PRRS virus for the development of neutralizing immunity
2. Cessation in new breeding stock introductions to facilitate the "dying out" of the virus. Stopping gilt introductions removes the population of susceptible animals in which the virus could continue to replicate.

Basics of PVC protocol include:

- Written plan approved by Health Director
- Whole herd "exposure" will be accomplished through either:
  - serum exposure/live virus injection, or
  - mass vaccination with commercially approved modified live PRRS vaccine
- Gilt pool will always receive serum/field virus exposure
- 250 day herd closure to gilt entries, beginning when gilts are exposed to field virus

Requirements at closure (Weeks 1–16):

1. Close farm to replacement gilt introductions
2. Virus exposure/"homogenization"
   a. Serum – expose all sows and gilts
   b. Vaccine –
      i. 2 doses vaccine to sows
      ii. Field virus exposure to non-pregnant gilts + PRRS vaccine
3. No pigs/V-boars retained on farm
4. Save serum samples used for gilt exposure for potential later analysis

Requirements mid/late closure (Weeks 17–36):

1. Begin pig movement restrictions in farrowing
   a. Block sizing
   b. Viremic pigs euthanized
2. Begin piglet testing no later than 16–18 weeks into closure
   a. Oral fluids and serum
   b. Fall behind pigs in oldest farrowing rooms
3. Change needles and disinfect nippers between litters
4. No partial washing of farrowing rooms
5. Foam hallways with Synergize prior to power washing following weaning events
6. No tubs or processing carts
7. Discontinue all pre-farrow feedback
8. Discard all serum retained on the farm
9. Discard all frozen/refrigerated feedback material retained on the farm
10. Defrost, clean, and disinfect all farm refrigerators and freezers
11. Discard all open bottles, needles, syringes, oral dispensers,etc from refrigerator and med storage area – any open bottles or delivery devices that may have been used when animals were still viremic on farm
12. Clean and disinfect all storage areas, hallways, water rooms, etc – good general farm cleaning

13. No manure pumping or agitation within 14 days from the end of herd closure (arrival of naïve gilts). All manure pumping following closure must be approved by Health Director
14. Discontinue oral dosing of piglets
15. GDU must be cleaned, disinfected, and inspected by Health Tech

Release from herd closure:

1. Requires approval of Pipestone System Health Director – make sure to contact before ordering gilts.
2. Testing program for release of closure:
   a. 3 bleeds of 30 head @ 2 week intervals – 100% PCR Neg
   b. 3 bleeds of 60 head @ 2 week intervals – 100% PCR Neg
   c. Oral fluid testing of piglets – collect 6 ropes (12 litters) on weeks opposite of serum collection

Post-closure testing

1. Monthly piglet PRRS PCR on OF or serum
2. Monthly PRRS PCR on naïve replacement gilts that have entered the G-barn
   a. 10 hd serum
   b. Test for 6 months

Following the above protocol, PVC has documented ~90% success rate at elimination of resident PRRS virus.

An interesting case – deeper knowledge helps avoid jumping to conclusions

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ORF-5 sequence information is commonly used by veterinarians in helping to understand the characteristics and dynamics of a client herds' PRRS status. Primarily this information is used in an effort to answer the question, "Is this my old virus or is this a new infection?" In addition, ORF-5 sequences may be used in an epidemiologic comparison of viruses in a pigflow or neighborhood. In a recent case example, neighborhood, pigflow, and ORF-5 sequences were used in elucidating a likely transmission pattern in lieu of an initially suspected route.

In early 2012, a Company-A isowean sow unit experienced a new PRRS infection. The virus matched very closely with a strain of PRRS identified at a Company-A finishing site found in late 2011 (Figure 1). Both sites were part of the same production system (although not part of the same pig flow) and were 15.7 miles apart. Due to the similarity of the two viruses, a Company-A, "in-system" route of infection was suspected (transportation, personnel, equipment, etc…).

Concurrent communication with the veterinarian servicing other area farms (Company-B) revealed additional information. Shortly after the initial Company-A finishing site became infected, a neighboring Company-B sow farm (1.9 miles away) became infected with a nearly identical virus. Following infection at the Company-B sow unit, pigs were weaned to the downstream Company-B sites. Two of these sites were 3.1 miles away from the Company-A sow unit (Figure 2). The timing of infection at the different sites and the sequence of pig movements, now suggested a multi-step route of transmission between Company-A's finisher and sow unit. With this more complete information, both

Figure 1:

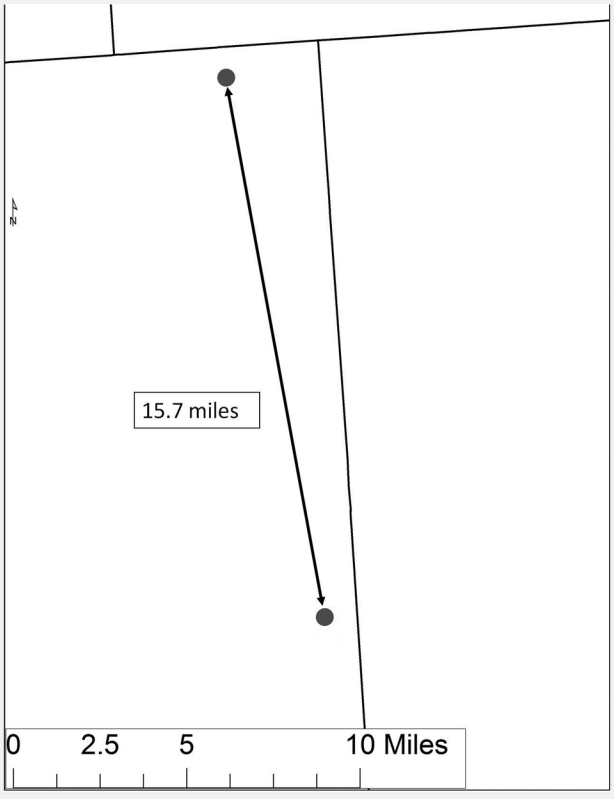
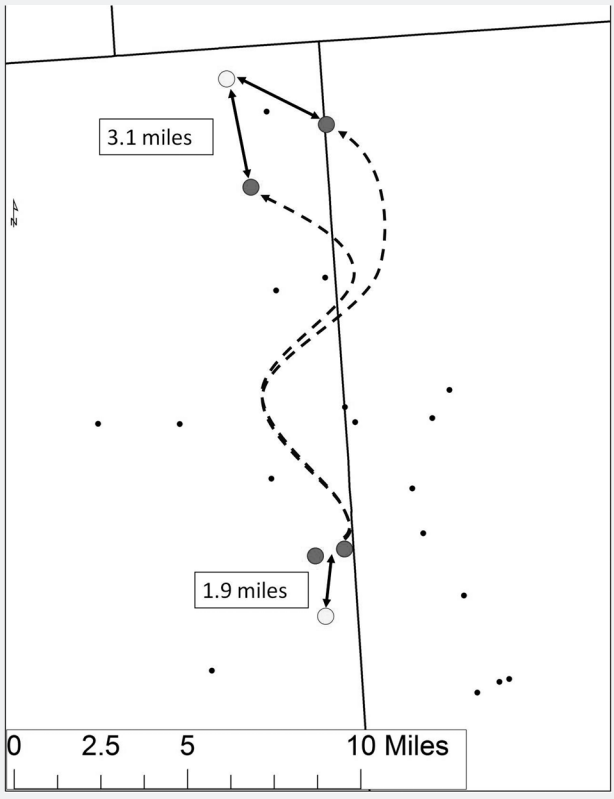


Figure 2:



companies gained a deeper understanding of the sources and outcomes of their PRRS infections, and ultimately were able to avoid making false assumptions.

This case illustrates the necessity of a thorough epidemiologic investigation in understanding PRRS transmission events. Throughout most of the major pig producing areas in the US, the landscape is characterized by a broad and diffuse criss-crossing of pig flows. The possibility of aerosol transmission is amplified by movements of growing pigs (weaned pigs and feeder pigs) between sites in multisite production systems. Not appreciating the potential combination of infection routes may lead to erroneous conclusions and unwarranted actions. Effective communication methods and professional goodwill between producers and between their veterinarians is essential in completing the epidemiologic picture in a PRRS investigation.

Why we cannot just use 2%

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PRRSV ORF5 sequencing is widely used by producers and veterinarians to help interpret the source of outbreaks and the effectiveness of biosecurity procedures. Sequence comparisons by percent difference or percent similarity provide a crude estimate of relatedness, but frequently provide inadequate guidance to decide if a new sequence indicates re-isolation of an existing virus or discovery of a newly introduced virus. Expert opinion also may not be helpful since "experts" often express a wide range of opinions. Some consider a 2% difference significant, whereas other consider 5% difference as still being equivalent.

So what is a person to do? We know that the PRRSV that comes out of a pig is the same or nearly the same as the virus that went into a pig. In direct comparisons the difference is usually between zero and six mutations (6 changes in ORF5 equals 1%). Sequential passage of an isolate from one pig to another and another gives a more-or-less additive increase in variation, so that a specific virus may change by 1% or more in a year's time. When a producer or veterinarian is using percent similarity to determine if sequences in a herd are re-isolates of a herd-specific sequence or are a new viral introduction, the interpretation requires a thorough historical knowledge of previous isolates circulating in the herd and their rates of variation.

A critical misinterpretation can occur in using percent similarity to judge the relatedness of field isolates. In ORF5 sequencing a 1% difference means that six bases are not the same due to mutation. Suppose you find PRRSV A in a herd. Six months later, virus B shows up, with six mutations. At 12 months, two new viruses are found. Virus C has the same 6 mutations as virus B plus six more. Virus C now is 2% different from virus A. Since it is 1% different from virus B, it is reasonable to conclude that A –> B –> C. The second virus at 12 months, virus D, also has 12 mutations compared to virus A, but they are all different from the mutations in viruses B and C. It could not have evolved from virus B, since the six mutations in B would have to revert to the virus A version, and then 12 new mutations would need to occur. This is highly unlikely. It is probable that virus D is a new introduction into the herd.

Since it is possible to get viruses that have the same percent similarity but acquiring independent and unrelated mutations, using percent similarity alone is unreliable for determining relatedness. The same problem occurs in using RFLP type to characterize PRRSV. It is well known that viruses that are genetically completely unrelated may have the same RFLP pattern. For example, the highly virulent 1-4-4 family of viruses that appeared in 2010 is not related at all to 1-4-4 viruses that were circulating in the 1990's.

The solution is to use phylogenetic trees (dendrograms) in addition to percent similarity for interpretation of PRRSV relatedness. Dendrograms show calculate the most simple path by which mutations in a group of isolates could have evolved. The value of a dendrogram and the resulting interpretation is enhanced by the regular monitoring to detect viruses that are present in the herd, awareness of clinical and production history of the herd, and knowledge of resident viruses in the region. We know that PRRSV is a difficult challenge, so it is important to have as much information as possible.

A decision tree to guide to answering the question, is this a new virus or not to the farm?

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Introduction

One of the hardest questions to answer is, could this be a new virus to the herd or is it the resident virus that has been in the herd before. In herds that are negative it is an easy question but for herds that have had previous virus exposure it is a harder question. Sequencing is a valuable epidemiological tool but can be difficult to interpret once the results are completed. One of the most challenging things to do is to determine if a virus that is found from a diagnostic workup is a virus the herd has or is it new. The standard convention is if 2 viruses are more than 2% different then they are different viruses.[1] The MJ classification system is another method to determine similarity of viruses.[2]

In herds that are class IV (by AASV PRRS herd classification system)[3] negative. When a virus is identified in these herds it is easy to interpret because all viruses in the herd are considered new.

Class III herds are similar to the class IV herds but need to make sure that it is not the resident virus that was being eliminated from the herd.[3] These herds have sentinel animals entered after the break and herd closure and these can be help in interpretation of diagnostic information.

The real problems in interpretation of sequencing results are in the class II and I herds.[3] These herds are known to have a resident virus and then it is more difficult to make the evaluation.

Determining if the virus is a resident virus or a new introduction is particularly important if the herd is in a herd closure program and this may extend the time before gilts can be re-introduced. There are also pig flow considerations on where can the pigs go and do changes need to be made.

Objectives

To develop a methodology that can be used to determine if 2 viruses with similar sequence patterns are the resident virus in the herd or a new introduction. A working group of the AASV PRRS task force was formed to define a way to make these determinations.

Materials and methods

The viruses that are more than 5% different are easy to determine that they are new introductions to the herd. The ones that are tough are the ones that are less than 2% different. The standard conventional wisdom is that less than 2% different is a similar virus and closely related.[1]

In a poll of expert opinion viruses that are more than 4% different are considered to be clearly different. Anything less than this may or may not be related. This just adds to the confusion around the conventional wisdom determining if this is a resident virus or not.

There are number of questions that need to be asked to help determine if the virus is a new introduction or not to use in conjunction with the sequencing information. The sequencing cannot be used as a standalone method to answer this question. This algorithm is designed as decision tree type of structure using other herd information once the sequence is determined and how use this information in an organized manner to help answer the question is this the herd virus or is it a new introduction.

Start with the sample collected and then work down the decision tree to determine if it is a resident or new virus (See Figures 1-3).

Conclusions

There is still much to be understood about virulence and cross protection with the PRRS virus. Until this is better understood practitioners and producers have to use the best information available to answer the epidemiological question of could this be a resident herd virus or is this a new introduction. There is not a simple answer the hope is this decision tree type algorithm is helpful in giving a structured way to use clinical information about the herd and sequence information and determine the status of the virus.

Figure 1: The next step is to work through the Location/Biosecurity/Sequence information.

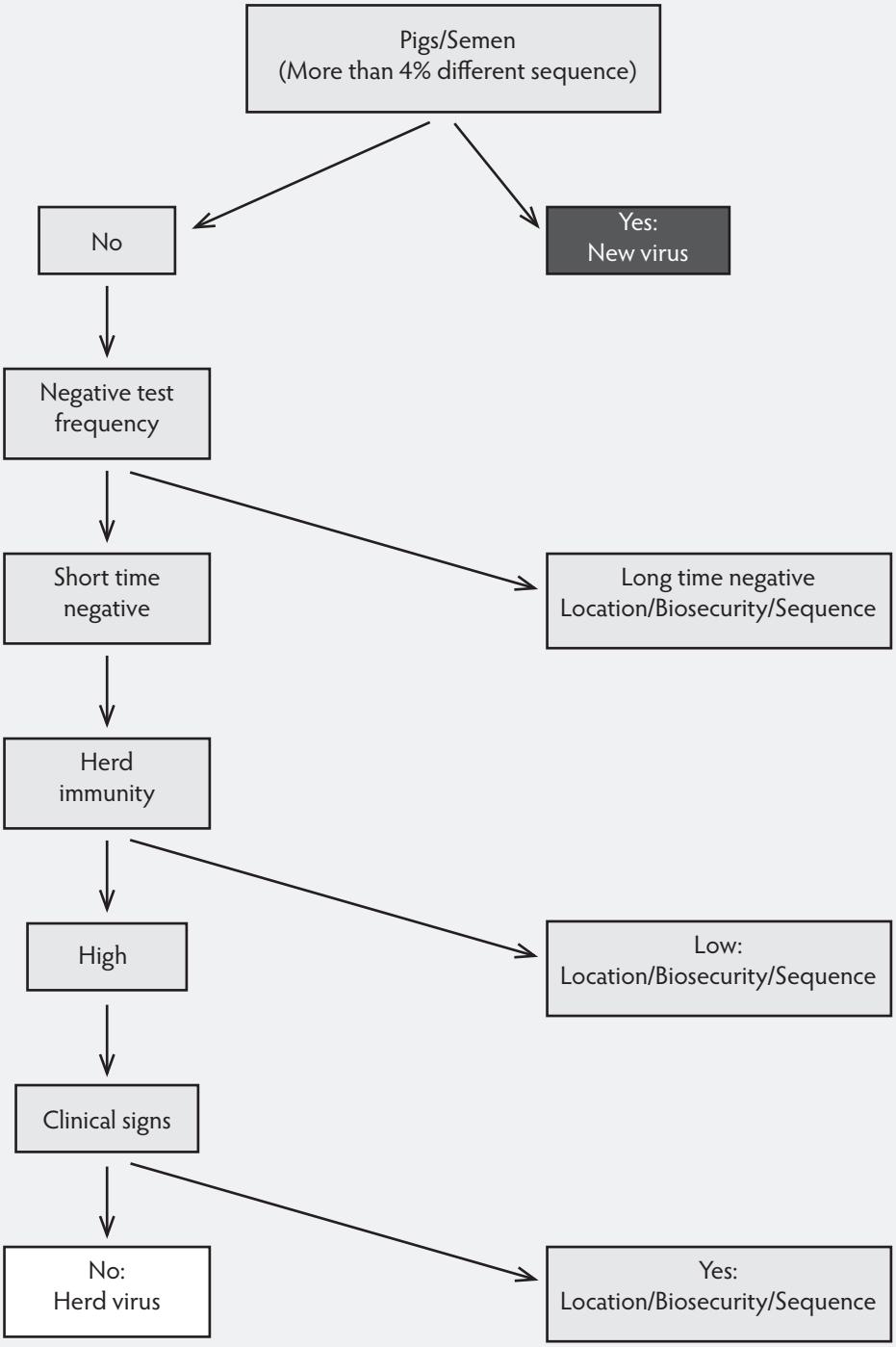


Figure 2: Biosecurity questions that need to be answered.

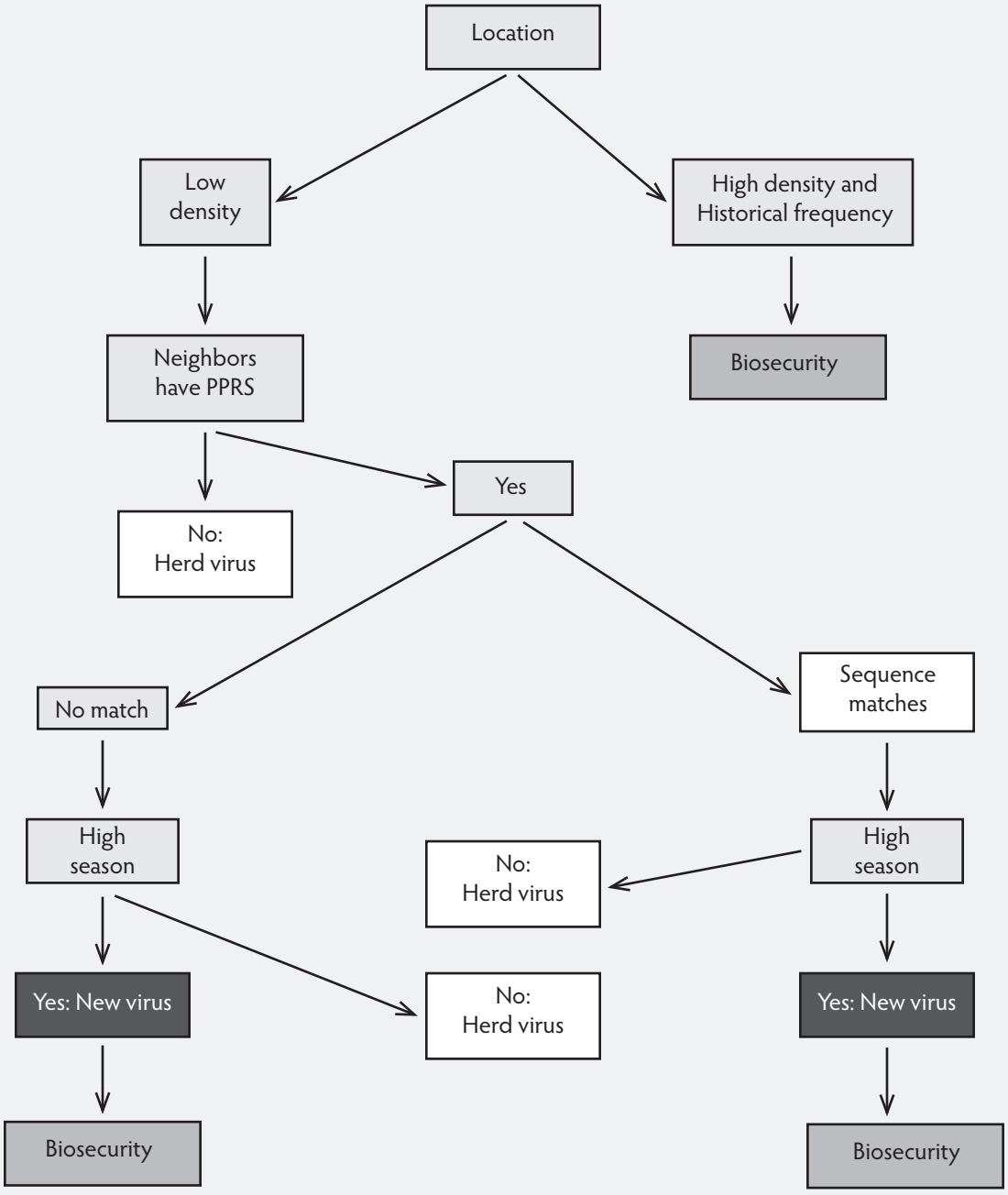
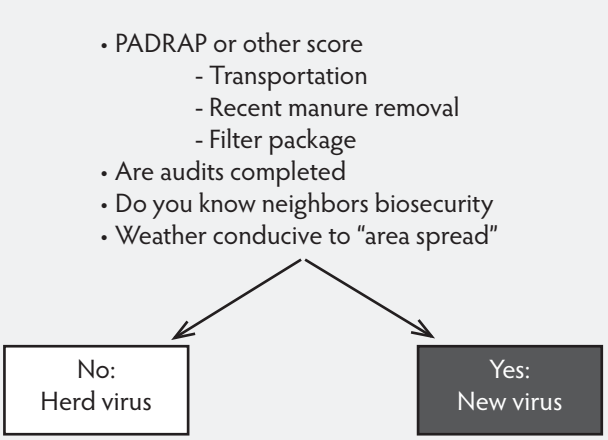


Figure 3: Biosecurity review



References

1. Murtaugh M.; Using Interpretation of sequencing in PRRSv control program: Inside the "black box" Proceedings AASV Preconvention session Full Circle Sequencing 2013 pp 19–24.

2. Snodgrass A.; Retrospective Analysis by Novel Methodology of PRRSv Field Strains in an Area-Regional Control and Elimination Project. Poster session AASV 2013

3. Holtkamp D.; Terminology for classifying swine herds by porcine reproductive and respiratory syndrome virus status; Journal of Swine Health and Production January/February 2011 pp 48–56.

Comparison of various sample types for detection of *Mycoplasma hyopneumoniae* in recently infected pigs

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Introduction

Mycoplasma hyopneumoniae (*M. hyopneumoniae*) infections continue to be a major problem for the swine industry worldwide. The detrimental effect of this bacterium in swine populations is not only due to the pneumonia and the associated decreased performance caused by the pathogen itself, but also by the animal's predisposition to secondary respiratory infections of bacterial and viral origin. Therefore, *M. hyopneumoniae* infections cause significant losses to swine producers each year.

Disease control, typically attempted through vaccination and/or medication of growing pigs, is expensive and does not consistently work in all herds. For these reasons, during the last few years many veterinarians and producers have chosen to completely eliminate *M. hyopneumoniae* from their herds. This has resulted in an increased number of herds that are free of this pathogen. Continuous serological surveillance of free herds by serum ELISA is a good strategy that most negative herds have in place. However, these ELISA-based surveillance protocols have two main limitations: 1. Current ELISAs lack the ability to detect early infections. 2. Current ELISAs produce false positives and no good confirmatory test is available. Usually another ELISA is used as a confirmatory test, which does not completely clear the uncertainty created by false positives. In many instances, the question of whether the original positive result was a false positive or the true early manifestation of a recent infection of the herd remains unanswered. In replacement breeding stock populations confirmation of the status of a population following questionable positive ELISA results often requires costly euthanasia of high value animals for tissue submissions. In addition, accurate detection of an early infection to prevent downstream spread is even more important.

The lack of tools to efficiently detect *M. hyopneumoniae* early infections in live pigs further complicates this scenario. *M. hyopneumoniae* diagnosis in live pigs, and especially during early stages of infection is very challenging. Therefore, there is a growing need for surveillance tools that complement ELISA tests to demonstrate that populations remain negative overtime or to accurately detect early infection to prevent further spread. In response to this need, several sampling and diagnostic tools to be used in live pigs have been proposed, for example, oral fluids, tracheal swabs, and tracheo-bronchial lavages. However, some of these techniques have not been properly evaluated and they have not been compared side by side. Therefore, we carried out an investigation by performing a side by side comparison of sampling and testing tools currently available for detection of *M. hyopneumoniae* during the early phase of infection, as well as the use of alternative procedures.

Materials and methods

Twenty three 8-week old conventional pigs were obtained from a source known to be negative to major swine pathogens, including *M. hyopneumoniae* and PRRSV. Pigs were housed in the Isolation Units at the St. Paul Campus of the University of Minnesota.

Pigs were ear-tagged at arrival and divided in two groups of 21 and 2 animals. Each pig in the group of 21 was inoculated with *M. hyopneumoniae* 7 days after arrival, and all were housed in experimental rooms (3 rooms of 7 pigs each). Pigs were intra-tracheally inoculated with *M. hyopneumoniae* strain 232. The remaining two pigs were mock inoculated with negative culture media and served as negative controls.

Each pig was sampled at 0, 2, 5, 9, 14, 21 and 28 days post-inoculation (dpi). At each sampling time, blood, nasal swabs, laryngeal swabs, tracheo-bronchial lavage and oral fluids were collected. All animals were euthanized at 28 dpi and *M. hyopneumoniae* infection was assessed by gross and microscopical lesions and by bronchial swab real time PCR.

Blood samples were separated to obtain serum, which was aliquoted and prepared for the following ELISA testing:

- IDEXX
- Oxoid
- Biochek

All serum samples were processed straight after collection and aliquots were stored at -80°C until analysis.

Nasal, laryngeal and bronchial swabs, as well as tracheo-bronchial lavage and oral fluids (by room) were submitted to the VDL for DNA extraction and *M. hyopneumoniae* real time PCR testing (VetMAX™, Life Technologies).

After euthanasia, macroscopic lung lesion evaluations were conducted as a single blind study. Microscopic lung lesion evaluation was done using the fixed lung tissues collected at euthanasia.

The percentage of positive pigs for each diagnostic procedure was compared with McNemar tests for paired samples at each sampling time.

Results

The two mock inoculated pigs in this study were negative for *M. hyopneumoniae* at all sampling times for all type of tests, ELISAs, real-time PCR and lung lesion evaluation.

The detection of *M. hyopneumoniae* genetic material in the experimentally infected pigs was as follows: All pigs were negative to *M. hyopneumoniae* at 0 dpi (prior to inoculation). All samples from 2 dpi resulted negative for detection of *M. hyopneumoniae* DNA. First positive real time PCR results were obtained at 5 dpi, in either nasal or laryngeal swabs, or in tracheo-bronchial lavages. *M. hyopneumoniae* genetic material was detected in various sample types from various pigs in all rooms at 9, 14, 21 and 28 dpi. The higher percentage of positive pigs was achieved with laryngeal swabs from 5 until 28 dpi. Proportion of positive pigs, as tested by tracheo-bronchial lavage increased from 5 dpi until 21 dpi, decreasing at 28 dpi. DNA detection in nasal swabs was similarly low from 5 until 21 dpi, increasing at 28 dpi.

Mycoplasma hyopneumoniae genetic material was detected in oral fluids in 2/3 samples (2/3 rooms) obtained at 9 dpi and 28 dpi, and was negative at all other sampling times.

Specific *M. hyopneumoniae* antibodies were detected in a proportion of experimentally infected pigs at 21 and 28 dpi. Detection rates were similar among the three ELISA assays compared in this study ($P > 0.05$).

Conclusions

Under the conditions of this study, laryngeal swabs showed the highest sensitivity for early detection of *M. hyopneumoniae* compared to other sample types. Tracheo-bronchial lavages were more sensitive than nasal swabs during the first 21 dpi, while nasal swabs showed lower sensitivity during the first 3 weeks post-inoculation, compared to laryngeal swabs and tracheo-bronchial lavage. Oral fluids showed low sensitivity for *M. hyopneumoniae* detection during the early stages of infection. Detection of *M. hyopneumoniae*-specific antibodies was similar by the 3 commercial kits used in this study. In summary, detecting pig exposure to *M. hyopneumoniae* was earlier achieved by real time PCR testing in laryngeal and nasal swabs, tracheo-bronchial lavage than by detection of antibodies by ELISA.

Acknowledgements

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References

1. Fablet C, Marois C, Kobisch M, Madec F, Rose N. 2010. Estimation of the sensitivity of four sampling methods for Mycoplasma hyopneumoniae detection in live pigs using a Bayesian approach. Vet Microbiol. Jul 14;143(2–4):238–45.

2. Maes, D., Segales, J., Meyns, T., Sibila, M., Pieters, M., Haesebrouck, F. Control of *Mycoplasma hyopneumoniae* infections in pigs. Vet. Microbiol. 2008. Jan 5;126(4):297–309.

3. 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.

4. Pointon, A.M., Davies, P., and Bahnson, P.B. 1999. Disease Surveillance at Slaughter. In: Diseases of Swine. 8th Edition. B. Straw, S. D'Allaire, W. Mengeline, and D. Taylor (editors). Iowa State Press. p. 1118.

5. Sibila, M., Pieters, M., Molitor, T., Maes, D., Haesebrouck, F., Segales, J. 2009. Current perspectives on the diagnosis and epidemiology of *Mycoplasma hyopneumoniae* infection. Vet. J. Sep:181(3):221–231.

6. Thacker, E.L. & C. Minion. 2012. Mycoplasmosis. In: Disease of Swine. Tenth Edition. Zimmerman, J (Editor). Hoboken, NY. USA.

7. Woolley LK, Fell S, Gonsalves JR, Walker MJ, Djordjevic SP, Jenkins C, Eamens GJ. 2012. Evaluation of clinical, histological and immunological changes and qPCR detection of Mycoplasma hyopneumoniae in tissues during the early stages of mycoplasmal pneumonia in pigs after experimental challenge with two field isolates. Vet Microbiol. Dec 28;161(1–2):186–95.

Molecular characterization of *Mycoplasma hyorhinis* field isolates

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Introduction

Since late 2008 *Mycoplasma hyorhinis* associated disease has been one of the main concerns of the US pork industry.[1] It appears that differences in virulence of the infecting *M. hyorhinis* strain, the host immune response, and concomitant infections may play a role on disease manifestation. Antigenic differences between different *M. hyorhinis* isolates have been shown by seroreactivity to specific antisera.[2] A few experimental challenge studies have shown differences in virulence *in vivo*.[3] 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 *M. hyorhinis* isolates circulating amongst swine populations. The molecular typing of *M. hyorhinis* would aid in better understanding transmission routes, in assessing sources of infection and also in evaluating interventions such as vaccination and use of antibiotics. MLST is considered a rapid, reproducible and inexpensive technique that can identify phylogenetic relationships amongst diverse organisms.[4,5] The objective of this study was to develop and validate a multi-locus sequence typing (MLST) protocol for the characterization of *M. hyorhinis* field isolates.

Materials and methods

Thirty-nine *M. hyorhinis* isolates from different geographic regions obtained from pigs with polyserositis and a clinical history suggestive of *M. hyorhinis* infection, together with one reference ATCC strain (17981D) were utilized in this study. Modified Hayflick's medium was used to grow all *M. hyorhinis* isolates. Purified, genomic DNA was obtained using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). The completed genome sequences of four *M. hyorhinis* isolates available in Genbank were utilized to identify potential target genes. Primers were designed with MEGA 5.2.1 (version 5; **www.megasoftware.net**) and Primer 3 software. Primers targeted different housekeeping and outer membrane protein gene segments dispersed throughout the *M. hyorhinis* genome. PCR was performed using an initial denaturating step at 95°C for 15 min, followed by 35 cycles of 94°C for 1 min, 43.5°C for 1 min 30 sec. Agarose electrophoresis was performed on the amplified products and bands were observed under ultraviolet light in the presence of ethidium bromide. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and bidirectionally sequenced by standard Sanger sequencing on an ABI 3730xl genetic analyzer (Applied Biosystems). Quality of the generated sequencing data was evaluated and sequences were aligned utilizing ClustalW and trimmed to equal sizes. Phylogenetic analysis mas carried out using MEGA 5.2.1.

Results

A total of 25 genes were evaluated as potential target genes. These included housekeeping genes: *pgmB*, fusA, gyrB, lepA, *metS*, *gltX*, *dnaA* and *pdhB*, as well as surface proteins: *p3*, *p95*, *p37*, hexosephosphate transport protein (*hexo*), ribonucleoside-diphosphate reductase (*nrdf*), uracil-DNA glycosylase (*ung*), mannitol-1-phosphate 5-dehydrogenase (*mtlD*), lipoprotein Signal peptidase II (*lspA*) and variable lipoproteins (*vlp*) *A*,*B*,*C*,*D*,*E*,*F* and *G*. The majority of the housekeeping genes and a few surface protein genes were found to be identical in all four annotated genomes, therefore were not included for further testing. On the contrary, the gene sequences for *vlpF* and *B* genes were found to be highly divergent among the four available genomes, with no conserved flanking regions hindering the possibility of primer placement. Primers for the remaining vlp genes were designed, however, an amplicon was not obtained for *vlpA*, *C*, *D* and *E* gene when testing 6 of the 39 available *M. hyorhinis* isolates. Although and amplicon was generated for the *vlpG* gene, the generated product had low quality and was not reproducible. Finally, a total of 7 target genes were included in the MLST protocol: *ung*, *pdhB*, *mtlD*, *hexo*, *p3*, *p95* and *p37*. Preliminary data suggests that there is variation at the nucleotide level amongst the 39 isolates tested, with varying degrees of nucleotide difference between isolates within each gene. Further analysis will be performed on the concatenated sequence data and sequence types will be assigned. The data will be evaluated to potentially identify epidemiological patterns, i.e, clustering of isolates by tissue, system, state and year.

Discussion and conclusions

Currently swine producers and veterinarian are faced with the challenge of controlling *M. hyorhinis* disease in affected post-weaning pigs. The development of a genotyping tool will result in a better understanding of the diversity of *M. hyorhinis* field isolates circulating in US swine herds. This tool will allow to 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 US swine industry will be better positioned to control a pathogen that is responsible for an important part of the mortality observed in the nursery.

References

1. Leuwerke B. (2009) Proceedings of the Allen Leman Swine Conference. Saint Paul, MN. 36:89–90

2. Friis N.F. 1971. *Acta Vet Scand*. 12(1):69–79.

3. Gois M. & Kuksa F. 1974. *Zentralbl Veterinarmed B*. 21(5):352–61.

4. Keim, P., et al., 2000. Multiple locus variable number tandem repeat analysis reveals genetic relationships within Bacillus anthracis. J. Bacteriol. 182:2928–2936.

5. Cooper J., Feil E. 2004. Multilocus sequence typing – what is resolved? Trnds in Micro 12(8), pp 373–377.

Mycoplasma hyosynoviae A Case Study: Quantitative assessment of incidence and severity across time alongside a diagnostic monitoring plan and intervention

Co-authored by Sarah Probst Miller (SPM), AgCreate Solutions; and Alejandro Ramirez (AR), Iowa State University (IS-CVM; Original case pathology and histology photographs by Kuldeep Singh (KS), University of Illinois (UICVM); Diagnostic plan coordinated with Paul Knoernschild and Everett Rosey from Zoetis, Monitoring diagnostics sponsored by Zoetis via STOMP and completed by IS-CVM

Review of *Mycoplasma hyosynoviae* as a pathogen (SPM)

Mycoplasma hyosynoviae is one of several agents capable of instigating arthritis in growing swine. This arthritis is typically 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 animal afflicted with infectious arthritis may make it to commercial markets, the concern lies in the welfare of afflicted animals as well as potential impact on pig 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. This case study reports the process of diagnosing and treating a gilt herd afflicted with aesthetic and clinical lameness.

Background (SPM)

In September of 2013 a wean to market gilts site of 7200 gilts sourced from an 1800 multiplication unit complained of reduced selects sold due to "lameness." Gilts are negative to PRRS, *Mycoplasma hyopneumoniae,* and APP. The reported "lameness" was purely aesthetic (Image 1) in many cases and clinical in others (Image Groups 2 and 3). Most females appeared to recover and not suffer lameness in the sow herd if they were in the aesthetic lameness category. Those farms receiving clinical lameness cases (Image Groups 2 and 3) reported improvement in some females and deterioration in others. An effort was made to weed out all clinical lameness cases during the selection process.

Past percent sold on the select market had ranged from 66 to 79% sold per group over the previous 6 months (Table 8 and 9). At the time of first assessment, the herd had reduced the percent sold per group to 51.9% in August 2012 followed by 38.6% in September 2012 of opportunity females (Table 8). This reduction was not due to demand but was due to true reduction in ability to select because of this condition.

Maternal barrows in a variety of wean-to market barns from this parent source also went through this "lameness." However, the "lameness" did not impact pigs sold at the commercial level nor did it appear to impact growth. In fact, at the commercial level, the "lameness" was not considered problematic just purely incidental. At the gilt grow out however, the height of the swelling and associated lameness occurred at the time when the majority of females were sold, 230 to 250 pounds, hence the strong impact on percent sold.

Clinical observations at first visit 09/19/2012 (SPM)

Clinically at the time of first visit, the observing veterinarian noted various levels of swelling surrounding the hock and/or shoulder region (See Image Groups 1, 2 and 3). Many females had one limb affected, however other females had two, three or all legs affected. Original incidence by age is shown in Table 1. Varying levels of lameness was observed ranging from no effect on mobility, to mild aversion to weight on the affected limb, to pigs with muscular tremors walking in an eggshell-like fashion and going down in back. Initially two females were delivered live to the University of Illinois Veterinary Diagnostic Laboratory.

Diagnostics (SPM and KS)

Diagnostics on sacrificed females by UICVM pathologist on call reported the following:

Morphologic diagnosis:

1. Moderate bilateral subacute to chronic tibiotarsal and hock joints tenosynovitis
2. Marked chronic tenosynovitis of the left carpal and right tibiotarsal region
3. Moderate acute synovitis of the left stifle joint

Microscopic report:

> Tendon sheaths were markedly thickened and expanded by mature fibrous connective tissue, as well as multiple, variably sized, occasionally coalescing foci with small to moderate numbers of reactive fibroblasts, newly formed blood vessels and small to moderate numbers of lymphocytes, plasma cells, and rare eosinophils and neutrophils. The synovial lining is multifocally thickened, eroded and partially replaced by proteinaceous fibrillar material fibrin. The underlying areas are multifocally to locally extensively infiltrated by small to moderate numbers of lymphocytes, plasma cells, few eosinophils and rare neutrophils. In other areas the synovial lining is multifocally thickened (hyperplastic). The tendon sheath lumen contains variably sized clumps of fibrin with minimal amounts of necrotic cellular debris. The synovial membrane lining is multifocally minimally thickened, forms rare, small papillary projections, and the underlying soft tissues are infiltrated a few aggregates of small numbers of lymphocytes, plasma cells, and rare eosinophils.

Microscopic summary:

1. A moderate to marked diffuse chronic lymphoplasmacytic tenosynovitis with fibrin formation and granulation tissue proliferation (Image Groups 3 and 4)
2. Minimal to mild multifocal chronic lymphoplasmacytic synovitis (Image Group 5)
3. Moderate to severe reactive lymphadenopathy with lymphoid hyperplasia. (Image Group 6)

In regards to long bone articular cartilage, the pathologist noted some lesions within the articular cartilage and physis, but said that gross lesions and histopathology did not support OCD. He also reported that the observed lesions on the cartilage were likely secondary to expansion of inflammation centered on tendons and that nutritional disease seemed unlikely.

M. hyosynoviae was isolated from Pig B's right tarsal tendon. Other affected tendons in Pig A were not positive but the pathologist said that lesions were typical of *M. hyosynoviae*. At a later date from subsequent diagnostic submissions, *M. hyorhinis* was isolated from affected pigs (lungs) but not from leg lesion sites. Post treatment implementation there were no non-treated females on site. No further isolates occurred on subsequent submissions (6) likely do to treatment impact or efficacy. All lesions on submitted females looked the same on morphologic and microscopic examination and remained consistent with findings in the original case submitted.

After these diagnostics, the veterinarian was tasked with development of a diagnostic, treatment, preventative and monitoring plan. The goal was to reduce lesion incidence and severity and increase the number of selects sold and to measure the effectiveness of the treatment/preventative plan.

First plan of action and results (SPM)

The first treatment program implemented had a dual preventative and treatment purpose. To accomplish this, a feed medication chlortetracycline (10 mg/lb.) pulsing program was initially instigated. Shortly after implementation the treatment/preventative plan, diagnostics showed the isolated *M. hyosynoviae* resistant to chlortetracycline, but sensitive to lincomycin. Lincomix® is not labeled for reduction or control of *Mycoplasma hyosynoviae* however it is labeled for control of ileitis.[2] This herd also needed a control plan for ileitis. Switching the treatment plan to Lincomix® achieved this end goal as well and also had a secondary benefit of assisting with the *Mycoplasma hyosynoviae* prevention and treatment plan. Details of the plan implemented in late November of 2012 involved concentration of Lincomix® therapy at 100 g per ton from 3 to 8 weeks post entry, with pulses of Lincomix starting at 11 weeks and again at 15 weeks post entry. Table 1 a and 1 b show the incidence results of lameness (Table 1) and diarrhea (Table 2) and Fallouts (Table 3) per age per monthly visit as we monitored the quantitative impact of this prevention/treatment program on diarrhea and fallout incidence as well as lameness.

Simultaneously, our isolate was forwarded to laboratories to be poised for potential production of autogenous vaccine.

Case communication to customers (SPM)

The goal with preventative and treatment implementation was not to hide the situation, but rather be transparent and clear regarding our diagnostic findings and preventative/treatment approach. As the plan was implemented, vet-to-vets were completed with all customers and diagnostics and preventative/treatment plan discussed. During the vet-to-vet's communication, we made it clear that this herd remained negative to *Mycoplasma hyo**pneumoniae*, but like many herds was positive to *Mycoplasma hyo**synoviae*, and to the best of our knowledge was now experiencing clinical expression of *Mycoplasma hyosynoviae*. Vet-to-vet's information gathered reported that most of gilts merged well with the sow herds that were negative to PRRS and *Mycoplasma hyopneumoniae*, however if the herd converted to PRRS or *Mycoplasma hyopneumoniae* during acclimation, the severity of the lameness would increase vs. decrease. When Lincomix®[2] was implemented at the time of PRRS and *Mycoplasma hyopneumonaie* conversion, this increase in severity was mitigated or

eliminated. During and post communication customers were concerned, but expressed desire to remain on the same gilt source. They reported good performance of gilts in spite of the situation we were addressing.

Further diagnostics plan started (SPM)

A diagnostic and monitoring plan was implemented to further work to evaluate the impact of the prevention/ treatment program and impact on *M. hyosynoviae* and to evaluate whether *M. hyorhinis* was impacting the signs as well. The veterinarian in charge, Sarah Probst Miller, coordinated with the Zoetis representatives, Drs. Paul Knoernschild and Everett Rosey from Zoetis as well as Drs. Alex Ramirez and Phil Gauger at ISU-CVM.

Background summary of diagnostic capabilities at the time of the plan development (AR)

Unfortunately not all swine diseases have well established validated diagnostic tools available. Such is the case for *M. hyorhinis* and *M. hyosynoviae*. Culture for *Mycoplasma spp* is not easy and is usually time consuming. Mycoplasmas tend to be overgrown by contaminants quite easily. There are several PCR assays available for both of these mycoplasmas. These PCRs are quite sensitive and specific but unfortunately difficult to quantify the amount of DNA present in the sample; especially for *M. hyosynoviae*. As such, sometimes we can rely on CT values as close proxy to help us compare amount of mycoplasma DNA between two samples. This is not a perfect science, especially when comparing CT values from different days, but with caution, CT values can provide some insight into possible pathogen load until quantitative PCR assays are developed and validated.

These PCR assays can be used on blood, tonsil scrapings as well as oral fluids. Past experience and research by ISU has shown that tonsil scrapings and oral fluids are the best samples for detecting *M. hyosynoviae*.

Serologically we still have the problem on having a validated assay. Currently the ISU-VDL has a Tween-20 ELISA assay for both *M. hyorhinis* and *M. hyosynoviae* that is still under research development. Although cutoff values have not been well established for these assays, the trend in increased in corrected optical density (OD) values can be used to demonstrate exposure through increased values. As a test under research development, OD values between dates can vary. Currently, based on past experience, we believe that the immune response detected by this Tween-20 ELISA assay occurs much later than the traditional 2 weeks post exposure we are used to with many other pathogens.

Diagnostic plan created (SPM)

The following diagnostic plan was created:

1. Quantitative assessments of incidence and severity levels:
   a. To monitor the incidence rate and severity (ABCE ranking levels described in b.), whole barns of gilts were monitored monthly by choosing 4 pens of gilts per barn and assessing all gilts per pen for signs of lameness and associated severity.
   b. In each pen, 4 gilts were tagged and their level of lameness was monitored over time. Gilts were given a severity ranking of A, B, C or E. (See Images 4, 5, 6 and 7),
      i. A pigs showed aesthetic swelling but would likely sell as sound due full mobility (Image 4).
      ii. 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 (Image 5).
      iii. C pigs would for sure end up a market hog vs. a select due to level of swelling and clinical lameness (Image 6).
      iv. E pigs were severely lame hogs that would either go on the cull/lite truck or be euthanized (Image 7).
2. To further assess presence and intensity of *M. hyosynoviae* and *M. hyorhinis* in the population, the following sampling methodology was completed:
   a. Every month rope sampling was done in the selected pens
   b. Every month serum sampling and tonsilar swabbing was done on the tagged gilts

Results (SPM and AR)

Quantitative results showing incidence per symptoms of concern per month are reported in Tables 1, 2, and 3. Total incidence does not reduce over time. In fact, it appears to increase. At first, only total incidence of each symptom was assessed. We implemented ranking by severity in February. We believe by inclusion of the aesthetic lameness category of A where these animals had mild visual signs, but no actual mobility lameness; we were more willing to put females into this category thus increasing our incidence totals. At this same time, as we started to have females sold that had been through the entire treatment program; our percent selects sold started to go up (Table 8 and 9). April 9, 2013 had our first group that had received 80% of the preventative treatment plan and gilts sold on

5/9/2013 through 7/9/2013 had received 90 to 100% of the preventative/treatment plan. Once females receiving the preventative/treatment plan for most of their life started to be sold the range of percent sold moved from 38 to 55% sold to 58 to 75% sold.

Quantitative results reporting the severity breakdown per incidence are shown in Tables 4a and b through Tables 7a and b. These charts show the percentage of A, B, C and E pigs in the total incidence reported. Since A pigs **could** be sold as selects, we also created a Linear Forecast Trend line showing the total pigs with symptoms minus the A pigs. Most of these forecasts in every age group are trending down indicating a trend towards more saleable females, except for the 15 to 18 week trend. We believe this 15 to 18 week trend may be skewed by potentially aberrant July results. At the time of the presentation we will have August quantitative numbers in and will be able to understand if this July spike is artifact or real. Of importance is that the trend line is headed in the right direction for animals in the 19 to 22 week range as this is the age that the majority of females are sold (Table 7b).

Serology results April, May and June 2013 testing results

In April, Tween 20 testing showed presence of both M. *hyosynoviae* and *M. hyorhinis*. Table 10a shows the average S/P ratio of *M. hyosynoviae* across age groups. Both pathogens peaked at 22 and 17 weeks respectively. Initial exposure Table 11 shows the average S/P ratios for pigs ranked as A,B or C and Normal (labeled as blank in table) pigs. Normal pigs had lowest S/P ratios while B (subacute) and C (chronic) had the highest.

In May and June we focused on just *M. hyosynoviae* to reduce total testing cost as *M. hyorhinis* was never isolated from a leg lesion and only from lung tissue. Also the height of symptoms matched better with the timing of typical *M. hyosynoviae* infections. May had similar results to April and June's Tween 20 results are be redone to check test accuracy due to unexpected variation from April and May.

Rope PCR results

Rope PCR results are reported in Chart 1a-c. By pen females appear to arrive with some pens positive. As they start Lincomix® treatment, the majority of pens are negative. As pigs move to non-medicated feed, pens start to move back positive again. This test lets us know that the Lincomix® program does appear to reduce the presence of the pathogen when under full treatment; but it does show that in any given pen, the pathogen does reappear over time once treatment is discontinued. This would be discouraging if not for the results from the tonsilar swabs from the individual females.

Tonsilar swabs of individual females

In Chart 2a and b we have *M. hyosynoviae* results from tagged females. Results show a reduction in the number of pigs who are individually positive in those females who had received a more complete preventative/treatment course. With this test we speculate that by continuing the preventative/treatment Lincomix® plan we appear to be reducing pathogen load over time.

Next steps

Based on these results, a decision has been made to hold the current preventative/treatment course and work to move the Lincomix® program as close to arrival as possible. With this plan we hope to continue to reduce the pathogen load at this site. Due to a K88 diarrhea at arrival the herd is currently reluctant to move their Mecadox program in the first two weeks post arrival. Lincomix®will go in the feed 2 weeks post entry for most females.

At this time the herd is poised for potential autogenous production, but does not have immediate intentions of starting autogenous production.

This report will be communicated to the sow farm and a possible pathogen reduction program at the sow farm level will be discussed.

Discussion and significance of the case study

M. hyosynoviae is hard to diagnose and prove correlation with lameness in certain circumstances. The novel rope tests for *M. hyosynoviae* involved in this case showed promise as a diagnostic tool for the industry to use in the future to help diagnose presence of *M. hyosynoviae* and treatment efficacy. In addition, tonsilar swabs of individual animals allowed us to better understand reduction in pathogen presence. When diagnostic testing is paired with regular intervals of quantitative assessment of incidence and severity of symptom, the presence and absence of clinical signs during therapeutic intervention can be potentially assist with diagnostics interpretation. This quantitative and diagnostic approach allowed us to achieve an early understanding of trends to know if we were on the right track.

Summary of what we learned about diagnostic capabilities from this case (AR)

One of the key points we have learned from this case study is that in working up a case like this using multiple diagnostic tools is essential; especially since we are dealing with tools that we are still learning how to use for this

particular pathogens. If we just take one individual test, say tonsil scraping PCR vs. oral fluid PCR, we would arrive at different conclusions based on which test we would have chosen. In this flow, tonsil scrapings were more likely to be positive in younger animals while oral fluids tended to be more positive in older animals. Using multiple tools has allowed us to have a better picture on what may be going on in this flow. Also incorporating be big overall clinical picture as well as having a keen understanding of when and how pigs are being medicated is a critical skill that attending herd veterinarians are able to bring to the table to help interpret diagnostic results. This re-emphasizes the importance of the clinical perspective and understanding necessary to interpret any diagnostic results.

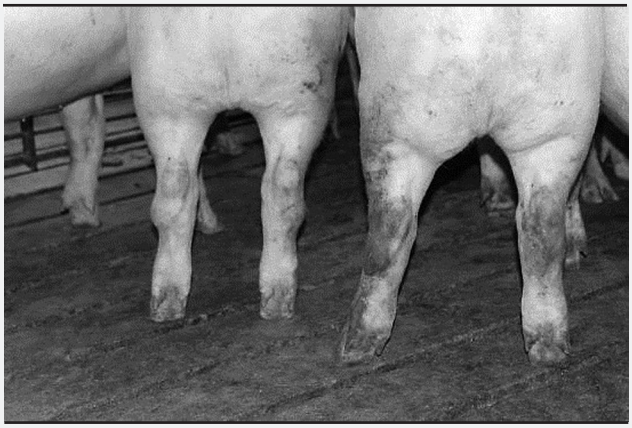
References

1. Gomes Neto JC, Gauger PC, Strait EL, et al. Mycoplasma-associated arthritis: Critical points for diagnosis. J Swine Health Prod.2012;20(2):82–86

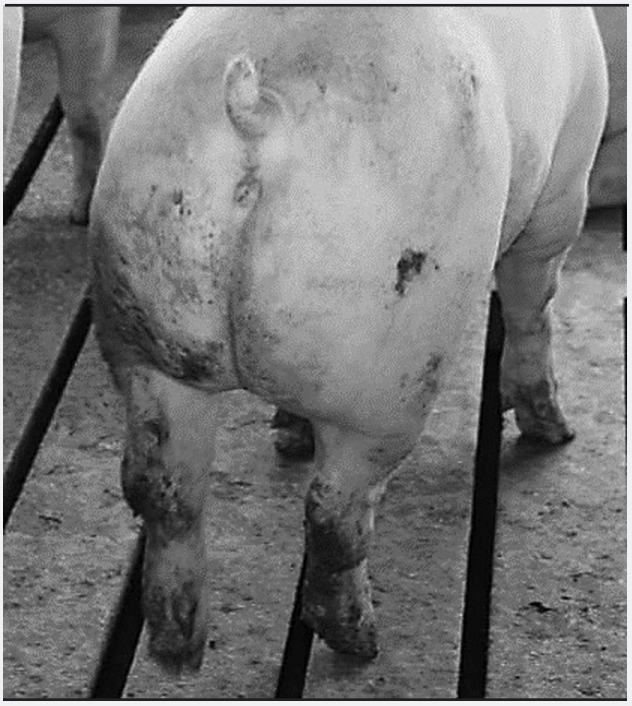
2. Lincomix® Label https://online.zoetis.com/US/EN/products/Pages/Lincomix _Feed.aspx

Images 1-7:

1



2A



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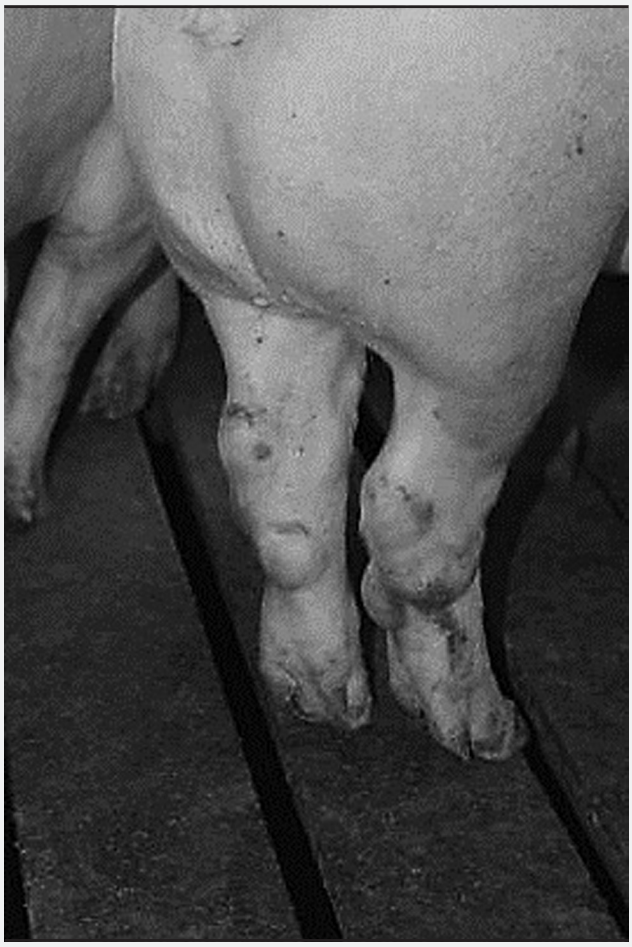
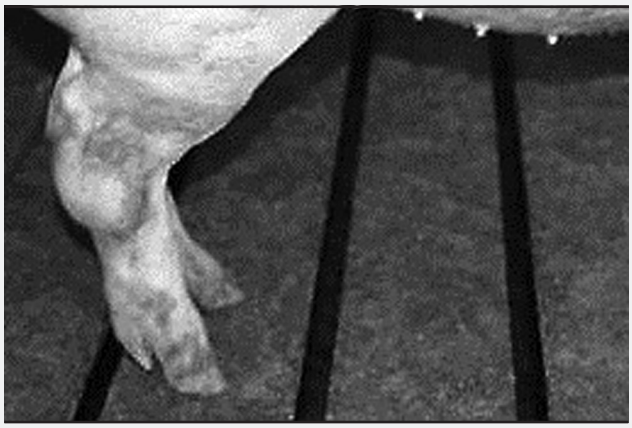
2B



2C

2D



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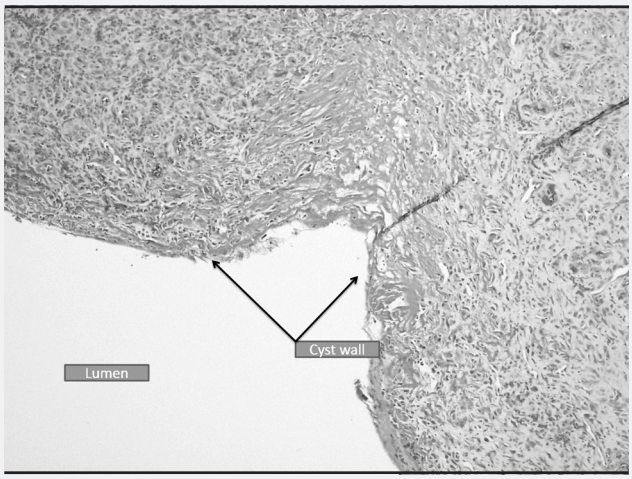
3A



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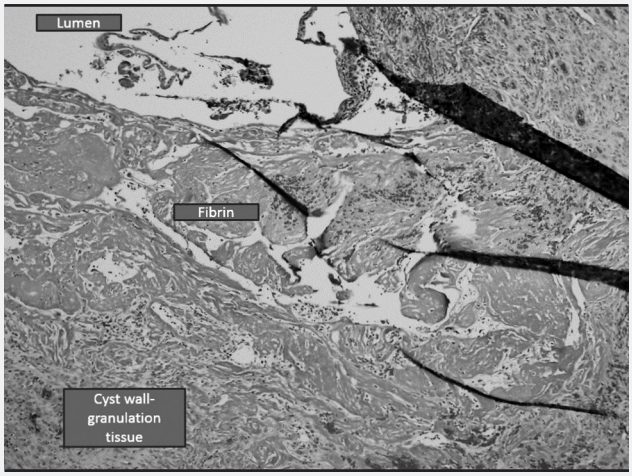

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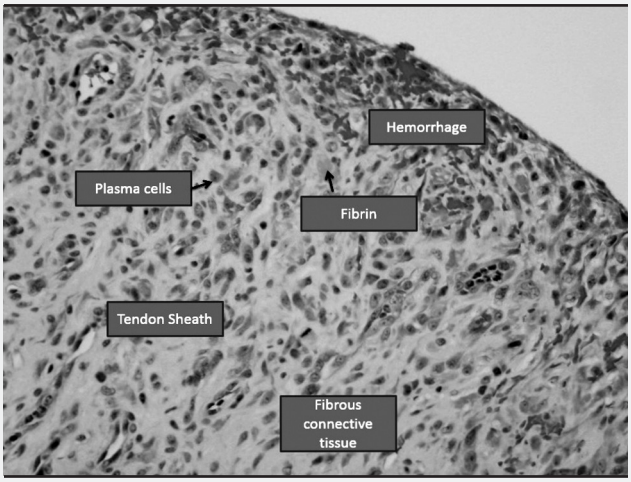

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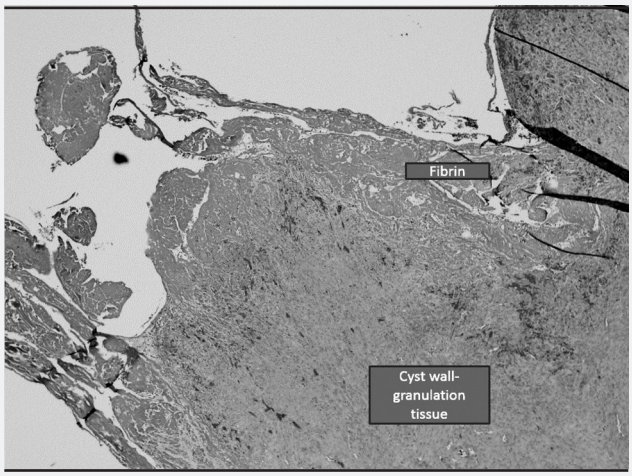

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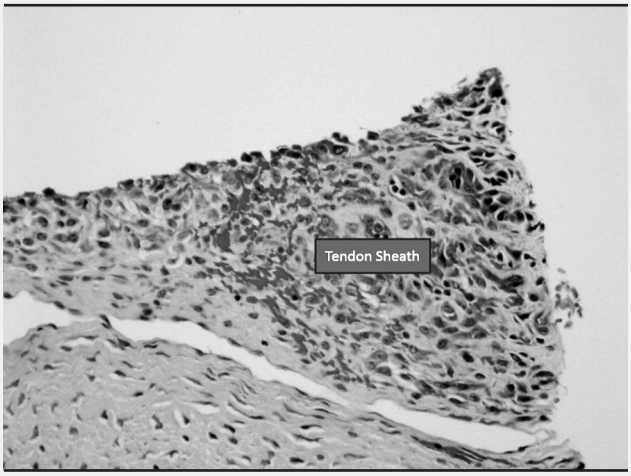

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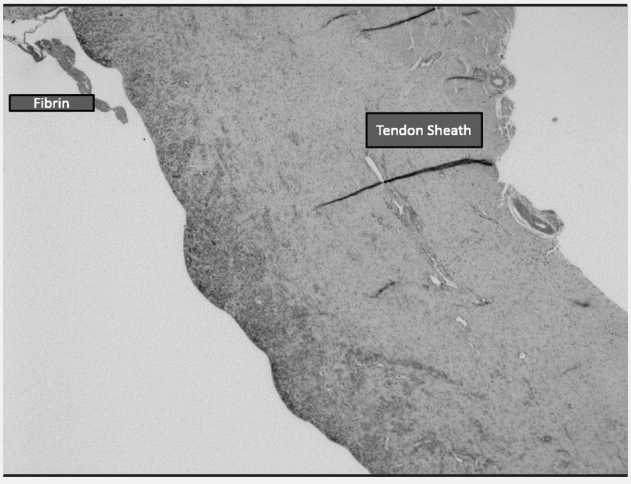
4C



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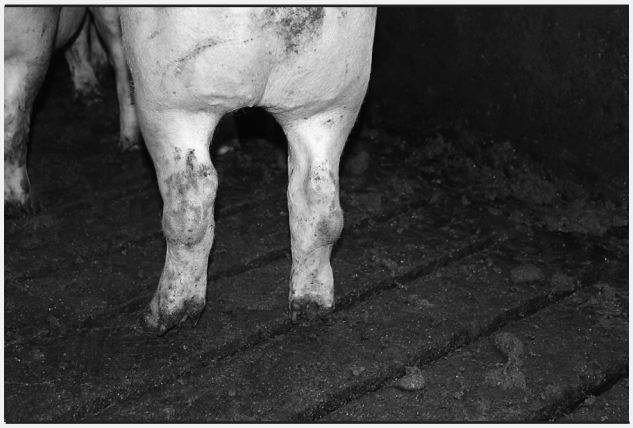

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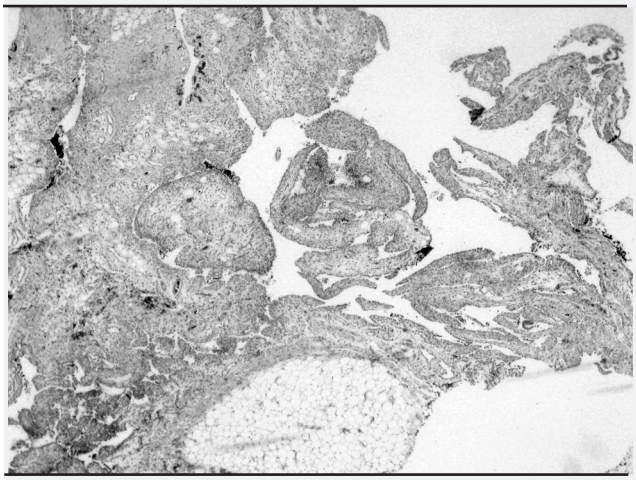

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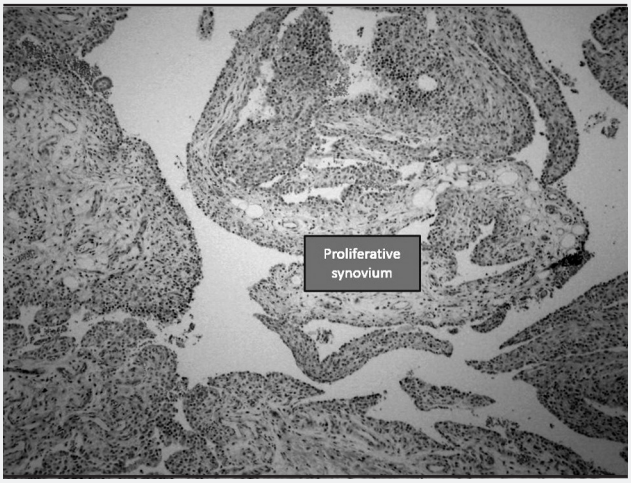
6A



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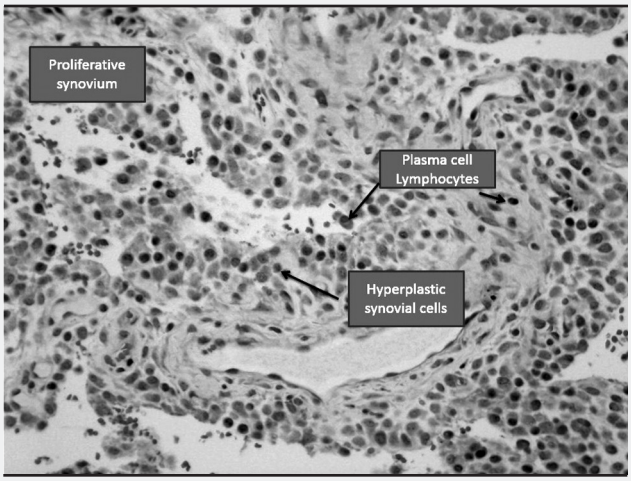

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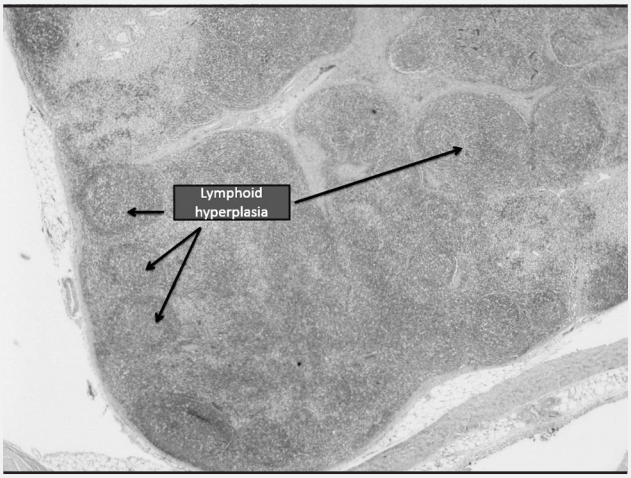


Images 1-7: Continued

7

Table 1: Lameness incidence per age per month.

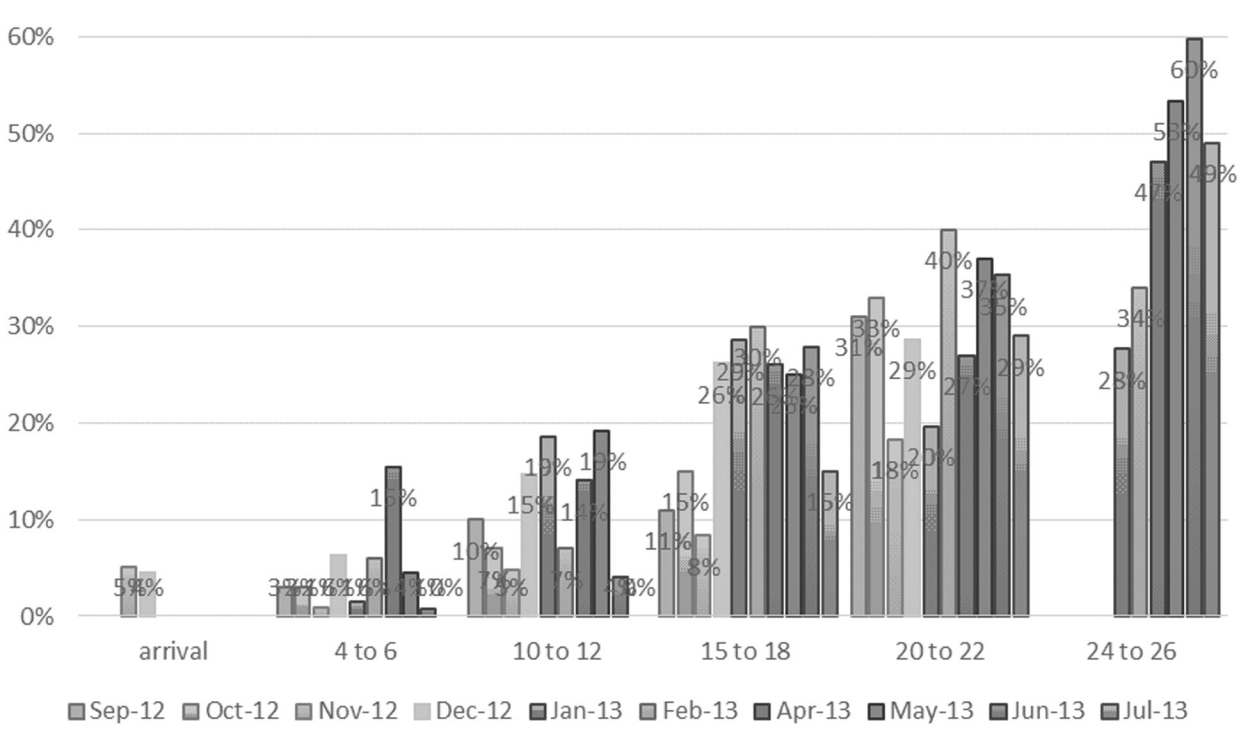


Table 2: Diarrhea incidence per age per month.

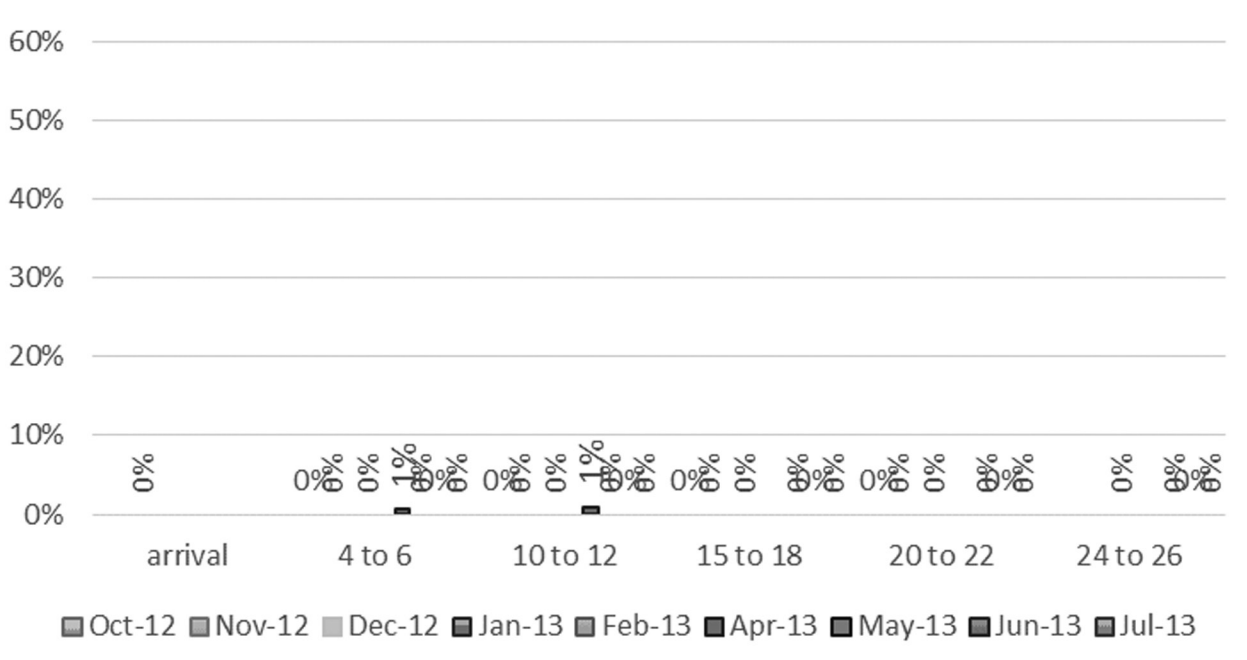
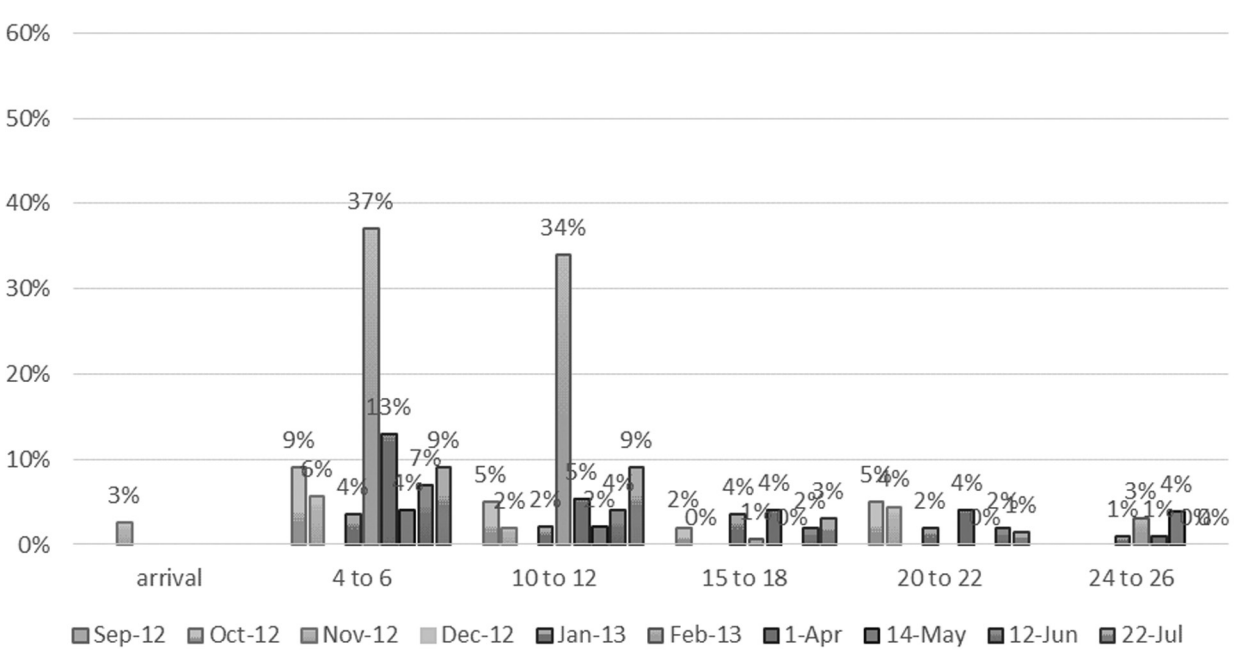


Table 3: Fallout incidence per age per month.



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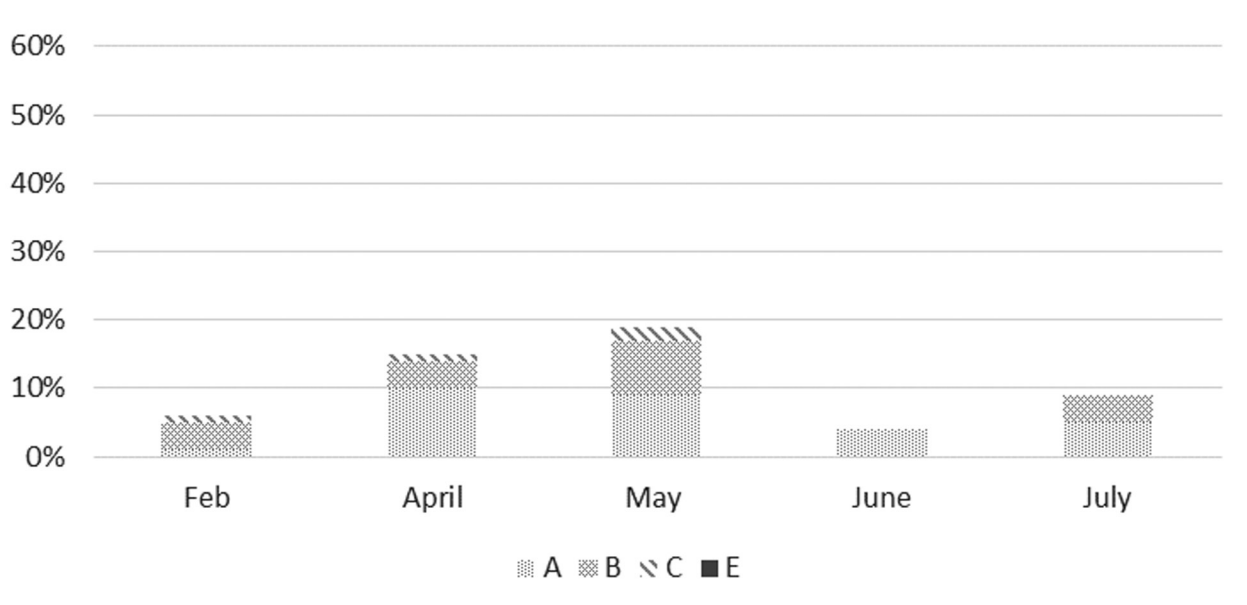

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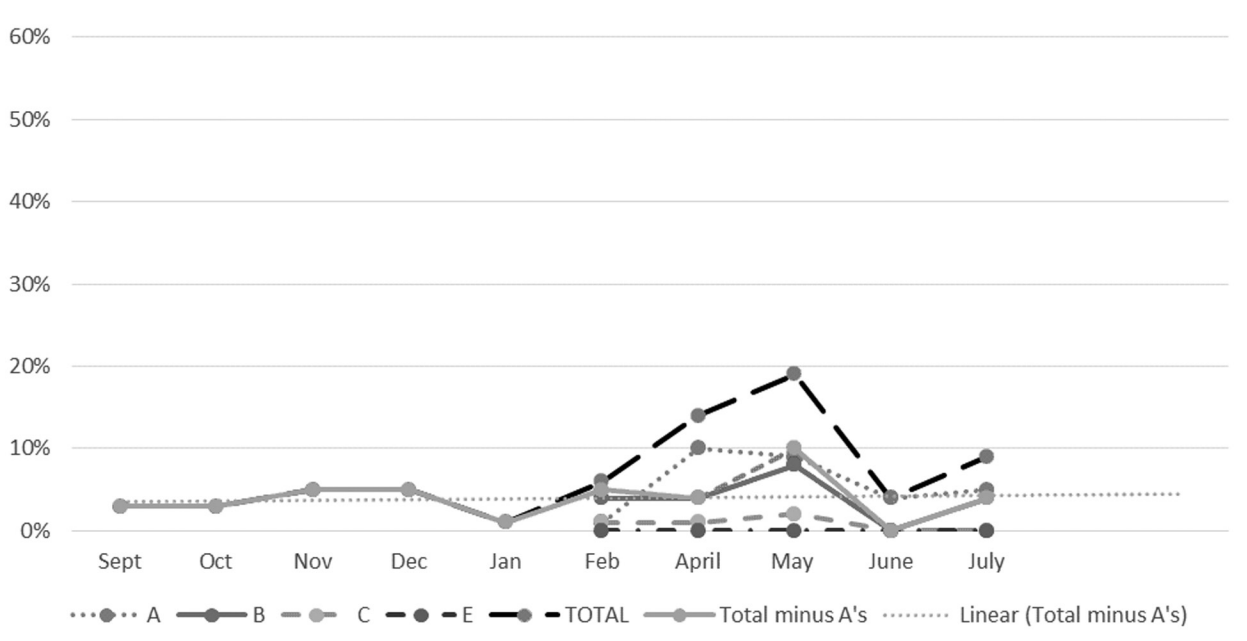

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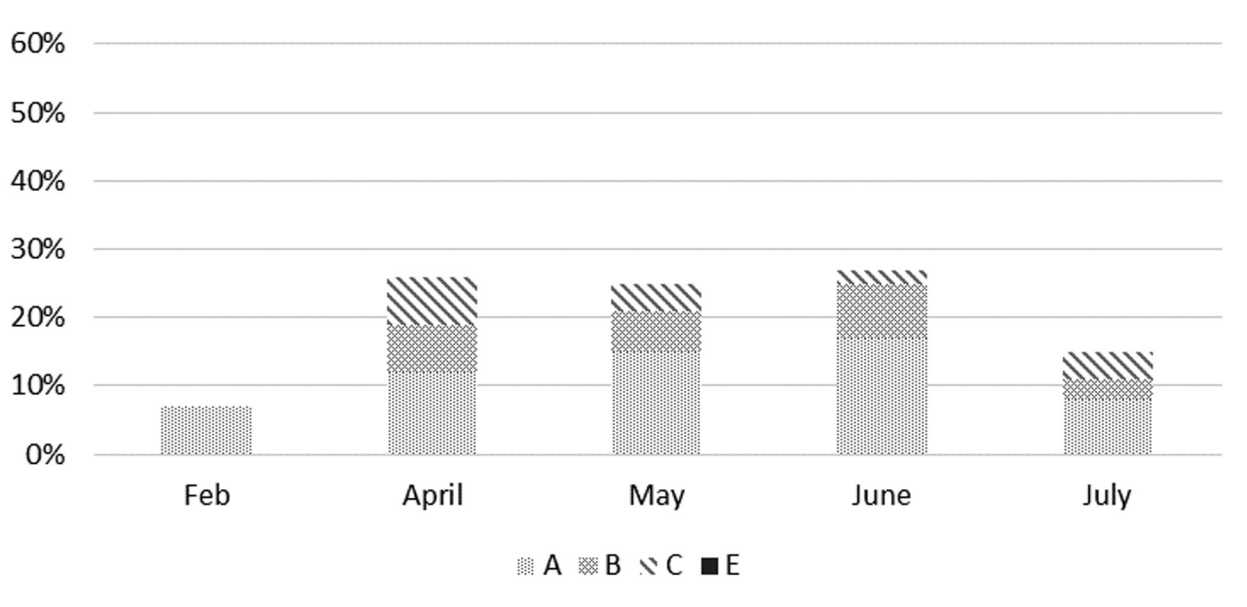

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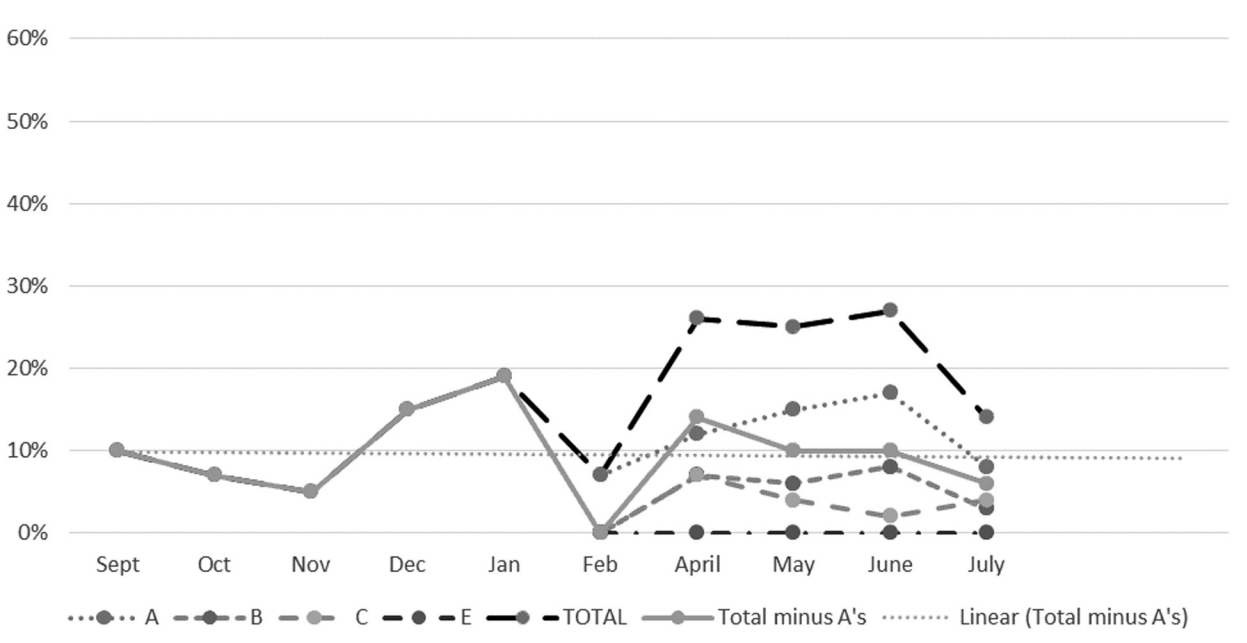

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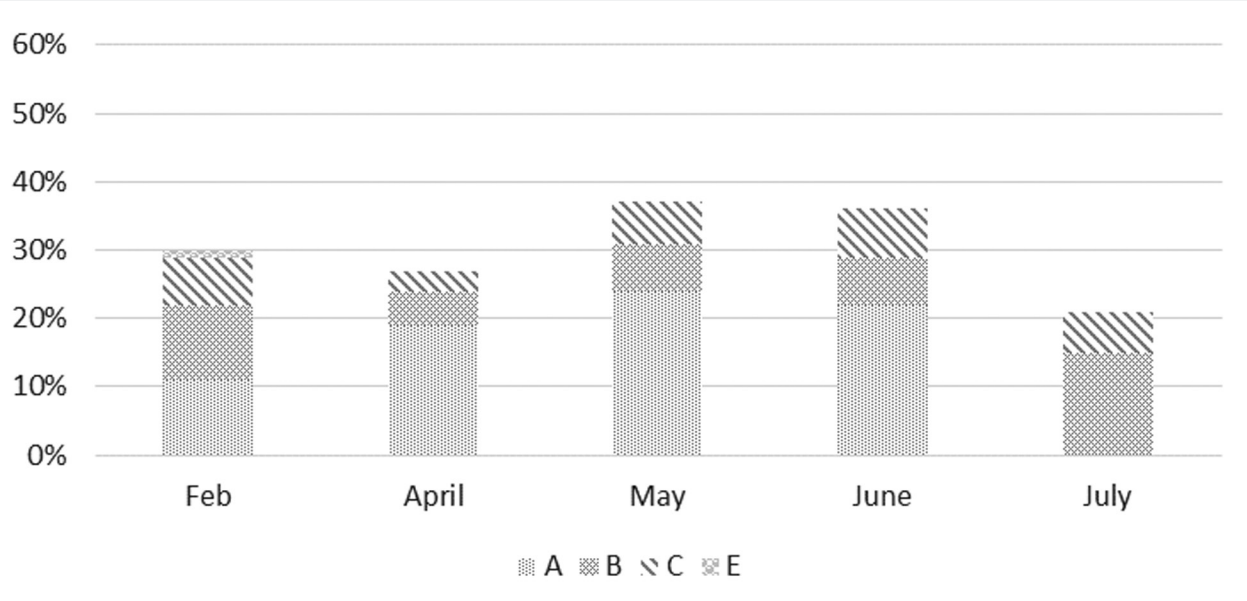

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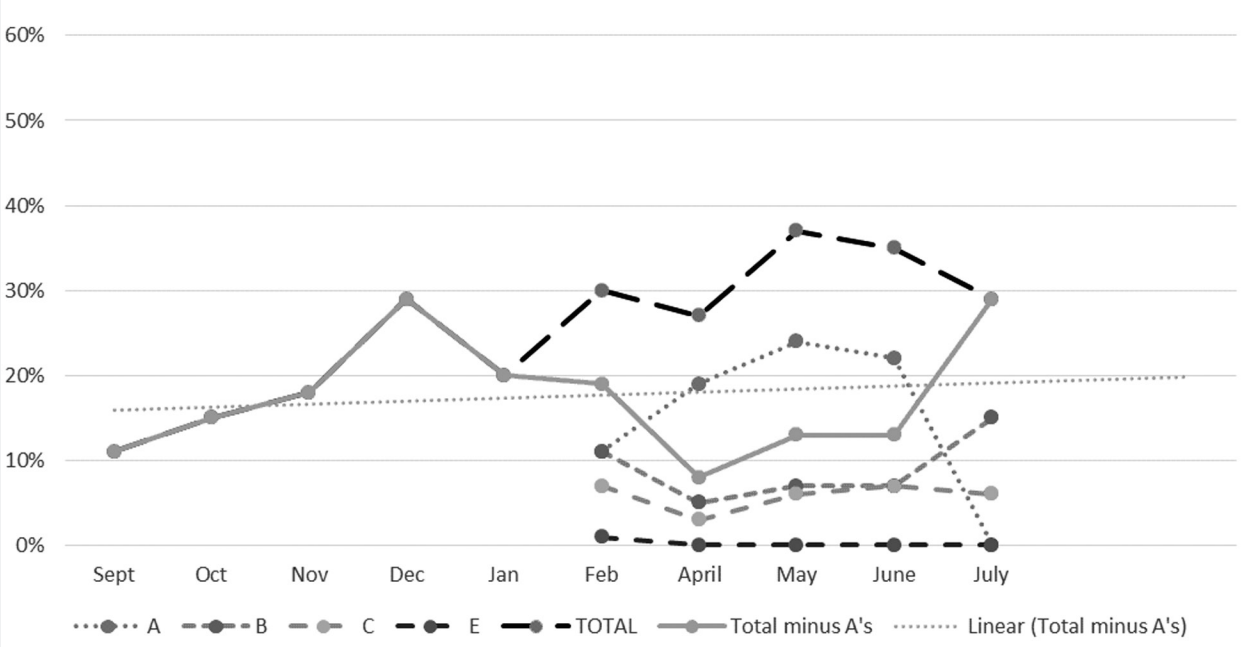

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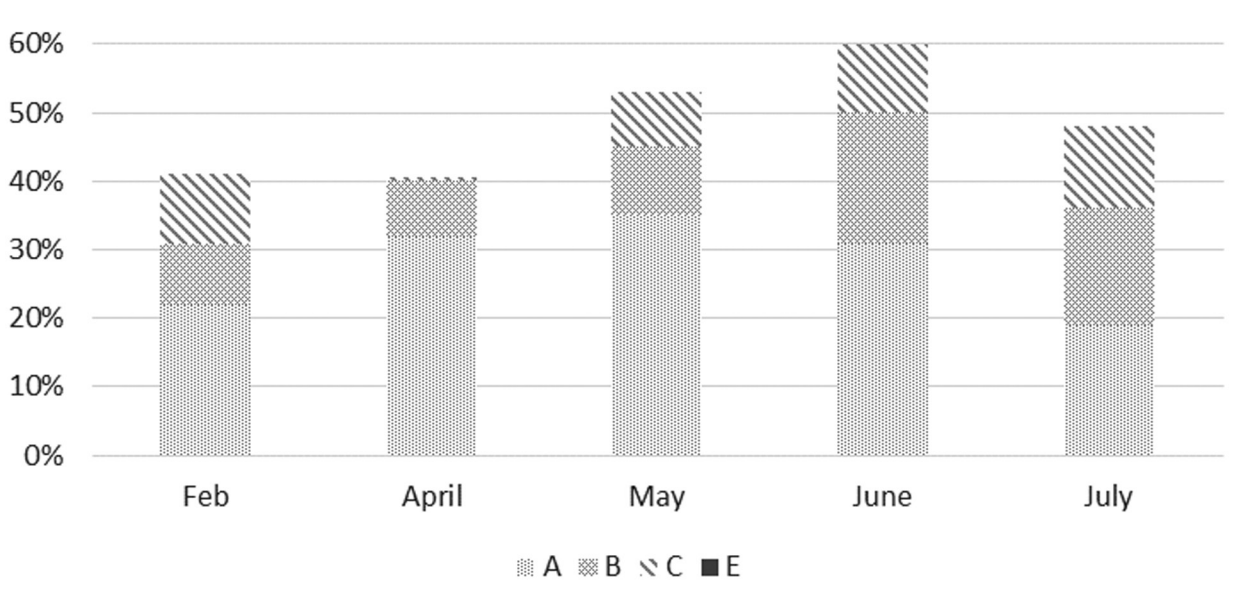

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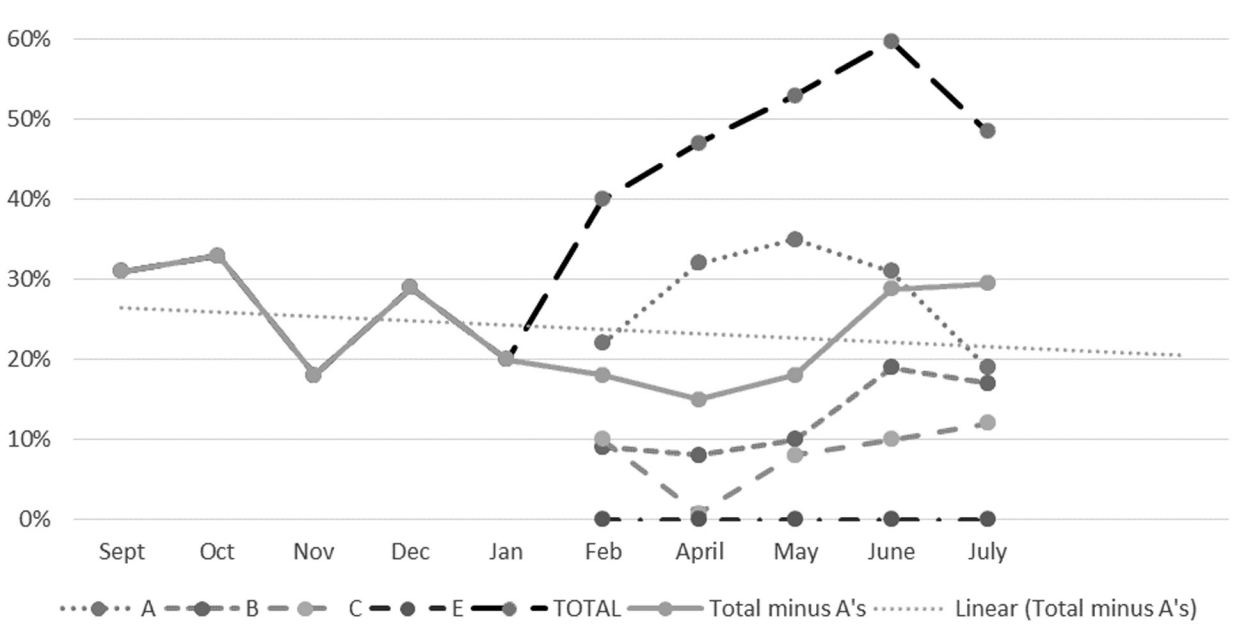

Table 8: Percent sold in categories over per sell group date.

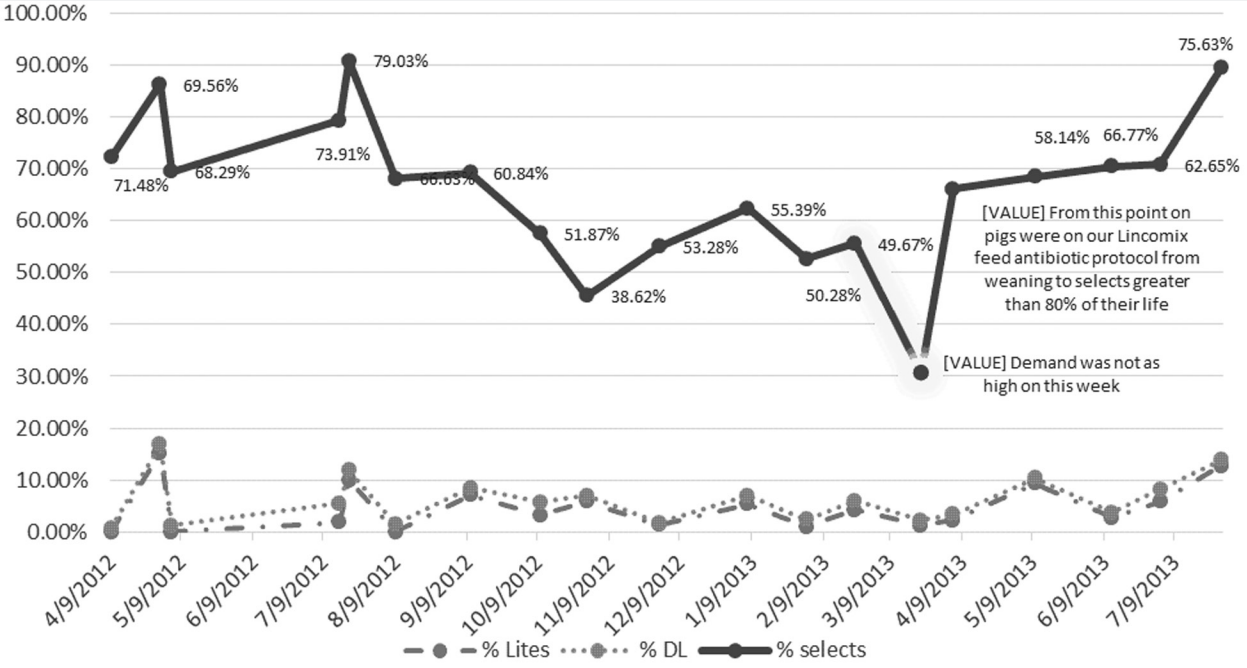

Table 9: Percent selects sold with a moving average trend line.

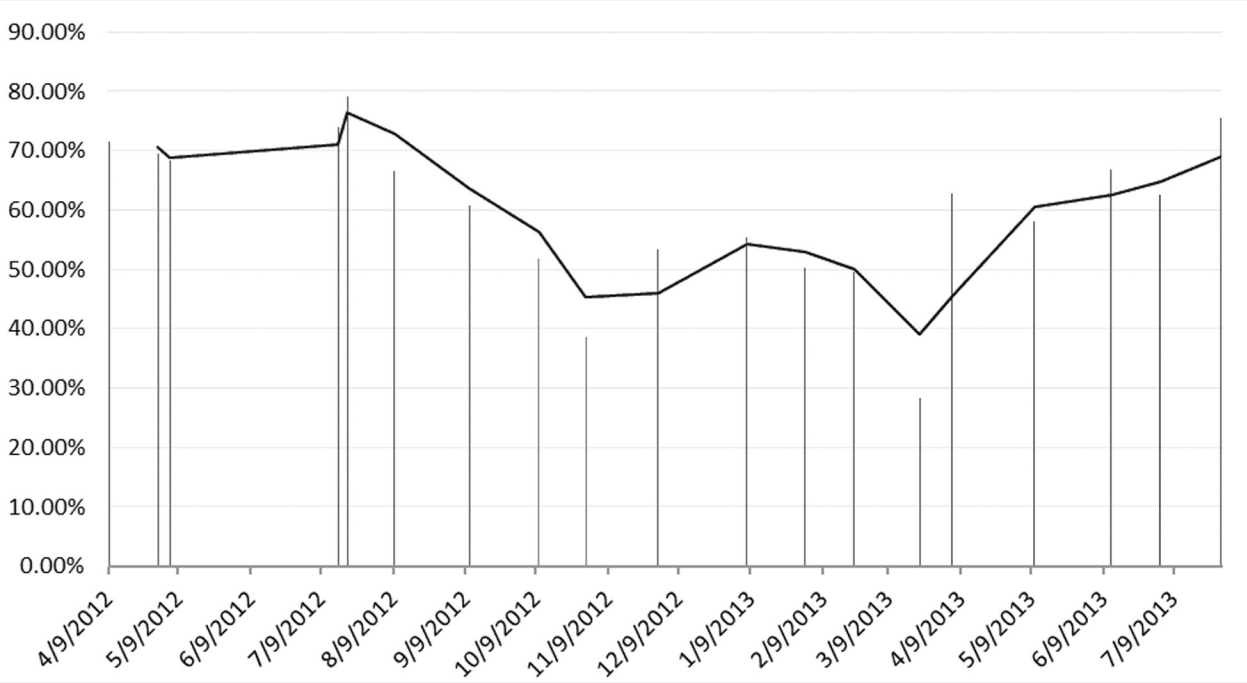

Table 10a: April Testing of *M. hyosynoviae* Tween 20 results average of S/P

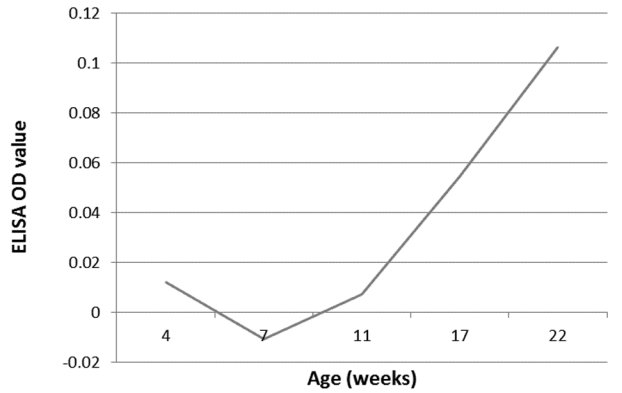

Table 10b: May Testing of *M. hyosynoviae* Tween 20 results average of S/P

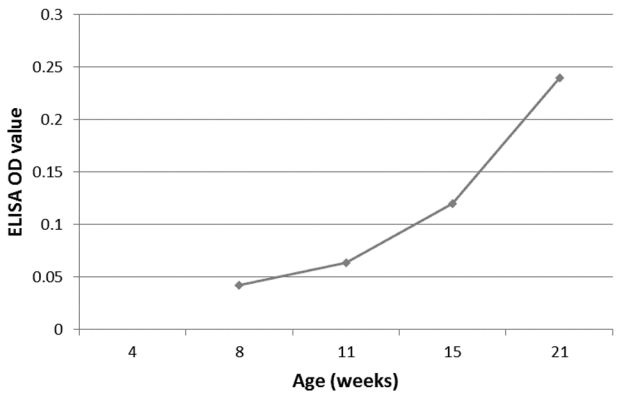

Table 10c: June testing results of *M. hyosynoviae* Tween 20 average of S/P

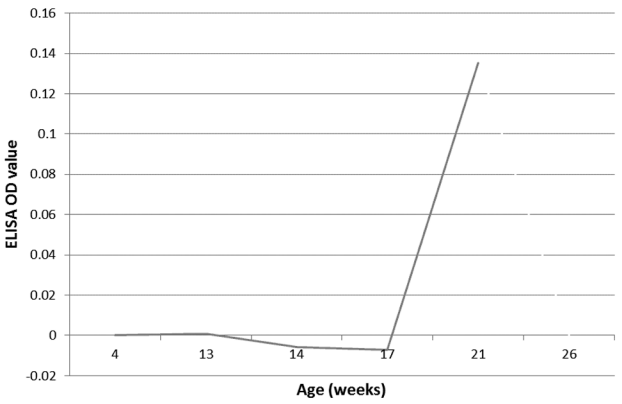

Table 11: S/P ratios of A, B, C and Normal (blank) pigs.

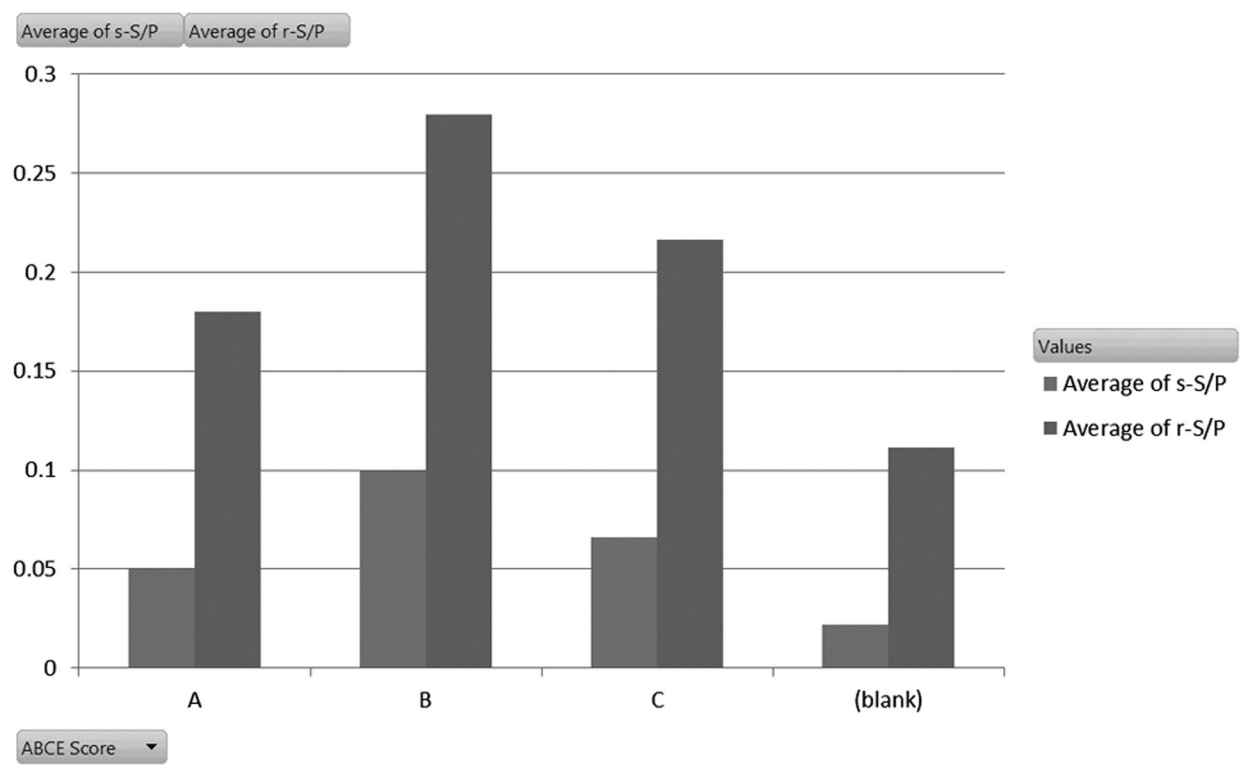

Chart 1a: April Rope PCR *M. hyosynoviae*

Count of R-s-R04	Column labels		
Row labels	**Neg**	**Pos**	**Grand total**
4	3	1	4
7	2	2	4
11	3	1	4
17	1	3	4
22		4	4
Grand total	**9**	**11**	**20**

Chart 1b: May Rope PCR *M. hyosynoviae*

Count of R-s-R05	Column labels		
Row labels	**Neg**	**Pos**	**Grand total**
4		2	2
8	4		4
11	2	2	4
15		4	4
21		4	4
Grand total	**6**	**12**	**18**

Chart 1c: June Rope PCR *M. hyosynoviae*

Count of R-s-R06	Column labels		
Row labels	**Neg**	**Pos**	**Grand total**
4	3	1	4
13	1		1
14	3		3
17	3	1	4
21		4	4
26			
3	3		3
Grand total	**13**	**6**	**19**

Chart 2a: April Tonsilar swabs on individual tagged pigs

Tonsilar swabs April	Exposure appears to be higher. These pigs received a partial preventative/treatment therapy in their lifetime.			
Count of s-R04	Column labels			
Row labels	**Neg**	**Pos**	**Sus**	**Grand total**
4	6		2	8
7	14	1	1	16
11	16			16
17	10	3	3	16
22	8	7	1	16
Grand total	**54**	**11**	**7**	**72**

Chart 2b: June Tonsilar swabs on individual tagged pigs

Tonsilar swabs June	Exposure appears to be less than in April. These pigs have received more of the preventative/treatment therapy in their lifetime.			
Count of s-R06	Column labels			
Row labels	**Neg**	**Pos**	**(blank)**	**Grand total**
4				
13	4			4
14	12			12
17	15			15
21	13	3		16
26				
Grand total	**44**	**3**		**47**

Influenza virus prevalence and risk factors in weaning-age pigs

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College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota

Introduction

Influenza virus continues to be a major respiratory pathogen in pigs. A recent active surveillance study identified 91% of enrolled growing pig farms as influenza virus positive during a 12 to 24 month study (Corzo et al., 2013). While many herd level risk factors have been described for influenza virus infection based on serologic surveillance, little information is available regarding influenza virus epidemiology in sow herds. Weaning age pigs have been identified as an important subpopulation for influenza virus infection, but little is known regarding factors influencing influenza virus prevalence in sow herds (Allerson et al., 2013). The objectives of this study were to assess the prevalence and temporal patterns of influenza virus infection in weaning-age pigs and to evaluate the association between sow herd attributes (including influenza vaccination) and the prevalence of influenza virus positive weaning-age pigs.

Materials and methods

Sow herds (breed to wean) from across the United States were invited to participate in this study. Sow herds with a previous history of influenza virus infection were preferentially enrolled. Each sow herd collected 30 individual nasal swab samples from weaning-age pigs each month for a six month period. All samples were tested for influenza virus RNA via a matrix gene based RRT-PCR in pools of 3 nasal swabs.

Results

Fifty-two sow herds were enrolled from 5 different states and 8 different swine production systems. To date, approximately 5,160 nasal swabs (1,720 pools) have been collected and tested via RRT-PCR. Of the 1,720 pools (pools of 3 nasal swabs) tested, 22% were positive or suspect. Of the 172 sampling events (30 nasal swabs from a sow herd), 30% have contained at least one positive or suspect pool. Finally, of the 52 sow herds tested to date, 42% have tested positive at least once for influenza virus.

Discussion

The common use of multi-site production systems in the United States has allowed for the strategic segregation of pigs in order to reduce disease pressure on a particular site. This also allows for the elimination of certain pathogens once the infection dynamics of those pathogens are understood. This large scale study has identified weaning age pigs as a significant subpopulation for influenza virus infection and transmission. This knowledge will better inform control strategies and herd elimination strategies. Further risk factor analyses will add to the understanding of influenza virus at the sow herd level.

Acknowledgements

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References

1. Corzo CA, Culhane M, Juleen K, Stigger-Rosser E, Ducatez MF, and Webby RJ, 2013. Active surveillance for influenza A virus among swine, midwestern United States, 2009-2011. Emerg Infect Dis. http://dx.doi.org/10.3201/eid1906.121637.

2. Allerson MW, Davies PR, Gramer MR, and Torremorell M, 2013. Infection Dynamics of Pandemic 2009 H1N1 Influenza Virus in a Two-Site Swine Herd. Transboundary and Emerging Diseases. DOI: 10.1111/tbed.12053.

Dynamics of influenza in a wean-finish population

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Introduction

Influenza A virus (IAV) infection is endemic in pigs worldwide. The epidemiology of IAV within and between species is extremely complex and not completely understood. The virus has a segmented genome, negative sense RNA, with 8 gene segments that translate up to 12 different proteins. The pig has been blamed to be the mixing vessel for IAV, but little is known of IAV virus evolution within swine populations. The objective of this study is to characterize IAV transmission in pigs after weaning, and to determine which factors allow IAV to persist in swine populations for prolonged periods.

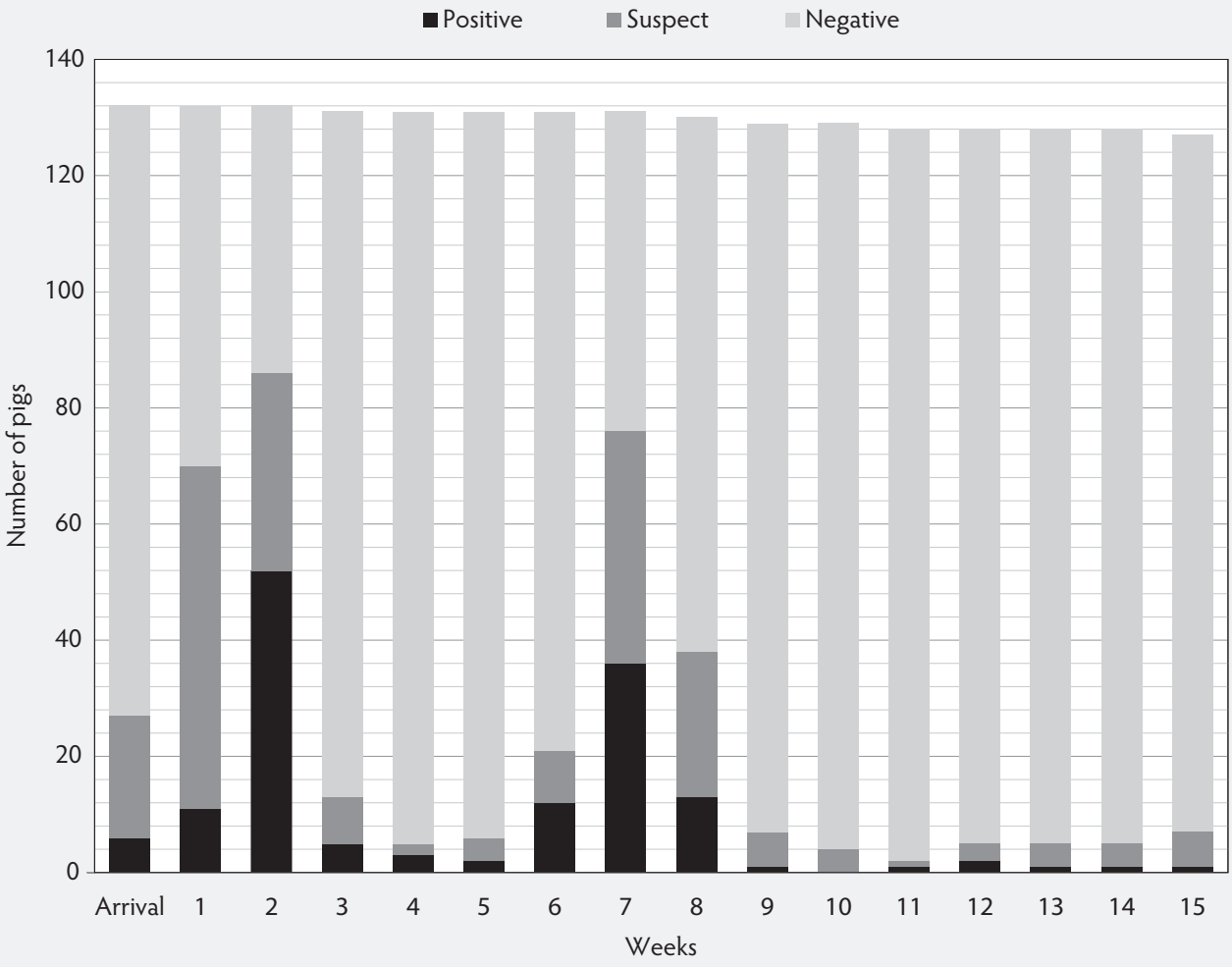
Materials and methods

One hundred and thirty two pigs out of two thousand and two hundred 3 week old pigs were randomly selected, and ear tagged, at arrival to a commercial wean to finish facility. The swine herd selected for the study was previously diagnosed as IAV positive, and received piglets from a single infected breeding herd. Nasal swabs were collected from each pig at arrival, and on a weekly basis for 15 weeks. Each swab was tested for IAV by RT-PCR targeting the M gene, and positive swabs were tested by RT-PCR to determine the subtype of IAV circulating within the population. The proportion of positive to negative pigs by RT-PCR were compared between weeks, and PCR results from individual animals were compared to assess the risk of re-infection with IAV over time. Serum samples were collected at arrival and every 4 weeks there after, and tested for antibodies to IAV by ELISA. The mean of the sample to negative ratios, (S/N) from the ELISA test, were compared between weeks using a paired t-test, and considered significant at 0.05.

Results and conclusions

Positive pigs were identified at arrival to the barn by RT-PCR indicating that weaned pigs can be a source of IAV to other swine populations. Additionally several animals tested positive after weaning. At least one animal tested positive to IAV for most of the weeks, (except for week 10 after weaning). There were 2 infection waves identified throughout the study period (Fig. 1). There were significant differences on the ELISA S/N result between weeks. Overall our results indicate that IAV in endemically infected growing pigs is very dynamic, that wean pigs can be a source of IAV to other pigs after weaning and that the levels of maternal antibodies may play a role on the maintenance and development of new infections.

Figure 1: Frequency distribution of the number of animals that tested positive to IAV by RRT-PCR



Getting a closer look at USDA swine influenza A surveillance: What can we learn from the genetic data that can help us on the farm?

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Introduction

Among the influenza A viruses (IAV) circulating in the US swine population from 1930 to present are at least ten genetically and antigenically distinct hemagglutinin (HA) lineages: three classical swine lineages H1α, H1β, H1γ; two lineages derived from human seasonal H1 viruses H1δ1, H1δ2; the H1pdm09; and H3 cluster I-IV viruses.[1,2] The primary implication of these antigenic differences is that controlling infection and transmission via vaccination may not be optimal. Current swine IAV vaccines use multivalent formulations of field-sourced virus, each component representing one of the different lineages, but not all lineages are included.[3] These vaccines elicit antibodies with a relatively narrow range of protection, and efficacy is equivocal for drifted strains.

Our best option in controlling disease is the development and appropriate application of effective vaccines. It is essential that these vaccine strains match viruses circulating in the swine population, and achieving this is predicated on availability of robust viral surveillance data. As a model, the global surveillance program administered by the World Health Organization for human influenza vaccine design conducts large-scale phylogenetic and antigenic analyses of thousands of HA1 sequences and hemagglutination inhibition (HI) results collected from >100 countries on a semi-annual basis to inform selection of strains for multivalent vaccine compositions.[4] Although vaccine mismatches occasionally occur, a robust surveillance system has resulted in vaccines that tend to protect well against circulating viruses, reducing morbidity and mortality in the human population.[5] Prior to 2009 such an approach would not have been feasible in the swine IAV system; however, capacity building efforts in North America, led by the United States Department of Agriculture (USDA) and implemented through the voluntary National Animal Health Laboratory Network (NAHLN) has redressed concerns about the insufficient quantity of virological and molecular surveillance of IAV in swine.[6] Consequently, it is now possible to provide insight into the patterns of swine IAV spread, genetic diversity throughout the year, and the dynamics of IAV evolution in North America. These data allow for the identification of intervention strategies such as timely vaccine and diagnostic updates, as well as providing insight into determinants of transmission that could be mitigated by changes in production practices or facility management.

Methods

From 2009-2013 samples were collected from swine across the US and processed upon: a) observation of swine with influenza-like illness (ILI); b) observation of swine epidemiologically linked to a human case of novel IAV; or c) observation of swine with signs of ILI at "comingling points." Up to 10 samples per laboratory accession, either from nasal swabs, lung tissues or oral fluids, were sent to a participating NAHLN laboratory and screened with a matrix (M) gene PCR assay specific for IAV. For those submissions positive for IAV, up to 2 positive samples were subjected to subtyping by PCR assays (H1 or H3, N1 or N2, and/or undetermined), and virus isolation. Successful virus isolations were further characterized by sequencing of the HA, neuraminidase (NA), and M genes and subsequently deposited into the Influenza Virus Resource, National Center for Biotechnology Information's online sequence repository.[7]

Nucleotide sequences from 1210 HA segments, 1175 NA segments, and 1197 M segments were analyzed from IAV from US swine during 2009-2013. Viruses were collected from swine in 25 US states (Arkansas, Colorado, Iowa, Illinois, Indiana, Kentucky, Michigan, Minnesota, Missouri, Mississippi, Montana, North Carolina, North Dakota, Nebraska, New York, Ohio, Oklahoma, Oregon, Pennsylvania, South Dakota, Tennessee, Texas, Virginia, Wisconsin, and Wyoming).

From these data, five sequence alignments were constructed using MUSCLE v.3.8.31:[8] an alignment of H3 and H1 HA sequences, an alignment of N1 and N2 neuraminidase sequences, and an alignment of the M sequences. Based upon the H1 phylogeny, H1N1 and H1N2 isolates were assigned to one of six previously described H1 antigenic lineages, H1α, H1β, H1γ, H1δ1, H1δ2, H1pdm09.[9,10] H3N2 isolates were assigned to one of four main clusters based upon the H3 phylogeny,[2] and H3 Cluster IV isolates to one of 6 recently designated "clades".[2] Within and between

clade nucleotide distances were calculated using MEGA.[11] To clarify the evolutionary history of the H3N2 viruses, sequences from randomly selected H3 Cluster I, II and III viruses in Genbank were included in addition to the USDA system H3N2 isolates. For each of the alignments a maximum likelihood tree was inferred using RAxML (v7.4.2;)[12] employing a general time-reversible (GTR) model of nucleotide substitution with G-distributed rate variation among sites. The starting tree was generated under parsimony methods, with the best-scoring tree and statistical support values obtained with the rapid bootstrap algorithm (1,000 replications).

To study the seasonal patterns of swine IAV in the US, we conducted statistical analyses using the number of influenza isolates aggregated by month from 2009 to 2013. Frequencies of monthly isolates were analyzed through decomposition of the time series into seasonal, trend and irregular components using Loess.[13] Swine population estimates were sourced from the Quarterly Hogs and Pigs report produced by the National Agricultural Statistics Service (USDA: `http://usda.mannlib.cornell.edu/`). In addition, using a Lotka–Euler framework, we relate observed growth rate in the number of swine IAV cases in the surveillance system to the reproductive number.[14] In this way the reproductive number, which represents generation intervals and seasonal epidemic growth, provides insight into the strategies and efforts required to control swine IAV.

Results and discussion

The three IAV subtypes (H1N1, H1N2, and H3N2) endemic in the US swine population were detected every year during our study period. The H1N1 and H1N2 subtypes were detected at similar frequencies across the 4 years, representing 37.4% and 36.8% of all isolates respectively. Although the H3N2 represented less than 25% of the identified viruses during the total time period, this subtype represented an increasing proportion of sequenced isolates, from 25% in 2010 to 33% in 2012. Among the H1N1 and H1N2 subtype viruses in our study, 1.1% were H1α, 3.4% were H1β, 32.8% were H1γ, 43.3% were H1δ1, 3.9% were H1δ2, and 13.9% were H1pdm09. Of note, is the rapid increase in the occurrence of H1δ1 in samples submitted, with a concurrent decrease in H1pdm09 since 2009. These data reveal year round circulation with a primary peak of sequenced isolates in October-November; and in H1N1 and H1N2, a secondary peak in March. We find that the reproductive number of H1N1, H1N2 and H3N2 across each year is larger than R = 1: in 2010 H1N1 = 2.31 (1.7-2.9); H1N2 = 3.14 (1.3-5.7); H3N2 = 3.55 (1.6-6.8) and in 2012 H1N1 = 2.75 (2.6-2.9); H1N2 = 2.67 (2.5-2.8); H3N2 = 6.05 (1.1-14.8).

We also detected a seasonal trend in clinical swine IAV within the US We observe a primary seasonal peak that starts in September and peaks during November, and a secondary peak that begins in February and peaks in March. These peaks are coincident with changes in farm management. In the fall of each year when temperatures drop and daylight hours decrease, farms move from open-ventilation to closed-ventilation systems, with a concomitant change in indoor environmental conditions (i.e., reduced air exchange, decreased relative humidity). It is likely these factors facilitate transmission by exposing pigs to different climatic, airflow, and/or behavioral stress conditions; and management and control of transmission should take this into consideration.

Swine IAV is commonly identified in diagnostic investigations of respiratory disease; these outbreaks and endemic infections likely cause significant economic burden on producers given the loss in growth potential following infection.[15] Vaccines that do not sufficiently cover or match the observed diversity of IAV in the swine population and, in relation, poorly timed vaccine use (i.e., when pigs have circulating maternal antibodies[16]) likely contributes to the difficulty in controlling IAV in swine. Given that we document seven genetically and antigenically distinct hemagglutinin lineages (H1α, H1β, H1γ, H1δ1, H1δ2, H1pdm09 and H3 cluster IV) circulating, it is essential that we reconsider our approach to vaccine updates and/or technology. Surveillance of IAV in US swine has progressed substantially since 2009, providing timely insight into co-circulating viral diversity. These data should be used to inform intervention strategies of vaccine and diagnostic updates and potential changes swine health management.

References

1. Lorusso A, Vincent AL, Gramer MR, Lager KM, Ciacci-Zanella JR. Contemporary Epidemiology of North American Lineage Triple Reassortant Influenza A Viruses in Pigs. Curr Top Microbiol Immunol 2013; 370:113-32.

2. Kitikoon P, Nelson MI, Killian ML, et al. Genotype patterns of contemporary reassorted H3N2 virus in U.S. swine. J Gen Virol 2013; 94: 1236-1241.

3. Van Reeth K, Ma W. Swine Influenza Virus Vaccines: To Change or Not to Change-That's the Question. Curr Top Microbiol Immunol 2013; 370:173-200.

4. Russell CA, Jones TC, Barr IG, et al. Influenza vaccine strain selection and recent studies on the global migration of seasonal influenza viruses. Vaccine 2008; 26 Suppl 4:D31-34.

5. Karlsson Hedestam GB, Fouchier RA, Phogat S, et al. The challenges of eliciting neutralizing antibodies to HIV-1 and to influenza virus. Nat Rev Microbiol 2008; 6:143-155.

6. Korslund JA, Pyburn DG, Swenson SL, et al. (2013) Summary of results: USDA surveillance for influenza A virus in swine. American Association of Swine Veterinarians, pp 507-512.

7. Bao Y, Bolotov P, Dernovoy D, et al. The influenza virus resource at the National Center for Biotechnology Information. J Virol 2008; 82:596-601.

8. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 2004; 32:1792-1797.

9. Vincent AL, Ma W, Lager KM, et al. Characterization of a newly emerged genetic cluster of H1N1 and H1N2 swine influenza virus in the United States. Virus Genes 2009; 39:176-185.

10. Vincent AL, Ma W, Lager KM, Janke BH, Richt JA. Swine influenza viruses a North American perspective. Adv Virus Res 2008; 72:127-154.

11. Tamura K, Peterson D, Peterson N, et al. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 2011; 28:2731-2739.

12. Stamatakis A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 2006; 22:2688-2690.

13. Cleveland RB, Cleveland WS, McRae JE, Terpenning I. STL: A seasonal-trend decomposition procedure based on Loess. J Off Stat 1990; 6:3-73.

14. Wallinga J, Lipsitch M. How generation intervals shape the relationship between growth rates and reproductive numbers. Proc Roy Soc B 2007; 274:599-604.

15. Brown IH. The epidemiology and evolution of influenza viruses in pigs. Vet Microbiol 2000; 74:29-46.

16. Vincent AL, Ma W, Lager KM, et al. Live attenuated influenza vaccine provides superior protection from heterologous infection in pigs with maternal antibodies without inducing vaccine-associated enhanced respiratory disease. J Virol 2012; 86:10597-10605.

Managing risk with employee safety and animal welfare

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Animal agriculture is a risky place to be. Machines, humans, and animals are busy at all hours of the day and night. Therefore, personal safety and good animal-handling techniques are pivotal pieces in managing risk in a sustainable operation.

Perhaps risk can be best understood by using the analogy of driving or riding in a car. All of us have done that in our lifetimes, probably as recently as today, even though we know it has the potential to cause significant injury or death. Yet, we are willing to take that risk in order to achieve the end result of arriving at our destination. We also want to be efficient with our time while driving. Have you ever seen someone talking on his/her cell phone? Or texting? Or reading? Or shaving? Or applying make-up? Or various other activities? Of course, we all have. When drivers are occupying their minds in ways other than paying attention to their driving, the risk of having an accident increases dramatically. There are laws against texting while driving, but millions of people still text while behind the wheel, which brings up the unanswerable question: How safe is safe enough?

This speaks to the point of effective safety programs and animal handling programs. Most employees on your farms know what is safe and what is not. They know what proper animal handling looks like and what is not appropriate. The programs with the most "thou shalt nots" are not the most effective. Those programs which touch the hearts of your employees are. Effective safety programs help people change their behaviors because they want to, not just because they have to. An effective program must also have a measurable goal and be very simple and easy to understand.

Naturally, the most important part of any program is communication. First, the specific, measureable goals and expectations must be communicated to all employees. But one safety meeting per year is not enough. The communication must be ongoing and consistent. Warning signs and safety reminders ought to be posted in visible areas, safety discussions should happen on a daily basis and a culture of candor and openness should be nurtured in order to hold one another accountable. The same can be said of animal handling. Open communication about the expectations, protocols, procedures and consequences must be continually cultivated.

Perhaps you only visit the operation once per month as an on-call veterinarian. What can you do? You can encourage the owner/manager to have employee safety and animal handling conversations with his/her employees. You may share ideas you have seen on other farms. You may visit with employees to help them see the importance of good animal handling techniques and safety protocols. You are part of the problem if you sit back and passively watch things happen that you know are not right.

As business owners/operators and veterinarians we have an obligation to our employees and animals. We must provide a work environment which promotes employee safety and the well treatment of animals. We must have measures in place to encourage good performance and discipline misbehavior. And we must ensure that all employees are aware of the expectations and consequences.

Injury prevention in swine production

Luis Torres

For the most part, safety hazards and injury types in a hog farm are similar to those found in other agricultural production units. Animal contact, operation of specialized and general equipment, tractors and other vehicles, electrical hazards, unsafe walking and working surfaces, chemical exposure, confined spaces, and extreme temperatures can result in injuries of various types. These include caught in or between two objects, animal etc.; slips, trips, and falls; electrocution; hit by or against and object, animal, etc.; chemical and fire burns; eye injuries; and oxygen deprivation, to mention some of the most common. The severity of these injuries also varies from minor abrasions and bruises to loss of limbs and lives. With that said, this document will review these risks and hazards specifically from the perspective of the people working in pig production environments.

Hazard ID and risk assessments

"A hazard is any existing or potential workplace condition that, by itself or by interacting with other variables, can result in death, injury, property damage, or other loss."[1]

The first step in understanding hazards and risks specific to the swine production industry is to review every one of the procedures performed by each individual production employee Currently, pig production is divided in three stages, and therefore into three different types of production units: sow production, boar studs, nurseries, and finishers (wean to finish). Although these types of units share the same general hazards and risks, there are differences between them in the potential frequency, likelihood, and severity of the resulting injuries. The differences are primarily due to the differences in the size of the pigs at each stage, but it is also due to the different processes and procedures performed by the workers. Because of those differences, it's recommended to perform hazard identification and risk assessment for each production stage. Hazard assessments should be conducted anytime a change is made to the workplace (processes, equipment, materials, construction modification, etc.).

The purpose of formal hazard identification is (1) to find patterns of unsafe conditions or behaviors; (2) to determine which of these hazards have a significant impact on the frequency, likelihood, and severity of workplace incidents; and (3) to use that information to prioritize the order in which potential hazards should be addressed. The hazards on that short list are deemed to be significant risks.

Significant risks in a swine production unit

- Animal contact

  Examples of farm tasks that present this risk:

  - Moving boars, sows, gilts, feeder pigs, and finishing hogs between and inside barns
  - Loading and unloading animals from live-haul trucks
  - Heat detection
  - Pregnancy checking in gestation barns
  - Vaccination or blood collection
  - Assisting sows during parturition
  - Tagging/tattooing
  - Euthanizing heavy hogs or breeding stock
- Walking/working surfaces (slips/trips/falls)

  Examples of farm tasks that present this risk:

  - Every task performed inside the barns
  - Climbing feed bins, flush tanks, and pens
  - Loading/unloading animals using loading chutes
  - Showering in and out of the farm
  - Any area of the farm with deficient housekeeping
  - Any area outside the barns with slippery conditions due to weather or spills
- Operating tractors and other farm vehicle

  Examples of farm tasks that present this risk:

  - Grass mowing
  - Dead pig disposal

[1] National Safety Council. Advance H&S certification manual.

- Weed spraying
- Cleaning feed or waste water spills

Other risks that have shown to produce incident patterns are:

- Electrical hazards due to exposed wires or deficient repairs and maintenance or lock-out tag-out programs.

  Examples of farm tasks that present this risk:

  - Pressure washing
  - Replacing feed motors
  - Operating fans, heaters, heat pads, curtain systems, and lights
  - Working around electric fences outside barns

- Exposure to Chemicals due to deficient Hazard Communication and personal protection equipment (PPE) programs

  Examples of farm tasks that present this risk:

  - Disinfecting farrowing rooms
  - Pesticide application
  - Reproductive hormone injections

- Caught in or between two objects, animal etc. due to deficient Training and Machine Guarding programs

  Examples of farm tasks that present this risk:

  - Operation of fans, feed motors, or tractor PTO
  - Dead cart operation
  - Hand feeding using feed carts
  - Composter and incinerator operation

- Injuries incurred during non-routine tasks due to deficient Hazard ID and Risk Assessment programs

  Examples of farm tasks that present this risk:

  - Cleaning waste-water recycle lines
  - Construction and building modifications
  - Live haul vehicle accident response
  - Any task performed less than once a month

Operational controls

Once the hazards existing in the work areas have been identified, analyzed, and prioritized, the next step is to implement controls to minimize them or eliminate them. This process should consider the specifics of each hazard, including the frequency of employee exposure and the availability of mitigating technology, materials, and human resources. Control methods can be based on engineering, administrative, or PPE processes.

Ideally, the solution to prevent injuries should result from enhanced engineering controls. This integrates safety protocols into the design of a work procedure before the workers are involved by modifying, substituting, or eliminating materials, processes, and equipment; or by installing barriers between areas of hazard exposure and the workers.

If engineering based solutions are not feasible, the next best thing to do is to eliminate or reduce risks using an administrative control. This category of controls eliminate or minimize worker's exposure to hazards by establishing a comprehensive program for housekeeping, workers training, job rotation, adequate rest periods, tools, equipment, and facility compliance audits and inspections.

Use of PPE should always be the last option of operational control to be considered. The reason is that control using PPE does not eliminate or even reduce workplace hazards. PPE provides only an immediate barrier between the worker and the hazard, and too many variables (quality, comfort, training, commitment, supervision) could affect the efficiency of the protection achieved using PPE.

Safety included in all training and development activities

Besides being an OSHA requirement and part of the administrative control category, including safety in all worker training is a crucial component of any effort to eliminate and prevent workplace injuries. Too many sectors of agriculture industry still train their workers on the most efficient way to perform a task and leave the worker the responsibility to figure out the safest way to do it. In a true safety culture, the best way to perform a task is also the safest one.

When workers receive training on the best and safest way to do a job simultaneously, they perceive the expectation from their supervisor is to fulfill the requirement as a whole, and not to ignore safety for the sake of efficiency. This ensures that the workers will learn the safety aspects for the specific tasks they are performing; nothing more, but most importantly, nothing less.

Finally, conducting appropriate safety training does not guarantee the elimination or reduction of incidents. A follow-up and compliance monitoring program that includes strict but fair and consistent disciplinary actions as well as meaningful acknowledgement of positive safety practices is an equally critical element for maintaining an appropriately safe workplace environment.

Near misses

Unfortunately, not even the best hazard identification program is 100% effective. There will always be unidentified hazards that result in close calls that almost lead to injuries, especially with non-routine tasks. Because it's painless and inexpensive, the investigation of near misses is the best way to prevent injuries in the workplace. However, this is only effective if the workers involved in the incidents take the time to report it; supervisors and workers conduct a thorough investigation that results in appropriate corrective action; those measures are communicated to all workers and recorded; and a timely follow-up is conducted to verify effectiveness and compliance.

Conclusion

Safety works, try it first

When it comes to safety, prevention is always better than reaction. A good safety program must include strong hazard identification and risk assessment protocol; an up-to-date and comprehensive set of hazard-reducing operational controls through the application of engineering, administrative and protective equipment solutions; a training program that includes safety in all standard operating procedures; an efficient tracking and investigating system for all workplace incidents that includes immediate and appropriate corrective action; a versatile communication system to maintain universal workplace awareness of the safety program; and production of simple but meaningful incentives to recognize and reward employee participation.

The Upper Midwest Agricultural Safety and Health Center: Worker health and safety in animal agriculture

Bruce H. Alexander, PhD; Jeffery B. Bender, DVM,MS, DACVP

The challenge of providing a consistent, affordable, and safe food supply for a growing population has lead to considerable change in how food is produced in the United States and globally. The agriculture industry continually evolves in response to economic conditions, social drivers, such as environmental and animal welfare concerns, and globalization. As the industry changes there can be both positive and negative consequences for the health of agriculture producers, workers, and their families. To ensure the health and well-being of agricultural populations is maintained, innovative approaches using multidisciplinary partnerships are required.

The Upper Midwest Agricultural Safety and Health Center (UMASH) was formed to develop multidisciplinary approaches to address existing and emerging workplace health and safety issues in agriculture. UMASH is dedicated to improving the health, safety, and well being of agricultural workers and their families by building partnerships to improve knowledge about safety and health in agriculture and to inform industry practices and policy. UMASH has adopted three guiding principles to address its mission.

- All people, organizations, and diverse perspectives are important to the work of UMASH.
- UMASH embraces and promotes a One Health philosophy that emphasizes the connections between human health, animal, and plant health and the health of the environment.
- UMASH partners seek to understand how changes in agriculture, to meet an increasing global demand for food and fiber at affordable prices and produced in a sustainable manner, can impact agricultural workers and their families.

The Upper Midwest produces more hogs than any other region in the country, thus the health and safety in swine production is a priority for the UMASH center. Some of the Center's work is directly related to health and safety in swine production, while other work may provide tangential information on health and safety.

Agriculture is recognized as a dangerous profession with some of the highest rates of fatal and non-fatal injuries. The type of agriculture and production methods largely determines the specific health risks related to agriculture. All types of animal agriculture production can create potentially hazardous environments. Exposure to organic dusts, bioaerosols gases, and injury hazards are common. In the pork industry there is a recognized potential for exposure to dusts, toxic gases, and endotoxin at levels sufficient to affect respiratory health (Charavaryamath et al. 2005; Dosman et al. 2006; Heederik et al. 2007; Mc Donnell et al. 2008; Von Essen and Donham 1999; Von Essen et al. 2010; Von Essen and Auvermann 2005; Wyatt et al. 2008). These exposures are modified by production practices, particularly confinement operations that bring many animals into a smaller space. Though the potential for exposure is well characterized, the impact on the production practice is not clear. Injury risk is another recognized hazard in agriculture and contact with animals is considered one of the major risk factors (Boyle et al. 1997; Erkal et al. 2008; Hard et al. 2002). Animal confinement operations, including pork, dairy, beef, and pork are known to be high injury risk work places in contrast to other agriculture (Kaustell et al. 2007; Mitloehner and Calvo 2008; Gordon and Rhodes 1993).

The trend toward fewer, larger and more specialized pork production enterprises has been accompanied by a continual rise in the pig inventory (National Pork Board 2010) and a greater percent of the labor force working for larger operations (Kliebenstein et al. 2005). By 2005 nearly 30 percent of employees worked on operations that produced more than 100,000 pigs, where as less than 9 percent of operations produced 10,000 or more pigs in 1990. Despite the trend toward the use of larger production facilities, there are still a substantial number of producers with smaller enterprises. A review of the industry in 2006 reported that 85 percent of swine come from operations marketing at least 10,000 hogs per year, while 86.1 percent of the operations marketed fewer than 1,000 hogs annually (Grimes and Lawrence 2006). The trends in employment also track the specialization occurring in the pork industry. There is a movement away from the farrow-to-finish operations where all phases of pork production occurs on one site toward specialization; 83.1% to 43.7% of producers (Kliebenstein et al. 2005). Specialization may impact the

potential for various exposures or injury such as repetitive motion injuries. In the past workers may have spent only a part of each day in barns caring for animals, but in specialized swine farms the workers are employed full-time in the pork enterprise. In the 2005 survey of pork producers and employees, employees indicated that they worked an average of 45.3 hours (Kliebenstein et al. 2005). With every other weekend or partial Saturdays and Sundays off, it is common for pork production workers to put in a 50-hour work week and 11.3% of employees indicate that they do not have any weekends off (Kliebenstein et al. 2005).

As the industry has moved toward greater intensification and specialization in pork production there has been an increase in consumer interest in, and concern about, food production, which has been described as "consumer driven agriculture" (Martinez and Davis 2002). The concern over animal welfare has lead toward changes in practices demanded by consumers and the food retail industry. For example, several states are following suit with the European Union and considering a ban on gestation stalls (Harper 2006). The EU is also planning to ban piglet castration by 2018 (PigProgress.net 2010). These trends are also being observed in the United States as several states have banned gestation stalls, and the largest pork producer in the United States agreed to voluntarily move their sows from stalls to penned housing (Harper 2006). Potential impacts on the workplace are possible from these production changes, such as more frequent interaction of workers with loose animals as well as contact with intact males that may be more aggressive than castrated males.

The type of facility or practice employed in the industry may reflect the economic and social influences. The confinement facilities are under scrutiny for environmental exposures and some efforts have been made to characterize the impact of production methods on workplace and environmental health. The modest efforts to date suggest potential differences in exposure by season (Kim et al. 2007; O'Shaughnessy et al. 2010). The influence of confinement facility type is not well characterized, and the studies that have explored the issue have been limited (Diefenbach et al. 2007; Lavoie et al. 2009; Letourneau et al. 2010).

The UMASH center has currently funded projects related to health and safety in the pork production industry, methicillin-resistant *Staphylococcus aureus* (MRSA) colonization and infection in swine veterinarians, surveillance of disease and injury in dairy farmers, surveillance of zoonotic diseases in agriculture workers, immigrant dairy worker health and safety, facilitating return to work in ill and injured workers, and establishing a multidisciplinary network to address agriculture worker health and safety issues. The center is also building partnerships to engage producers in conversations about health and safety of their employees as the workforce and other drivers such as animal welfare, economics and emerging pathogens influence their operations.

In this conference we will discuss the characterization of risks to pork production workers for injuries and airborne exposures in the working environment in a study comparing concentrations of airborne contaminants and potential injury risk in operations using gestation crates or gestation pens. We will also summarize information related to the risk of needlestick injuries in livestock workers and potential methods for prevention.

References

1. Boyle D, Gerberich SG, Gibson RW, Maldonado G, Robinson RA, Martin F, et al. 1997. Injury from dairy cattle activities. Epidemiology 8(1): 37–41.

2. Charavaryamath C, Janardhan KS, Townsend HG, Willson P, Singh B. 2005. Multiple exposures to swine barn air induce lung inflammation and airway hyper-responsiveness. Respir Res 6: 50.

3. Diefenbach H, Hartung E, Sundrum A, Lohmeyer M, Rieger MA. 2007. Airborne biological hazards in different pig fattening systems. Int 20(2 Suppl 2): 45–49.

4. Dosman JA, Fukushima Y, Senthilselvan A, Kirychuk SP, Lawson JA, Pahwa P, et al. 2006. Respiratory response to endotoxin and dust predicts evidence of inflammatory response in volunteers in a swine barn. Am J Ind Med 49(9): 761–766.

5. Erkal S, Gerberich SG, Ryan AD, Renier CM, Alexander BH. 2008. Animal-related injuries: a population-based study of a five-state region in the upper Midwest: Regional Rural Injury Study II. J Safety Res 39(4): 351–363.

6. Gordon RL, Rhodes S. 1993. Injuries to workers in a swine confinement facility. J Occup Med 35(5): 518–521.

7. Grimes G, Lawrence J. year. Production and marketing characteristics of U.S. pork producers. Department of Agricultural Economics Working Paper No. AEWP 2007–5. Available: http://agebb.missouri.edu/mkt/porkind06.htm 2011.

8. Hard DL, Myers JR, Gerberich SG. 2002. Traumatic injuries in agriculture. J Agric Saf Health 8(1): 51–65.

9. Harper AF. year. Gestation Stalls, Animal Welfare and People. Available: http://www.thepigsite.com/articles/1/pig-health/1757/gestation-stalls-animal-welfare-and-people.

10. Heederik D, Sigsgaard T, Thorne PS, Kline JN, Avery R, Bonlokke JH, et al. 2007. Health effects of airborne exposures from concentrated animal feeding operations. Environ Health Perspect 115(2): 298–302.

11. Kaustell KO, Mattila TEA, Rautiainen RH. 2007. Safety performance of animal confinement floors: slip, trip, and fall injuries in Finland. J Agric Saf Health 13(4): 395–406.

12. Kim KY, Ko HJ, Kim HT, Kim YS, Roh YM, Lee CM, et al. 2007. Influence of extreme seasons on airborne pollutant levels in a pig-confinement building. Arch Environ Occup Health 62(1): 27–32.

13. Kliebenstein J, Hurley T, Orazem P, Miller D, May S. 2005. A look a the employment in the United States Swine Production Industry. A.S. Leaflet R2168..Iowa State University http://www.econ.iastate.edu/research/webpapers/paper_12616.pdf

14. Lavoie J, Godbout S, Lemay SP, Belzile M. 2009. Impact of in-barn manure separation on biological air quality in an experimental setup identical to that in swine buildings. J Agric Saf Health 15(3): 225–240.

15. Martinez S, Davis D. 2002. Farm Business Practices Coordinate Production with Consumer Preferences. In: FoodReview : Consmer-Driven Agriculture (Morrison R, ed). Washington D.C.: U.S. Department of Agriculture, 33–38.

16. Mc Donnell PE, Coggins MA, Hogan VJ, Fleming GT. 2008. Exposure assessment of airborne contaminants in the indoor environment of Irish swine farms. Ann Agric Environ Med 15(2): 323–326.

17. Mitloehner FM, Calvo MS. 2008. Worker health and safety in concentrated animal feeding operations. J Agric Saf Health 14(2): 163–187.

18. National Pork Board. year. Quick Facts: The Pork Industyr at a Glance. Available: http://www.pork.org/MediaLibrary/FlipBooks/QuickFacts2010/.

19. O'Shaughnessy PT, Donham KJ, Peters TM, Taylor C, Altmaier R, Kelly KM. 2010. A task-specific assessment of Swine worker exposure to airborne dust. J Occup Environ Hyg 7(1): 7–13.

20. PigProgress.net. year. EU banning piglet castration by 2018. Available: http://www.pigprogress.net/news/eu-banning-piglet-castration-by-2018-6786.html 2011].

21. Von Essen S, Donham K. 1999. Illness and injury in animal confinement workers. Occup Med 14(2): 337–350.

22. Von Essen S, Moore G, Gibbs S, Larson KL. 2010. Respiratory issues in beef and pork production: recommendations from an expert panel. J 15(3): 216–225.

23. Von Essen SG, Auvermann BW. 2005. Health effects from breathing air near CAFOs for feeder cattle or hogs. J 10(4): 55–64.

24. Wyatt TA, Sisson JH, Von Essen SG, Poole JA, Romberger DJ. 2008. Exposure to hog barn dust alters airway epithelial ciliary beating. Eur Respir J 31(6): 1249–1255.

Processing of high fiber feedstuffs for swine

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Introduction

Escalating costs of typical swine feed ingredients such as corn and soybean meal has created a demand for producers to consider feeding diets containing higher levels of by-products to nursery and finishing pigs to reduce feed cost. However, these alternative feed ingredients are generally higher in fiber and lower in dietary energy compared to the cereal grains normally utilized. This decrease in energy along with increased fiber and decreased bulk density can lead to decreased performance and an increase in the time needed for hogs to reach target market weights.

In light of these circumstances, more emphasis is being placed on feed processing technologies to improve utilization of the higher fiber diets. Fine grinding and pelleting are the two primary feed processing technologies that exist that will improve feed efficiency. First, reducing particle size of individual ingredients or whole diets is commonly understood to improve their digestibility and consequently feed efficiency, but little research has explored the effects of fine-grinding high fiber alternative ingredients or the entire diet containing these high fiber ingredients. Secondly, pelleting high by-product diets will improve diet bulk density, reduce feed wastage, and potentially improve diet digestibility. When used in tandem, reducing particle size of ingredients or complete diets and then pelleting could potentially be useful strategies to improve feed efficiency and subsequently reduce the cost per pound of gain.

Adding the necessary infrastructure to fine grind and pellet diets has a high initial cost and necessitates increased energy usage, which leads to a higher cost of production of the feed. However, these extra costs should provide more economic return through improved feed utilization and growth performance. Thus, the economics associated with the increased production costs of grinding and pelleting need to be studied and compared with pig performance benefits.

In this paper we briefly review recent studies conducted at Kansas State University investigating the effects of fine grinding high fiber ingredients such as dried distillers grains with solubles (DDGS), wheat middlings (midds), and soybean hulls as well as the interactive effects of fine grinding and pelleting on both nursery and finishing pig growth performance.

Nursery pigs

Recently, four studies were conducted to investigate the effects of fine grinding high-fiber ingredients and/or complete diets as well as the effects of pelleting on growth performance of nursery pigs.

Experiment 1 – Pelleting and particle size

De Jong et al. (2013a) used a total of 675 nursery pigs in a 21-d study to evaluate the effects of varying ingredient particle size and diet form on nursery pig growth performance and caloric efficiency. Pigs were fed 1 of 8 experimental diets including 3 corn-soybean meal–based diets consisting of: (1) corn fraction ground to an average of 620 µ and fed in meal form, (2) corn fraction ground to an average of 352 µ and fed in meal form, and (3) corn ground as in diet 2 but fed in pellet form. The remaining 5 diets were high by-product diets containing 20% midds and 30% DDGS. Diets 4 to 8 consisted of: (4) corn fraction ground to an average of 620 µ, midds and DDGS unground (as received from the supplier) with an average particle size of 534 µ and 701 µ, respectively, and fed in meal form; (5) diet 4 but corn fraction ground to an average of 352 µ and fed in meal form; (6) diet 5 but fed in pellet form; (7) corn, soybean meal, DDGS, and midds ground to average particle sizes of 352 µ, 421 µ, 377 µ, and 357 µ, respectively, fed in meal form; and (8) diet 7 but fed in pellet form. The diets were not balanced for dietary energy, thus energy was lower for treatments 4 to 8 than for treatments 1 to 3.

Overall, pelleting diets improved ADG and G:F as expected, but reducing corn particle size reduced ADG as a result of decreased ADFI. Pigs fed the high-by-product diet had reduced ADG, ADFI, and final BW and poorer G:F, but caloric efficiency (defined as the amount of calories needed to produce one unit of gain) was similar to pigs fed the corn-soybean meal–based diet. Finally, grinding the by-products to a smaller particle size further reduced ADG, ADFI, and final BW and did not influence feed efficiency.

Experiment 2 – Particle size pelleting and grinding DDGS

In the second experiment De Jong et al. (2013b) used 687 nursery pigs in a 21-d study to evaluate the effects of fine grinding corn or DDGS in diets fed in meal or pellet form on nursery pig performance. The 10 experimental diets fed included 4 corn-soybean meal-based diets consisting of: (1) corn ground to ~638 µ in meal form, (2) treatment 2 in pellet form, (3) corn ground to ~325 µ in meal form, and (4) treatment 3 in pellet form. The remaining 6 diets contained 30% DDGS. Diets 5 to 10 consisted of: (5) corn and DDGS ground to ~638 and 580 µ, in meal form, (6) diet 5 in pellet form, (7) corn and DDGS ground to ~638 and 391 µ, in meal form, (8) diet 7 in pellet form, (9) corn and DDGS ground to ~325 and 391 µ, in meal form, and (10) diet 9 in pellet form.

A corn particle size (regardless of DDGS addition) × diet form interaction was observed as a result of increased ADFI when corn was ground and fed in pellet form but decreased intake when corn was finely ground and fed in meal form. Pelleting the diets decreased ADG, ADFI, and final BW and increased G:F and caloric efficiency on both an ME and NE basis. Fine grinding corn decreased ADG as a result of numerically decreased ADFI. Also, feeding 30% DDGS decreased ADG, ADFI, and NE caloric efficiency, and tended to decrease final BW.

Experiment 3 – Corn particle size, complete diet grinding and pelleting

A third trial conducted in a commercial setting was completed to evaluate the corn particle size results found in the first two experiments. De Jong et al. (2013c) used 996 nursery pigs in a 21-d study to evaluate the effects of corn particle size, complete diet grinding, and diet form on nursery pig growth performance. The same corn-soybean meal-based diet containing 30% DDGS and 10% midds were used for all treatments. The 6 treatments were: 1) roller-mill ground corn (737 µ) fed in meal form; 2) treatment 1 fed in pellet form; 3) hammer-mill ground corn (324 µ) fed in meal form; 4) treatment 3 fed in pellet form; 5) complete mixed diet reground through a hammer-mill (541 µ fed in meal form; and 6) treatment 5 in pellet form. All diets contained 2% added fat prior to pelleting.

Overall ADG and ADFI decreased when corn was finely ground and fed in meal form, but increased when fed in pelleted form. Pelleting diets improved ADG, G:F, ME and NE caloric efficiencies, and final BW. The interaction of diet form × corn µ and numerical decreases in intake of pigs fed fine ground complete diet, suggests that finely ground feed fed in meal form may reduce palatability of the diet; however, improved performance from fine grinding may be realized if the diet is fed in pelleted form. Thus data from this trial shows that pelleting diets significantly improved performance and fine grinding corn numerically improved performance when fed in pelleted form. No additional improvements were found when the complete mixed diet was finely ground compared to only grinding corn, which agrees with earlier nursery studies.

Results from these three studies suggest that reducing the particle size of either corn or high fiber ingredients in complete feed when fed in meal form decreases performance in nursery pigs. While previous research has shown improvements in reduced particle size, most of this work only showed a response to approximately 500 µ and no additional benefit by grinding to a smaller particle size. The available research is consistent with our findings where fine grinding corn did not result in further improvements and in fact negatively impacted feed intake and gain when fed in meal form. Interestingly, as all major ingredients were ground in the high-by-product diet and fed in meal form, feed intake for these pigs was reduced even further, which suggests that feeding a finely ground complete diet may negatively influence palatability.

The varying response to pelleting on nursery pig performance in these three trials supports previous research where not all, but the majority of trials have shown a positive effect on growth performance.

Medel et al. (2004) showed inconsistent results when pigs fed diets that were pelleted had improved G:F from d 22 to 42 post-weaning but had no differences in ADG. Traylor et al. (1996) conducted an experiment feeding meal and pelleted diets to nursery pigs. They also observed improved G:F when pigs were fed pelleted feed; however, there were no differences in ADG. Hansen et al. (1992) also showed improved G:F of nursery pigs when fed a pelleted diet compared to the same diet in meal form immediately following weaning with no changes in ADG. This trend held true in our current nursery experiments as there was an improvement in G:F for all three experiments, but pigs in the first experiment had improved ADG and pigs in the second and third experiments had decreased ADG.

We believe the reduction in ADG from feeding pellets that was associated with a similar decrease in ADFI may be a result of increased pellet hardness. We observed a high pellet durability index which is an indicator of a hard pellet. Mavromichalis et al. (2002) conducted 2 experiments to determine the effects of pellet hardness on nursery pig growth performance. In the first experiment they showed that harder pellets decreased ADFI and ADG but had no effect on G:F and in a second experiment, ADFI tended to decrease leading to numerically decreased ADG.

Experiment 4 – Grinding and pelleting soy hull diets

A fourth experiment was conducted in a commercial setting to determine the effects of fine grinding or pelleting diets containing soybean hulls. Goehring et al. (2013a) used a total of 1,100 nursery pigs in a 42-d growth trial to determine the effects of increasing soybean hulls (10 or 20%) and soybean hull particle size (unground or ground) in nursery pig diets fed in both meal and pelleted forms. The average particle size of the unground and ground soybean hulls were 617 and 398 µ, respectively.

Soybean hulls worsened G:F when fed at 20% compared with 10%; however, pelleting these diets resulted in little change in G:F compared with the 20% inclusion fed in meal form. Pelleting diets resulted in improved ADG, but also increased feed intake, which resulted in no change in feed efficiency compared with pigs fed a meal diet. The increased feed intake could be the result of providing a more dense feed, because soybean hulls in a meal diets reduced diet bulk density. For fine grinding soybean hulls, ADG and ADFI were reduced which resulted in no change in feed efficiency.

Nursery summary

Pelleting and fine grinding had few positive and even some negative effects on nursery pig performance. Possible reasons include decreased palatability of finely ground ingredients, limited or no benefit to grinding grain finer than 600 µ for nursery pigs, or limited biological benefits of fine grinding other ingredients for nursery pigs. We speculate that the lack of improvement to pelleting in the second experiment may have been a result of increased pellet hardness. It is clear; however, that more research needs to be conducted to determine the optimum particle size of cereal grains and complete diets when fed to nursery pigs as well as the effects of pellet hardness on nursery pig performance.

Finishing pigs

Two studies were conducted to investigate the effects of feed processing technologies on high fiber diets for finishing pigs.

Experiment 1 – Processing techniques for diets with high fiber ingredients

The first trial was conducted in a commercial setting investigating the effects of corn particle size, complete diet grinding, and diet form of high fiber diets on finishing pig growth performance. De Jong et al. (2013d) used a total of 855 finishing pigs in a 111-d trial. All pigs were fed the same corn-soybean meal–based diet containing 30% DDGS and 20% midds. Different processing techniques were used to achieve the 5 dietary treatments: (1) roller-mill ground corn to approximately 650 µ with the diet fed in meal form; (2) hammer-mill ground corn to approximately 320 µ with the diet fed in meal form; (3) treatment 2 pelleted; (4) corn initially roller-mill ground to approximately 650 µ, then the complete mixed diet reground through a hammer-mill to approximately 360 µ with the diet fed in meal form; and (5) treatment 4 fed in pellet form.

Overall diet form × portion ground interactions were observed for ADG, ADFI, final BW, percentage of pigs removed per pen, and HCW. These interactions occurred because fine grinding the complete diet reduced each variable when fed in meal form, whereas pigs fed that same diet in pellet form had increased responses for each of the measurements. Reducing particle size of the corn from 650 to 320 µ did not affect ADG or ADFI but improved G:F and caloric efficiency. Pelleting the diet improved ADG, G:F, caloric efficiency, final BW, HCW, and loin depth but tended to increase BF.

Particle size reduction improved G:F and caloric efficiency; however, fine grinding the complete diet was actually detrimental to performance when fed in meal form. Particle size reduction of corn has been consistently shown to improve performance in finishing pigs. Hedde et al. (1985) observed that pigs fed finer particle corn compared to simple cracked corn had improved ADG and G:F. Wondra et al. (1995 a,b) found that reducing particle size from 1,000 to 400 or 800 to 400 µ improved G:F and apparent digestibility of diets.

It is commonly understood that fine grinding will influence feed mill production parameters. Wu et al. (1985) found that decreasing particle size increased the total particle surface area but worsened mill production rate and grinding efficiency. Thus, feed mill operators and producers must balance decreased mill efficiency with growth benefits.

We hypothesized that grinding the corn, complete diet, and pelleting would have allowed for more calories to be available to the pig, thus improving caloric efficiency. However, only grinding the corn portion and pelleting the diets improved caloric efficiency. Interestingly, grinding the entire diet post-mix did not improve caloric efficiency over that of the diet containing fine ground corn. This means that fine grinding the other major ingredients (soybean meal, DDGS and midds) did not create additional dietary energy for the pig regardless of feed form. In addition, this study showed that ADFI decreased when the complete diet was finely ground. The decrease in ADFI may have been a result of decreased palatability of the diet when finely ground. Work by Mavromichalis et al. (2000) and Wondra et al. (1995a) both showed that finely ground ingredients in diets decreased ADFI.

Ulceration of the pig's esophageal region of the stomach is a concern when feeding finely ground ingredients or

diets. There were increased removals off test for pigs fed the diet that was post-mix ground and pelleted. However, no clear link was found between the removals and feed processing in this study. Most removals reported during the study appeared to be caused by reasons other than experimental treatment effects. More research needs to be conducted to evaluate whether this effect was diet-related.

Fine grinding ingredients and complete diets can also potentially lead to feed handling issues. In the current experiment, angle of repose was used as an indicator of the flow ability of the diet. Angle of repose is the measure of the internal angle created by feed allowed to flow freely onto a horizontal surface. As the angle increases (becomes steeper) the feedstuff is associated with having decreased flow ability. Fine grinding only the corn fraction of the diet didn't affect angle of repose when compared to the control and this was most likely caused by the limited concentration of corn (30-40%) in the diet. However, when the complete diet was reground there was an increase in the angle of repose when compared to the control or the meal diet with finely ground corn.

In conclusion, fine grinding corn and pelleting the diet improved performance and carcass characteristics, as well as caloric efficiency of finishing pigs. However, grinding the complete diet and feeding it in meal form had detrimental effects on all criteria measured which may be due to reduced palatability of a finely ground diet presented in meal form. Finally, post-grinding a complete diet did not provide any advantage in growth performance to that of a diet with only finely ground corn. This indicates that fine grinding DDGS, midds and soybean meal does not provide an additional benefit.

Experiment 2 – Particle size and inclusion rate of soy hulls

In the second experiment Goehring et al. (2013b) used 1,235 finishing pigs in a 118 d growth trial to determine the effects of 7.5 and 15% ground or unground soybean hulls on growth performance and carcass characteristics of pigs raised in a commercial environment. Treatments were arranged in a 2 × 2 factorial with a positive control. Main effects were soybean hull particle size (unground or ground, 787 and 370 μ, respectively) and amount of soybean hulls (7.5 or 15%) in corn-soybean meal–based diets. The fifth treatment was a positive control, corn-soybean meal–based diet with no added soy hulls. Diets were fed in meal form and were formulated to a constant SID lysine level.

Overall, increasing dietary soybean hulls did not impact ADG, ADFI, or final live BW; however, G:F decreased. Caloric efficiency improved on an ME and NE basis as soybean hulls were added. Feeding pigs diets with the ground soybean hulls did not influence ADG or ADFI, but resulted in poorer G:F and caloric efficiency on an ME and NE basis compared to pigs fed the diets with unground hulls.

A study in South America conducted by Moreira et al. (2009) observed an improvement in DE and ME when soybean hulls were ground through a 2.5 mm screen as compared to a screen size up to 3.5 mm. However, soybeans are processed differently in South America than in the United States. In South America the soybean hulls are separated before roasting and trypsin inhibitors may still be present in the hull. The improvement in digestibility observed by Moreira et al. (2009) could be the result of reducing the negative effects of trypsin inhibitors and not improving digestibility of soybean hulls. In our study grinding soybean hulls resulted in no difference in ADG or ADFI but feed efficiency and caloric efficiency were worsened with soybean hull grinding. These results imply that grinding soybean hulls did not improve pig performance by means of improving digestibility and in fact, the opposite may have occurred. It has been observed that increasing the amounts of fiber in the diet will increase the rate of passage of digesta (Ehle et al., 1982; Stanogias and Pearce, 1985). It could be possible that an increased rate of passage caused by smaller particles of fiber occurred which resulted in decreased digestibility of the feed and consequently poorer caloric efficiency.

In summary, increasing soybean hulls reduced G:F as expected due to lowering diet energy density. The hypothesis of reducing the particle size of soybean hulls to improve their energy value was proven false. Grinding soybean hulls reduced ADG and ADFI, but pelleting improved ADG and ADFI regardless of particle size. It appears that fine grinding soybean hulls when fed in meal form is detrimental to performance but performance can be restored if the diets are pelleted.

Overall summary

- Grinding ingredients other than corn or complete diets resulted in little or no improvements in feed efficiency (Table 2).
- Fine grinding ingredients to resulted in no improvements and even negative effects on growth performance in nursery pigs.
- In nursery pigs some variability exists, but our data combined with that available in the data base suggests a positive response to pelleting (Table 1).
- In finishing pigs, pelleting appears to elicit a more consistent positive feed efficiency response.
- Pelleting diets for finishing pigs appears to mitigate any negative effects of the finely ground ingredients.

Table 1: Effects of diet form on nursery and finishing pig growth performance.

Item:	ADG, g		ADFI, g		G:F	
	Meal	Pellet	Meal	Pellet	Meal	Pellet
Nursery						
Exp. 1	578	597	914	916	0.633	0.652
Exp. 2	594	560	952	864	0.626	0.648
Exp. 3	413	445	641	643	0.645	0.692
Exp. 4	470	491	707	737	0.665	0.667
Finishing						
Exp. 1	920	970	2,510	2,520	0.370	0.390

Table 2: Effects of particle size on nursery and finishing pig growth performance.

Item:	Diet µ	ADG, g	ADFI, g	G:F	Research notes
Nursery					
Exp. 1	696	648	1001	0.648	620 µ corn, corn-soy formulation
	517	621	963	0.647	352 µ corn, corn-soy formulation
	679	585	935	0.626	620 µ corn, high fiber formulation
	551	564	917	0.615	352 µ corn, high fiber formulation
	397	548	861	0.637	397 µ complete diet, high fiber formulation
Exp. 2	724	621	989	0.631	638 µ corn, corn-soy formulation
	619	589	932	0.633	325 µ corn, corn-soy formulation
	709	588	958	0.616	638 µ corn, high fiber formulation
	703	595	955	0.625	391 µ DDGS, high fiber formulation
	550	576	926	0.620	325 µ corn and 391 µ DDGS, high fiber formulation
Exp. 3	656	421	650	0.649	742 µ corn, high fiber formulation
	425	402	632	0.636	335 µ corn, high fiber formulation
	540	416	641	0.650	540 µ complete diet, high fiber formulation
Exp. 4	436	487	731	0.667	617 µ soy hulls
	412	474	713	0.666	398 µ soy hulls
Finishing					
Exp. 1	596	920	2,580	0.355	647 µ corn, high fiber formulation
	487	930	2,530	0.369	322 µ corn, high fiber formulation
	365	900	2,480	0.365	365 µ complete diet, high fiber formulation
Exp. 2	573	840	2,150	0.387	787 µ soy hulls, 7.5% inclusion
	595	850	2,180	0.384	787 µ soy hulls, 15% inclusion
	546	840	2,210	0.381	370 µ soy hulls, 7.5% inclusion
	543	820	2,180	0.375	370 µ soy hulls, 15% inclusion

References

1. De Jong, J. A., J. M. DeRouchey, M. D. Tokach, R. D. Goodband, S. S. Dritz, and J. L. Nelssen. 2013a. Effects of varying ingredient particle size and diet form on nursery pig growth performance and caloric efficiency. J. Anim. Sci. 91(Suppl. 2):111–112 (Abstr.).

2. De Jong, J. A., J. M. DeRouchey, M. D. Tokach, R. D. Goodband, S. S. Dritz, and J. L. Nelssen. 2013b. Effects of fine grinding corn or DDGS in diets fed in meal or pellet form on 25–50 lb nursery pig performance. In Kansas Swine Industry Day Report of Progress 2013. Manhattan, KS. (Unpublished at time of printing).

3. De Jong, J. A., J. M. DeRouchey, M. D. Tokach, R. D. Goodband, S. S. Dritz, J. L. Nelssen and C. Hastad. 2013c. Effects of corn particle size, complete diet grinding, and diet form on 11- to 20-kg nursery pig growth performance. Manipulating Pig Production Australasian Pig Science Association, Werribee, Victoria. (Unpublished at time of printing).

4. De Jong, J. A., J. M. DeRouchey, M. D. Tokach, R. D. Goodband, S. S. Dritz, and J. L. Nelssen. 2013d. Effects of corn particle size, complete diet grinding, and diet form on pig growth performance, caloric efficiency, and carcass characteristics. J. Anim. Sci. 91(Suppl. 2):70 (Abstr.).

5. Ehle, F. R., J. L. Jeraci, J. B. Roberson and P. J. Van Soest. 1982. The influence of dietary fiber on digestibility, rate of passage and gastrointestinal fermentation in pigs. J. Anim. Sci. 55:1071–1081.

6. Goehring, D., J. M. DeRouchey, M. D. Tokach, R. D. Goodband, S. S. Dritz, J. L. Nelssen, and B. W. James. 2013a. The effects of dietary soybean hulls, particle size, and diet form on nursery pig performance. J. Anim. Sci. 91(Suppl. 2):114 (Abstr.).

7. Goehring, D., J. M. DeRouchey, M. D. Tokach, R. D. Goodband, S. S. Dritz, and J. L. Nelssen. 2013b. The effects of dietary soybean hulls and their particle size on growth performance and carcass characteristics of finishing pigs. J. Anim. Sci. 91(Suppl. 2):113 (Abstr.).

8. Hansen, J. A., J.L. Nelssen, M.D. Tokach, R.D. Goodband, L.J. Kats, and K.G. Friesen. 1992. Effects of a grind and mix high nutrient density diet on starter pig performance. J. Anim. Sci. 70(Suppl. 1):59 (Abstr.).

9. Hedde, R. D., T. 0. Lindsey, R. C. Parish, H. D. Daniels, E. A. Morgenthien, and H. B. Lewis. 1985. Effect of diet particle size and feeding of HZ-receptor antagonists on gastric ulcers in swine. J. Anim. Sci. 61:179–186.

10. Mavromichalis, I., D. R. Cook, M. M. Ward, and N. D. Paton. 2002. Effect of pellet hardness on growth performance of weaned pigs. J. Anim. Sci. 80(Suppl. 1):29. (Abstr.).

11. Mavromichalis, J., D. Hancock, B. W. Senne, T. L. Gugle, G. A. Kennedy, R. H. Hines, and C. L. Wyatt. 2000. Enzyme supplementation and particle size of wheat in diets for nursery and finishing pigs. J. Anim. Sci. 78:3086–3095.

12. Medel, P., M. A. Latorre, C. de Blas, R. Lázaro, and G. G. Mateos. 2004. Heat processing of cereals in mash or pellet diets for young pigs. Anim. Feed Sci. Technol. 113:127–140.

13. Moreira, I., M. Kutschenko, D. Paiano, C. Scapinelo, A. E. Murakami, and A. R. Bonet de Quadros. 2009. Effects of different grinding levels (particle size) of soybean hull on 47 starting pigs performance and digestibility. Braz. Arch. Biol. Technol. v.52 n.5:1243–1252.

14. Stanogias, G. and G. R. Pearce. 1985. The digestion of fibre by pigs. 1. The effects of amount and type of fibre on apparent digestibility, nitrogen balance and rate of passage. Br. J. Nutr. 53:513–530.

15. Traylor, S. L., K. C. Behnke, J. D. Hancock, P. Sorrell, and R. H. Hines. 1996. Effect of pellet size on growth performance in nursery and finishing pigs. J. Anim. Sci. 74(Suppl. 1):67(Abstr.).

16. Wondra K. J., J. D. Hancock, K. C. Behnke, R. H. Hines, and C. R. Stark. 1995a. Effects of particle size and pelleting on growth performance, nutrient digestibility, and stomach morphology in finishing pigs. J. Anim. Sci. 73:757-763.

17. Wondra, K. J., J. D. Hancock, K. C. Behnke, and C. R. Stark. 1995b. Effects of mill type and particle size uniformity on growth performance, nutrient digestibility, and stomach morphology in finishing pigs. J. Anim. Sci. 73:2564-2573.

18. Wu, J. F. 1985. Effects of particle size of corn, sorghum grain, and wheat on pig performance and nutrient digestibility. PhD Dissertation. Kansas State Univ., Manhattan.

The effect of regrinding major feed ingredients to improve pellet quality and pig performance

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Introduction

Pelleting is a technology that is widely used in southeastern US feed mills and gaining popularity in the Midwest as it offers economic, physical and nutritional benefits. The physical benefits of pelleting include improved ease of handling, reduced ingredient segregation, less feed wastage and increased bulk density. Nutritional benefits of pelleting, as measured through feeding trials, are increased growth rate and increased nutrient utilization, resulting in improved feed efficiency. Pelleting research has tended to focus mainly on the benefits of pelleted diets in comparison to mash. However, pellet quality has become more important as people recognize the economic benefits of pelleting.

Stark (2011) reported the factors affecting pellet quality are diet composition (40%), particle size (20%), conditioning temperature (20%), die specification (15%) and cooling (5%). Recently, production throughput has been added to the list of factors that impact pellet quality (Stark, 2011). Therefore, the current study chose to focus on two of the most important factors that explain variation in pellet quality, diet composition and particle size.

Diet composition has been demonstrated by several studies to impact pellet quality. Wheat based diets have been reported to have greater pellet durability index (PDI) in comparison to corn diets (Stevens, 1987; Winowiski, 1988; Abdollahi, 2011). Inclusion of dried distillers grains with solubles (DDGS) has been shown to both increase (Feoli, 2008) and decrease (Stender et al., 2008; Fahrenholz et al., 2013; Loar et al., 2010) PDI. Briggs et al. (1999) reported increasing the protein content of the diet increased PDI. Similarly, Buchanan and Moritz (2009) reported the inclusion of small amounts of soy protein isolate or soybean meal improved PDI. The same authors showed adding small amounts of fiber (cellulose) resulted in greater pellet durability.

Reducing particle size has been shown to improve PDI. In corn-soy diets, Wondra et al. (1995) reported PDI improved from 79% to 86% as corn particle size was reduced from 1000 to 400 microns. Also using corn-soy diets, Stark (1994) reported PDI improved from 97% to 99% when the entire diet was ground from 543 to 233 microns. In diets containing DDGS, Fahrenholz et al. (2013) reported modified PDI improved 1% to 3% when DDGS particle size was reduced from 692 to 508 microns in diets containing 10 to 30% DDGS.

Pelleting is well known to improve swine feed conversion in comparison to mash diets (Stark, 1994; Wondra et al., 1995). Yet, little public information exists associating pellet quality characteristics and pig performance. Schell and van Heugten (1998) showed feed efficiency improved from 2.10 to 2.01 as the level of fines decreased from 37% to 3% in grower pigs. In finishing pigs, Stark (1994) reported feed conversion improved from 2.82 to 2.65 as percent fines decreased from 60% to 0%. Also in finishing pigs, Nemechek et al. (2013) reported feed efficiency was 2.67 to 2.55 for pigs fed 50% and 0% fines, respectively. Collectively, these results suggest improving pellet quality by increasing pellet durability and reducing percent fines has economic value. Therefore, the objective of the current study was to evaluate the effect of regrinding major feed ingredients on pellet quality and pig performance.

Methods and materials

Experiment 1. The effect of regrinding major feed ingredients on pellet quality

Feed was produced at the North Carolina State University Feed Mill Educational Unit (Raleigh, NC) June 11 and 12, 2013. Grow-finish diet formulations (Table 1) were provided by Murphy-Brown, LLC. Treatments consisted of two levels of DDGS (0% & 30%), two particle sizes of DDGS (650 & 450 microns) and two particle sizes of soybean meal (1000 & 450). Corn was ground to 400 microns in all diets. Particle size was measured using the approved method by ANSI/ASAE (ASABE, 2009) using sieve agitators and 0.5 g dispersing agent. Each treatment was replicated 4 times, with each batch being 909 kg and representing an experimental unit. Treatments were randomly ordered within replication. A 30 HP California Pellet Mill (Crawfordsville, IN) model 1112-2 pellet mill equipped with a 4.4 mm (11/64") × 31.75 mm (1 1/4") pellet die. Feed was steam conditioned prior to pelleting using a 30.5 cm (12") by 121.9 cm (48") steam conditioner. All

Table 1: Diet formulations used in experiment 1, the effect of regrinding major feed ingredients on pellet quality.

	Diets	
Ingredient	**Corn-soy**	**Corn-soy with DDGS**
Corn, %	69.4	53.0
DDGS, %	-	30.0
Soybean meal, %	22.3	8.0
Poultry fat, %	6.5	6.5
Limestone, %	0.9	1.7
Deflourinated phosphate, %	0.5	-
Premix[1], %	0.4	0.8

[1] Premix contains synthetic amino acids, salt, trace minerals, selenium, vitamins and phytase.

treatments were steam conditioned with the same retention time and set to the same temperature of 82°C (180°F). Production rate, conditioning temperature and hot pellet temperature were monitored during pelleting. A representative sample from each batch was reserved for determination of pellet quality, as measured by pellet durability index and modified pellet durability index. Pellet quality was assessed on the day of manufacture via a tumbling box according to ASAE standard S269.4 (ASAE, 1997). Modified PDI was determined by the addition of three 19.1 mm hexagonal nuts to each chamber of the tumbler box to simulate movement of feed from the feed mill to the barn. Data were analyzed by analysis of variance using Proc GLM in SAS (SAS Institute, Inc, Cary, North Carolina). Contrast comparisons were used to evaluate the impact of regrinding soybean meal and DDGS on pellet quality. Models for PDI and modified PDI included diet and time of day as fixed effects. A value of $P < 0.05$ was considered statistically significant in all tests. Summary statistics for experiment 1 are shown in Table 2.

Experiment 2. The effect of regrinding DDGS on finishing pig performance

Pigs (n = 760) will be housed at Murphy-Brown's research facility near Rose Hill, NC starting August 2013. The test period will be from 27 to 123 kg. Pigs will be weighed at the start of the trial and at the end of each dietary phase. The formulation for experiment 2 will consist of a corn-soy diet with 30% DDGS. Four treatments will be randomly assigned to pens with 10 replicates per treatment. A 2 × 2 factorial will be used, with two particle sizes of DDGS (450 vs. 650 microns) and two levels of removing fines after pelleting (yes & no). Data collection will include feed milling characteristics, growth rate, feed intake, feed efficiency and carcass weights.

Results

Pellet durability index and modified PDI differed ($P \leq 0.05$ and $P \leq 0.01$, respectively) between treatments in experiment 1 (Table 3). Corn-soy diets had 1.6% lower ($P < 0.05$) PDI and 9.5% lower modified PDI ($P < 0.01$) in comparison to diets containing 30% DDGS. Regrinding soybean meal in corn-soy diets tended ($P = 0.07$) to improve PDI by 2.3% and tended ($P = 0.06$) to improve modified PDI by 5.8%. Within DDGS diets, regrinding soybean meal did not improve ($P = 0.19$) PDI, but improved ($P \leq 0.05$) modified PDI by 4.3%. Across all treatments, regrinding soybean meal improved ($P < 0.05$) PDI by 1.5% and improved ($P < 0.05$) modified PDI by 4.7%. Regrinding DDGS had no effect ($P > 0.05$) on PDI or modified PDI. In fact, regrinding DDGS numerically reduced both PDI and modified PDI.

Time of day impacted both PDI and modified PDI. Batches pelleted in the morning had greater ($P < 0.01$) PDI and greater ($P < 0.05$) modified PDI in comparison to those pelleted in the afternoon (91.4 vs. 89.4 and 70.5 vs. 65.9, respectively).

Results for experiment 2 will be available fall of 2013.

Summary

- Diets containing 30% DDGS improved PDI and modified PDI by 1.6 and 9.5%, respectively in comparison to corn-soy based diets
- Regrinding soybean meal from 1000 to 450 microns improved PDI and modified PDI by 1.5% and 4.7%, respectively
- Regrinding DDGS from 650 to 450 microns did not impact PDI or modified PDI

Table 2: Descriptive statistics from experiment 1, the effect of regrinding major feed ingredients on pellet quality.

Trait	Mean	Standard deviation
Production rate, kg per hour	849	10.0
Conditioning temperature, °C	82.2	0.90
Hot pellet temperature, °C	85.8	1.07
Standard pellet durability index, %	90.4	2.1
Modified pellet durability index, %	68.2	6.8

Table 3: The impact of regrinding DDGS and soybean meal on pellet quality.

	Treatment							
	1	2	3	4	5	6	SE	*P*-value
Level of DDGS, %	0	0	30	30	30	30		
DDGS particle size, microns	–	–	650	650	450	450		
Soybean meal particle size, microns	1000	450	1000	450	1000	450		
Pellet durability index, %	88.3^{a}	90.5^{abc}	91.3^{bc}	92.0^{c}	89.5^{ab}	91.0^{bc}	1.16	0.05
Modified pellet durability index, %	59.0^{a}	64.8^{ab}	71.5^{cd}	73.5^{d}	67.0^{bc}	73.5^{d}	2.28	< 0.01

^{abcd} Means within a row with different subscripts differ ($P < 0.05$).

References

1. Abdollahi, M. R. 2011. Influence of feed processing on the performance, nutrient utilization and gut development of poultry and feed quality. Ph.D. Dissertation, Massey University, New Zealand.

2. American National Standards Institute/American Society of Agricultural Engineers (ANSI/ASAE). 2009. Method of determining and expressing fineness of feed materials by sieving. ANSI/ASAE Standard ANSI/ASAE S319.4. American Society of Agricultural and Biological Engineers, St. Joseph, MI.

3. American Society of Agricultural Engineers. 1997. ASAE S269.4: Cubes, pellets, and crumbles—Definitions and methods for determining density, durability, and moisture. ASAE Standards 1997. American Society of Agricultural Engineers, St. Joseph, MI.

4. Buchanan, N. P. and J. S. Moritz. 2009. Main effects and interactions of varying formulation protein, fiber, and moisture on feed manufacture and pellet quality. J. Appl. Poult. Res. 18:274–283.

5. Briggs, J. L., D. E. Maier, B. A. Watkins, and K. C. Behnke. 1999. Effect of ingredients and processing parameters on pellet quality. Poult. Sci. 78:1464–1471.

6. Loar II, R. E., J.S. Moritz, J. R. Donaldson, and A. Corzo. 2010. Effects of feeding distillers dried grains with solubles to broilers from 0 to 28 days posthatch on broiler performance, feed manufacturing efficiency, and selected intestinal characteristics. Poult. Sci. 89:2242–2250.

7. Fahrenholz, A. C., K. C. Behnke, L. J. McKinney. 2013. Processing of pelleted feeds using pelleted DDGS as an ingredient. Appl. Eng. in Agric. 29(1): 89–92.

8. Feoli, C. 2008. Use of corn- and sorghum-based distillers dried grains with solubles in diets for nursery and finishing pigs. Ph.D. Dissertation, Kansas State University, Manhattan.

9. Nemechek, J., M. Tokach, E. Frugé, E. Hansen, S. Dritz, R. Goodband, J. DeRouchey, and J. Nelssen. 2013. J. Anim. Sci. 91(Suppl. 2):69.

10. Schell, T.C. and E. van Heugten. 1998. The effect of pellet quality on growth performance of grower pigs. J. Anim. Sci. 76(Suppl. 1):185.

11. Stark, C.R. 1994. Pellet quality I. Pellet quality and its effects on swine performance. Ph.D. Dissertation, Kansas State University, Manhattan.

12. Stark, C. R. 2011. Feed processing to maximize feed efficiency. International Conference on Feed Efficiency in Swine. Nov. 8 to 9, Omaha, NE.

13. Stender, D. and M.S. Honeyman. 2008. Feeding pelleted DDGS-based diets to finishing pigs in deep-bedded hoop barns. J. Anim. Sci. 86 (Supp. 3):50

14. Stevens, C. A., 1987. Starch gelatinization and the influence of particle size, steam pressure and die speed on the pelleting process. Ph.D. Dissertation. Kansas State University, Manhattan, KS.

15. Winowiski, 1988. Wheat and pellet quality. Feed Management. 39 (9):58–64.

16. Wondra, K.J., J.D. Hancock, K.C. Behnke, R.H. Hines, and C.R. Stark. 1995. Effect of particle size and pelleting on growth performance, nutrient digestibility, and stomach morphology in finishing pigs. J. Anim. Sci. 73:757–763.

Effects of reducing the particle size of corn on the digestibility of energy and nutrients and growth performance and carcass characteristics of growing - finishing pigs[1]

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Introduction

Grinding of feed ingredients is used to reduce the particle size and increase energy and nutrient digestibility (Wondra et al., 1995d; Laurinen et al., 2000; Mavromichalis et al., 2000; Kim et al., 2002) and it is usually accomplished with the use of either roller mills, hammer mills, or a combination of roller and hammer mills. It is currently recommended that corn grain be milled to an average particle size of approximately 640 - 650 μm (Wondra et al., 1995b; Kim et al., 2002), but it has also been documented that the apparent total tract digestibility (ATTD) of energy, DM, and N in corn fed to finishing pigs or sows increases as the particle size of the grain is reduced (Healy et al., 1994; Wondra et al., 1995a-d). It was also reported that there is a tendency for an increase in the SID of AA in soybean meal as particle size is reduced (Fastinger and Mahan, 2003), but there is no effect of particle size on the ATTD of P in distillers dried grain with solubles (DDGS; Liu et al., 2012). However, there are no data that demonstrate the effects of particle size on the standardized ileal digestibility (SID) of AA, the apparent ileal digestibility (AID) of starch and GE, or the standardized total tract digestibility (STTD) of P in corn grain.

Therefore, the objectives of the present experiments were to determine the concentration of DE and ME, the STTD of P, the AID of starch and GE, and the SID of AA and CP in corn grain that was ground to different particle sizes and fed to growing pigs. The hypothesis that there is a linear increase in the concentration of DE and ME and in the digestibility of AA, starch, GE, and P in corn, as particle size is reduced was tested.

Materials and methods

Four experiments were conducted. The same batch of corn (Pioneer P0528) was used in all diets in both experiments and the corn was grown in IA in 2011. The corn grain was first rolled using an automatic roller mill (Model CSU 500, 2 stage; Automatic Equipment Mfg. Co., Pender, NE). The rolled grain was then divided into 4 batches that were ground using a hammer mill (Model #EL-9506-TF; Bliss Industries, Ponca City, OK) with 3, 10, 24, or 40 mm screens to obtain average final particle sizes of 339, 485, 677, and 865 μm, respectively. The grain was milled at the Pioneer Hi-Bred Feed Mill in Johnston, IA, and stored at 15ºC until used (Table 1).

Exp. 1: Ileal digestibility of CP and AA

Ten growing barrows (initial BW: 29.2 ± 1.35 kg) were equipped with a T-cannula in the distal ileum according to procedures adapted from Stein et al. (1998). Pigs were allotted to a replicated 5 × 5 Latin square design with 5 diets and 5 periods in each square. Pigs were housed in individual pens (1.2 × 1.5 m) in an environmentally controlled room. A feeder and a nipple drinker were installed in each pen. Five diets were formulated (Tables 2 and 3). Four of the diets each contained one of the 4 batches of corn (96.55%, as-fed basis) and corn was the only ingredient contributing AA, CP, starch, and GE to the diet. The last diet was a N-free diet that was used to measure basal endogenous losses of AA and CP. Ileal digesta were collected on d 6 and 7 of each period following standard procedures and values for the standardized ileal digestibility of CP and all AA were calculated (Stein et al., 2007).

Exp. 2: Total tract digestibility of GE and P

Experiment 2 was designed to determine the concentration of DE and ME, the ATTD of GE, and the ATTD and STTD of P in the 4 batches of corn that were used in Exp. 1. Forty barrows (initial BW 22.8 ± 2.13 kg) were allotted to a randomized complete block design with 4 diets and 10 replicate pigs per diet and placed in metabolism cages that were equipped with a feeder and a nipple drinker, fully slatted floors, a screen floor, and urine trays, which allowed for the total, but separate, collection of urine and fecal materials from each pig.

[1] Financial support for this research was provided by the National Pork Board (Des Moines, IA). Appreciation is also extended to Pioneer Hi-Bred (Johnston, IA) for donation of the corn grain used in these experiments and for grinding the grain to the specified particle sizes.

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Table 1: Chemical and physical composition of corn with different particle sizes, as-fed basis

	Corn particle size			
Item	**339 μm**	**485 μm**	**677 μm**	**865 μm**
GE, kcal/kg	3,870	3,914	3,900	3,920
DM, %	86.30	86.71	86.40	86.54
CP, %	7.00	7.25	7.23	7.08
Ash, %	1.10	1.23	1.39	1.15
AEE1, %	3.57	3.53	3.51	3.45
NDF, %	9.25	9.29	10.01	11.06
ADF, %	1.91	2.24	2.27	2.41
Starch, %	64.42	62.73	61.19	62.90
P, %	0.29	0.30	0.34	0.31
Ca, %	0.03	0.03	0.03	0.03
Indispensable, AA %				
Arg	0.35	0.35	0.37	0.35
His	0.20	0.20	0.21	0.20
Ile	0.24	0.25	0.26	0.24
Leu	0.83	0.83	0.84	0.85
Lys	0.25	0.25	0.26	0.25
Met	0.14	0.13	0.14	0.14
Phe	0.35	0.35	0.35	0.35
Thr	0.25	0.25	0.24	0.25
Trp	0.05	0.05	0.05	0.06
Val	0.35	0.36	0.38	0.35
Dispensable, AA %				
Ala	0.51	0.51	0.52	0.51
Asp	0.49	0.49	0.50	0.49
Cys	0.15	0.14	0.15	0.15
Glu	1.26	1.26	1.25	1.28
Gly	0.30	0.30	0.30	0.30
Pro	0.63	0.64	0.62	0.64
Ser	0.31	0.30	0.30	0.32
Tyr	0.21	0.20	0.22	0.20
Total AA	**6.87**	**6.86**	**6.96**	**6.93**
Physical characteristics				
Standard deviation of particle size	1.89	2.92	3.20	3.15
Surface area (cm^2)/gram	164.5	166.6	132.1	101.4
Angle of repose, °	57.4	54.9	50.7	46.8
Bulk density, g/L	564.5	601.4	631.5	650.6
Water binding capacity, g/g	2.3	2.3	2.2	2.2

[1] AEE = acid hydrolyzed ether extract.

Four corn-based diets that contained 97.7% corn were formulated. The 4 diets were similar with the exception that the corn used in each diet was ground to a different mean particle size (i.e., 339, 485, 677, and 865 μm) as explained for Exp. 1. The experimental diets were fed to pigs for 12 d. The initial 5 d were considered an adaptation period to the diet, while urine and fecal samples were collected during the following 5 d according to standard procedures using the marker to marker approach (Adeola, 2001). Feces were collecting twice daily and stored at -20°C immediately after collection. Urine buckets were placed under the metabolism cages to permit total collection. They were emptied in the morning and afternoon and a preservative of 50 mL of 6*N* HCL was added to each bucket when they were emptied. The collected urine was weighed and a 20% subsample was stored at -20°C. Samples were analyzed for energy and P and the DE and ME in each source of corn was calculated according to Adeola (2001) and the STTD of P was calculated as described by Rojas and Stein (2013).

Exp. 3: Growth performance of growing finishing pigs

Experiment 3 was conducted to confirm that the increase in the ME of corn that was observed in Exp. 2 can be used in practical diet formulation. A total of 36 gilts and 36 barrows with an average initial BW of 32.00 ± 1.58 kg were used. All pigs were housed individually in pens (0.9 × 1.8 m) with fully slatted concrete floors. A feeder and a nipple drinker were provided in each pen. Feed and water were provided on an ad libitum basis throughout the experiment. Pigs were fed a 3 phase program (Table 2) with phase 1 diets being offered from 30 to 65 kg, phase 2 diets from 65 to 100 kg, and phase 3 diets from 100 to 135 kg. Within each phase, 4 corn-SBM based diets were formulated to meet or exceed current nutrient requirements (NRC, 2012). The same batch of corn and SBM was used in all diets. The only difference among diets within each phase was that the corn that was used was ground to a different particle size (i.e., 339, 485, 677, and 865 μm) and diets were formulated using values for ME, standardized total tract digestibility of P, and standardized ileal digestibility of CP and AA in corn with different particle size that were determined in Exp. 1 and 2. Diets were formulated to contain the same ME and addition of soybean oil was adjusted to compensate for the reduction of ME as particle size of corn increased. Diets containing corn ground to the larger particle sizes, therefore, contained more fat than diets containing corn with the reduced particle sizes. The inclusion of soybean oil was 2.00% in phase 1, 2, and 3 diets containing corn ground to 339 microns; 2.78, 2.85, and 2.92% in diets containing corn ground to 485 micorns; 3.12, 3.23, and 3.30% in diets containing corn ground to 677 microns; and 3.60, 3.74, and 3.87% in diets containing corn ground to 865 microns. Pigs were randomly allotted to the 4 dietary treatments in a 2 × 4 factorial design with sex (gilts and barrows) and diet as factors. There were 9 gilts and 9 barrows allotted to each diet. Pigs were allowed ad libitum access to feed and water throughout the experiment. When pigs reached an average BW of 135 kg, they were harvested and carcass measurements were recorded.

Results and discussion

Exp. 1: Ileal digestibility of CP and AA

The SID of CP and all indispensable and dispensable AA was not affected by the particle size of corn (Table 2). The average SID of indispensable AA was also not different, among diets, but the average SID of dispensable AA decreased linearly ($P < 0.05$) as particle size of corn increased.

The fact that particle size of corn did not influence the SID of AA concurs with observations by Fastinger and Mahan (2003) who reported that a reduction in particle size of soybean meal from 949 to 185 μm has no effect on the SID of indispensable AA. The implication of this observation is that the same values for digestibility of AA can be used for corn regardless of particle size.

Exp. 2: Total tract digestibility of GE and P

The ATTD of GE was reduced (linear and quadratic, $P < 0.01$) as the particle size increased from 339 to 865 μm, respectively (Table 3). The concentration of DE (as-fed and DM basis) decreased (linear and quadratic, $P < 0.05$) as the particle increased from 339 to 865 μm. Likewise, the ME concentration, calculated on an as-fed or on a DM basis, decreased from 3,432 to 3,311 kcal/kg and from 3,964 to 3,826 kcal/kg, respectively, when corn particle size increased from 339 to 865 μm.

The increase in DE and ME that was observed as the particle size was reduced is less than the increase reported by Wondra et al. (1994c), when grain with different particle sizes were fed to sows, but in agreement with data reported by Oryschak et al. (2002). The reason for this difference is most likely that sows have a greater ability to ferment fiber and nutrients compared with growing pigs (Noblet and Shi, 1993). However, the fact that the ME increased as particle size was reduced indicates that dietary energy concentration will be increased if particle size of corn is reduced.

There were no differences in ADFI and P intake among pigs fed the 4 experimental diets. The concentration of P in feces decreased linearly ($P < 0.01$) as corn particle size increased from 339 to 865 μm. However, there were no differences in P output and absorbed P among diets. Likewise, the ATTD and STTD of P did not change as particle size of corn changed. This observation concurs

Table 2: Standardized ileal digestibility (SID) of CP and AA (%) in corn that was ground to different particle sizes and fed to growing pigs, Exp. 1[1]

	Corn particle size					*P*-value	
Item	**339 μm**	**485 μm**	**677 μm**	**865 μm**	**Pooled SEM**	**Linear**	**Quadratic**
CP, %	75.5	77.2	73.7	72.8	5.63	0.28	0.83
Indispensable AA, %							
Arg	89.2	93.3	94.9	92.0	2.69	0.33	0.07
His	80.9	83.2	84.3	82.3	2.73	0.47	0.17
Ile	73.9	76.3	77.7	76.4	4.85	0.68	0.38
Leu	84.1	85.1	86.3	83.0	2.45	0.63	0.14
Lys	74.8	75.8	73.6	74.3	6.53	0.60	0.98
Met	82.7	82.9	83.1	81.5	3.04	0.47	0.76
Phe	80.6	82.7	82.8	81.6	3.33	0.97	0.34
Thr	66.9	70.7	74.5	66.4	7.19	0.99	0.15
Trp	70.6	78.2	80.4	71.7	6.78	0.86	0.03
Val	74.1	77.4	79.8	78.9	4.73	0.22	0.29
Mean	77.8	80.6	82.0	78.6	4.06	0.79	0.36
Indispensable AA, %							
Ala	82.5	81.6	81.6	80.0	3.62	0.23	0.91
Asp	77.5	77.6	79.0	78.1	4.67	0.95	0.79
Cys	74.0	76.1	79.3	76.6	3.95	0.25	0.25
Glu	84.3	84.8	85.5	82.7	2.65	0.42	0.30
Gly	84.8	95.5	98.1	87.2	9.04	0.75	0.08
Ser	81.7	82.5	85.4	79.6	3.69	0.68	0.15
Tyr	76.6	78.3	78.2	76.4	4.63	0.56	0.44
Mean	80.2	82.3	83.9	80.1	3.87	0.01	0.43
All AA	**78.8**	**81.3**	**82.8**	**79.2**	**3.72**	**0.33**	**0.47**

[1] Data are least squares means of 10 observations.

[2] Values for SID were calculated by correcting the values for AID for basal ileal endogenous losses. Basal ileal endogenous losses were determined (g/kg of DMI) as CP, 21.50; Arg, 0.84; His, 0.18; Ile, 0.37; Leu, 0.60; Lys, 0.56; Met, 0.09; Phe, 0.37; Thr, 0.59; Trp, 0.13; Val, 0.51; Ala, 0.77; Asp, 0.86; Cys, 0.18; Glu, 1.06; Gly, 2.20; Ser, 0.51; and Tyr, 0.32.

with observations by Liu et al. (2012), who reported that reduction of particle size in distillers dried grains with solubles did not influence the ATTD of P. Thus, it appears that reduction in particle size or increases in surface area are not effective in improving P digestibility in pigs.

Exp. 3: Growth performance of growing finishing pigs

The overall G:F was increased (liner, $P < 0.05$) as the particle size increased from 339 to 865 μm (Table 4), but there were no differences (linear, $P > 0.05$) in the starting weight or in final weight among dietary treatments. Likewise, no differences (linear, $P > 0.05$) among treatments were observed for overall ADG and ADFI. The observation that no differences in growth performance among treatments were observed confirms that the differences in ME among the 4 batches of corn that were observed in Exp. 2 are of practical importance and can be used in diet formulations.

There were no differences (linear, $P > 0.05$) in live BW, carcass fat-free lean (**FFL**), and HCW among dietary treatments (Table 5). However, the dressing percentage was reduced (linear, $P < 0.01$) for pigs fed the diet corn ground to the greatest particle size compared with pigs fed the diet with corn ground to the least particle size. The back fat, LEA, and pH of LEA were not different (linear, $P > 0.05$) among dietary treatments.

Table 3: Concentration of digestible and metabolizable energy, and apparent total tract digestibility (ATTD) of energy and P and standardized total tract digestibility (STTD) of P that was ground to different particle sizes, as-fed basis, Exp. 2[1]

Item	Corn particle size					*P*-value	
	339 µm	485 µm	677 µm	865 µm	Pooled SEM	Linear	Quadratic
ATTD of GE, %	91.6	90.3	89.2	88.7	0.51	< 0.01	< 0.01
DE, kcal/kg	3,547	3,492	3,441	3,402	24.58	0.01	0.01
DE, kcal/kg DM	4,097	4,035	3,978	3,932	28.40	0.02	0.02
ME, kcal/kg	3,432	3,371	3,346	3,311	19.54	< 0.01	< 0.01
ME, kcal/kg DM	3,964	3,895	3,868	3,826	22.58	< 0.01	< 0.01
ATTD of P, %	29.6	31.0	31.1	31.3	3.32	0.67	0.81
STTD of P[2], %	37.8	37.1	37.3	37.4	2.99	0.99	0.87

[1] Data are means of 10 observations per treatment.

Table 4: Growth performance of pigs fed diets containing corn ground to different particle sizes[1]

Item	Corn particle size, µm					*P*-value		Sex			
	339	485	677	865	Pooled SEM	Linear	Quadratic	Barrows	Gilts	Pooled SEM	*P*-value
BW, kg											
Day 0	32.00	32.19	31.93	31.97	0.44	0.47	0.64	32.57	31.49	0.27	0.01
Day 29	61.86	62.33	62.07	62.81	1.18	0.40	0.84	64.87	59.67	0.58	< 0. 01
Day 58	93.72	93.83	94.10	94.72	2.16	0.53	0.85	99.38	88.80	0.98	< 0. 01
Day 93	129.81	130.25	128.58	129.97	2.90	0.88	0.75	136.24	122.98	1.41	< 0. 01
ADG, kg/d											
Day 0 - 29	0.99	1.00	1.01	1.03	0.03	0.30	0.79	1.07	0.94	0.01	< 0. 01
Day 29 - 58	1.06	1.05	1.07	1.06	0.04	0.80	0.93	1.15	0.97	0.02	< 0. 01
Day 58 - 93	1.03	1.04	0.98	1.01	0.17	0.29	0.71	1.05	0.98	0.02	< 0. 01
Day 0 to 93	1.03	1.03	1.02	1.03	0.03	0.97	0.76	1.09	0.96	0.01	< 0. 01
ADFI, kg/d											
Day 0 - 29	2.15	2.13	2.13	2.11	0.07	0.50	0.98	2.29	1.97	0.03	< 0. 01
Day 29 - 58	3.11	3.05	3.03	3.01	0.12	0.27	0.70	3.35	2.74	0.05	< 0. 01
Day 58 - 93	3.47	3.44	3.32	3.24	0.12	0.04	0.85	3.63	3.10	0.06	< 0. 01
Day 0 to 93	2.94	2.90	2.85	2.81	0.10	0.09	0.98	3.12	2.63	0.04	< 0. 01
G:F											
Day 0 - 29	0.46	0.47	0.47	0.49	0.01	0.03	0.52	0.47	0.48	0.01	0.38
Day 29 - 58	0.34	0.35	0.36	0.35	0.01	0.12	0.54	0.34	0.35	0.01	0.12
Day 58 - 93	0.30	0.30	0.30	0.31	0.01	0.19	0.31	0.29	0.32	0.01	< 0. 01
Day 0 to 93	0.35	0.36	0.36	0.37	0.01	0.02	0.56	0.35	0.37	0.01	< 0. 01

[1] Data are means of 18 observations per treatment, except for the treatment with corn particle size of 677 µm, which had only 17 observations.

[2] There was not interaction effect between diet and sex for all the variables except for ADFI d 0 to 29, G:F d 29 to 58, and G:F d 0 to 93.

The reduction in dressing percentage that was observed for pigs fed diet containing corn ground to a greater particle size is mainly due to the increase in the intestinal weight for pigs fed the diet with the greatest particle size compared with pigs fed the other diets. This observation is in agreement with data published by Wondra et al. (1995a).

Overall, results of the growth performance experiment confirmed that the values for ME that were determined in Exp. 2 can be used in practical diet formulations without negatively impacting pig performance or carcass quality. Thus, there were no indications that the reduced inclusion of soybean oil in the diets had any negative influence on pig performance. The savings in diet formulations that are achieved by reducing the amount of added fat in the diets, therefore, will result in increased profit form the pigs.

References

1. Adeola, O. 2001. Digestion and balance techniques in pigs. In: A. J. Lewis and L. L. Southern, editors, Swine Nutrition. 2nd. CRC Press, Washington, DC. p. 903–916.

2. Fastinger, N. D., and D. C. Mahan. 2003. Effect of soybean meal particle size on amino acid and energy digestibility in grower-finisher swine. J. Anim. Sci. 81:697–704.

3. Healy, B. J., J. D., Hancock, G. A. Kennedy, P. J. Bramel-cox, K. C. Behnke, and R. H. Hines. 1994. Optimum particle size of corn and hard and soft sorghum for nursery pigs. J. Anim. Sci. 72:2227–2236.

4. Kim, I. H., J. D. Hancock, J. W. Hong, M. R. Cabrera, R. H. Hines, and K. C. Behnke. 2002. Corn particle size affects nutritional value of simple and complex diets for nursery pigs and broiler chicks. Asian-Aust. J. Anim. Sci. 15:872–877.

5. Laurinen, P. H., Siljander-Rasi, J. Karhunen, T. Alaviuhkola, M. Nasi, and K. Tuppi. 2000. Effects of different grinding methods and particle size of barley and wheat on pig performance and digestibility. Anim. Feed Sci. Technol. 83:1–16.

6. Liu, P., L. W. O. Souza, S. K. Baidoo, and G. C. Shurson. 2012. Impact of DDGS particle size on nutrients digestibility, DE and ME content, and flowability in diets for growing pigs. J. Anim. Sci. 90:4925–4932.

7. Mavromichalis, I., J. D. Hancock, B. W. Sense, T. L. Gugle, G. A. Kennedy, R. H. Hines, and C. L. Wyatt. 2000. Enzyme supplementation and particle size of wheat in diets for nursery and finishing pigs. J. Anim. Sci. 78:3086–3095.

8. Noblet, J., and X. S. Shi. 1993. Comparative digestibility of energy and nutrients in growing pigs fed ad libitum and adult sows fed at maintenance. Livest. Prod. Sci. 34:137–152.

9. NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

10. Oryschak, M. A., P. H. Simmins, and R. T. Zijlstra. 2002. Effect of dietary particle size and carbohydrase and/or phytase supplementation on nitrogen and phosphorus excretion of grower pigs. Can. J. Anim. Sci. 82, 533–540.

11. Rojas, O. J., and H. H. Stein. 2013. Phosphorus digestibility and concentration of digestible and metabolizable energy in corn, corn co-products, and bakery meal fed to growing pigs. J. Anim. Sci. Submitted.

12. Stein, H. H., B. Sève, M. F. Fuller, P. J. Moughan, and C. F. M. de Lange. 2007. Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: Terminology and application. J. Anim. Sci. 85:172–180.

13. Stein, H. H., C. F. Shipley, and R. A. Easter. 1998. Technical Note: A technique for inserting a T-cannula into the distal ileum of pregnant sows. J. Anim. Sci. 76:1433–1436.

14. Wondra, K. J., J. D. Hancock, K. C. Behnke, R. H. Hines, and C. R. Stark. 1995a. Effects of particle size and pelleting on growth performance, nutrient digestibility, and stomach morphology in finishing pigs. J. Anim. Sci. 73:757–763.

15. Wondra, K. J., J. D. Hancock, K. C. Behnke, and C. R. Stark. 1995b. Effects of mill type and particle size uniformity on growth performance, nutrient digestibility, and stomach morphology in finishing pigs. J. Anim. Sci. 73:2564–2573.

16. Wondra, K. J., J. D. Hancock, G. A. Kennedy, K. C. Behnke, and K. R. Wondra. 1995c. Effects of reducing particle size of corn in lactation diets on energy and nitrogen metabolism in second parity sows. J. Anim. Sci. 73:427–432.

17. Wondra, K. J., J. D. Hancock, G. A. Kennedy, R. H. Hines, and K. C. Behnke. 1995d. Reducing particle size of corn in lactation diets from 1,200 to 400 micrometers improves sow and litter performance. J. Anim. Sci. 73:421–426.

Table 5: Weights of carcass of growing pigs fed diets containing corn ground to different particle sizes[1]

Item	Corn particle size, µm					*P*-value		Sex			
	339	485	677	865	Pooled SEM	Linear	Quadratic	Barrows	Gilts	Pooled SEM	*P*-value
Live wt, kg	127.38	127.35	126.96	127.45	2.88	0.99	0.88	133.70	120.74	1.42	< 0.01
Dressing[2], %	80.29	79.82	79.78	79.30	0.31	< 0.01	0.97	80.27	79.32	0.17	< 0.01
FFL[3], %	52.74	51.71	53.06	53.29	0.99	0.31	0.46	50.68	54.77	0.49	< 0.01
Hot carcass wt, kg	102.31	101.67	101.30	101.10	2.44	0.57	0.87	107.35	95.76	1.15	< 0.01
Back fat, cm	2.25	2.48	2.22	2.23	0.19	0.53	0.51	2.69	1.88	0.09	< 0.01
LEA[4], cm	52.92	51.32	53.44	54.52	1.43	0.19	0.35	52.46	53.65	0.89	0.35
pH at LEA[5]	5.62	5.63	5.57	5.60	0.03	0.25	0.65	5.63	5.58	0.02	0.07

[1] Data are means of 18 observations per treatment, except for the treatment with corn particle size of 677 µm, which had only 17 observations.

[2] Dressing, % = HCW / live wt × 100.

[3] FFL, % = carcass fat-free lean as calculated from NPPC (1999): pounds fat free lean = 8.588 – (21.896 × 10th rib fat depth, in.) + (0.465 × HCW, lb.) + (3.005 × 10th rib loin muscle area, sq. in.), (pounds FFL / HCW) × 100 = %FFL.

[4] LEA = Loin eye area.

[5] pH = It was took at the 10th rib in the loin eye (longissimus dorsi).

Nutrition and health interactions: A practitioner's perspective

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Historically, diets for growing pigs have been formulated for optimal lean tissue accretion. Since the 1970's many trials have been done to establish levels for amino acids, energy, macro-nutrients and micro-nutrients to optimize growth. Some of these trials would include whole body tissue analysis which involved grinding the complete pig up in order to measure all the building block pieces. These values with the known or assumed absorption and losses (via urine, fecal and etc.) would be used to determine the feeding requirements for each of these nutrients. The increase capability of measurement techniques has increased our ability to get these assumed requirements down to very precise levels. Many nutritionists have been willing to narrow the margins of error because of the perceived accuracy of our knowledge. The inherit problem with these trials is that they did not always account for the increase requirements for other issues the pig may encounter in its life in the "real world" such as environmental stressors or disease challenges.

It has long been known that the stimulation of the immune system has growth implications. These have been believed to be a combination of decrease intakes and likely an increase in nutrient reallocation but the exact process was not completely understood. These effects were seen and measured as decreases in growth, feed efficiency and lean tissue accretion. These effects were seen whether the immunological challenge was disease or vaccination in origin. The level and duration of the changes were normally longer during the disease challenges but we would expect the response to be organism specific. The specific immunological by-products released that decrease appetite are assumed to be there for some protective reason but the effect on either the digestibility or absorption of nutrients during these health challenges may be reversible. The trials done to determine growth requirements may easily have missed these survival requirements due to the nature of most research facilities conducting these trials would have had very clean pigs. The decrease performance was accepted as normal and we just tried to make them all healthy.

In the Midwest we have spent most of our lives feeding diets to pigs that were comprised mainly of corn and soybean meal. Crude protein levels were always consider high due to formulating primarily for lysine needs for optimal growth, feed efficiency and lean tissue accretion. Years ago technology brought us products (synthetic AA's) that gave us the ability to decrease SBM in pig diets. Lysine, Methionine, Threonine and Tryptophan along with the use of dried distiller grains allowed diets to be formulated with SBM completely out. It was during a research trial using synthetic AA's that while experiencing a disease challenge the pigs on the highest SBM were the least affected for growth rate and feed efficiency. At first the data looked like it may not be useable due to the health challenge but then the fact that there was such a difference in the clinical response prompted us to ask some questions. The experts we spoke with looked at the data and then went back in some of their own work and found similar results that had been unexplained. Many others gave their opinions as to what may actually be present SBM that would help support the beneficial immunological response these pigs appeared to express. This prompted some controlled studies to be done in an effort to validate the hypothesis. We all have seen groups of pigs that have suffered 15–20% yet there are pigs present in the population that appear to have not been affected. Why?

Gene Gourley and I decided to put together a group of experts that could try and explain what was really going on in these pigs. The mission was to find out what may be happening in these pigs that might be helping them through these challenges, was it a missing nutrient, AA or something else that when SBM was too low they did not possess. This required the development of a disease challenge model that would consistently create a consistent clinical response every time so that possible solutions could be applied and tested. Different disease challenges were discussed and a PRRS challenge model was decided upon. We approached the process looking at multiple physiological parameters and the differences between the top performers of a challenge group and the bottom ones. I will leave the discussion of these first trials to the lead researchers in this session. Once the model is established and specific parameters are defined we should be able to apply possible solutions to see if we can eliminate or minimize the negative effects of the disease challenges.

The issue with trying to determine if we should or can feed pigs differently during health challenges is still being

debated but without the ability to measure the differences we will get nowhere. There are defensive reasons why pigs react they way they do to certain health challenges that we may not what to decrease or divert. The reduction in appetite may be a way to restrict the growth of organisms in the animal thus protecting it. But if part of the growth performance reduction is due to inadequate availability of a specific nutrient, the inability to absorb a nutrient or the lack of ability to digest the source of that nutrient maybe we can formulate diets that provide a solution to one or more of these or maybe we should just feed very basic diets and save the money? The answer here may help us prepare animals better for the immunological response required during a vaccination. Maybe less adjuvant can be used to get the same protection? The answers to this immunological response question is becoming more and more pertinent as we are put under more and more pressure to reduce the use of antibiotics in food animal production.

The impact of PRRSV on feed efficiency, digestibility and tissue accretion in grow-finisher pigs

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The economic losses caused by Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) infection are estimated to cost the US swine industry more than $640 million annually (USDA, 2008). While significant advances have been made through research efforts to enhance our understanding of PRRSV at the animal health, immunological and genomic level, this disease still remains a significant issue in the US swine industry. Although we clearly know that PRRSV attenuates ADG of production pigs, its direct impact on feed efficiency, nutrient and energy digestibility, and whole body lean and fat accretion in grow finisher pigs has been poorly characterized. Therefore, the overall objective of this project was to characterize the impact PRRSV challenge has on grow-finisher pig feed efficiency, energy and nutrient digestibility, and tissue accretion rates.

To achieve this objective, 60 Choice Genetic maternal line gilts (33 ± 3 kg BW) where selected. These gilts came from 30 sows and consisted of live weight matched littermate pairs. After a nursery period, the litter mates were then split into two separate barns and placed into five pens (n = 6 pigs/pen). Pigs in both barns were fed corn-soybean-DDGS diets containing the non-digestible marker titanium dioxide. All pigs had ad libitum feed and free access to water. Pigs were of high health status and PRRSV naïve. One barn was then inoculated intramuscularly with a live field strain of PRRSV (PRRVS+) and the other barn with saline (PRRSV-). Blood samples were collected for PRRSV titers and feed intake and body weight gains were recorded for each pen over a 70-80 day performance period. At 19-21 days post inoculation (dpi), total tract fecal samples were collected for nitrogen and energy digestibility analysis. To longitudinally assess the impact of PRRSV on tissue accretion, at -1 and 80 dpi, all 60 pigs were sedated and scanned using a dual X-ray absorptometry machine (DXA). The DXA scan enabled us to measure whole body fat, lean, protein, water, and bone mineral content in a non-destructive way. DXA scan data was then corrected to account for gut and bladder fill using regression equations.

All pigs were PRRSV naïve before the study started and the 30 PRRSV- pigs remained PRRSV negative throughout the study. Within the first seven dpi, the PRRSV+ gilts had a log adjusted PRRSV genomic titer of 4.6 and as expected they had all seroconverted by 35 dpi. No mortalities or secondary infections were reported in the study. The impact of PRRSV challenge on pig performance is shown in Table 1. As expected, body weights where negatively affected by the virus challenge and growth rates were reduced by 20% compared to the PRRSV- pigs ($P < 0.01$). PRRSV+ gilts also had significantly ($P < 0.05$) reduced ADFI and G:F compared to their healthy counterparts over the test period (-7% and -15%, respectively, Table 1). This was especially apparent over the first 28 dpi (data not shown). Proximate analysis of feed and fecal grab samples highlight that apparent total tract digestibility of dry matter, nitrogen and energy were all significantly reduced at 19-22 dpi ($P < 0.001$). The digestibility coefficients for dry matter (81.5 vs. 79.8), nitrogen (80.1 vs. 78.0) and energy (78.1 vs. 76.3) were all reduced by 3-5% for PPRSV- vs. PRRSV+ pigs, respectively. Knowing that PRRSV infection alters ADG, we wanted to know if the composition of gain was affected. Longitudinal DXA scan analysis showed that PRRSV+ gilts had significantly lower lean, protein, fat and bone accretion rates compared to their PRRSV- counterparts (Table 1).

In summary, this ongoing project is clearly demonstrating that PRRSV infection reduces not only ADG, but also ADFI, coefficients of apparent total tract digestibility, and feed efficiency in grow-finisher gilts. Furthermore, lean/protein and fat accretion rates all appear to be affected to a similar extent. Additional blood analysis and carcass data from this project will allow us to better understand the metabolic impact of PRRSV in pigs and to calculate the economic impact of this health challenge in a grow-finisher production setting. This work has been supported by the National Pork Board grant #12-163 and Choice Genetics.

Table 1: Overall growth performance and whole body accretion rates of gilts challenged with PRRSV

Parameter	PRRSv-	PRRSv+	SE	*P*-value
Start BW[1], kg	33.0	34.2	0.15	0.66
End BW[1], kg	94.8	85.7	0.02	< 0.05
ADG[1], kg/d	0.90	0.72	0.07	< 0.01
ADFI[1], kg/d	1.98	1.84	0.046	< 0.01
G:F[1]	0.46	0.39	0.021	< 0.05
Lean[2], g/d1	633	547	13.5	0.001
Protein[2,3], g/d	126	109	2.53	0.001
Fat[2], g/d	205	169	7.05	0.001
Bone mineral[2], g/d	12.0	11.0	0.35	0.006

[1] n = 5 pens/trt, 6 pigs/pen

[2] n = 30 gilts/trt

[3] Predicted based on DXA lean (Chemical protein = DXA Lean x 0.2006 - 0.6611)

Feeding options to fit pig health

Don Giesting

Introduction

The challenge of keeping young pigs healthy and growing after weaning can be complex and bewildering. Pigs are quite naïve in terms of digestive and immunological development, at the same time they are being stressed by removal from the sow, mixing with other pigs, traveling, changes in feed source and form, and exposure to new organisms in their environment. The common problems of delayed and low feed intake, diarrhea, and short or longer term disruptions in growth are commonly recognized. There remains much debate about which aspects of the challenges to address, and how best to do so. This presentation will seek to provide some review and some perspectives on how pig health and performance in the starter period might be improved.

The pig at 2½ to 4 weeks of age

Pigs are born quite naïve and have evolved to develop immunity, intake, and digestive capacity over the first 8-10 weeks of their lives, while still benefiting from maternal protection and nutrient supplementation. Commercial production does not afford these options, as we try to optimize efficiency of the overall enterprise. Thus, we try to support the early-weaned pig with an artificial rearing system that is warm, sanitary, and able to provide all required nutrients in palatable and digestible forms, balanced to meet the pig's rapidly changing requirements. Unfortunately, pigs vary in their adaptability to the rigors of weaning, unfamiliar and less digestible nutrient sources, and microbial challenges in their new environment. Failure to strive is apparent, when diarrhea hits, pigs fail to eat, uniformity of the group is destroyed, and morbidity and mortality spike.

What we control

Weaning age is a crucial fundamental factor establishing the degree of challenge for us to meet the needs of young pigs. Weaning generally causes loss of mucosal integrity and tight junctions that increase gut permeability and reduce nutrient absorption and reduce brush-border enzymes. In general, the younger the pig, the more vulnerable they are to these changes after weaning. Recent research shows that younger pigs not only are more affected by weaning, including lower barrier function, more inflammation, and gut mucosa atrophy, but that the insults of weaning persist longer in earlier weaned pigs. Increasingly, producers are determining that pushing back weaning to avoid weaning pigs less than three weeks of age, improves outcome in many systems.

Creep feed exposure has been viewed from many directions, as a terrible waste of money and nutrients, to a crucial tool to introduce pigs to dry feed as a necessary source of nutrients when the sow is removed. While pigs consume very low amounts of creep feed prior to about 18 days of age, studies suggest that the learning value and the stimulation of digestive and immune functions in the pig may well over-ride the cost and labor challenges of providing creep feed before weaning. Of course, as weaning age increases beyond 21 days, the importance of creep feeding to provide supplemental nutrients to support the rapidly increasing appetite and digestive capacity of the unweaned pig becomes more apparent.

Group size, handling, sorting practices, feeder and water design all affect the stress experienced by young pigs. From the pig's point of view, weaning is a bewildering experience. The loss of maternal security, the need to learn not only the location of food and water, but the necessity to consume dry feed and water in reasonable meal sizes and proportions are not learned until after weaning, during a highly stressful period. Not surprisingly, the time it takes for pigs to consume feed after weaning, the pattern of meal size, and the time between meals is seen to be highly variable among pigs. Delays in food and water intake have been shown to increase the severity of gut damage, lower the rate of gain, destroy feed efficiency, and increase the risk of disease. Any adjustments in management that can limit stress and delay in feed and water intake can be expected to help commercial success of the enterprise.

Assuming we have made the best choices we can to limit stress (social and environmental), to allow pigs' easy access to feed and water, and that we are weaning at the oldest practical age for our system, we now need to grapple with the feed itself.

Don Giesting

Customizing diets to enhance pig success

As with other aspects of post-weaning management, feed design is a continuous effort to strike the balance among numerous variables that affect pig performance, health, and cost of production. When we venture too far from optimal nutritional balance and high digestibility, we increase risks of digestive and performance disruption and poor efficiency. If we recognize the risks and the options, we can better determine the solution we should pursue.

In simple terms, pigs need proper levels of digestible and absorbable amino acids and energy to match their digestive development. Anti-nutritional factors, including mycotoxins, antigenic proteins, protease inhibitors, and some types of poorly digested carbohydrates and proteins that may be of little consequence to older pigs, can disrupt newly weaned pigs. Proteins that are not digested in the stomach and small intestine can be fermented in the lower bowel, producing ammonia, amines, phenols, and indoles that cause looseness and poorer efficiency.

Some amino acids which are not considered essential for older pigs, may be essential during the post-weaning period. Glutamate and glutamine are important energy sources for the gut, enhancing gut wall integrity and repair. They may also have roles as precursors for glutathione, a crucial endogenous antioxidant molecule. They also seem to stimulate both innate and adaptive immunity. Alanine, glycine, and arginine sometimes show benefits during post-weaning recovery from gut damage.

Protein sources are more critical to young pigs, whose digestive enzyme levels are limited and who have rapid digesta passage rate compared with older pigs. Dried animal plasma has been shown to greatly enhance the intake and gain, and often gut health, of young pigs. The benefit goes beyond provision of digestible nutrients or simply passive immunity from antibodies in the plasma. Recent research suggests that cytokines and factors that limit inflammation support better gut integrity. Properly processed milk or other animal proteins or specially processed soy products have shown benefits in the first 2 weeks, or so, after weaning.

Some fiber sources, including some relatively purified soluble fibers may support increased and more stable commensal bacterial populations that help reduce overgrowth of pathogens. They produce increased volatile fatty acids, especially butyrate that may help repair the mucosa of the intestine. Modest levels of insoluble fiber may help adaptation to dry diets by stimulating intake and gain, increasing brush border enzymes responsible for digestion of partially digested peptides and starches to allow them to be absorbed, and reducing E. coli attachment and proliferation in the small intestine. On the other hand, some partially digested starches and fibers, especially from small grains, may inhibit digestion of proteins, and favor gas production in the lower tract.

Starch is commonly the primary energy source in young pig diets, but some starch, including corn starch is not rapidly digested by young pigs and may arrive in the lower tract where it is fermented. The value of processing starches by pelleting or other method to increase rate of digestion, especially by a gut hindered by post-weaning damage, generally, is most clearly beneficial to performance, in young pigs.

Additives to support young pig performance

Additives of many types have been shown to improve health, gain, and efficiency in young pigs. However, it is not always straight-forward to determine which additives to use, which additives are complementary to each other, or to know which additives may be only effective in unusual situations. A great effort is ongoing to understand more about the physiological and immunological effects of different additives for young pigs. Newer methods are helping to elucidate the many impact points additives can provide.

Traditional application of antibiotics and pharmacological levels of zinc oxide and copper have generally been credited with controlling deleterious bacteria. While these effects are clear, the modes of action are still being untangled and vary among these compounds. Some impacts appear to be direct impacts on the gut or the immune system. Recent research shows zinc oxide increases immune system development and improves gut barrier function in young pigs.

Prebiotics are an additive type providing conditions that directly or indirectly reduce the opportunity for pathogenic bacteria to become established, especially gram negative species of E. coli and salmonella. Perhaps, the most effective prebiotic for pigs is an ingredient commonly viewed not as a prebiotic, but as an energy source. Shortly after weaning, most diets contain lactose (milk sugar). At least part of dietary lactose is fermented by lactic acid bacteria to produce lactic acid and other metabolites that favor commensal bacteria populations and inhibit pathogens. Probiotics may populate the gut (Enterococci, Lactobacilli) or in the case of some bacilli, may favor lactic acid bacteria, and produce enzymes, or other useful metabolites. Organic acids used individually or more often in combinations, exert antimicrobial effects on pathogens, but may not impede favorable commensal bacteria, thus enhancing the stability and health of the pig's digestive tract.

Plant derived additives, including essential oils or other extracts, are being studied and showing promise to address some of the fundamental challenges in the gut of early weaned pigs. Plant-based antioxidants may help pigs overcome losses of antioxidant function, at the gut level, that occur with weaning and bacterial overgrowth. Other plant extracts have been shown to reduce inflammatory responses by reducing cytokines that stimulate cascade effects, resulting in gut damage. Some plant extracts show promise in the face of PRRS or other viral challenges, not as a primary treatment, but to enhance the ability of the pig to cope with the effects of virus and limit immune system over response.

On-going research using newer techniques, supported by practical feeding experiments and field validations will continue to help us clarify not only the modes of action, but the relative importance of additives and nutritional sources to help young pigs through the rigors of post-weaning growth. The day may come, when this information is well-enough defined that young pig models can be advanced and applied and allow us to predict the effect of a change in production condition on the pig, and to compensate with an appropriate nutritional adjustment to maintain or improve enterprise profitability.

What we are seeing at the diagnostic lab

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Introduction

Swine diets are tailored for maximum growth and not necessarily for optimal bone strength. Complicating this potential issue, that can result in clinical lameness, is ingredient cost, alternative feed sources and variability in feedstuffs. Not to mention NRC values for bone composition maybe outdated with today's industry practices or genetics.

Bone is a dynamic organ and continues to remodel after growth is complete. Besides mechanical strength, bone is crucial to body calcium homeostasis. Over 99% of body calcium is stored within bone.[1] Following extracellular depletion of calcium for numerous cellular functions; a symphony of intricate events allows calcium to be pulled from existing bone. This finely regulated system will ultimately result in increased calcium absorption from the diet if adequate vitamin D is available. Phosphate, also a large component of bone matrix, is required for mineralization and is released when body demands increase.

In the not so distant past, metabolic bone disease issues arose within the swine industry resulting in clinical lameness and even increased mortality. Metabolic bone disease broadly categorizes disturbances related to bone formation and remodeling.[2] Disease can be gradual or abrupt and is associated with inadequate calcium, phosphorus, vitamin D, or hormonal problems related to parathyroid hormone.

Mechanisms of metabolic bone disease

1. Inadequate dietary supplementation of vitamin D_3
2. Inadequate absorption of phosphorus due to low phosphorus in diet, phosphorus bound to phytate and therefore unavailable, and inadequate or ineffective phytase usage
3. Inadequate dietary calcium
4. Imbalance of feed calcium to phosphorus ratio; improper formulation of Ca:P ratio in diet (should be roughly 1.2:1)[1]

Terminology

Rickets and osteomalacia are the disease names associated with metabolic bone disease in growing animals and adult animals, respectively. The cause and pathogenesis of these two diseases are similar; rickets involves growing bones while osteomalacia occurs with mature bone (remodeling issues).[2]

Osteoporosis is disorder of bone that is classified under the umbrella of metabolic bone disease as well. This term is a lesion rather than a process as in rickets or osteomalacia, and denotes overall decreased bone mass. Bones are structurally normal, but become increasingly brittle (easily snap). Osteoporosis results from decreased caloric intake, decreased dietary calcium, lactation, or from increased stress.[2]

Fibrous osteodystrophy (FOD) is a consequential lesion that is common in pigs with bone disorders. Primary FOD is caused by excessive production of parathyroid hormone. Parathyroid hormone is responsible from initiating the release of calcium from bone. Secondary FOD is associated with dietary or renal problems which result from inadequate dietary calcium or loss of calcium through the kidney. Secondary FOD is usually what is occurring in pigs with metabolic bone disease. In short, bone is replaced by fibrous connective in a response to add structural support.

What was seen?

Clinical presentation

Clinical signs of swine metabolic bone disease depend on the age of pigs, the specific mechanism involved, and the magnitude and the duration of the insult. Growing pigs with classic rickets will have weak bones that bend before they break, clinical lameness, and enlarged growth plates that can give the appearance of swollen joints. Limb conformation may be abnormal because of bending or bowing of bones, but this was not typical in swine cases. Of note, long hair and decreased appetite have also been reported for vitamin D deficient pigs and there is substantial evidence suggesting "humpback" formation is a manifestation of rickets.[3]

Gross abnormalities can take up to 4-6 months to develop in swine that are feed vitamin D deficient diets. The onset of disease is dependent on how severe the deficiency is.

However, chronic nutrient imbalances can have acute clinical manifestations other than pathologic fractures or rubbery or weak bones. Somewhat more unusual is the rather abrupt onset of clinical signs associated with acute hypocalcemia. Clinical signs of swine hypocalcemia include: tremors, tetany, seizure-like muscle fasciculations, weakness, lameness, painful gait with reluctance to move, and bone fractures (macroscopic and/or microscopic).

Gross lesions

Gross lesions, as with clinical signs, are highly variable. Gross lesions are often not detected. Bone fractures, rib calluses from previous fractures, rachitic lesions in growth plates may be apparent in other cases. Rachitic rosary lesions on rib bones are widened at the distal growth plate. Caudal ribs typically have more pronounced rachitic changes because these ribs are growing faster than cranial ribs.[2] Widened growth plates can be seen in few or multiple bones, and frequently there are no grossly widened plates. The most useful post-mortem finding is measured by accessing bone strength by breaking ribs. In the absence of overt lesions, spending time to assessing rib strength is recommended

Diagnosis

Diagnosing hypovitaminosis D or metabolic bone disease in swine is not difficult if disease is suspected clinically. It is difficult to diagnose if one is not aware or unintentionally excludes metabolic disease from the differential list. Serum levels of calcium, phosphorus, and vitamin D are a vital diagnostic inquiry in disease scenarios. Low values of calcium and/or phosphorus are often diagnostic, but normal values do not exclude the disease process. Low, reliably measured, serum levels of vitamin D are diagnostic for vitamin D deficiency (Table 1). Liver analyses for calcium or phosphorus do not reflect blood levels and are not a good estimate of overall calcium or phosphorus status since bone is the reservoir organ.

Microscopic examination of bone growth plates in growing animals can confirm and/or add confidence to the diagnosis of rickets. Microscopic changes in bone can take weeks to months to develop. Rickets lesions will resolve in 1-3 months after the deficiency is corrected. Bone can be used post clinically to indicate previous metabolic disturbance because microscopic changes take time to return to normal and/or remodel. In acute cases, bones changes may not be seen or location dependent.

Bone density and total bone ash are very useful diagnostic tools for confirmation of deficiency. Bone ash and density are abnormally low in clinical cases of rickets, osteomalacia and osteoporosis. Abnormal ash and density may take several weeks to develop during a deficient state and will gradually return to normal after dietary correction and restoration. This is similar to microscopic lesions.

For laboratory submission of bone for both histopathology and bone analysis, it is recommended that the entire 2nd rib be used for these analyses**.** Analysis of consistent specimen (e.g. 2nd rib) is essential to interpreting bone ash and density (Table 2). However, it is always recommended to submit lesions (rachitic rosaries) if visually present. Disarticulate the rib at its insertion to the vertebral body and make sure the costochondral junction and growth plate are included from the ventral attachment to sternum.

When metabolic bone disease is demonstrated, dietary factors are implicated as causal irrespective of infectious diseases present in the population. A problematic caveat to feed testing is that the feed associated with a clinical problem is seldom the feed that is on-site during an investigation. Routinely saving a feed sample from each feed delivery in the freezer is recommended. Retained samples can be evaluated, depending on results of initial testing of tissue or serum samples. The important point is that there is feed retained for testing.

Vitamin D research

Recently, Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) broadly surveyed serum vitamin D levels in 3-4 week-old pigs, 10-12 week-old pigs, late finishing, sows, and boars in Iowa and adjacent Midwestern states to try and determine if hypovitaminosis D is an industry wide problem. From each age group, 15 farms were identified on serum submission to the ISU-VDL. Serum

Table 1: Suggested serum vitamin D levels for by pig age.

Normal reference range for serum vitamin D	
Age of animal	**25-OH-D3 (ng/ml)**
Neonate	5-15
10 days	8-23
Finishing pigs	30-35
Mature	35-70
At parturition	35-100

Table 2: Normal reference intervals for bone ash, bone density, bone calcium and bone phosphorus in swine

Swine; bone	
Bone ash	58-62%
Bone density	1.4-1.5 g/ml.
Bone ash calcium	32-39%
Bone ash phosphorus	13-22%

from 8 pigs per farm were assayed for serum vitamin D. Samples were collected from pigs that had no history of metabolic bone disease. A total of 600 serum samples were tested in January 2011 during the short photoperiod time of year. Another 600 samples were randomly tested in June of 2011. In addition, serum samples were collected from groups of outdoor pigs in June of 2011as a non-confinement raised comparison. Ten samples from nursery, grower, finisher and sows were collected.

Mean serum vitamin D results were lower than both reference values (Table 1), and outdoor pig samples in all age categories (Table 3). Trends in both January and June confirm mature animals having greater vitamin D levels than younger animals. Results from both time periods indicate that nursery pigs, as a group, have a lower than expected serum vitamin D compared to reference values. There was considerable variation in all age groups, with individual pigs within groups deemed deficient. When January submissions were compared to June submissions, there was a significant increase in serum 25, (OH) D levels in both nursery and boar age categories ($P < 0.01$). When June submissions were compared to outdoor submissions, all age categories (with the exclusion of boars) had significantly lower serum 25, (OH)D levels than outdoor animals ($P < 0.01$).

Interpretation is more difficult because this is only a survey. Besides calcium absorption of the small intestine, vitamin D has been shown to have anticancer properties, reduce the risk of heart disease, and more importantly vitamin D is known to have a role in immunity. How this translates to swine is unknown.

Following the serum vitamin D survey, a bulk source vitamin D assessment was conducted through collaboration with feed suppliers. Bulk source vitamin D supplement samples were collected monthly from November 2011 through July 2012. Information recorded for each sample included: date of collection, date of manufacturing, expected concentration, manufacturer name, manufacturer country, lot number and supplier name. Each sample was then split, and sent to two separate laboratories (DSM and Heartland Assays, LLC) for vitamin D assays. A total of 45 supplement samples were collected, resulting in 90 assays completed.

The bulk source vitamin D assessment results showed no difference between manufactures or country of origin. No samples were found to be statistically lower than the expected concentration of 500,000 IU/lb. However, a month effect was revealed indicating vitamin D degradation during extended storage.

Confounding problems

Metabolic bone disease has heightened the sensitivity of lameness issues within the industry. But obviously not all lameness cases in grow-finish pigs are related to the above. In the past 18 months, the ISU-VDL has seen a slight percentage increase in the numbers of cases compared to before the "metabolic bone disease rise". Submitted cases run the gamut from acute clinical outbreaks of infectious disease to varied surveillance of the issue. The two etiologies that occur most frequently in these submissions to the ISU-VDL are osteochondrosis/osteochondrosis dissecans and *Mycoplasma hyosynoviae*.

Osteochondrosis is a common growth cartilage disorder in pigs and can affect both articular or physeal cartilage. The exact pathogenesis is sketchy, but multiple risk factors including rapid growth, heredity, trauma, and dietary factors are often implicated. Common lesion locations in swine are the distal humerus and femur, but can be seen elsewhere.[4]

Table 3: Combined serum 25, (OH) D results by age and month. Comparison of outdoor pig serum 25, (OH) D is also included.

	Serum 25, (OH)D levels (ng/ml)								
	January			June			January vs. June	Outdoor	June vs. Outdoor
Age category	Min	Avg	Max	Min	Avg	Max	(*P*-values)	Avg	(*P*-values)
Nursery (2-4wks)	2.1	8.42	56.2	3.0	13.75	62.4	< 0.01	58.54	< 0.01
Grower (10-14wks)	3.4	21.80	54.1	5.3	18.04	50.5	0.013	61.03	< 0.01
Finisher (6-8mon)	3.7	27.66	77.9	66.6	28.18	68.5	0.048	85.98	< 0.01
Sows	8.6	35.70	83.4	4.7	36.33	94.5	0.792	57.17	< 0.01
Boars	8.9	31.56	64.5	17.3	45.42	93.8	< 0.01	n/a	n/a

Mycoplasma hyosynoviae is vertically transmitted for dam to offspring and subsequently transmitted horizontally in the grow-finish phase of production. The bacteria can localize in one or multiple joints resulting in proliferative synovitis with excessive red-tinged and watery synovial fluid. In my experience, the stifle and elbow are common joints that are affected. In addition, PCR detection of *Mycoplasma hyosynoviae* is common practice, yet caution in the diagnosis is advised; detection does automatically yield cause.

Thoughts to ponder and diagnostic advice

1. A common diagnostic sampling mistake is submitting only rear legs without a clinical examine for localization purposes. Lame pigs lying on concrete with often form hygromas lateral to the hock. This is often misinterpreted as a swollen hock. The actual cause of lameness is elsewhere.
2. *Mycoplasma hyosynoviae* can be a primary etiologic agent for lameness; however, it can also be secondary to joint instability. It is sometimes difficult to determine which process occurred fist. It is like the old saying, "which came first the chicken or the egg"?
3. Would bone strength issues result in osteochondrosis? In other words, if a pig has subclinical nutritional issues with calcium, phosphorus, or vitamin D in the growing phase, would this lead to late clinical osteochondrosis issues? A similar thought could be made for *Mycoplasma hyosynoviae*. If there is reduced bone strength causing joint instability, would this be an advantage for *Mycoplasma hyosynoviae* replication and disease?

With the last thought above, I can't help but believe that there is an intricate web or etiology associated with bone strength, infectious disease, and osteochondrosis.

Conclusion

As of late, the number of metabolic bone disease case submissions has been declining. The decline is suspected do be related to awareness within the swine industry while improving quality control methods related feed ingredients, changes in diet formulations to enhance bone formation, and prophylactically treating with commercial supplements (i.e. oral vitamin D). However, there are still periodic cases or instances where metabolic bone disease issues are causing reduced profitability in the barn today.

When working through lameness cases, a good clinical examination followed by a complete diagnostic submission will aid in determining the pertinent etiology for the issue. Bear in mind though, that other lameness disorders may be a consequence of previous nutritional issues arising from decreased bone strength at some point during grow-out.

References

1. Crenshaw TD: 2001, Calcium, phosphorus, vitamin D and vitamin K. *In:* Swine Nutrition, Lewis, A. J. and Southern, L. L., 2nd: pp. 187–212. CRC Press LLC, Boca Raton, FL.

2. Thompson K: 2007, Bones and joints. *In:* Pathology of domestic animals, Maxie, MG, 5th: pp. 1–184. Elsevier, Philadelphia, PA.

3. Limeback H, Schlumbohm C, Sen A, Nikiforuk G: 1992, The effects of hypocalcemia/hypophosphatemia on porcine bone and dental hard tissues in an inherited form of type 1 pseudo-vitamin D deficiency rickets. J Dent Res 71:346–352.

4. Ytrehus B, Carlson CS, Ekman S: 2007, Etiology and pathogenesis of osteochondrosis. Vet Pathol 44:429–448

Clinical diagnosis of skeletal integrity in swine

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Introduction

Lameness and mortality issues in the swine industry have escalated in recent years (Madson et al., 2012). Many of the recent cases were attributed to hypovitaminosis D. The issues have spurred interest and questions about bone composition and the 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, but more definitive descriptors are needed to establish guidelines that are useful for the establishment of a balance between nutrient inputs required for animal well-being and the environmental issues that often pressure nutrient formulation strategies. The goal is to prevent deficiencies without contributing to excess nutrient supplementation. This review briefly summarizes basic principles involved in bone composition, the impact of dietary nutrients on bone composition, and critically assesses methods used to quantify bone integrity in clinical and research settings.

Bone tissue composition

As a wet tissue, bone is composed of 45% water, 10% fat, 20% organic matrix, and 25% inorganic mineral (Crenshaw, 2001). The water and fat content of bone varies with age, type of bone, and nutrient input. Thus, the mineral content expressed as a percentage of the dry, fat-free weight describes the extent to which the organic matrix has become saturated with mineral. On a dry, fat-free basis, approximately 56% of the entire skeleton is ash. However, the percent ash varies from 62 to 72% in cortical bone from mature sows (Crenshaw et al., 2012), but in young pigs the percent ash may be only 44 to 46% in bones that are mostly trabecular such as ribs (Rortvedt et al., 2012a) or vertebrae (45% in pigs at birth, 35% at day 25, Vanderwerff et al., 2013). Thus, the selected bone sample and animal age are critical determinants if percentage ash is used for diagnostic assessment of the mineral adequacy.

Despite the 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. The amount of inorganic material and the extent of mineralization of the organic matrix (percent ash) may vary, but the Ca and P composition of ash will not change (Crenshaw, 2001). If molar corrections of phosphorus for phosphate are calculated, approximately 90% of the inorganic material in bone can be attributed to Ca and P. The remaining minerals include Mg, Na, K, Cl, and trace minerals.

Roles for trace minerals in bone

As discussed by Crenshaw et al., 2012, trace minerals are integral nutrients involved in bone and connective tissue. In skeletal tissues, the roles of these nutrients are mostly associated with roles as cofactors for proteins involved in the synthesis and maintenance of the organic matrix rather than a role in the formation of mineral crystals per se. Primary roles for trace minerals include: Cu, a cofactor for lysyl oxidase and formation of collagen cross-links; Mn, glycosyltransferases involved in the formation of glycosaminoglycans that are structural components of the extracellular matrix; Zn, a cofactor for alkaline phosphatase and collagenases, with more recent identification of a role as an antagonist to the RANK and RANKL ligands involved in the coupling of osteoblast and osteoclast function to balance bone formation with resorption; and Fe, a co-factor lysine and proline hydroxylase enzymes involved in collagen cross-links.

Trace mineral deficiencies, excesses, or imbalances will result in bone integrity issues, but homeostatic mechanisms allow animals to adjust to a relatively wide range of trace mineral nutrient inputs. Bone integrity issues in commercial production animals are not commonly associated with trace mineral deficiencies. Over-supplementation or an imbalance of trace minerals are more common issues with trace minerals. With exception of Fe, the best method to clinically assess trace mineral status involves measurements of the trace mineral composition of bone ash. The trace minerals should be expressed as a percentage of the bone ash to remove variable concentrations relative to water, fat, and organic matrix content.

Which bone to sample?

Decisions on the selection of a single bone to represent the entire animal have been speculative and were based on which bone responded to dietary treatments (Crenshaw

et al., 1981), or whether the fibulae were easier to collect than femurs or metacarpal bones (Biehl and Baker, 1996). With development of techniques, such as dual energy x-ray absorpitometry (DXA), to accurately measure the ash content of the entire growing pig (Schneider et al., 2005) or feet (Hoffman et al., 2007), different inferences about responses to dietary treatments were identified if inferences were based on the fibula vs the femur of young pigs (Crenshaw et al., 2009). The conclusion that more accurate inferences were obtained with assessments of the femur was based on observations that the femur ash content provided a better fit to dietary P inputs than the fibula ash content relative to responses in the entire pig. 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.

In 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 bone mineral content. Thus, selection of limb bones from pigs at market weight (120 kg) are not as critical as the bone selected at younger ages (< 40 kg), consistent with earlier conclusions (Crenshaw et al., 1981).

Bone growth and maintenance - synthesis and accumulation

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 that affect the rate and accumulation of the organic matrix, but do not act directly to drive 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 compression load. Rather, most forces imposed on live animals involve a combination of the two loads to produce a bending load. One surface of the bone is compressed while the other surface is under tension. Thus, the combination of materials, collagen and mineral crystals, provide a cumulative response to forces that cannot be explained by a single material.

Guidelines to identify bone strength properties that reflect an acceptable range 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). Thus, the ash content of bone is not directly proportional to the strength properties. Additionally, procedures used to measure bone mechanical traits are not standardized across laboratories. The time required for mechanical

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 equations	
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

* 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.

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 issues, rather such test methods remain as a research technique for assessment of hypothesis-driven research projects.

Nutrient inputs alter bone ash and integrity

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 that 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.

The recent escalated lameness issues associated with vitamin D have highlighted concerns for quality control issues in diet formulations (Madson, 2013). Failures in quality control procedures for feed management may contribute to delivery of diets with un-intentional deficiencies. In recent studies we have focused on the ability of growing pigs to recover skeletal mineral content after a brief period of mineral deficiency. 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 (Aiyangar et al., 2010). However, femurs collected after the 8-wk recovery period had apparently regained equal strength properties as those in pigs fed control diets throughout the entire trial. Recovery of femur strength, but not whole body bone mineral content 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 bone mineral (ash) measurements and bone mechanical test properties. Similar results have also been reported in nursery pigs (Rortvedt et al., 2012b, growing-finishing pigs (Iwicki et al., 2011) and to an extent by other researchers (Létourneau-Montminy et al., 2011).

Which method to assess mineral adequacy?

The results about the selection of a bone specimen and the depletion-repletion studies illustrated differences in conclusions based on methods used. 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 three procedures, with exception to 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 applications often require rapid turn-around diagnosis 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 test require a longer turn-around time for results and are more expensive.

Conclusions

Clinical cases that involve lameness issues often lead to questions about the adequacy of nutrient formulations, especially dietary Ca and P, and more recently vitamin D concentrations. Attempts to correct the increased incidence of lameness by over-supplementation of nutrients most often do not resolve the problems and may contribute 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 bone mineral content.

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

References

1. 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.

2. Biehl, R. R and D. H. Baker. 1996. Efficacy of supplemental 1a-hydroxycholecalciferol and microbal phytase for young pigs fed phosphorus- or amino acid-deficient corn-soybean meal diets. J. Anim. Sci. 74:2960–2966.

3. Crenshaw, T. D., E. R. Peo, Jr., A. J. Lewis, B.D. Moser and D. Olson. 1981. Influence of age, sex, and calcium and phosphorus levels on the mechanical properties of various bones in swine. J. Anim. Sci. 52:1319–1329.

4. 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.

5. 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.

6. 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.

7. Crenshaw, T. D., D. K. Schneider, C. S. Carlson, J. B. Parker, J. P. Sonderman, T. L. Ward, M. E. Wilson. 2012. 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.

8. Hoffman, L. E., T. Burgers, D. K. Schneider, and T. D. Crenshaw. 2007. DXA scans of pig feet accurately predict bone ash content. J. Anim Sci 85 (Suppl. 1):511.

9. 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.

10. 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.

11. 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. Diagn. Invest. 24:1137–1144.

12. Madson, D. M. 2013. The rise and fall of clinical cases of vitamin D deficiency in commercial swine operations. J. Anim. Sci. 91 (e-Suppl.): *In-press.*

13. Rortvedt, L. A., P. M. Cline, C. Ryer, B. Frederick, K. J. Retallick, D. K. Schneider, and T. D. Crenshaw. 2012a. An oral dose of vitamin D at birth increased serum 25-OH D at weaning but failed to alter bone mineral density in pigs. J. Anim. Sci. 90 (e-Suppl. 2):115.

14. Rortvedt, L. A., D. K. Schneider, and T. D. Crenshaw. 2012b. 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.

15. Schneider, D. K. and T. D. Crenshaw. 2005. GE Lunar Prodigy DXA accurately predicts skeleton ash but not fat and lean composition in growing (1 to 60 kg) pigs. J. Bone Mineral Res. 20 (Suppl. 1):S332.

16. Vanderwerff, L. M., L. A. Rortvedt-Amundson, and T. D. Crenshaw. 2013. Kyphosis induced by maternal vitamin D intake is not explained by a reduction in vertebrae body mineral content of pigs at birth or weaning. J. Anim. Sci. 91 (e-Suppl.): *In-press.*

Nutritional requirement for calcium, phosphorus, and vitamin D in pigs

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Introduction

The objective of this manuscript is to review recent research information related to dietary requirements and utilization of calcium (Ca), phosphorus (P), and vitamin D (VitD) to optimize bone health, pig growth, farm profitability, and minimize environmental pollution. Nutritional programs for swine must take into account the essential roles of Ca, P, and VitD in: 1) metabolism and overall lean tissue and bone growth, 2) contributions to total diet cost, and 3) managing excess concentrations of P in manure to minimize environmental pollution (Crenshaw, 2001). Calcium and P are required for optimal bone mineralization, while VitD is required for proper Ca absorption and potentially pig health. Phosphorus is the third most expensive nutritional component of swine diets after energy and amino acids, and excess P in runoff manure may account for 33% of the P load into fresh water resources (UN-FAO, 2006).

The dietary requirements of nutrients for pigs has evolved from a static value expected to match all production circumstances to a dynamic value that accounts for multiple factors affecting the physiological processes related to maintenance, growth, and efficiency of nutrient utilization. Animal growth and subsequent nutrient requirements are affected by multiple factors such as growth rate, feed intake, and type of diet. Therefore, it is not correct to assume that nutritional recommendations will fit all animals in all production conditions.

Several published references provide a review of current nutrient requirement recommendations based on the revised "Nutrient Requirements of Swine" from the National Research Council (NRC, 2012). The NRC, 2012 along with newer mechanistic models of the fate of dietary P in the gastrointestinal tract and metabolic pathways in pigs allow for more precise estimates of dietary concentration and requirements of P for pigs (Schulin-Zeuthen et al., 2007; Létourneau-Montminy et al., 2011; Létourneau-Montminy et al., 2012a,b). The NRC (2012) and the newer mechanistic models are guidelines for nutritional recommendations and are useful to illustrate the variety of options that nutritionists use to meet requirements for Ca, P, and VitD in diets for growing pigs and sows.

Physiological functions of Ca, P, and Vitamin D

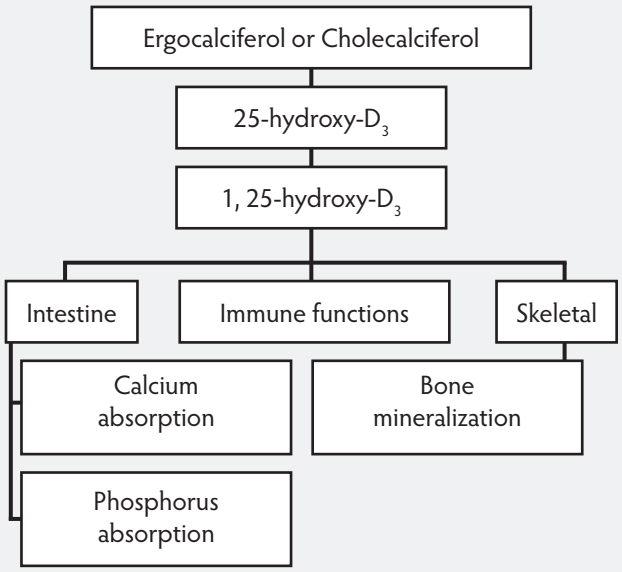
Despite their high concentrations in skeletal tissue (>90% of Ca, and > 60% of P), Ca and P also have important roles not only on bone structure, but also in nutrient metabolism, cell signaling, blood clotting, among others (Crenshaw, 2001). Calcium is deposited in skeletal tissue as hydroxyapatite, $Ca_5(PO_4)_3(OH)$, which may constitute about 50% of dry weight of bones. Deposition of both elements is necessary for proper bone formation and one mineral will not be deposited without the other (Crenshaw, 2001). Calcium also has metabolic regulatory properties. For example, Ca forms complexes with proteins allowing for reversible conformational changes and regulating the function of proteins. Phosphorus has structural functions in bone mineralization, is an intrinsic part of hydroxyapatite, and it is also constituent of phospholipids (Ewing and Charlton, 2007). Phosphorus also functions to regulate metabolism. Phosphorylation activates and deactivates enzymes, and energy transfer requires formation of P bonds.

The main link of VitD to Ca, P, and bone health is the role of VitD on intestinal absorption of Ca. Calcium is absorbed in the small intestine by active-transcellular and passive-paracellular pathway and VitD causes formation of Ca binding proteins for the active absorption of Ca (Wasserman and Taylor, 1966; Crystakos et al., 2011). The role of VitD in Ca absorption is key for bone mineralization. However, VitD has other non-structural roles in regulation of immune cells (Figure 1). Therefore, the nutritional requirements of these 3 nutrients depend on the balance and utilization of Ca, P, and VitD in all functions.

Concentration, utilization, and measures of Ca, P, and Vitamin D in feed ingredients

The concentration of Ca and P in feed ingredients, concentrates, and complete diets can be expressed on the basis of: 1) total dietary concentration, 2) availability, or 3) digestibility (Joengbloed et al., 1991; Carlson et al., 2008, Richert, 2012). It is well establish that total concentration of Ca and P is not a reliable measurement of the potential

Figure 1: Schematic presentation of systems and functions affected by vitamin D



use of the nutrient in feed ingredients and diets (Jongbloed et al., 1991). The reason for the unreliable use of total Ca and P as measure of requirement is that these minerals can be found bound to phytic acid in most plant raw materials (Table 1). The standardized total tract digestibility (STTD) of P bound to phytic acid in plant raw materials is only about 20% compared with 80% in most sources of inorganic P. Only transgenic pigs producing salivary phytase are capable of utilizing Ca and P bound to phytic acid (Goloban et al., 2001).

The differences in digestibility and utilization of P among raw materials can be calculated by measuring the availability of P and Ca in raw materials. The availability of Ca and P are expressed as a value relative to a reference raw material (typically monocalcium or dicalcium phosphate). The inorganic standard raw material is assumed to be 100% bioavailable for metabolic functions and bone mineralization. Therefore, bone strength and bone ash are the major outcomes of measuring bioavailability of Ca and P in feed ingredients. The availability system is a major improvement over formulating diets for pigs, especially when using alternative feed ingredients (Hanson et al., 2012).

The major problem with the bioavailability system is that digestibility and subsequent availability of "a standard feed ingredient" varies between and within ingredients (Table 2). This results P availability values not being additive, and will cause errors in nutrient concentration in the final diet (Pedersen et al., 2011). In addition, expression of nutrient concentration of feed ingredients on digestible basis allows for calculations of nutrient excretion and use of digestibility values to determine necessary amounts of phytase enzymes to be added to the diet. Diets can also be formulated to minimize indigestible P concentrations, which is an advantage of digestibility system over other methods.

The digestibility of P can be expressed on apparent, standardized, or true total tract digestibility (NRC, 2012). Therefore, the concentration of P in diets for pigs can be calculated using a digestibility estimate times the concentration of total P. The recent NRC (2012) adopted the nomenclature and methodology of standardized total tract digestibility (STTD). The value of STTD represents the concentration of digestible P in a diet or feed ingredient after has been corrected for basal endogenous losses, which is the same concept comparable to that used for amino acids (Stein et al., 2007). Basal endogenous P loss is the amount of P that pigs excrete daily consuming a diet devoid of P (Petersen et al., 2012). It is assumed that an adjustment of digestibility values for endogenous losses of P will make diet formulation for P more additive because it corrects for losses of similar amounts across all feed ingredients, which is an advantage over the availability system.

The concentration of vitamins can also be expressed on their total concentration usually in international units/kg. The international unit is a measurement based on the biological activity of the specific chemical form of the vitamin (Baker and Stein, 2012). Given that there are many chemical forms, preparations, and sources of VitD, it is necessary to express their concentration on IU. The IU of VitD is the equivalent of the biological activity of 0.025 μg of cholecalciferol ($VitD_3$) a main form of the vitamin found in animals (NRC, 2012). The other prominent form is ergocalciferol (vitamin D_2) found in plant materials. Pigs can synthetize VitD by isomerication of 7-dehydrocholesterol (7-DHC) in the skin to $VitD_3$ following exposure to sun light or from ingestion of vitamin D_2 or D_3 in the diet. There are numerous factors that affect the synthesis of VitD such as availability of precursors and amount of exposure to sun light. Once formed, VitD undergoes 2 hydroxylations steps in liver in kidney at the 1 and 25th positions to form $1,25(OH)_2D_3$.

The nutritional concentration of vitamins for requirements for vitamins has been expressed on the basis of their bioavailability in a similar manner that used to express P bioavailability relative to a standard source (Baker and Stein, 2012). The bioavailability of vitamins in cereal grains and most ingredients is disregarded when formulating diets for pigs, while all requirements are met by vitamins supplied in the form of a premix added to the final diet. There are many factors that affect the bioavailability and degradation of VitD in premixes such as humidity, sun light exposure, heat, pH, presence of oxidative agents, and type

Table 1: Concentration of total calcium, total phosphorus, and phytate bound phosphorus in common raw materials fed to growing pigs

	Calcium, %	Phosphorus, %	Phytate, %	Avail. of P, %[1]	STTD of P[2], %
Corn	0.02	0.26	0.21	–	34.0
Soybean meal	0.33	0.71	0.38	–	48.0
DDGS, 10% oil	0.12	0.73	0.26	–	65.0
Dicalcium phosphate	24.8	18.8	–	49.5	81.4
Monocalcium phosphate, 70	16.9	21.5	–	68.9	53.4
Monocalcium phosphate, 85	–	–	–	66.3	–
Monocalcium phosphate, 100	–	–	–	84.9	–

[1] Availability of phosphorus, values modified from Pedersen et al. (2011).

[2] Standardized total tract digestible phosphorus.

Table 2: Example of diets formulated with variable concentrations of total phosphorus but a similar concentration of standardized total tract digestible (STTD) phosphorus (P)

Item	Diet 1	Diet 2	STTD of P, %
Ingredient composition, %			
Corn, yellow dent	64.5	43.7	26
Soybean meal	32.6	13.2	39
Distillers dried grains with solubles	–	40	65
Monocalcium phosphate	1.07	0.4	83
Nutrient composition			
Total phosphorus, %	0.61	0.64	51
STTD of P, %	0.31	0.31	48

of inorganic trace minerals (Shurson et al., 2011; NRC, 2012). Therefore, for many practical applications, vitamin concentrations provided by premixes are at levels several times greater than recommended levels in the final diet to account for these uncertainties while ensuring adequate vitamin nutrition (Richert, 2012).

Models to determine Ca, P, and VitD requirements

Nutritional requirements for Ca and P of growing pigs, gestating and lactating sows can be obtained from published values or from newer predictive models. In the newest nutritional recommendations from NRC (2012), the P requirement for growing pigs is calculated using a modeling approach of factorial and dynamic estimations. It is difficult to quantify each physiological function of these nutrients to determine a dietary requirement. Therefore, an alternative approach is to partition the animal's nutritional requirement into the functions that P has on basal metabolism and production (growth or lactation). Maintenance requirements for P are based on the amount of P that would need to be consumed to keep basal metabolic functions and replace those amounts lost in feces and urine (Jongbloed et al., 1991). The requirement for production purposes are the amount of P needed to support growth or milk production (Jongbloed et al., 1991; NRC, 2012). The sum of maintenance and production requirements are additive and account for all P intake required per day (Baker and Stein, 2012; NRC, 2012).

For growing pigs and sows, the main determinants of P requirements are: 1) maximum rate of retention (calculated from whole body P mass), 2) P retention in products of conceptus, 3) P output in milk, 4) basal endogenous loses of P, 5) minimum urinary losses of P, and 6) marginal efficiency of using STTD P intake for P retention. In addition, for growing pigs, the NRC estimates that the P requirement for maximum growth performance is 85% of the P requirement for maximum P retention. This suggests that optimal

pig growth can be achieved by feeding diets containing 85% of the P that would be required for optimal bone mineralization. The requirement of P on a STTD basis can be calculated as follows:

$$\text{STTD P req (g/day)} = 0.85 \times [(\text{max WB P retention})/0.77 + 0.19 \times \text{DMI} + 0.007 \times \text{BW}]$$

Where 0.85 is the maximum P required for growth as a proportion from the maximum requirement for P retention, Max WB P retention is the maximum whole body P retention, DMI is the dry matter intake of pigs, and BW is the body weight of pigs that is dynamically changed with phase of feeding. From the above requirement model it is implied that pigs require less dietary intake of P for optimal growth that for optimal bone mineralization. The optimal level of P for maximal profitability is then based on animal growth at 0.85 of the requirement for optimal bone mineralization (Cromwell et al., 1970). Under commercial conditions, pigs are generally fed greater dietary levels of P than actually required for optimal growth to avoid the risk for rickets in young or osteomalacia in adult pigs. Therefore, a 5-10% safety margin is generally applied to calculated dietary requirements to account for variation in requirements and feed intake among the population of pigs. The other solution is to formulate diets using stochastic models (Pomar et al., 2011). Stochastic models of nutrient requirements take into account growth and P requirements of individual pigs, but they are rarely use under commercial conditions at this moment.

The requirements for Ca, are expressed on total concentration of dietary Ca and are not expressed on STTD basis in NRC (2012). Therefore, the current NRC (2012) calculates the requirement of Ca as a linear function of the requirements for STTD P as 2.15. It is expected that new values for endogenous losses of Ca will be available to recalculate nutrient concentration of feed ingredients on STTD of Ca in the future (Gonzalez-Vega, 2012). A ratio of 0.9:1 for true total tract digestible Ca to true total tract digestible P was calculated, but not included in the current models of NRC (Fan and Archbold, 2012).

The nutritional recommendations for the concentration of VitD in diets of pigs between 5 and 25 kg in NRC (2012) remained the same at 220 IU/kg (Table 3) compared with previous revisions of the requirements since 1968 (NRC, 2012). Despite the fact that pig's genetics, feed intake, and environment has changed since 1968, the NRC 2012 has kept requirements similar because very few studies have been conducted to establish a new requirement. Furthermore, most of the studies suggesting a benefit from increasing dietary VitD concentrations have considered only a few dietary levels (Jang et al., 2012). Experimentally, using only a few dietary levels to measure responses makes impossible to establish an objective rather subjective requirement for nutrients (Baker, 1986). It is also necessary to use the most appropriate response criteria for experiments designed to establish a nutritional recommended level of VitD, and in general, any essential nutrient (Baker, 1986). Common response criteria used to establish VitD requirements are measurements of bone health, as well as incidence and severity of rickets in young animals and osteomalacia and in adult animals that are fed increasing levels of VitD under experimental conditions (Combs et al., 1966). These types of experiments are usually conducted under clean farm environments with minimal pathogen load. However, under field conditions it is possible that greater nutritional requirements are needed when animals are exposed to pathogens (Figure 1).

What is the role of the immune system on Vitamin D requirements? New areas of utilization

Vitamin D has being demonstrated to have a modulatory role in the immune system of pigs (Tougsignant et al., 2013). These modulatory effects of the immune system suggest that pigs may benefit from greater dietary levels of VitD. However, the dietary levels of VitD that are necessary for improving immune function or improving pig growth under disease challenge conditions are not well defined for several reasons. First, as mentioned above most studies have used limited dietary or supplemental levels of VitD, which makes impossible to establish a correct dietary requirement (Baker, 1986). Second, most studies have measure only a few physiologic responses, either immune parameter or growth, but none has tested the balance among all functions for VitD (Figure 1). The optimal supplement or dietary concentration of VitD will depend on the most profitable balance between all variables.

In conclusion, dietary requirements for Ca, P, and VitD are dynamic requirements that need constant monitoring and adjustment in diet formulations to optimize animal growth and minimize environmental impact of pork production. Nutritional models are a useful tool for determining objective and dynamic requirements, but new parameters in to account for differences in animal health may need to be included in these models to increase the precision of estimating requirements.

References

1. Abbott, J., D. Madson, and P. Arruda. 2012. Survey in vitamin D levels in swine serum across different stages of production. In Proceedings of the 2012 American Association of Swine Veterinarians Annual Meeting: Integrating Science, Welfare, and Economics into Practice, pp. 113–116.

2. Baker, D. H. and H. H. Stein. 2013. Bioavailability of minerals and vitamins in feedstuffs. Pages 341–365 in Sustainable Swine Nutrition. Chiba, L. I., ed. John Wiley & Sons Inc., Ames, IA.

Table 3: Summary of effect of age, vitamin D supplementation, and sun exposure on serum levels of vitamin D[1]

Age	Body weight, kg	NRC requirements, IU/kg	Serum $25(OH_2)D_3$, ng/mL		
			No supplement	40,000 IU[1]	Reared outdoor[2]
Birth	1.5	–	2-3	4	–
Day 10	3	–	8-9	60-80	–
At weaning	5-6	200	5-6	20-30	58.5
Grower	25-70	150	15-20	–	61.0
Finisher	70-140	150	28	–	86.0

[1] Tousignant et al. (2013), Jang et al. (2012).
[2] Abbott and Madson (2012).

3. Baker, D. H. 1986. Problems and pitfalls in animal experiments designed to establish dietary requirements for essential nutrients. J. Nutr. 116:2339–2349.

4. Baldwin, R. L., and R. D. Sainz. 1995. Energy partitioning and modeling in animal nutrition. Annual review of nutrition 15:191–211.

5. Carlson, M. S., S. D. Carter, J. M. DeRouchey, D. J. Meisinger, D. E. Reese, B. T. Richert, K. J. Stalder, H. H. Stein, E. van Heugten, and M. H. Whitney. 2008. Survey of swine nutrition and feed industry practices in the United States for development of a national swine nutrition guide. J. Anim. Sci. 86, E-Suppl. 3:59.

6. Christakos, Sylvia, Puneet Dhawan, Angela Porta, Leila J. Mady, and Tanya Seth. 2011. Vitamin D and intestinal calcium absorption. Mol. and Cel. Endocrinol. 347:25–29.

7. Combs, G. E., T. H. Berry, H. D. Wallace, and R. C. Crum, Jr. 1966. Levels and sources of vitamin D for pigs fed diets containing varying levels of calcium. J. Anim. Sci. 25:827:830.

8. Crenshaw, T. D. 2001. Calcium, phosphorus, vitamin D, and vitamin K in swine nutrition. Pages 187–212 in Swine Nutrition. 2nd ed. A. J. Lewis, and L. L. Southern, ed. CRC Press, Boca Raton, FL.

9. Cromwell GL, Hays VW, Chaney CH and Over?eld JA 1970. Effects of dietary phosphorus and calcium level on performance, bone mineralization and carcass characteristics of swine. J. Anim. Sci. 30:519–525.

10. Ewing, W., and S. J. Charlton. 2007. Calcium. Pages 5a-5f, and 19a in The Minerals Directory. 2nd ed. Context Products Ltd, Leicestershire, UK.

11. Fan, M. Z. and T. Archbold. 2012. Effects of dietary true digestible calcium to phosphorus ratio on growth performance and efficiency of calcium and phosphorus use in growing pigs fed corn and soybean meal diets. J. Anim. Sci. 90:254–256.

12. Golovan, S. P., R. G. Meidinger, A. Ajakaiye, M. Cottrill, M. Z. Wiederkehr, D. J. Barney, C. Plante, J. W. Polland, M. Z. Fan, M. A. Hayes, J. Laursen, J. P. Hjorth, R. R. Hacker, J. P. Phillips, and C. W. Fosber. 2001. Pigs expressing salivary phytase produce low-phosphorus manure. Nat. Biotechnol. 19:741–745.

12. Gonzalez Vega, J. 2012. Aspects of calcium digestibility in pigs. MS Thesis. University of Illinois, Urbana, IL.

13. Hanson, A. R., G. Xu, M. Li, M. H. Whitney, and G. C. Shurson. 2012. Impact of dried distillers grains with solubles (DDGS) and diet formulation method on dry matter, calcium, and phosphorus retention and excretion in nursery pigs. Anim. Feed Sci. and Technol. 172:187–193.

14. Jang, Y. D., H. J. Monegue, and M. D. Lindemann. 2012. The vitamin D conundrum. Page 201–212. Proceedings of the 73rd Minnesota Nutrition Conference. Owatonna, MN.

15. Jongbloed, A. W., H. Everts, and P. A. Kemme. 1991. Phosphorus availability and requirements in pigs. Pages 65–80 in Recent Advances in Animal Nutrition. E. R. Heiemann, ed. Butterworth, London, UK.

16. Létourneau-Montminy, M. P., C. Jondreville, D. Sauvant, and A. Narcy. 2012a. Meta-analysis of phosphorus utilization by growing pigs: effect of dietary phosphorus, calcium and exogenous phytase. Animal 6:1590.

17. Létourneau-Montminy, M. P., A. Narcy, C. Pomar. 2012b. Development of a model simulating the metabolic fate of dietary phosphorus and calcium in growing pigs. J. Recherche Porcine 44:153–158.

18. Létourneau-Montminy, M. P., A. Narcy, P. Lescoat, M. Magnin, J. F. Bernier, D. Sauvant, C. Jordreville, and C. Pomar. 2011. Modeling the fate of dietary phosphorus in the digestive tract of growing pigs. J. Anim. Sci. 89:3596–3611.

19. NRC, 2012. Nutrient requirements of swine. 11th Ed. The Nat Acad. Press Washington, DC.

20. Petersen, G. I., C. Pedersen, M. D. Lindemann and H. H. Stein. 2011. Relative bioavailability of phosphorus in inorganic phosphorus sources fed to growing pigs. J. Anim. Sci. 89:460–466.

21. Pomar, C., L. Hauschild, G. H. Zhang, J. Pomar, and P. A. Lovatto. 2011. Precision feeding can significantly reduce feeding cost and nutrient excretion in growing animals. In Modeling nutrient digestion and utilization in farm animals, pp. 327–334. Wageningen Acad. Publishers.

22. Richert, B. T. 2012. Macro Minerals for Swine Diets. Pages 53–64 in National Swine Nutrition Guide. Meisinger, D. J. Ed. US Pork Center of Excellence. Aimes, IA.

23. Schulin-Zeuthen, M., E. Kebreab, W. J. J. Gerrits, S. Lopez, M. Z. Fan, R. S. Dias, and J. France. 2007. Meta-analysis of phosphorus balance data from growing pigs. J. of Anim. Sci. 85:1953–1961.

24. Shurson, G. C., T. M. Salzer, D. D. Koehler, and M. H. Whitney. 2011. Effect of metal specific amino acid complexes and inorganic trace minerals on vitamin stability in premixes. Anim. Feed Sci. and Technol. 163:200–206.

25. Stein, H. H., B. Sève, M. F. Fuller, P. J. Moughan, and C. F. M. De Lange. 2007. Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: Terminology and application. J. of Anim. Sci. 85:172–180.

26. Tousignant, S. J. P., S. C. Henry, A. Rovira, and R. B. Morrison. 2013. Effect of oral vitamin D_3 supplementation on growth and serum 25-hydroxy vitamin D levels of pigs up to 7 weeks of age. J Swine Health and Prod. 21:94–98.

27. U. N. Food and Agriculture Organization. 2006. Livestock's long shadow – environmental issues and options. FAO, Rome, Italy.

28. Wasserman, R. H., and A. N. Taylor. 1966. Vitamin D3-induced calcium-binding protein in chick intestinal mucosa. Science 152:791–793.

Rise up: 2013

Paul McKellips

"Rise Up" is an inspiring and powerful multimedia presentation that traces the evolution of the extreme animal rights movement and reveals the impact it's having today on the biomedical research community worldwide. McKellips uses a litany of animal rights videos to uncover and identify the real agenda; it's not about the animals – it's about anarchy.

When animal rights extremists conduct direct action violence or hold public protests with non-traditional stunts, they receive "earned media" coverage from news outlets, bloggers, radio and newspapers. Often times the animal rights claims are so outrageous that the research community doesn't respond. Many voices fall silent because no one wants to put a target on their back.

This presentation identifies how the Foundation for Biomedical Research has used the mass market, international media reach of the *Bench to Bedside*™ television series, national TV ads, a magazine, and mass market commercial novels, to increase public support for the humane and responsible use of animals in research.

"Rise Up" ends with a motivational component that's guaranteed to leave every audience – at all levels of research and support staff – feeling empowered, energized and proud of their work.

About the Speaker:

McKellips has written, directed and produced three motion pictures and numerous television shows. He covered the Pentagon and State Department as national TV news correspondent and the Middle East desk at Voice of America before serving in Iraq and Afghanistan as a media trainer. McKellips has been the EVP at the Foundation for Biomedical Research since 2007 where he has earned eight Emmy™ nominations and 28 Telly Awards for the *Bench to Bedside*™ series and he has written two biomedical thrillers, UNCAGED and JERICHO 3. His third novel will be released in 2013 through the Penguin Group.

The growing diversity of H3N2 influenza A virus in swine and the impact on control in swine and at the human animal interface

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Introduction

H3N2 influenza A viruses (IAV) were recognized as endemic infections in the USA swine population following the 1997-98 incursion of the triple reassortant viruses with gene segments from human- (HA, NA, and PB1), swine- (NP, M, and NS) and avian- (PB2 and PA) adapted viruses (reviewed in).[1] The significance of the constellation of triple reassortant internal genes (TRIG) would be realized from that critical turning point into the present day, during which time we have observed the TRIG viruses reassort numerous times to acquire new surface genes (β-, γ-, δ1-, and δ2-H1N1 and H1N2; H2N3) in the USA, to be exported to new regions (China and South Korea for example), as well as to donate genes to the first human pandemic virus of the 21st century, H1N1pdm09.[2] Concurrent with frequent reassortment has been a dramatic evolution of IAV in swine in the USA, resulting in multiple antigenic variants that co-circulate and severely challenge our control measures. Recently, efforts within the public and animal health sectors to increase monitoring of IAV in populations of interest have been implemented, with the ultimate goals of improved detection and control interventions. The public health efforts are linked to a mandate instituted in 2005, requiring all human cases of infection with novel influenza viruses be reported to the World Health Organization under the International Health Regulations. Consequently, investment in resources for domestic detection and reporting mechanisms for novel IAV in humans has increased. This includes all zoonotic infections of humans with swine IAV, termed "variant" IAV and denoted by a lower case "v" following the subtype. On the animal health side, the USDA implemented a swine influenza surveillance system in 2009 in response to the growing concern over endemic swine IAV and the emergence of the H1N1pdm09 and subsequent spillover from humans to pigs in the USA. Data from both sectors have provided invaluable insights into the nature of IAV adapted to swine and the implications of spillover that occurs in both directions between humans and pigs. Although sporadic infection of humans with H1N1 and H1N2 swine IAV were reported since 2005, none have occurred with the magnitude of the recent H3N2v cases,[3] thus H3N2 in swine and H3N2v in humans will be the primary focus of this presentation.

Evolution of IAV in swine

Recent improvements in influenza surveillance in swine populations in the USA allow for timely epidemiologic, phylogenetic, and virologic analyses that monitor emergence of novel viruses and assess changes in viral population dynamics. A phylogenetic analysis was conducted on sequences from IAV isolated from US swine during 2009-2012 through voluntary and anonymous submissions into the USDA IAV swine surveillance system (T. K. Anderson, submitted). These analyses revealed changes in population dynamics among multiple clades of H1N1, H3N2, and H1N2 co-circulating in US swine populations during 2009-2012. Viral isolates were categorized into one of seven genetically and antigenically distinct hemagglutinin (HA) lineages: H1α, H1β, H1γ, H1δ1, H1δ2, H1pdm09 and H3 cluster IV. Over this period there was a dramatic increase in occurrence of H1d1 in samples submitted, with a concurrent decrease in H1pdm09. H3 cluster-IV exhibited increasing diversification, with multiple distinct phylogenetic branches emerging and being sustained since 2010.[4,5] Although H3N2 represented 25% of identified viruses, this subtype was reported in increasing proportion of sequenced isolates since late 2011. In addition to the phylogenetic clusters of H3, at least 10 distinct reassorted H3N2/H1N1pdm09 (rH3N2p) genotypes were identified in studies at NADC[5] and by others.[6,7] Nearly all of the H3N2 reassortants characterized in the USA contain the matrix gene from the H1N1pdm09 virus (pM) alone (genotype 1) or in combination with other H1N1pdm09 gene segments (genotypes 2-9). Genotype 1 was most frequently detected in swine and all human H3N2v viruses clustered within a single genotype 1 and HA clade. These data suggested that the genetic requirements for transmission of H3N2 to humans might be restricted to a specific subset of swine viruses. Mutations at putative antigenic sites as well as reduced serologic cross-reactivity among the H3 sub-clusters suggested antigenic drift of these contemporary viruses in swine that may impair currently available vaccines.

Phenotypes of H3N2v and contemporary swine H3N2 in pig challenge studies

The pathogenic, transmission, genetic, and antigenic properties of a human H3N2v isolate and two swine H3N2 isolates, H3N2-TRIG and rH3N2p, were compared.[8] Our *in vivo* study demonstrated no increased virulence in the H3N2v- or rH3N2p-infected pigs. On the contrary, all of the rH3N2p we assessed caused relatively mild clinical disease and pathology compared to H3N2 with the TRIG backbone. However, the rH3N2p isolates were as efficient as the H3N2-TRIG in nasal replication and transmission to contact pigs. Two additional rH3N2p isolates with high sequence identity to the human H3N2v were used in subsequent vaccine studies with similarly low pathogenicity phenotypes (P. Kitikoon, submitted and).[9] Initial studies characterizing antigenic phenotypes of contemporary H3N2 by hemagglutinin-inhibition (HI) assays indicated that although antibodies to a 2010 H3N2-TRIG virus cross-reacted to rH3N2p viruses, the H3N2v virus had reduced cross-reactivity to other H3N2 antisera tested. These results prompted broader studies into antigenic properties of additional representative H3N2 viruses and vaccine efficacy experiments.

Efficacy of vaccines to control H3N2 in swine

Vaccines against IAV in swine provide a primary means to limit disease but may not be effective at neutralizing infection or transmission. Populations with variations in immune status, such as pigs with waning maternal antibodies or varying levels of heterologous cross-reactivity, provide continual reservoirs for endemic infections with IAV as well as repeated outbreaks in naïve pigs. Antisera from 3 commercial vaccines were tested in HI assays against contemporary H3N2 viruses. Since HI cross-reactivity demonstrated that contemporary swine rH3N2p viruses had drifted from viruses in current swine IAV vaccines, a series of *in vivo* vaccine efficacy studies were performed to assess commercial swine IAV vaccine efficacy against rH3N2p similar to H3N2v from a human cases in 2011-12. Vaccine 1, with the highest HI cross-reactivity, was selected to test for efficacy against rH3N2p virus infection in pigs with or without maternally derived antibodies (MDA) (P. Kitikoon, submitted). In addition, efficacy of vaccines derived from a monovalent whole inactivated virus (WIV) was compared to experimental live attenuated influenza virus (LAIV) against rH3N2p. Vaccine 1 with the highest level of HI cross-reactivity significantly protected pigs without MDA. However, the presence of MDA at vaccination completely blocked vaccine efficacy. The performance of WIV and LAIV was comparable in the absence of MDA. LAIV was not tested in the face of MDA in this particular rH3N2p study, but previous studies have shown superior performance over WIV the presence of MDA.[10] A critical review of the use of WIV in sows is required in the context of the current IAV ecology and vaccine application in pigs with MDA.

A second study was completed to evaluate the efficacy of commercial inactivated swine IAV vaccines and LAIV vaccines against infection with H3N2 virus and subsequent indirect transmission to naïve pigs.[9] The rH3N2p virus evaluated was similar to the H3N2v detected in humans in 2012. Commercial vaccine 1 again provided significant partial protection in MDA negative pigs as in the other study, measured by reduced nasal shedding; however, indirect naïve contacts became infected, indicating the reduction in nasal shedding did not prevent aerosol transmission. An LAIV vaccine with a 2004-era HA and NA provided complete protection and none of the indirect contact pigs became infected. Clinical disease was not observed in any group including non-vaccinated animals, a consistent observation in pigs infected with contemporary rH3N2p swine viruses. Serum HI titers against the challenge virus were not predictive of efficacy: titers following vaccination with the LAIV that provided sterilizing immunity were below the level considered protective; yet titers in a commercial vaccine group that was not protective were above the cut-off. While vaccination with currently approved commercial inactivated products did not fully prevent transmission, vaccines tested and proven against contemporary rH3N2p may provide benefit for reducing shedding, transmission and zoonotic spillover of antigenically similar viruses at agriculture fairs, *when administered in the absence of MDA*. Vaccine application in growing pigs should be monitored for appropriate observation of label recommendations, but it is also critical to monitor the response in pigs against the targeted IAV strains for an indication of expected efficacy. Expectations for WIV must be realistic, placed in the right context, and used in conjunction with additional control measures.

H3N2v in humans during 2011-13

Of the approximate 360 detections of variant viruses of swine lineage in humans in the USA since 2005, 333 have been H3N2v: 12 in 2011, 309 in 2012, and 12 so far in 2013 (**http://www.cdc.gov/flu/swineflu/variant-cases-us.htm**). Many factors in the swine and human populations are likely to have impacted the increase in frequency of H3N2v detections in humans, but the precise factors have been difficult to discern with current epidemiologic information from exhibited swine. Certainly, the increase in monitoring and attention on H3N2v in the fair setting has played a role in the detection of new cases. However, difficulty in control of contemporary IAV in swine and

overall viral burden of IAV in swine is likely an important contributor. Although there has been an increase in detection of H3N2 in swine with the same genetic signature as the H3N2v in humans, this cannot be the sole explanation since there have also been increases in other genetically dissimilar H3N2 as well as in subsets of H1 viruses in the USDA surveillance data set. There may be specific virus properties that confer a better fit for the H3N2v to infect humans, and indeed the H3N2v was capable of airborne transmission in ferrets,[11] the standard laboratory model for human IAV. However, other variant human isolates and swine IAV were also shown to successfully infect and transmit in ferrets. An additional and very likely major factor is the lack of human population immunity against subtypes of IAV that have evolved independently in swine away from their respective human seasonal precursor viruses and the strains used in contemporary human seasonal vaccines. A substantial proportion of adolescents and young adults were shown to have cross-reactive antibodies against H3N2v; however, children and older adults lacked such protective antibodies.[12,13] The current human seasonal vaccines containing H3N2 do not appear to protect against the H3N2v.[13,14] Since the vast majority of cases of H3N2v have been in children with close contact and long periods of exposure time at agricultural fairs, all of these factors point to a unique set of circumstances that collectively increase the odds for H3N2v in these spillover events. This does not necessarily diminish the epidemic or pandemic risk of these viruses to humans if H3N2v gained the ability to efficiently transmit from human to human, allowing the virus further opportunity to mutate and adapt back to the human host.

Sustained collaborative efforts from all facets of human and animal health, research, and regulatory entities are required to define and intervene against the problem of IAV in swine and humans in the USA and globally.[15] To exemplify this type of cross-sector partnership, a national working group met in January 2013 and drafted guidelines aimed to minimize influenza transmission at agricultural fairs and swine exhibits in the USA (**http://nasphv.org/Documents/NASAH0-NASPHV-InfluenzaTransmissionAtSwineExhibitions2013.pdf**). The focus of the group's guidance document is beyond the scope of this presentation and will be discussed by others. The current situation of IAV in swine and spillover into humans highlights yet again the need for continuation of the USDA surveillance system in swine, improvements in swine vaccine technology and systematic updates, and expansion of research efforts to control this economically important and zoonotic virus in swine.

References

1. Lorusso, A., et al., *Contemporary Epidemiology of North American Lineage Triple Reassortant Influenza A Viruses in Pigs.* Curr Top Microbiol Immunol, 2012.

2. Dawood, F.S., et al., *Emergence of a novel swine-origin influenza A (H1N1) virus in humans.* N Engl J Med, 2009. 360(25): p. 2605–15.

3. Epperson, S., et al., *Human Infections With Influenza A(H3N2) Variant Virus in the United States, 2011–2012.* Clin Infect Dis, 2013. 57 Suppl 1: p. S4-S11.

4. Nelson, M.I., et al., *Evolution of novel reassortant A/H3N2 influenza viruses in North American swine and humans, 2009–2011.* J Virol, 2012. 86(16): p. 8872–8.

5. Kitikoon, P., et al., *Genotype patterns of contemporary reassorted H3N2 virus in US swine.* J Gen Virol, 2013. 94(Pt 6): p. 1236–41.

6. Ducatez, M.F., et al., *Multiple reassortment between pandemic (H1N1) 2009 and endemic influenza viruses in pigs, United States.* Emerg Infect Dis, 2011. 17(9): p. 1624–9.

7. Liu, Q., et al., *Emergence of novel reassortant H3N2 swine influenza viruses with the 2009 pandemic H1N1 genes in the United States.* Arch Virol, 2012. 157(3): p. 555–62.

8. Kitikoon, P., et al., *Pathogenicity and transmission in pigs of the novel A(H3N2)v influenza virus isolated from humans and characterization of swine H3N2 viruses isolated in 2010–2011.* J Virol, 2012. 86(12): p. 6804–14.

9. Loving, C.L., et al., *Efficacy of inactivated and live-attenuated influenza virus vaccines in pigs against infection and transmission of emerging H3N2 similar to the 2011–2012 H3N2v.* J Virol, 2013.

10. Vincent, A.L., et al., *Live attenuated influenza vaccine provides superior protection from heterologous infection in pigs with maternal antibodies without inducing vaccine-associated enhanced respiratory disease.* J Virol, 2012. 86(19): p. 10597–605.

11. Pearce, M.B., et al., *Pathogenesis and transmission of swine origin A(H3N2)v influenza viruses in ferrets.* Proc Natl Acad Sci U S A, 2012. 109(10): p. 3944–9.

12. *Antibodies Cross-Reactive to Influenza A (H3N2) Variant Virus and Impact of 2010–11 Seasonal Influenza Vaccine on Cross-Reactive Antibodies - United States.* MMWR Morb Mortal Wkly Rep, 2012. 61: p. 237–41.

13. Skowronski, D.M., et al., *Cross-reactive and vaccine-induced antibody to an emerging swine-origin variant of influenza A virus subtype H3N2 (H3N2v).* J Infect Dis, 2012. 206(12): p. 1852–61.

14. Houser, K.V., J.M. Katz, and T.M. Tumpey, *Seasonal trivalent inactivated influenza vaccine does not protect against newly emerging variants of influenza A (H3N2v) virus in ferrets.* J Virol, 2013. 87(2): p. 1261–3.

15. Vincent, A., et al., *Review of Influenza A Virus in Swine Worldwide: A Call for Increased Surveillance and Research.* Zoonoses Public Health, 2013.

Pork industry update on flu and fairs 2012 and 2013 follow-up

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Management and challenges of dealing with swine influenza

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Introduction

Swine influenza continues to present a health challenge of pigs. Recent active surveillance project of growing pigs in the Midwest identified 90.6 percent of the rural farms as influenza A positive at least once based on monthly testing for 12-24 consecutive months.[1] Neonatal pigs play a key role in transmission of the virus. The approach to eliminating endemic influenza in a breed-to-wean population lacks standardization. Strategies include natural virus circulation, mass vaccination of breed-to-wean population, pre-farrow group vaccination, and combinations of all these strategies. Research suggests that piglets with maternally derived antibodies and challenged with homologous or heterologous influenza strains may have reduced clinical signs but viral shedding may still occur.[2] The strategies are frequently changed based on empirical data. Because of the potential for reintroduction of the virus through human-to-pig contact, aerosol transmission, or endemic circulation, the cost of influenza is highly variable. Oftentimes, even though the cost of influenza is low in sow populations in herds that transfer wean pigs to multiple owners or to different owners, the frequency of even a low prevalence influenza cough is frequently not appreciated. With the perceived variability in weaning weights caused by influenza infection and with frequent co-infections of PRRS, producers frequently request that aggressive steps be taken to eliminate influenza from suckling pigs.

This paper discusses a multi-herd approach to reducing and/or eliminating endemic influenza from suckling pigs in a geographical area of the Midwest.

Background

A production system in the Midwest has experienced influenza viruses within the system over the past three years. Over that time period, various strategies from pre-farrow vaccination through mass vaccination have been implemented in an effort to decrease the endemic transmission. These herds vary from 1800 to 6400 sows. Each herd has replacement facilities adjacent to and connected to gestation. Replacement gilts are received every 28 days from a designated multiplier within the system at 21 days of age. Gilts are quarantined in an isolation nursery facility for 54 days during which time they are statistically sampled twice for PRRS and before movement to continuous flow gilt developer facility from 11-27 weeks of age. Entry population is sampled for influenza either by nasal swabs or oral fluid collection only if the source herd is active for influenza or there are clinical signs within the population.

Our historical strategy for this system was to mass vaccinate the breed-to-wean populations with commercial vaccines quarterly during the flu season. Populations were vaccinated in late August, December, and March. Vaccination was skipped during the summer months. Developing gilts were administered commercial flu vaccine after selection at 151 days of age with a second dose administered at 165 days of age. In herds that had clinical increased flu activity or were endemic within the suckling pigs, the developing gilts were also vaccinated at 63 and 84 days of age.

Description

In the summer of 2012, discussion led to implementing various vaccines within herds of the system (15 herds were enrolled and two influenza negative herds were not vaccinated). Each herd was allotted to one of three strategies based on influenza strains within the herd and the genetic source herd. Vaccines administered were Newport Monovalent H1N2, Sirrah Monovalent H1N2, and FluSure™ XP. (Table 1)

Protocol

Mass vaccination of the entire herd with assigned product. This includes the sows and gilts on breed row to preg check. Mass vaccination will be repeated. Gilts will be vaccinated every three weeks. Depending on age, some gilts may receive a fourth vaccination.

- Newport Monovalent H1N2
  - Week 35: Mass vaccinate
    - Sows and gilts
- Week 44: Vaccinate gilts in GDU
  - This includes gilts in isolation

Table 1: Source farms

Flow 3	**Newport Monovalent H1N2**
Herd 1	Sirrah Monovalent H1N2
Herd 2	FluSure™ XP
Herd 3	Newport Monovalent H1N2
Flow 2	**Sirrah Monovalent**
Herd 1	FluSure™ XP
Herd 2	Newport Monovalent H1N2
Herd 3	Newport Monovalent H1N2
Herd 4	Sirrah Monovalent H1N2
Herd 5	FluSure XP
Flow 1	**FluSure™ XP**
Herd 1	Sirrah Quadvalent
Herd 2	FluSure™ XP
Herd 3	Newport Monovalent H1N2
Herd 4	FluSure™ XP
Herd 5	Sirrah Monovalent H1N2

  - Week 41: Surveillance begins
  - Week 41: Mass vaccinate
    - Sows and gilts
- Sirrah Monovalent H1N2 and Sirrah Quadvalent
  - Week 35: Mass vaccinate
    - Sows and gilts
  - Week 37: Surveillance begins
  - Week 38: Mass vaccinate
    - Sows and gilts
- Week 41: Vaccinate gilts in the GDU
- FluSure™ XP
  - Week 35: Mass vaccinate
    - Sows and gilts
  - Week 37: Surveillance begins
  - Week 38: Mass vaccinate
    - Sows and gilts
  - Week 41: Vaccinate gilts in the GDU

Monitoring

- Start two weeks after second vaccination is completed (week 41).
- Repeat every two weeks.
  - Ten nasal swabs from due to wean piglets.
    - Sample five pigs per swab.
  - Seven oral fluid (rope) samples from the GDU.
    - Hang one rope per lot.
    - The rope should be shared between two pens of the same lot.
    - Label the tubes with the lot number.
- Any clinical signs of flu in the sows or gilts in an off test week will be tested.
  - The farms should contact the PM and vet team if clinical signs develop.
    - i.e., off feed sows, deep barking cough, nasal discharge, lethargy.

Summary

The results of the three interventions will be reported. Two mass vaccinations were beneficial in reducing influenza circulation in herds that were positive for H1N2 but not successful in eliminating the virus in 100 percent of the herds.

References

1. Corzo, Cesar A; Gramer, Marie; Kuhn, Michael; Mohr, Marty; Morrison, Robert. Observations regarding influenza A virus shedding in a swine breeding farm after mass vaccination, *Journal of Swine Health and Production* 2012;20.

2. Corzo, Cesar; Allerson, Matt; Gramer, Marie; Morrison, Robert. Detection of Airborne Influenza A virus in experimentally infected pigs with maternally derived antibodies. 2013. SDEC Partners Research Update.

Swine influenza: The human animal interface at Minnesota fairs and live animal markets

Joni Scheftel

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Using the Myers-Briggs Type Indicator to improve your own communication skills

Lisa Tokach

The Myers-Briggs Type Indicator (MBTI) is a useful method of understanding people by looking at eight different personality preferences that people use in everyday life. There are many personality profiling tools available to those who work in human resources. Some are just for fun, some are downright silly, but several are scientifically researched to prove their predictability and accuracy. Our office has used MBTI since 1991 as a tool to improve communication, help in conflict resolution, and improve teamwork skills. Other valuable tools are certainly out there such as TKI (Thomas-Kilmann Conflict Mode Instrument), FIRO (Fundamental Interpersonal Relations Orientation), KTS (Keirsey Temperament Sorter), and DiSC (Dominance, Influence, Steadiness and Conscientiousness). Certainly each one offers insight into how people think and react. The MBTI has proven to be a relatively easy to understand model that participants can learn and hopefully apply to their everyday lives in order to understand and improve their communication skills.

When applied correctly, use of the MBTI in an organization can help individuals and teams to:

1. Understand themselves better first, why they act and react the way they do.
2. Appreciate the skills of others so that they can be applied in constructive ways.
3. Approach problems in a different way so as to avoid the same chronic conflict issues.

What MBTI does not do:

1. Label people as a certain "type" that indicates skills they either have or don't have
2. Fix underlying health issues such as depression, anxiety, alcoholism, extreme OCD or the like
3. Reveal deep, dark secrets of the people taking the inventory and reveal them to a group
4. Cause everyone on your team to join hands and sing "Kumbaya".

MBTI works by having the participants take a 96 question survey that helps to sort them into one of two preferences in four different categories. Through a variety of questions, MBTI asks: where you get your energy, how you take in information, how you make decisions, and how you deal with the outer world. This gives 16 different four letter combinations, one of which is your MBTI type.

Ideally, a person who takes the MBTI, learns and understands their type can use that information to modify their communication to others, planning, and conflict resolution to make their own work environment have less conflict. Regardless of your MBTI type, everyone agrees that less conflict is better.

The goal of this session is to demonstrate the four dichotomies of MBTI and the two preferences of each. We will use some group exercises with those who have willingly agreed to take the MBTI in advance to demonstrate the differences. We will then give real life examples of how these differences can work together in an organization or how they can tear it apart depending on how we use them.

A MAJOR GENE FOR HOST RESPONSE TO PRRSV INFECTION

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Host genetics is an additional tool for controlling the costly disease of Porcine Reproductive and Respiratory Syndrome (PRRS). The objective of this work was to discover the genetic basis of host response to PRRS virus infection by estimating genetic parameters and conducting a genome-wide association study.

Eight groups of ~200 commercial crossbred pigs were infected between 25 and 35 days of age with virus isolate NVSL 97-7985. Breeds represented in the crosses included Large White, Landrace, Yorkshire, Duroc, and Pietrain. Blood samples and body weights were collected up to 42 days post infection (dpi). Experimental pigs and their parents were genotyped for 60,000 single nucleotide polymorphisms (SNPs) across the genome with Illumina's Porcine 60k Beadchip. Phenotypes analyzed were viral load (VL = area under the curve for log-transformed qRT-PCR based serum virus from 0-21 dpi) and weight gain from 0-42 dpi (WG).

Heritabilities estimated using pedigree were moderate at 0.44 for VL and 0.29 for WG. Genomic regions associated with VL were identified on chromosomes 1, 4, and X, and on chromosomes 4, 5, and 7 with WG. Apart from the SSC4 region, the other regions explained less than 3% of the genetic variance. The 1 Mb region on chromosome 4 (SSC4) explained 14.6% of the genetic variance for VL and 9.1% for WG. Effects of the most significant SNP in the region, WUR10000125 (WUR), acted in a dominant manner, with the favorable allele estimated to decrease VL by 4 units (0.53 phenotypic sd) and increase WG by 2 kg (0.49 phenotypic sd). The effect was present irrespective of parental breeds involved in the crosses.

In conclusion, the 1 Mb region on SSC4 explained a sizable proportion of genetic variation in response to experimental challenge with a specific strain of the PPRS virus. Heritability estimates were moderate and, with a frequency of 0.15 for the favorable allele at the WUR SNP on chromosome 4, genetic improvement of host response to PRRSv infection appears possible. However, additional research on the effect of the identified genomic regions during the reproductive phase and in the field is needed before selection can be implemented. This research is underway, with support from Genome Canada, Genome Alberta and USDA-NIFA grant number 2013-68004-20362.

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EVALUATION OF FOSTERA™ PRRS VACCINE SHEDDING IN GROWING PIGS FOLLOWING VACCINATION

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) is the most economically important disease affecting the US swine industry. Modified live vaccines have the ability to replicate in the animal after administration with subsequent shedding of the virus into the environment and to naïve swine in contact with vaccinates. In this study, we evaluated the magnitude and duration of shedding of the modified live Fostera™ PRRS vaccine virus in growing pigs following vaccination.

Materials and methods: Twenty (3-week-old) pigs confirmed free of PRRS virus (PRRSV) were divided into a Vaccinate group (n=10) and a Sham control group (n=10), each group occupying a separate room. Pigs in the Vaccinate group were vaccinated with 2 mL Fostera™ PRRS intramuscularly and pigs in the Sham control group were intramuscularly administered 2 mL of the vaccine diluent. Pigs were observed for clinical signs throughout the trial. Rectal temperature was recorded daily for 1 week and every-other-day for 1 additional week. Serum and feces were collected from individual pigs, and oral fluids were collected per pen, at 0, 3, 7, 10, 14, 21, 28, 35, 42, 49, 56 and 63 days post vaccination (DPV). Pigs were necropsied at 63 DPV. A quantitative PRRSV PCR (Life Technologies) was conducted on the lung and tonsil at necropsy and serum, oral fluids and feces collected throughout the trial. Virus isolation (VI) was attempted on selected serum, oral fluids, lung and tonsil on Marc-145 cells. Selected samples were sequenced for PRRSV ORF5 to confirm the virus identity. Serum and oral fluid samples were tested for anti-PRRSV antibody using PRRSX3 ELISA (IDEXX). Neutralizing antibodies in serum against the Fostera™ PRRS vaccine virus was determined. Rectal temperature data were analyzed by a repeated measure ANOVA. A *P*-value ≤ 0.05 was considered significant.

Results: Neither the Vaccinate nor the Sham control pigs demonstrated clinical signs of systemic disease, anorexia or lethargy after vaccination. Body temperatures were within the normal range of growing pigs (102-105°F) during 0-13 DPV and there were no significant differences between the two groups. As tested by quantitative PRRSV PCR, all 10 Vaccinate pigs became viremic at 3 DPV. All pigs remained virus positive in serum through 35 DPV. Viremia was detected in sera of 7/10, 2/10, 1/10 and 5/10 pigs at 42, 49, 56 and 63 DPV, respectively. Virus levels peaked at 21 DPV in serum with a mean titer of 4.4 $\log_{10}$ genomic copies per ml of serum and quickly declined thereafter. All oral fluids collected from Vaccinate pigs were PRRSV PCR positive throughout the study 3-63 DPV. One pig feces at 3 DPV and four pig feces at 7 DPV from the Vaccinate group were positive by PRRSV PCR. The lungs and tonsils were PRRSV PCR positive for vaccine virus at necropsy from 2/10 and 10/10 Vaccinate pigs, respectively. The vaccine virus was isolated from most sera of Vaccinate pigs at 3-7 DPV and from some sera till 21 DPV. VI was negative from oral fluids, lungs and tonsils. Anti-PRRSV ELISA antibodies were detected in serum of 5/10 Vaccinate pigs at 10 DPV and 10/10 pigs from 14-63 DPV, and in oral fluids from 7-63 DPV. Neutralizing antibodies were detected in serum of 2/10 at 10 DPV, 6/10 at 14 DPV and 10/10 Vaccinate pigs from 21-63 DPV. Virus was not detected in any sera, oral fluids, feces, lungs, or tonsils of Sham control pigs throughout the study. PRRSV-specific antibody was not detected from any Sham control pigs throughout the study.

Conclusions and Discussion: Fostera™ PRRS vaccine did not result in clinical disease or febrile responses in vaccinated growing pigs. The vaccine virus can be shed at low levels and short periods in feces but can be shed in serum and oral fluids for longer than 63 DPV in spite of the presence of neutralizing antibodies in serum. These data emphasize the importance of implementing proper biosecurity programs to prevent the spread of PRRS vaccine to naïve populations at risk, such as pregnant females and regional nursery and finisher swine.

FACTORS ASSOCIATED WITH N-SPECIFIC IgG RESPONSE IN PIGLETS EXPERIMENTALLY INFECTED WITH PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

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Porcine reproductive and respiratory syndrome (PRRS) costs the U.S. pork industry $664 million per year, affecting all stages of production (Holtkamp et al 2013). Vaccines, biosecurity measures, and proposed methods for eradication have only been partially successful. Our emphasis is on identification of genomic markers and pathways associated with host response. These markers could potentially be used for genetic selection of pigs for increased resistance or reduced susceptibility. The objective of this study was to identify factors associated with total antibody (tAb) response, determine whether a heritable genetic component was present, and subsequently perform a genome wide association study (GWAS) to find QTL associated with tAb response following experimental infection with PRRS virus (PRRSV). The data were from 464 Large White-Landrace crossbred piglets from three separate experimental infection trials of the PRRS Host Genetics Consortium. All piglets were experimentally challenged with PRRSV isolate NVSL-97-7895 at 28-35 days of age. Serum samples were periodically collected until 42 days post infection (dpi) and viremia determined by qPCR assays. Serum samples collected at 42 dpi were assayed for tAb response using the fluorescence microsphere immunoassay to detect virus N protein-specific Immunoglobulin G, whereby a negative control was included to adjust for background during analysis. Results, reported as the mean fluorescence intensity (MFI), were obtained from MAGPIX. Viral load was defined as the area under the curve of Log viremia from 0-21 dpi. A piglet was classified as having a virus rebound based on statistical evidence of a secondary viremia peak after 21 dpi. Weight gain from 28 to 42 dpi was assessed. Neutralizing antibody activity (nAb) was defined as the last 1:2 serial dilution of serum incubated with 200 50% tissue culture infectious doses of PRRSV without cytopathic effects in MARC-145 cells (titer). All piglets were genotyped using the 60k SNP chip. Heritability of tAb response was estimated at 18±14%. Pigs that did not experience virus rebound had a 1216±573 lower MFI ($p=0.036$), and each additional nAb titer was associated with a 360±132 unit increase in MFI ($p=0.007$). A one standard deviation increase in viral load was also associated with a 719±260 unit decrease in MFI ($p=0.006$). Additionally, a one kg increase in weight gained 28-42 dpi was associated with 250±101 less MFI ($p=0.014$). The GWAS discovered SNPs in the MHC class I antigen gene region on chromosome 7 to be associated with tAb response. This 1 Mb region was estimated to explain 30.5% of genetic variation, indicating the presence of a major QTL associated with IgG anti-N levels. An intronic SNP in the DHX16 gene explained all genetic variance observed in this window. Heterozygotes (AB) for this SNP had 4315±604 lower MFI ($p<0.001$) and gained 0.81±0.29 more kg between 28 and 42 dpi ($p=0.005$), as compared to AA individuals. These results suggest that AB individuals may be less susceptible to the effects of the virus. This SNP did not have a significant effect on viral load, virus rebound, or nAb, suggesting that this may be a QTL for general anti-PRRSV antibody response.

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References

1. Holtkamp, D. J., et al. Assessment of the economic impact of porcine reproductive and respiratory syndrome virus on United States pork producers. J Swine Health Prod 2013;21(2):72-84

FOSTERA™ PRRSV EXPOSURE IN GESTATING SWINE AND CONGENITAL INFECTION

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) is the most economically important disease affecting the US swine industry[1]. Modified live virus PRRS vaccine can behave similar to field virus regarding horizontal or transplacental transmission and congenital infection[2]. The safety of Fostera™ PRRS accidental exposure in naive pregnant swine and piglets born to exposed dams has not been reported.

Materials and methods: Fifteen pregnant dams confirmed free of PRRS virus (PRRSV) were divided into the following: Group 1 (*N*=10) dams were vaccinated with 2ml Fostera™ PRRS intramuscularly at 90 days of gestation. Group 2 (*N*=5) dams served as sham vaccinated controls. Dams were monitored daily for anorexia and lethargy post-vaccination. Rectal temperatures were recorded daily for 1 week and every-other-day for 1 additional week. At farrowing, total born, born alive, stillborn, mummified and nonviable piglets were recorded with piglet birth weight. Dams were necropsied at 46 days post vaccination (DPV). Pre-suckling serum was collected from the umbilicus or by venipuncture of 96 piglets and tested for PRRS specific antibodies by PRRSX3 ELISA (IDEXX) and viremia by a commercial PRRSV quantitative PCR assay (Life technologies). Piglet mortality data, weaning weight, weight gain and average daily gain were recorded from 5 litters in each group. Reproductive and piglet production and mortality data were analyzed using the student's *t* test (JMP, SAS institute). A *P*-value ≤ 0.05 was considered significant.

Results: Group 1 (G1) and Group 2 (G2) dams did not demonstrate clinical signs of illness, anorexia or lethargy post-vaccination. Significant differences in rectal temperatures were not observed. One G2 dam aborted nine PRRSV negative piglets at ninety-eight days of gestation. Significant differences between G1 and G2 (*N*=4) mean gestation length, total born, total born alive, birth weight, stillborn, mummified and non-viable piglets were not observed. Lung and tonsil from 4/10 and 10/10 G1 dams, respectively, were PRRSV PCR positive for vaccine virus at necropsy confirmed by ORF5 sequencing. Eighty-four percent (81/96) of presuckle piglets demonstrated PRRSV viremia at birth ranging from 0.7 – 7.2 Log_{10} genomic copies/ml of serum and anti-PRRSV presuckle antibodies were detected in 11.5% (11/96) of the piglets. Significant differences between G1 and G2 average weaned and percent pre-weaning mortality were not detected. In contrast, average weaning weight was significantly lower in G1 piglets (Table 1). Overall weight gain and average daily gain recorded for 5 G1 and 4 G2 litters was also significantly lower in G1 piglets from vaccinated dams. Clinical disease was observed in G1 piglets starting at 10 days post-farrowing.

Conclusions and Discussion: Fostera™ PRRS did not cause clinical signs or affect reproductive performance between 90 and 117 days of gestation under the conditions of this study. Dam lung and tonsil may remain PRRSV PCR positive for longer than 46 DPV. Fostera™ PRRS vaccinated sows give birth to viable piglets with variable levels of vaccine virus in piglet serum. Pre-wean mortality was unaffected by congenital vaccine viremia. In contrast, a significant reduction in growth performance and ADG was detected at weaning in congenitally infected piglets compared to the non-vaccinated controls.

Table 1. Mean weaning weight, weight gain and average daily gain per piglet at weaning.

	Average/pig at weaning± standard error of the mean				
Group	***N***	**Wean wgt***	***N***	**Wgt gain***	**ADG***
G1	92	11.2±0.29[a]	50	7.6±0.31[a]	0.36±0.01[a]
G2	41	13.8±0.47[b]	41	10.2±0.40[b]	0.47±0.02[b]

*Significant difference at $P < 0.0001$.

References:

1. Holtkamp DJ et al.: 2013, J Swine health Prod 21:72-84.
2. Mengeling WL et al.: 1996, Am J Vet Res 57(6):834-839.

GWAS FOR REPRODUCTION TRAITS AND ANTIBODY RESPONSE IN PRRS INFECTED SOWS

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Porcine Reproductive and Respiratory Syndrome (PRRS) causes increased abortions, stillbirths and mummies in reproductive sows, and chronic respiratory problems in pigs at all stages of production. The objective of this work was to study the genetic architecture underling a PRRS outbreak in a commercial pig herd.

Data available for the study included reproductive data on ~2,500 purebred sows from a fully pedigreed herd. The first day of herd PRRS exposure was estimated based on rolling averages of farrowing traits (Lewis et al., 2009) and used to split the data in a Pre- and a Post-PRRS outbreak phase. A total of 646 sows were in the herd during the PRRS outbreak and all had blood samples collected 46 days after the estimated outbreak for ELISA, allowing semi-quantification of PRRSV IgG antibody levels (reported as sample-to-positive (SP) ratio). The available phenotypic data for each sow included parity and numbers of piglets born alive, stillborn, mummified, and alive at 24 hours of age. Genetic parameters were estimated separately for the Pre- and Post-PRRS data sets. All 646 sows were genotyped for the PorcineSNP60 BeadChip. The genotype data was used to perform GWAS for all traits using the BayesB method with π=0.995, and a model with the fixed effects of parity, the genotype at a SNP (WUR) on SSC4 that has been found to be associated with PRRS response in growing pigs (Boddicker et al., 2012), and the rolling average for the trait being analyzed (except for SP ratio).

Pre-PRRS heritabilities for the farrowing traits ranged from 3% (number of mummified piglets) to 18% (number born alive). In the Post-PRRS data, estimates tended to be lower, ranging from 6% (number stillborn) to 10% (number alive at 24 hours). SP ratio showed the highest heritability (53%) and genetic correlations with farrowing traits, ranging from -0.75 (number mummified) to 0.74 (number born alive and alive at 24 hours). The GWAS detected several genomic regions associated with number stillborn and SP ratio. For number stillborn, two regions on SSC1 explained ~4.5% of the genetic variance. For SP ratio, one region on SSC2 and six regions on SSC7 were found to be associated. The six regions on SSC7 jointly accounted for 25% of the genetic variance. One region close to the Major Histocompatibility Complex (MHC) region explained 11.4% of the genetic variance. Another region at the end of SSC7, harboring several candidate genes and in high linkage disequilibrium, explained 7.8% of the genetic variance. These results indicate that there is a significant genomic component associated with PRRS antibody response in this PRRS outbreak sow herd, and that antibody response is genetically correlated with reproductive traits in pigs during the outbreak. Additional research is necessary to better refine the genomic regions for SP ratio identified here.

The work presented here was supported by a grant from Genome Canada, the Canadian Swine Health Board, and PigGen Canada.

1. Boddicker NJ et at.: 2012, J Anim Sci 60:1733.
2. Lewis CRG et al.: 2009, J Anim Sci 87:876.

PIGS SELECTED FOR INCREASED FEED EFFICIENCY ARE LESS AFFECTED BY EXPERIMENTAL INFECTION WITH THE PRRS VIRUS

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Feed efficiency is of great importance to the swine industry, yet little research has been performed to evaluate the robustness of pigs selected for increased feed efficiency to disease. Recent resource allocation theories suggest that such animals may be more susceptible to disease as a result of having less available energy to mount an immune response. Since Porcine Reproductive and Respiratory Syndrome (PRRS) is the most economically devastating disease to the US swine industry, the objective of this study was to analyze the effect of PRRS on lines of pigs divergently selected for low and high residual feed intake (RFI), a measure of feed efficiency. We hypothesized that the efficient low RFI pigs will have a greater reduction in average daily gain (ADG) and higher serum viremia upon infection with the PRRS virus, compared to pigs selected for high RFI (reduced feed efficiency).

One hundred piglets, each from the first parity of the 8th generation of the ISU high and low RFI selection lines, were shipped to Kansas State University upon weaning (between 18 and 28 days of age) and received an intranasal/oral dose of the NVSL 97-7985 PRRS strain one week post-arrival. Serum samples were collected on days 0, 4, 7, 11, and 14 post-infection and weekly thereafter. Body weights were collected weekly and used to calculate ADG as the regression of weight on day. For comparison, body weights were also collected on 489 non-challenged pigs, which were from parity two of generation 8 and were either full or half siblings of the pigs in the PRRS challenged data set. The non-challenged pigs were housed at the Iowa State Swine breeding farm throughout the duration of the data collection period. At least two weights (in addition to a weaning weight) were collected for each piglet before leaving the nursery and weights were estimated using linear regression for days that weight was not recorded. ADG was then calculated as the regression of these estimated weights on age. Viremia was quantified as viral load (VL) as area under the curve for 0-21 days post-infection of the log of PRC-based serum viremia.

In the challenged pigs, VL tended to be slightly lower and ADG slightly higher for the more efficient low RFI line ($p=0.11$ and 0.08, respectively). In contrast, for the non-challenged pigs, ADG was slightly lower for the low RFI line but again not significantly ($p=0.09$). A joint analysis of challenged and non-challenged pigs showed a significant interaction between RFI line and challenge status ($p=0.02$), demonstrating that growth of the low RFI line was less affected by PRRS challenge than growth of the high RFI line.

These findings demonstrate that selection for increased feed efficiency based on RFI does not increase the impact of PRRS infection on serum viremia and weight gain. In fact, our evidence shows that growth of the more efficient pigs was less affected by PRRS infection than that of the inefficient line. These findings may provide commercial farmers with additional incentives to invest in feed-efficient pigs. The work presented here was supported by USDA ARS and NIFA Awards 2012-38420-19286 and 2011-68004-30336 and the breeding companies of the PRRS Host Genetics Consortium.

BROADLY NEUTRALIZING ANTIBODIES TO PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

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Introduction

Following infection with PRRSV, the neutralizing antibody (nAb) response is generally described as weak and delayed. Moreover, the nAb response is primarily directed against genetically related PRRSV isolates. The limited ability to produce nAb to genetically diverse isolates is a major impediment for the development of broadly effective PRRSV vaccines. A unique class of antibodies have been described for several viruses that are capable of neutralizing a broad range of genetically diverse isolates. These antibodies are known as broadly neutralizing antibodies (bnAb). Based on these observations, we hypothesize that bnAb exist for PRRSV.

Methods

This work was conducted as part of the PRRS Host Genetics Consortium (PHGC). Serum samples were obtained from 1,211 pigs challenged with either the Type II isolates NVSL (854 pigs) or KS06 (357 pigs). Serum samples, collected 42 days after infection, were analyzed for virus neutralizing activity using a standard PRRSV serum neutralization assay (NA) against the homologous (challenge) isolate, and three genetically diverse isolates. Briefly, NA involved incubation of 1:2 sera dilutions with 200 50% tissue culture infectious doses of PRRSV in 96-well plates. Following one hour incubation, contents were transferred to confluent MARC-145 cells and incubated for four days. The neutralizing titer was determined as the last dilution without PRRSV CPE. Measurements of NA activity incorporated four genetically diverse PRRSV isolates, which possessed 5 to 11% nucleotide level identity in ORF5. Based on the results, samples were placed into one of four groups: Grp1, no nAb; Grp2, homologous nAb (reactivity against only the homologous virus); Grp3, heterologous nAb (reactivity against 1 or 2 additional isolates); and Grp4, bnAb (reactivity against all isolates). Selected serum samples from Grp1 and Grp4 were tested against 7 additional PRRSV isolates, including a Type I virus.

Results

The results show the percentage of pigs in each group: Grp1, NVSL-8.9%, KS06-24.9%; Grp2, NVSL-57.3%, KS06-46.5%; Grp3, NVSL-29.6%, KS06-24.1%; and Grp4, NVSL-4.3%, KS06-4.5%. Four Grp1 samples with high homologous neutralizing titers showed no neutralizing activity against seven additional isolates, which confirmed the strict homologous nature of the nAb response. Assays incorporating four Grp4 serum samples showed that two samples neutralized all eleven viruses isolates, including a genetically diverse Type I virus.

Conclusions and Discussion

These results demonstrate that all neutralizing antibodies are not equal. Furthermore, within a population, there exists a subgroup of pigs with the capacity to produce a response against multiple isolates, which we term a broadly neutralizing antibody response. Future work is directed at investigating the unique properties of bnAb, including the contribution of host genetics. We predict that epitopes recognized by bnAb are highly conserved. The results create the opportunity to design new vaccines and breed animals that favor a bnAb response.

This work was supported by National Pork Board Grant #12-120 and the PHGC

CHARACTERIZATION OF AGE-RELATED SUSCEPTIBILITY OF MACROPHAGES TO PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

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Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) is the most economically important disease affecting swine production in the United States today. Age dependent resistance to Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) has been observed, with young pigs experiencing longer periods and higher levels of viremia compared to older pigs.[1] PRRSV targets pulmonary alveolar macrophages (PAMs) and cellular surface receptors CD163 and CD169 have been identified as important for infection.[2] However, mechanisms underlying age-related differences as well as the specifics of the immune response to PRRSV remain unclear. Preliminary evidence suggests PAMs belonging to older pigs are more resistant to PRRSV compared to those from younger pigs (Li, 2010, *unpublished data)*. We hypothesized that age-related resistance to PAM infection results from decreased infection of cells due to differential expression of surface receptors for PRRSV.

Materials and Methods

PAMs isolated from six pigs of different age groups (3 days old, 10-12 weeks, and adult) were infected with PRRSV. Level of PRRSV infection and expression of CD163 and CD169 were analyzed by flow cytometry at 12 hours post-infection (HPI). Viral replication was compared at 12, 24, and 48 HPI by qRT-PCR.

Results

A higher percentage of PAMs from younger pigs were infected and yielded greater amounts of virus compared to those from older pigs. Level of infection for PAMs from 10-12 week old pigs was more similar to that of PAMs from the adults. CD163 and CD169 expression was not different between age groups.

Conclusions

PAMs isolated from older pigs are more resistant to PRRSV infection compared to those from younger pigs. This age-related PAM resistance to PRRSV infection is not due to differential levels of CD163 and CD169 expression. In the future, we hope to identify differences responsible for the age-related resistance. These may include cellular receptor polymorphisms, innate anti-viral gene response, and differential macrophage polarization.

Acknowledgements

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References

1. Klinge KL, et al.: 2009, Virol J 6:177.
2. Van Gorp H, et al.: 2008, J Gen Virol 89: 2943-53.

INFLUENCE OF PIG AGE ON THE NEUTRALIZING ANTIBODY RESPONSE TO PRRSV

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Introduction: There are inconsistencies in our understanding of the role of neutralizing antibodies in immune protection to Porcine reproductive and respiratory syndrome virus (PRRSV). After initial exposure to the virus, neutralizing antibodies are produced, but not until after peak viremia has abated. This suggests they are not the primary immunologic mechanism for viral clearance from the circulation; however the role these neutralizing antibodies play in protection against subsequent exposure to PRRSV is unclear.

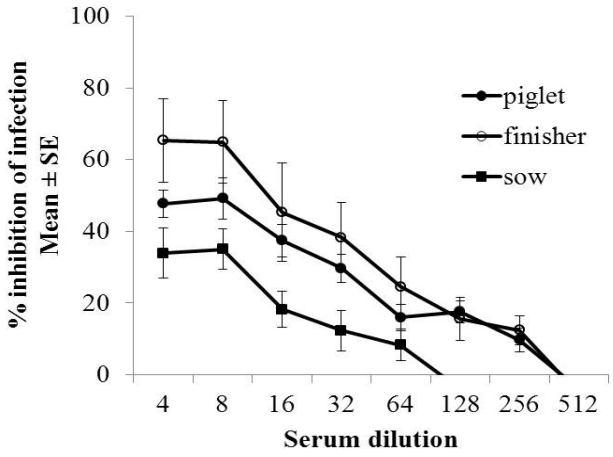
Age-dependent resistance to PRRSV has been observed, whereby younger pigs exhibit higher levels and longer duration of viremia compared to older pigs despite similar timing and magnitude of antibody production against the virus[1]. Most research investigating neutralizing antibodies to PRRSV has been conducted in young pigs, however in this study, we observed that older sows show high neutralizing antibody titers.

Materials and Methods: Using a high-throughput ELISA-based assay to examine the neutralizing antibody response to PRRSV, we sought to distinguish whether these high titers are a function of age at which pigs are first infected with PRRSV or a response to cumulative exposure to multiple PRRS viruses over time.

Results: Sows exposed to multiple field viruses over time have high neutralizing antibody titers against PRRSV VR2332, whereas sows from the experimental inoculation study had lower neutralizing titers.

Unexpectedly, sows experimentally infected with PRRSV JA142 had lower neutralizing titers compared with younger pigs (Figure 1).

Figure 1: Sows have lower neutralizing titers on first exposure to PRRSV compared with younger pigs.



Conclusions and Discussion: Findings suggests high titers observed in field sows *may* be more related to cumulative exposure, possibly to differing strains than to age at first exposure. Though the underlying factors responsible are not known, the importance of high neutralizing titers for protection against diverse PRRS viruses in older pigs can now be investigated.

Acknowledgements: Thanks to Dr. Paul Yeske and Dr. Mark Wagner for providing field sow serum samples and to Diem Ngo for technical assistance with serum neutralization assays. Serum samples for age comparisons were originally collected as part of the Klinge et al. study[1].

References: 1. Klinge KL *et al.*: 2009, *Virology Journal* **6**:177

THE EFFECT OF INGELVAC PRRS® ATP VACCINATION OF GROWING PIGS ON SEVERITY OF RESPIRATORY DISEASE CAUSED BY CO-INFECTION OF PRRSV AND SIV

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Introduction

Respiratory disease caused by porcine reproductive and respiratory syndrome virus (PRRSV) and swine influenza virus (SIV) causes dramatic losses for swine producers every year. Conditions that favor the transmission of these viruses are similar; therefore, simultaneous infection within herds is common. Vaccination of growing pigs with a modified-live PRRSV vaccine is performed to reduce the effects of PRRSV infection. This practice reduces mortality, culls and increases performance in herds that become infected with PRRSV.[1] Vaccination for SIV is less common due in part to timing constraints brought on by maternal immunity and limitted cross-protective immunity. The objective of this study was to assess whether vaccination with a modified-live PRRSV vaccine could reduce respiratory disease in pigs co-infected with both PRRSV and SIV.

Materials and methods

An experimental study was performed on 30, ten-week-old pigs that were PRRS and *Mycoplasma hyopneumoniae* negative. The study consisted of three groups. Group 1 (n=6) served as unvaccinated/unchallenged control. Group 2 (n=12) remained unvaccinated and was challenged with PRRSV and SIV. Group 3 (n=12) was vaccinated on day 1 and challenged with PRRSV and SIV. Pigs were ramdomly allocated to groups 2 and 3 and inoculated on day 32, via the intranasal route with 4ml of H1N2 SIV ($TCID_{50}$=1.33x10^6/ml) and PRRSV RFLP pattern 1-4-4 ($TCID_{50}$=1x10^4/ml). Weights were taken at days 1 and 39. Serum and nasal swabs were collected at 7 days post-inoculation (DPI) and rectal temperature was measured at 7 DPI. All pigs were necropsied at day 39 of the study. Lung lesions were scored as a percent of total lung affected for each pig individually. Parameters were compared using the non-parametric Mann-Whitney U Test.

Results

Fever and respiratory disease was identified by 2 DPI in groups 2 and 3. Temperature at 7 DPI was significantly higher in group 2. Although numerical differences were observed in favor of group 3 in average lung lesion score and average daily gain (ADG), no statistical significant differences were detected for these parameters between groups 2 and 3 (Table 1).

Table 1 Comparison of measured variables

	GP1	GP2	GP3	Diff 2vs3	P-value 2vs3
ADG 1-39 days	1.66[a]	1.57[a]	1.63[a]	0.06	0.58
Lung Score (%)	0.0[a]	10.2[b]	8.1[b]	2.10	0.40
Temp (F°) 7 DPI	NA	105.6[a]	104[b]	1.60	0.002

Different superscripts within row indicate statistical significant difference between groups.

Conclusions and discussion

In this study there was a significant reduction in fever at 7 DPI for the group receiving Ingelvac PRRS ATP vaccination. All pigs in group 2 and 3 were PRRS PCR positive at 7 DPI. Group 3 had 6 pigs SIV positive at 7 DPI compared to group 2 having 3 remaining SIV PCR positive. Although not statistically significant, vaccinates had a numerical reduction in lung lesions and greater ADG indicating a potential performance benefit to PRRS vaccination for those pigs challenged with PRRSV and SIV that should be evaluated in a follow up study with higher statistical power.

References

1. Waddell J. *Proc Am Assoc Swine Vet*. 2008, 257-260.

Acknowledgements

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EVALUATION OF AIRBORNE SHEDDING OF FOSTERA PRRS MLV VACCINE FROM GROWING PIGS RAISED UNDER COMMERCIAL CONDITIONS

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Current Status of the Problem
Herds have been successful at PRRS elimination and the ability to generate negative pigs. These negative pigs in high pig density regions are being vaccinated with PRRS MLV vaccines. Vaccinating these pigs has shown to improve performance when challenged with field virus[1,2]. Even though previous studies demonstrated limited airborne shedding[3] there is still concern with the use of PRRS MLV that it will shed and spread to other herds

Project Objectives
Identify the amount and duration of aerosol shedding of Fostera PRRS MLV inside a commercial wean to finish barn, outside the barn directly outside the pit fan and 1 mile down wind of the barn.

Procedures to achieve Objectives
1. 3600 PRRS negative pigs were housed in a tunnel ventilated double-sided wean-to-finish barn and split in 2 rooms with separated air spaces and entries.
2. Pigs in room one were vaccinated with Fostera PRRS MLV labeled dose at 4 weeks of age. Pigs in the other room were not vaccinated.
3. Air samples were tested for PRRS PCR.
4. Liquid cyclonic collectors were used to collect daily air samples for 28 days.
5. 30 sentinel pigs per room were tested periodically by PRRS PCR and ELISA. Six oral fluid samples were also collected per room from the pens where the sentinel pigs were located and tested by PRRS PCR and ELISA.

Results
No virus was detected in any of the air samples collected either in the barn, outside the pit fan or 1 mile in the down wind direction.
Viremia was detected in vaccinated pigs. PRRS virus was detected in oral fluids post-vaccination.
PRRS virus RNA and ELISA antibody were detected in serum or oral fluids in the non-vaccinated pigs following pit pumping and there is no evidence of a biosecurity breech in the system during this same time. Sequencing was completed and was 99.7% homology to vaccine virus. A limited number of air samples were collected when the control side seroconverted and these were negative as well.

Discussion
Vaccine virus was not found in air samples at all 3 collection sites. There was no spread via aerosol that can be detected. The non-vaccinated control room turned positive following pit pumping. Implication would be less likely to have virus spread following vaccination pigs in wean to finish sites.

References
1. Oppressing T. et.al.; Demonstration of Protection Against Heterologous PRRS Challenge; J Swine Health and Production 13:246-253.
2. Polson, D., et.al, 2000. Evaluating the Beneficial Performance and Financial Effects of Pig Vaccination with Ingelvac® PRRS MLV in a PRRS-Positive Multi-Site Production System. Proceedings of the 16th IPVS. Pg: 152.
3. Yeske P: Evaluation of Airborne Shedding of Ingelvac PRRs MLV from growing pigs under commercial conditions. Proceedings AASV 2012 pp219-222

NO INDICATION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) INFECTION IN BRAZILIAN SWINE HERDS

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is a globally important pathogen of economic and veterinary concern. Recent studies estimate a cost of US$664m yearly to United States swine industry [3]. PRRS is present throughout the world, with the exception of Australia, New Zealand, Finland, Norway, Sweden and Switzerland [5]. Although Brazilian swine production is expressive (fourth pork producer and exporter), there has been no evidence of PRRSV infection in those herds [1]. Most of the analysis used serology by commercial ELISA tests in breeding herds to perform prevalence surveys. The objective of this work was to detect PRRSV in samples of sera, plasma or oral fluids (OF) from swine herds, quarantined imported boars and feral pigs from 2008 – 2012.

Material and Methods

A total of 113 commercial pigs herds with a history of respiratory problems from 8 Brazilian states (SC, RS, PR, MG, SP, MS, MT and GO) were included. Farms were divided in 51 farrow to finish farms (1000 plasma and 257 OF samples) collected between 2008-2009; 62 commercial nursery farms (1860 sera and 232 OF paired samples from 8-12 week old pigs) collected between 2009-2012. Moreover, quarantined imported pigs (471 sera and 52 OF paired samples) collected in 2011 and 148 feral pigs serum samples collected from captured pigs in 5 farms in the sub-regions of Nhecolândia and Abobral, in the Pantanal of MS. This serum sample collection was done in two periods, from August to September 2009 and from January to August 2010. Thus, a total of 2479 serum samples, 1000 plasma samples and 541 OF samples were collected from 2008 to 2012 and were sent to Embrapa Swine and Poultry for processing. All samples were tested using IDEXX HerdChek* PRRS Antibody Test Kit. Positive samples were processed for viral RNA extraction by MagMAX® 1836-5 (Applied Biosystems). Real-time RT-PCR reactions were performed using specific primers targeting the ORF7 gene sequences of North-American PRRSV as described [4]. Positive control RNA was *in vitro* transcribed using RiboMAX™ (Promega), provided by NADC/ARS/USDA (Ames, USA) [2].

Results

4020 samples (serum, plasma and OF) from domestic or feral pigs from eight Brazilian states and quarantined imported boars were tested by IDEXX HerdChek* PRRS Antibody Test Kit. All OF samples resulted negative, 1/1000 plasma samples and 3/2479 sera samples resulted positives. Both sera and plasma samples were submitted to real-time RT-PCR and resulted negative as well. Real-time RT-PCR was able to detect $2.6x10^3$ molecules/uL of PRRSV positive control [2].

Discussion and Conclusion

Herein are presented different categories of sampling aiming to detect PRRSV infection in Brazilian pig herds. Although this does not characterize a surveillance study, it demonstrates the absence of PRRSV antibodies or RNA at analyzed samples. The main route in which PRRSV has been introduced into previously free countries is undoubtedly via pig movements [6]. Semen imports have also played a part, in some cases [6]. Thus, no evidence of PRRSV infection in those pigs was observed, indicating the importance to implement diagnostic tools and a monitory program to prevent the entry and further distribution of PRRSV in Brazil.

Acknowledgements

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References

1. Ciacci-Zanella JR et al.:2004, Ciência Rural 34:449.
2. Ellingson JS et al.: 2010, Vaccine 28:2679.
3. Lundeen, T. 2011: Feedstuffs (USA).
4. Nicholson TL et al. 2011,JClin Microb 49:4.
5. OIE. http://www.oie.int
6. Zimmerman JJ et al. 2012, Disease of Swine, 10th ed.

EVALUATION OF LATE-GESTATION GILTS FOLLOWING ACCIDENTAL EXPOSURE TO FOSTERA™ PRRS VACCINE VIRUS

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Introduction

Modified-live Porcine Reproductive & Respiratory Syndrome viruses (PRRSV) have the ability to replicate in the animal after administration with subsequent shedding of virus to the environment and to naïve swine in contact with previously vaccinated animals. Natural exposure of PRRSV in pregnant swine can be devastating depending on the virulence of the strain. It is vital to understand how accidental exposure with Fostera PRRS modified-live vaccine virus, may affect pregnancy and/or piglet viability and production if naïve, gestating gilts were exposed to the vaccine virus.

Materials and Methods

The study involved 76 healthy pregnant gilts at 90 days of gestation that were purchased from a SPF herd in Minnesota. All animals were confirmed free of PRRSV and PCV2, and were *Mycoplasma hyopneumoniae* positive but stable (ELISA or PCR). Animals were housed in a commercial farrowing unit with separate airspaces. Gilts were randomly allocated to 1 block of 3 animals each, and within blocks each animal was then randomly assigned either control or exposed groups in a 1:2 ratio, respectively (randomized complete block design). One day later, animals in each treatment group received the following injections (study day 0): Fostera PRRS: 2 mL IM (n=51); Control: 2 mL vaccine diluent IM (n=25). Data collected during the study included gilt morbidity and mortality, PRRSV serology, and litter productivity parameters (abortions, mummified/still-born piglets, live births, birth weights, pre-weaning mortality, weaning weights, birth-to-weaning daily gains). Serum samples collected from gilts (days -7, 0, 7, 48) and liveborn piglets were evaluated for anti-PRRSV antibody using a commercial ELISA test. Piglets that died were necropsied and were tested by PCR for detection of PRRSV. Air samples were also collected at the exhaust fans daily during the experiment.

Results and Discussion

Serology results showed that gilts in the Fostera PRRS vaccine virus group generated higher ($P \leq 0.0075$) PRRSV antibody titers on days 7 and 48 compared to control animals. All exposed gilts demonstrated PRRSV at 7 days post-exposure with PCR. PCR cycle times (CT) ranged from 23.9 to 33.0 with an average of 27.34 ± 1.9. These data indicate that administration of Fostera PRRS was successful in challenging gilts with the vaccine virus for the purpose of evaluating safety responses. None of the gilts injected with Fostera PRRS vaccine virus demonstrated any clinical signs of systemic disease associated with PRRSV, anorexia, or lethargy, nor did any control animals. Most notably, **no abortions** occurred in any of the 51 gilts that received the Fostera PRRS vaccine virus. In addition, no differences ($P > 0.56$) were observed between treatment groups in the average number of pigs born/litter or pigs born live/litter. Similar rates of mummies occurred in each group, but a higher rate of still births was noted in the Fostera PRRS vaccine virus group. A higher rate of pre-weaning mortality was also observed in the Fostera PRRS vaccine virus group. During the trial there were only two days that air samples from the exhaust fans were positive and this was after 98% of the farrowings had occured. The PCR cycle times were both greater than 35.

Though piglets born to gilts exposed to the vaccine virus 53% were viremic at birth (evidence of transplacental virus transmission), they appeared clinically unaffected. Dyspnea was noted in some animals at 7 to 10 days of age, and some were thin, gaunt, and rough-haired. Average daily gain (ADG) during the birth-to-weaning period was reduced ($P \leq 0.0001$) compared to piglets born to control gilts.

In summary, this trial demonstrated that inadvertent exposure of Fostera PRRS vaccine virus to naïve gilts at 90 days of gestation posed no threat to farrowing success, with the most notable observation being the absence of abortions that often result from PRRSV field infections.

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

Discussion and Conclusion

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.

PRRSV FIELD EXPOSURE AND MORTALITY RELATIONSHIP ANALYSIS IN NON-VACCINATED AND VACCINATED FINISHER PIG BARNS

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Introduction

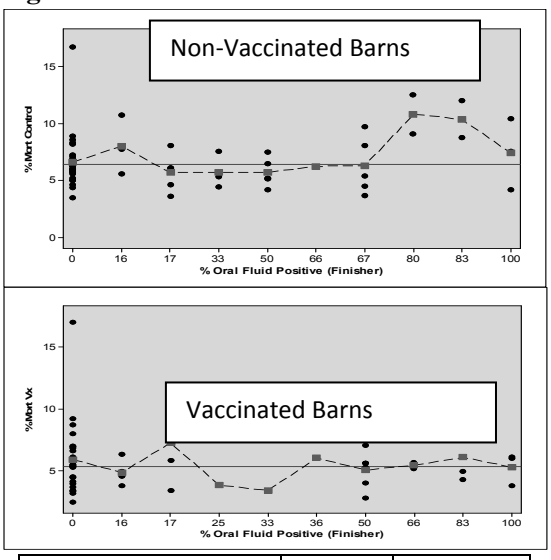
Modified live vaccination has been used as a primary tool for minimizing mortality rate due to PRRSv[1,2]. Vaccination reduces shedding and excretion of wild-type virus in vaccinated pigs, but did not prevent infection[2]. The objective of this study was to analyze mortality rate in non-vaccinated control and vaccinated finisher barns with different levels of wild type PRRS (WT PRRSv) exposure in a large production system.

Materials and Methods

A barn-level side by side study was conducted in a large production system located in the USA. The pig flow was selected from PRRSv Stable Sow Farms, defined as PRRSv negative or ≤20% positive PRRS PCR 5:1 pooled serum samples at weaning. Pigs in the vaccinated groups were vaccinated with Ingelvac PRRS® MLV (2ml) at nursery placement. Treatment group and matched pair integrity was maintained upon movement of pigs from the nursery to the finisher sites. The study evaluated 60 vaccinated finishing barns and 59 non-vaccinated finishing barns within the same finisher sites. The sampling protocol during the finisher phase utilized oral fluids from 6 ropes per barn, for PRRS PCR at 15-16 wks of age. All PCR positive samples were sequenced (ORF5). Percent mortality from the Finisher group/barn closeouts was used for the analysis. For each finisher barn in the study, the proportion of positives at 15-16 weeks of age and its respective mortality were analyzed through a run chart using MINITAB 16.2 with WT PRRSv proportion on axis X and mortality on axis Y. The run chart included two tests for randomness (No. of runs about the median & No. of runs up and down) to indicate whether there were any statistically significant patterns or evidence of non-randomness.

Results

Figure 1. Run Charts for Finisher Barns



	Control	Vaccinated
P-Value for clustering	**0.022***	0.910
P-Value for Mixtures	0.978	0.090
P-Value for Trends	0.391	0.710
P value for Oscillation	0.609	0.290

Conclusions and Discussion

Results showed that in control barns, the higher the percentage of OF sample positive for WT PRRSv, the higher the mortality (clustering P-value=0.022), however, vaccinated barns showed no indication of a relationship between high mortality and PRRSv WT proportion (clustering P value 0.910) confirming that vaccination does not prevent infection, but significantly reduces mortality due to PRRSv.

References

1. Philips R. et al. 2006. *Proc IPVS* O.46-06
2. Cano et al. 2007. Vaccines, 25 4383-4391

REDUCTION OF WILD-TYPE PRRS VIRUS SHEDDING IN AEROSOL OF GROWING PIGS BY MODIFIED-LIVE VIRUS VACCINATION AT WEANING

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Introduction

The risk of area-spread of porcine reproductive and respiratory syndrome virus (PRRSV) continues to be high in swine-dense regions potentially because of PRRSV shedding from large populations of growing pigs. The therapeutic use of modified-live virus (MLV) vaccine in infected pigs has been shown to reduce the duration of wild-type virus (WTV) shedding to sentinels and in aerosol.[1,2] The objective of this study was to quantify the effect of MLV vaccine on performance and measure WTV shedding in pigs vaccinated at weaning and challenged 4 weeks later.

Materials and Methods

A total of 2100 PRRS-negative weaned pigs were randomly allocated to either a non-vaccinated control (NVC) or to a MLV vaccinated group, each housed in separated rooms. Biosecurity protocols were implemented to avoid PRRSV transmission between rooms. Pigs in the MLV group were IM vaccinated with Ingelvac PRRS® MLV (Boehringer Ingelheim Vetmedica, Inc) at 4 weeks of age. Four weeks post-vaccination 10% of the pigs in each group were IM inoculated with 1 mL of PRRS WTV RFLP pattern 1-18-2 at a concentration of $4.2x10^7$ RNAc/mL. Infection dynamics was monitored by PCR and ELISA tests on serum and oral fluid (OF) samples. Daily air samples were collected from each group at 8 AM using Liquid Cyclonic Collectors (Midwest MicroTek, Brookings, SD) placed in front of exhaust fans for 30 minutes and tested by PCR. Mortality, cull rate, ADG and feed conversion (FC) were recorded for wean to finish performance.

Results

Mild clinical signs developed in both groups following inoculation with PRRS WTV. No significant differences between groups were detected in PCR or ELISA results in serum or OF samples. MLV vaccine was not detected in the NVC group. The duration and frequency of PRRSV RNA detection in air was significantly higher in NVC than in the MLV group ($p<0.0001$) (Table 1). Performance is summarized in Table 2.

Table 1. Detection of PRRSV in air by PCR

Parameter (days)	NVC	MLV
Frequency post-vaccination	0/28	5/28
Duration post-vaccination	0	6
Frequency post-inoculation	21/118	4/118
Duration post-inoculation	36	6

Table 2. Vaccination to market performance

Parameter	NVC	MLV
Mortality %	4.8	5.1
Cull rate %	5.9	2.8
ADG (lbs)	1.57	1.63
FC (lbs)	2.38	2.40

Conclusions

The prophylactic use of PRRS MLV vaccine in growing pigs at risk of infection represents a valuable tool to reduce the risk of transmission between herds in swine-dense-areas. The observed performance benefits as well as shedding reduction in MLV vaccinated pigs challenged with WTV support the recommendation of MLV vaccination of growing pigs at risk of infection.

References

1..Cano JP, *et al. Vaccine.* 2007 (25) 4382-4391.
2. Linhares DC, *et al. Vaccine,* 2012 (30) 407-413.

TRANSPORT TRUCK SURVEILLANCE FOR PRRS VIRUS IN SUMMER AND WINTER MONTHS

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Objectives

PRRS virus prevalence in central Missouri is low; however, the risk of contamination of trucks at slaughter facilities is hypothesized to be potentially moderate to high. The study objectives were to determine if PRRS virus could be detected via PCR from transport trucks arriving at a central Missouri site to load pigs for market. A second objective was to determine if the risk of PRRSv detection increased in transport trucks during the winter months compared to summer months.

Materials and Methods

Trucks were swiffered from June 6th to July 5th 2012 (summer, n=42) and from Nov 14th 2012 to Feb 20th 2013 (winter, n=40). During the summer period there were 3 transport companies and 10 drivers identified. During the winter period there were 3 transport companies and 9 drivers identified. Drivers completed a short questionnaire about their specific truck wash protocol upon arrival to the farm. Three, 6 foot diameter areas, were swiffered in the truck trailer on all 82 trucks. The swiffered areas consisted of the trailer floor near the rear of truck, trailer loading ramp, and a random area observed to be wet or noticeably dirty in the truck trailer. A 6 foot diameter area was swiffered in each location. Gloves were changed between each sample. Individual Swiffer Sweepers® were pre-packed with 20 ml of transport media. The transport media was squeezed from the swiffer prior to swabbing the trailers (media remained in the bag). Immediately following sampling, the swiffer was returned to media. The media was reabsorbed back into the swiffer and squeezed to release the fluid three times. The fluid was transferred to a snap-top tube, frozen and later shipped to the BI-HMC for PRRS PCR evaluation (246 PCR samples total).

Results

All trucks were washed and disinfected prior to entering the farm (Table 1). Seven trailers were observed to have standing water and 19 contained an observable dirty area on the floor. No PCR positive PRRS samples were obtained from any of the 3 locations sampled in each of the 82 truck trailers.

Table 1: Questionnaire responses for truck cleaning procedures prior to entering farm

	Truck driver response	
Activity	Yes	No
Washed truck prior to arrival	82/82 (100%)	0/82 (0%)
Disinfected truck prior to arrival	82/82 (100%)	0/82 (0%)
Commercially power washed truck	79/82(96%)	3/82 (4%)
Truck dried prior to arrival	74/82 (90%)	8/82 (10%)

Conclusions

Study limitations prevented sampling of trucks upon exit from the slaughter facilities so prevalence of PRRSv prior to washing is unknown. Although no PRRSv positive samples were obtained during this study, it can not be concluded that there is no risk of PRRSv transport within transport trucks. However, the various truck wash protocols utilized by drivers in this study appeared to reduce the risk of PRRSv contamination from transport vehicles.

PRRS PCR and PRRS ELISA Responses in Feeder Pigs After Vaccination with Fostera™PRRS

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Introduction

Recent estimates regarding Porcine reproductive and respiratory syndrome virus (PRRSV) show that more than half of weaning-age PRRSV-negative pigs become infected with PRRSV before going to market.[1] Vaccination and other control measures are required to help growing pigs defend themselves against PRRSV. A field study was undertaken in order to examine post-vaccination responses to Fostera™ PRRS™ as assessed by PCR and ELISA, comparing responses in pigs mass-vaccinated by a vaccination crew to those of pigs individually vaccinated.[2] To evaluate economic return, close-out results from this vaccinated group were compared to the previous group of pigs placed at this site.

Materials and Methods

The non-clinical vaccine-response study involved 5000 feeder pigs approximately 8 weeks of age that were sourced from a PRRS-naïve sow farm and placed at a commercial finishing site located in Iowa. After 1 week in the finisher, pigs were vaccinated with Fostera PRRS on study day 0. The vaccine was either mass-administered by a vaccination crew or pigs were individually vaccinated. For monitoring of vaccine responses, 10 pigs in each of 5 separate pens (total of 50 pigs) were randomly selected and tagged for blood sampling during the study. Of these 50 pigs, 5 pigs were unvaccinated controls, 18 pigs were individually vaccinated, and 27 pigs were mass vaccinated with the rest of the pigs on the site. Blood samples were collected from designated pigs on study days 0, 8, 16, 21, and 29 and analyzed for PRRSV status using PCR and ELISA. Close-out production data was compared to the previous grow-finish cycle at the same site involving feeder pigs from the same source that did not receive PRRS vaccination. During both production cycles, pigs experienced a natural challenge with PRRS field virus at approximately week 14 of finishing.

Results

By 8 days post-vaccination 100% of the individually vaccinated pigs were PCR positive compared to 96% of the crew-vaccinated pigs. Non-vaccinated control animals were 100% PCR-positive on day 21. By day 16 100% of individually vaccinated pigs were ELISA positive compared to only 70% of crew-vaccinated pigs. At day 21, 81% of crew-vaccinates were fully ELISA positive, but the response rate rose to 93% by day 29. For non-vaccinated controls, sero-conversion did not occur until day 29. Based on close-out comparisons, mortality was reduced and ADG, feed/gain, and marketing parameters were improved during the study finishing period that included administration of Fostera PRRS vaccine compared to the previous production cycle involving non-vaccinated pigs.

Discussion

In this study differences in effective immunization achieved by typical mass vaccination compared to more careful individual vaccination were shown as evidenced by delayed PCR and ELISA responses in crew-vaccinated pigs. Improved performance of the study group was noted when pigs were vaccinated with Fostera PRRS prior to natural PRRSV challenge compared to non-vaccinated pigs comprising the previous production cycle resulting in an estimated 10:1 return-on-investment at this commercial operation.

References

1. Holtkamp D, Kliebenstein J, Zimmerman J, et al. Assessment of the economic impact of porcine reproductive and respiratory syndrome virus on U.S. pork producer. *National Pork Board Research Report* 2011; NPB #10-158.
2. Data on file, Study Report No. 12PETBIO01, Zoetis Inc.

PRESENCE OF PORCINE CIRCOVIRUS TYPE 2 ANTIBODIES AND VIRUS IN FINISHING PIGS AFTER WIDESPREAD USE OF PCV2 VACCINATION.

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Introduction

Porcine circovirus 2 (PCV2), one of the most economically important pathogens of pigs, is the causative agent of porcine circovirus associated disease (PCVAD). Widespread availability and use of PCV2 vaccines, starting in 2006, ameliorated PCVAD in finishing pigs so successfully that nearly all pigs in the US are currently vaccinated at or before weaning. In a commercial setting, vaccination of piglets around the time of weaning eliminates disease, decreases the level of PCV2 in serum, and increases production performance, but does not eliminate infection. Thus suggesting that nearly all finishing pigs are infected with PCV2 which has been confirmed in recent studies. However, widespread use of PCV2 vaccination may decrease the PCV2 viral load in pigs. The aim of this study is to examine and compare the PCV2 viral load and antibody levels in finishing pigs after years of widespread PCV2 vaccination and compare this with levels obtained in 2006, prior to vaccine use.

Materials and Methods

Serum samples were collected as part of the USDA National Animal Health Monitoring System's (NAHMS) Swine 2012 study focusing on operations with 100 or more pigs in 13 states. Approximately 15 serum samples from each of 100 farms were chosen from this collection for the analysis of both PCV2-specific antibody levels and PCV2 viral levels in serum.

PCV2-specific antibody levels were determined by indirect ELISA using either recombinant PCV2 capsid or replicase protein. PCV2 viral DNA levels were determined using a PCV2 quantitative real-time PCR assay. Results from both antibody and viral load analysis were compared to that of the NAHMS 2006 study.

Results

PCV2 viral loads in serum were similar between animals on the same farm. However, between farms, viral loads varied from barely detectable in many of the farms to low viral levels present on other farms. High viral levels were not observed in animals from any of the farms, contrary to serum loads observed in farms from just a few years ago.

PCV2 capsid-specific antibodies were found to be present in the majority of animals, but at much lower levels than that seen in animals sampled just a few years ago. Antibodies to the PCV2 replicase protein were observed low levels, but some animals had high levels of PCV2 replicase antibodies.

Conclusions and Discussion

Widespread use of PCV2 vaccines has been able to control PCVAD and has greatly decreased, but not eliminated PCV2 virus in swine herds throughout the US. PCV2 viremia today, if observed at all, is observed at low levels in finishing pigs. PCV2 viremia levels before widespread vaccination (in 2006) showed high levels of virus in serum from the majority of animals and all farms were infected. As compared to a few years ago, antibodies to PCV2 are still present in the majority of animals, but the antibody levels are lower than that observed previously. Thus, widespread vaccination of animals for PCV2 is able to decrease the viral load on farms, while still maintaining protective antibody levels. This decrease in viral load may be able to lead to the production of PCV2-free animals in the future.

Acknowledgements

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INTERPRETATION OF PCV2 ELISA RESULTS, A NEW APPROACH.

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Introduction

PCV2 is endemic in almost all swine herds in the world and therefore antibodies against PCV2 can easily be found in animals. Serology is commonly used to establish herd profiles to evaluate the PCV2 dynamics. Since there are no DIVA vaccines for PCV2 on the market, one cannot easily discriminate between field infected or vaccinated animals. This paper describes an approach to discriminate between vaccinated and field infected animals.

Materials and methods

Sera and ELISA

Sera from natural (field) infected animals and from vaccinated animals where evaluated with the BioChek Porcine Circovirus 2 (PCV2) Antibody Test Kit. Sera were obtained from different herds, different breeds and different ages. For vaccination different commercial vaccines were used.

Calculation

S/P ratios were recalculated in titres using the formula:

Log10 Titre = 1.1 * Log (SP) + 3.361
Antilog = Titre

From one herd or group of animals a mean titre and standard deviation was calculated using Microsoft Excel. Percentage of coefficient variance was calculated using the formula:

(Standard deviation/mean titre)*100= %cv

Results

In table 1, a summary of the results is given, showing mean titre, %cv and number of positives.

Table 1. Summary of results in field infected and vaccinated animals.

Group	Mean Titre	CV%	Number Positive	Number Negative
vaccinated*	3000 - 6000	<30%	>95%	<5%
non vaccinated	0 - 7000	>30%	varies	varies

*Different vaccines and vaccination programs can give different mean titres.

Discussion

From the results it is clearly evident that although the mean titre can be the same in field infected and vaccinated animals, the %cv is different. The vaccinated animals are showing a lower %cv indicating a more uniform immunity. The differences between field infected and vaccinated animals were consistent for almost all commercial vaccines, proving that the approach for interpretation is meaningful. Establishing more specific baselines for different vaccines and vaccination protocols is on-going.

Conclusion

When using mean titre and %cv it is possible to discriminate between PCV2 field infected animals and PCV2 vaccinated animals. Baselines for this are essential for interpretation.

FIELD COMPARISON OF TWO COMMERCIAL VACCINES FOR CONTROLLING MUTANT PORCINE CIRCOVIRUS TYPE 2 VIREMIA

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Introduction- Recently a new strain of porcine circovirus Type 2 has been identified in the US based on genetic sequencing.[2] This virus has a similar sequence pattern to a virus previously identified in China and is often referred to as the "Chinese mutant" or mutant PCV2 (mPCV2). Some concern has been expressed regarding the ability of current US commercial vaccines to protect against this new strain. As part of a producer initiated PCV2 vaccine evaluation, we were provided the opportunity to monitor the PCV2 viremia and antibody status of pigs undergoing field exposure to mPCV2.

Materials and Methods- The pigs originated from a herd free of PRRSv and *Mycoplasma hyopneumoniae* (Mhp) and were part of a larger field evaluation comparing the performance between two commercial PCV2 vaccines: Fostera™ PCV (FOST) (Zoetis, Florham, NJ) and Circumvent® PCV (CVENT) (Merck Animal Health, Summit, NJ). This study used a "barn level" design. The pigs that were monitored for PCV2 viremia by PCR and PCV2 antibody by 4-dilution IFA were housed in two adjacent finisher barns. The FOST pigs were vaccinated once at weaning (3 weeks of age). For CVENT vaccination, the producer elected to administer the two vaccinations at processing (3 days of age) and at weaning. The pigs were tagged after arrival to separate nurseries and the same pigs were sampled at 4, 11, 16 and 19 weeks of age. Forty pigs from the source sow herd were sampled at 10 days of age. All laboratory testing was performed by routine methods at the ISU-VDL. Samples for PCR were tested in pools of 5. To confirm the presence of mPCV2 in each barn, oral fluids from 5 pens and blood from 10 light weight, non-tagged pigs were collected at 19 weeks of age. Several positive samples were sequenced and all sequences indicated mPCV2. Based on serotesting at 19 weeks of age, the pigs remained free of PRRSv and Mhp. Statistical analysis was performed by ANOVA and a P value <0.05 was considered significant.

Results- The 10-day-old pigs from the source herd were not viremic and had a geomean IFA titer of 190.3. The following table presents the PCR and IFA results from the tested serum:

Age (wks)	PCR – Pools Pos./Tested		Geomean IFA Titers	
	CVENT	FOST	CVENT	FOST
4	0/5	0/5	498.7[a]	131.8[b]
11	0/5	0/5	560.8[a]	86.9[b]
16	0/4	5/5	266.6[a]	139.3[b]
19*	0/5	5/5	216.3[b]	844.3[a]
19**	1/2	2/2	139.3[b]	735.2[a]

[a,b] If different within a row, $P < 0.05$. * Tagged pigs. ** Light weight pigs.

The average cycle times (CTs) for the positive pools from the FOST pigs at 16 and 19 weeks of age were 27.90 and 23.84, respectively. For the light weight pigs, the two FOST pools had CTs of 25.00 and 22.10, and the CT of the single CVENT positive pool was 26.4. For the oral fluids, the average CTs were 23.54 for the FOST barn and 33.53 for the CVENT barn.

Conclusions and Discussion- The data presented clearly illustrates the ability of CVENT to protect against mPCV2 viremia. In addition, the declining IFA titers in the CVENT pigs indicate protection. This finding was reported in a previous field study that compared non-viremic, CVENT vaccinated pigs (declining tiers) to viremic, non-vaccinated controls (rising titers).[1] The oral fluid results indicate a lower level (approximately 1,000 times less) of virus shedding in the CVENT barn.

References

1. Thacker, B. et al (2013) Proc AASV, 217.
2. Xiao, C. et al (2012) J Virol 86:12469.

LONGITUDINAL EVALUATION OF PORCINE CIRCOVIRUS TYPE 2 ANTIBODY LEVELS IN FIELD STUDY PIGS USING THREE ASSAYS

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Introduction- Porcine circovirus Type 2 serotesting is performed to determine the time of infection, evaluate maternal antibody levels and measure post-vaccination responses. Several types of tests are available worldwide. In the US, IFA performed by several laboratories and an ELISA performed by the ISU-VDL are the main tests used. In a previous report and subsequent field experiences, the ISU-VDL ELISA and IFA test results sometimes disagree, especially with regard to maternal antibody status and the antibody response following vaccination with Circumvent® PCV (Merck Animal Health, Summit, New Jersey).[1] To provide insight into these discrepancies, sera from a vaccination field study was evaluated by IFA, the ISU-VDL ELISA and a commercially available ELISA not commonly used in the US.

Materials and Methods- The sera were obtained from a field study to evaluate the growth and viremia status of pigs until market weight.[2] Pigs were vaccinated at 3 and 6 weeks of age with Circumvent® PCV M (Merck Animal Health, Summit, New Jersey) or kept as non-vaccinated controls. Field exposure began as early as 1 week of age based on PCV2 PCR testing. In addition to the 4-dilution IFA testing previously reported[2], the samples were tested by the ISU-VDL in-house ELISA (ISU-ELISA) and a commercial ELISA (COM-ELISA; Ingezim Circo IgG, Ingenasa, Madrid, Spain). All testing was performed at the ISU-VDL. Titer results are presented for each sampling age as the group geometric mean and S/P ratios as averages. Each group contained 20-25 pigs.

Results- In general, the tests provided similar results. At 1 week, the maternal antibody levels measured by the COM-ELISA were at low-to-moderate levels while the IFA showed low levels. With regard to post-vaccination titers, all three assays detected a slight rise in titers after the first vaccination. Following the second vaccination, titers peaked at 8 weeks for the ISU-ELISA and at 10 weeks for the COM-ELISA and IFA. For all assays, titers in the control group started to increase at 10 weeks and peaked at 19 or 25 weeks. Titers in the vaccinated group were declining by 25 weeks, especially with the COM-ELISA and IFA.

Test: Unit	Age (Wks)	Group	
		PCVM	CONT
Ingenasa ELISA: Titer	1	2,231	2,070
	3	633	639
	6	998	274
	8	20,172	268
	10	15,010	996
	19	1,563	8,017
	25	878	5,706
ISU ELISA: SP Ratio	1	ND	ND
	3	0.398	0.381
	6	0.942	0.387
	8	1.911	0.417
	10	1.714	0.664
	19	1.269	0.878
	25	1.209	1.261
4-dilution IFA: Titer	1	35	53
	3	ND	ND
	6	303	127
	8	893	143
	10	1,178	604
	19	640	983
	25	338	1,076

Conclusions and Discussion- Each test has positive and negative attributes. IFA is labor intensive and tends to be subjective. The ISU-ELISA sometimes provides confusing results and the scale of S/P ratios is less discriminating than a titer. The COM-ELISA appears to work well with regard to evaluating post-vaccination responses but more evaluation is needed.

References

1. Thacker, B. et al (2008) Proc AASV, 153.
2. Thacker, B. et al (2013) Proc AASV, 217.

COMPARATIVE STUDY EVALUATING THE IMPACT OF TWO DIFFERENT PCV2 VACCINES ON AVERAGE DAILY GAIN (ADG) AND BODY TEMPERATURE POST-VACCINATION

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Introduction

PCV2 piglet vaccination is widely used in the swine industry today. While protective efficacy remains the most relevant criteria to choose a PCV2 vaccine, it has been shown that differences in safety between vaccines may also have an impact on production performance (1). In addition, it has been reported that the immediate period after weaning has a significant impact on the pigs' lifetime performance, influencing growth and variability of weights through the end of finishing (2,3). The objective was to evaluate the impact of two different PCV2 vaccines on body temperature and performance post-vaccination.

Materials and Methods

The study was conducted on a 1,600 sow herd positive for PRRS, PCV2 and M hyo located in the southeast of Spain. Pigs are weaned at 3 weeks of age. One week after weaning (4 weeks of age) 500 clinically healthy pigs were ear-tagged, individually weighed and randomly allocated to one of two treatment groups. One day later piglets received either one dose of Ingelvac CircoFLEX® (1 ml) or Suvaxyn® PCV (2 ml, marketed in North America under the trade name Fostera® PCV) and were weighed. Both vaccines were used in 50ds presentations and vaccines were allowed to reach room temperature (23.6°C) before application. All pigs were vaccinated on the left side of the neck. Needles were changed every 25 piglets (pen size) and between treatments. On the vaccination day, body temperature was measured before and 8h post vaccination in 70 animals per treatment group. Piglets were weighed individually again at 24h and 48h post vaccination to calculate ADG. Body temperature and ADG were analyzed by ANOVA using GLM analysis with treatment as fixed effect. Differences between least square means were compared through Tukey test. Fisher's exact test was used to evaluate the association between the presence of injection site lesions and treatment. Level of significance was set at $p < 0.05$.

Results

Results are summarized in Tables 1 and 2.

Table 1: Body temperature and ADG.

Parameter	Circo-FLEX®	Chimeric vaccine	P-value
Number of pigs	250	250	
Weight d 0 (kg)	6.04 [a]	6.07 [a]	0.79
Body temp. before vaccination*	39.9[a]	40.0[a]	0.25
Body temp.8h post-vaccination*	40.1[a]	40.7[b]	<0.001
ADG 0-24h (g/day)	167[a]	79 [b]	0.001
ADG 0-48h (g/day)	160[a]	125 [b]	0.003

a,b value with a different superscript, differ significantly ($p < 0.05$), *subset of 70 animals/group

Table 2: Prevalence of injection site lesions on day 9 post-vaccination

Parameter	Circo-FLEX®	Chimeric vaccine	P-value
% Piglets with cervical lesion	0.41[a]	3.54[b]	0.02

a,b value with a different superscript, differ significantly ($p < 0.05$)

Conclusions and Discussion

Animals vaccinated with the chimeric PCV2 vaccine showed a significantly higher body temperature (+0.62ºC) at 8h post vaccination compared to the CircoFLEX vaccinated group. In addition, there was a significant difference observed in injection site lesions on day 9 post vaccination. This is in line with previous publications indicating a higher reactivity of the chimeric vaccine.

The different impact of the two vaccines resulted in significant differences in ADG post-weaning, as measured for the first 48h post-vaccination. These results confirm that Ingelvac CircoFLEX® is very safe and less reactive than the chimeric PCV2 vaccine. The time immediately after weaning is a critical period in the pig's life and can affect performance up to slaughter. In addition to efficacy, safety should be considered when applying vaccines around weaning.

References

1. Potter et al.(2012)J Anim Sci: 90(11):4063-71
2. Cole and Varley (2000) Proc 5th Semin. Int. Porc., Spain, p. 37-52
3. Tokach et al. (1992) Report of Progress 667. KSU Agricultural Experiment Station and Cooperative Extension Service, Manhattan, KS. p 15-17.

MONITORING OF PCV2 MATERNAL DERIVED ANTIBODIES LEVELS IN 3 WEEKS OLD PIGLETS IN 6 SPANISH COMMERCIAL FARMS

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Introduction

Protective immune response in face of high levels of MDA has been repeatedly demonstrated for Ingelvac CircoFLEX, an aqueous 1-ds ORF2 based subunit vaccine (1,2), and no restrictions apply with regard to MDA levels at vaccination according to the product information (3). However, for other PCV2 vaccines, the benefits of vaccination in pigs with high levels of mAb have not been demonstrated or the vaccines must to be administered a second time in pigs with high levels of MDA (4,5). Typically, antibody levels > 8-10 $\log_2$ are classified as 'high' (6). The objective of this study was to evaluate the prevalence of high levels of maternal antibodies at weaning in piglets deriving from non-vaccinated sows.

Materials and Methods

The study was conducted on 6 commercial farms located in different regions of Spain. Five two-site and one multisite production system were selected. Overall 270 blood samples, 45 samples per sow herd were collected as follows. Five piglets (3 weeks of age) from 3 sows of each of the following three parity groups: parity 1-2, parity 3-4 and parity 5-6.

Sows and piglets were unvaccinated against PCV2 at the time of sampling and cross-fostered piglets were excluded. Piglets were vaccinated against PCV2 after the blood sampling.

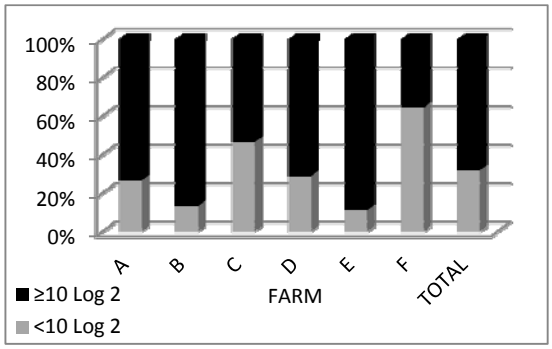
The serum samples were analyzed for PCV2 antibodies with the Serelisa® PCV2 Ab Mono Blocking ELISA (Synbiotics Corp). The results are expressed as log2 titers with titers of 10 or above classified as 'high'.

Results

Figure 1 reveals that, in total, 68% of the piglets showed high MDA levels (≥10 $\log_2$) and piglets with high MDA levels were present in each of the sampled farms (ranging from 36% in farm F to 89% in farm E). Only in one of the 6 farms (farm F) less than 50% of the piglets had high titer levels.

All parity groups (across herds) had a prevalence of high titers in piglets of more than 50%. While 61% of piglets from younger sows (parity 1&2) had high levels of MDA, 72% of pigs from parity 3&4 and 73% of the pigs from the older sows (parity 5&6) displayed those.

Figure 1. Percentage of piglets (%) with high MDA titers (≥10 $\log_2$, dark gray) and MDA titers < 10 $\log_2$ (light gray) per farm (A to F) and across all farms (Total).



Conclusions and Discussion

This study indicates that in Spanish commercial sow herds the majority of piglets deriving from non-vaccinated sows have high levels of MDA at the time of weaning (3 weeks of age). Across all farms and parity groups 68% of the piglets had a titer of 10 or above. For some vaccines (including a 2-ds product) a possible interference with high levels of maternal antibodies is suggested based on the product information or field data. In contrast, Ingelvac CircoFLEX®, a single dose ORF2 based subunit vaccine, has demonstrated to provide solid and consistent protection independent of maternal antibody titres (1, 2).

References

1. Baysinger et al. (2010) Proc 41st AASV, p. 281.
2. Grosse Liesner et al. (2008) Proc. Leman Swine Conf, Rec Research, p. 9.
3. Ingelvac CircoFLEX, accessed 11th July 2013 http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/veterinary/000126/WC500062388.pdf
4. Suvaxyn PCV, accessed 11th July 2013 http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/veterinary/000149/WC500069200.pdf
5. Porcilis PCV, accessed 11th July 2013 http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/veterinary/000135/WC500061523.pdf
6. Martens et al. (2010) Proc. IPVS, Vancouver, 132.

POSITIVE IMPACT OF PCV2 VACCINATION OF GILTS AND SOWS ON REPRODUCTIVE PERFORMANCE DEMONSTRATED IN A SIDE-BY-SIDE FIELD OBSERVATION

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Introduction and Background

PCV2 is one of the viral pathogens with the most important economical impact in the pork industry. In the last six years vaccination of pigs against PCV2 has become routine worldwide. In addition, it has been experimentally shown that PCV2 can cause reproductive failure[1,2]. Some field studies have demonstrated a positive effect of PCV2 gilt and sow vaccination on reproductive parameters[3,4]. The aim of this study was to investigate the effectiveness of vaccinating gilts and sows against PCV2 in a commercial farm without obvious clinical signs of PCVD in the reproductive herd.

Materials and Methods

The study was conducted between April and September 2012 in a 2-site, PRRS-negative, 950 sow farrow-to-finish farm situated in the South of Russia. Since January 2012, pigs were vaccinated at 19 days of age against PCV2 with Ingelvac CircoFLEX® (Boehringer Ingelheim). In April 2012, 98 clinically healthy gilts and 326 sows were randomized into treatment and control groups. Gilts and sows in the treatment group were vaccinated with 1 dose (1 ml) of Ingelvac CircoFLEX® at 3 weeks before insemination. The control animals were not vaccinated. Vaccinate and control pigs were housed side-by-side in alternating pens in the same air space and under the same management and climatic conditions. Pigs from the subsequent litter were weaned at 22-23 days of age. Cross-fostering of piglets was only allowed within treatment groups.

Results

Results are shown in Tables 1 and 2.

Discussion and Conclusions

In this study, PCV2 vaccination of gilts and sows with Ingelvac CircoFLEX® improved reproductive performance compared to non-vaccinated animals and provided an additional 62.7 weaned pigs for each 100 reproductive animals. Similar results were described in Mexico and Canada[3,4]. However, to our knowledge this is the first large scale side-by-side study demonstrating a positive effect of PCV2 vaccination on reproductive performance.

Table 1: Vaccination results for gilts

Parameters	Vacc.	Control	Diff.
Inseminated, n	48	50	
Pregnancy rate,%	83.3	82.0	1.3%
Abortion rate,%	2.5	2.4	0.1%
Farrowing rate,%	81.3	80.0	1.3%
Total born/litter, n	9.8	9.3	0.5
Born alive/litter, n	9.3	8.6	0.5
Stillborn/litter, n	0.5	0.7	0.2
Mummified/litter,n	0.1	0.1	0.0
Weak/litter, n	0.3	0.3	0.0
Weaned/litter, n	8.4	7.6	0.8
Survivability,%	90.6	88.9	1.7%

Table 2: Vaccination results for sows

Parameters	Vacc.	Control	Diff.
Inseminated, n	166	160	
Pregnancy rate,%	91.6	88.8	2.8%
Abortion rate,%	0.7	2.8	-2.2%
Farrowing rate,%	91.0	86.3	4.7%
Total born/litter, n	10.6	10.4	0.2
Born alive/litter, n	10.2	9.8	0.4
Stillborn/litter, n	0.3	0.5	-0.2
Mummified/litter,n	0.0	0.1	-0.1
Weak/litter, n	0.2	0.3	-0.1
Weaned/litter, n	9.7	8.8	0.9
Survivability,%	94.6	89.5	5.1%

References

1. O'Connor (2001) Can Vet J 42: 551-553.
2. Opriessnig (2007) J Vet Diagn Invest 19: 591-615.
3. Diaz et al. (2011) Proc. 6th Int Symp ERPD: 149.
4. Menard J. (2011) Proc. 6th Int Symp ERPD: 241.

COMPARING PLACENTA AND PRESUCKLE PIGLET PCV2 STATUS BETWEEN TWO BREEDING SITES

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Objectives

The study objectives were to determine if the placenta PCV2 status could be compared across farms to determine PCV2 status in pre-suckling pigs.

Materials and Methods

Site A (avg. parity 2.8) and B (avg. parity (2.3) are 1,600 and 5,000 head commercial farrow-to-wean herds, respectively, where PCVAD had been diagnosed in weaned pigs from Site A but not Site B. Assorted-parity litters were identified at Site A (n=21) and Site B (n=26). Farrowings were attended and eight pre-suckling pigs per litter were sampled. Piglet handling procedures included: new born piglets were towel-dried, tails were docked using side-cutters and tail-stump blood samples were collected using a flocked swab in Amies' solution. The expelled placental material was collected a minimum of four hours after expulsion at Site A and immediately after expulsion at Site B. Three placental umbilical cords from each placenta were milked out into a single blood tube to create a single sample per placenta. Swiffer® Sweeper Dry Cloths were used to collect environmental samples from the farrowing crate. Blood was collected from the 47 sows and tested on PCV2 PCR.

Results

PCV2 diagnostic results for Site A and Bs' pigs, placentas, dams and farrowing crate environments are shown in Table 1.

Table 1: PCV2 diagnostic results

Sample	PCV2 Test	Site A	Site B
Placental umb. cord serum	PCR	17/21 81.0%	3/26 11.5%
Presuckle piglet serum		54/168 32.1%	5/246 2.0%
Environmental cloth		20/20† 100%	10/24† 41.7%
Sow serum		5/21 23.8%	1/26 10.0%
Litters with ≥ 1 positive pigs		16/21 76.2%	3/26 11.5%

† 20 of 21 and 24 of 26 crates sampled due to on-site supply avail.

Table 2: Comparing PCV2 PCR results on pig and placenta level per dam

		Litter status*	
		-	+
Dam's placenta	-	**24**	**3**
	+	**4**	**16**

*positive litter contained at least one positive pig

Conclusions

Differences in PCV2 status of litters, placenta, sows and farrowing crate environment exist between breed-to-wean sites. Placenta and piglet percent positives closely match one another in both herds. This supports the potential use of the placenta as an indicator of the PCV2 status. It is noted that a consistent protocol for placental sampling should be developed as environmental contamination is a possibility. Sow serum PCR was not as sensitive as placenta or piglet testing in determining PCV2 status. PCV2 sow herd testing, utilizing the placenta, may be useful when evaluating interventions that may impact PCV2 stability status. These studies are currently being conducted.

DIAGNOSTIC INVESTIGATION TO DETERMINE HERD STABILITY OF A SOW HERD WITH DOWNSTREAM PCVAD IN NURSERY PIGS

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Objectives

The objective was to determine if the sow herd supplying a nursery flow demonstrating PCVAD was stable for PCV2. Blood collected from placental umbilical cords, pre-suckle piglets, and sows were evaluated to determine the prevalence of neonatal PCV2 viremia at birth and dam PCV2 viremia. Additionally, environmental samples were collected to assess presence of PCV2 in the environmental.

Materials and Methods

The 1,200 sow farrow-to-wean operation was selected based on nursery pigs experiencing PCVAD. The average parity was 2.82. Twenty-one mixed-parity litters due to farrow in a 48 hour period were identified. Immediately post-farrowing and pre-suckle, eight new-born piglets per sow were towel-dried, approximately one-third of their tails were removed using a side cutter, a tail-stump blood samples were collected on a flocked swab, and placed into a test tube containing transport media. The expelled placental material was collected a minimum of four hours after expulsion. The placenta was manipulated to expose the glossy, 'fetal' surface and fluid samples were manually expressed into a blood collection tube from a minimum of 3 placental umbilical cord stumps. Each placenta was handled with new gloves to minimize transfer of PCV2 between placentas. Swiffer® Sweeper Dry Cloths were used to collect environmental samples from the exposed surface of the farrowing crate deck. Dam's serum was tested for PCV2 antibodies and virus via ELISA and PCR, respectively.

Results

Serum and environmental test results are shown in Table 1. Table 2 illustrates that 15 of the 16 positive litters were associated with at least 1 PCV2 PCR positive piglet.

Table 1: PCV2 antibody and virus results

Sample	PCV2 Test	Results
Umb. cord serum	PCR	17/21 (81.0%)
Presuckle serum		54/168 (32.1%)
Environmental cloth		20/20 (100%)
Sow serum		5/21 (23.8%)
	ELISA	21/21 (100%)

Table 2: Comparing PCV2 PCR results on pig and placenta level per dam

		Dam's piglets	
		-	+*
Dam's placenta	-	3	1
	+	2	15

* positive litter contained at least one positive pig sample

Conclusions

This sow herd was shedding PCV2 to its intrauterine piglets at a prevalence higher than expected. Previous diagnostic work in this system would suggest that <9% of sows were PCV2 positive via PCR. This diagnostic investigation suggests that serum from placental umbilical cords could be a good indicator of the PCV2 status of the pre-suckle piglets at birth. The comparable findings of this diagnostic investigation suggest that additional studies are indicated to determine the future application of the placenta and environmental testing for predicting PCV2 stability status of a sow herd.

PCV2 VACCINE PROTOCOLS REDUCE PCV2 VIREMIA IN LOW PCV2 CHALLENGES

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Introduction and Objectives
Previous studies indicate that PCV2 viremia levels do not influence ADG in PCV2 vaccinated pigs; however, veterinarians still inquire about viremia.[1,2,3] An objective of these trials was to compare the average PCV2 log levels in non-vaccinated and vaccinated pigs under field conditions.

Materials and Methods
Two trials were conducted. Both included non-vaccinated control groups and two PCV2 vaccination protocol groups (Table 1). Vaccination occurred per label instructions for all products. All pigs were serially sampled at 6 time points (Table 1). Serum was evaluated using an updated PCV2 qPCR protocol (Health Management Center, Ames, IA). The low end cut-off for percent positives of the updated protocol was 2 logs at 45 cycles. Average log level of viremia was calculated for each group at each time period. Results were analyzed by MANOVA repeated measures. Preset contrast were utilized to compare differences between vaccinated and non-vaccinated pigs and to compare vaccinated groups in trial A and Tukey's HSD was used to discern differences in trial B.

Table1: Trial group description

Trial	Group	N serially bled	Weeks of age sampled
A	NVC†	60	3, 10, 13, 16, 19, 22
	FLEX‡	60	
	PCVM^	60	
B	NVC†	10	3, 7, 10, 14, 19, 23
	CF*	30	
	PCV#	30	

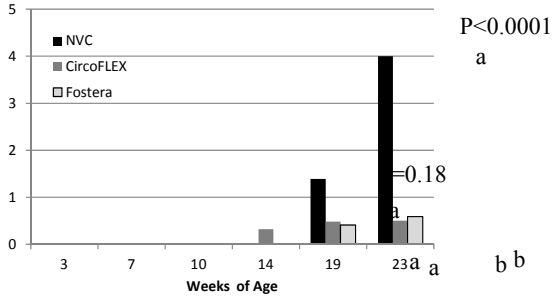
†Sterile saline; ‡Ingelvac CircoFLEX-MycoFLEX® (Boehringer-Ingelheim Vetmedica, In., St. Joseph, MO); ^Circumvent PCVM (Merck Animal Health, Summit, NJ); *Ingelvac CircoFLEX® (Boehringer-Ingelheim Vetmedica, In., St. Joseph, MO); # Fostera PCV (Pfizer, New York, NY)

Results
Vaccination statistically decreased viremia compared to non-vaccinates in trial A on weeks 10, 13, 19, and 22 (P=0.01, P=0.02, P<0.0001, P<0.0001, respectively) and in trial B, week 23 (P<0.0001). In trial A, percent positive samples peaked at 19 weeks and was 75% (NVC), 41% (FLEX) and 39% (PCVM). Peak percent positive in trial B occurred at 23 weeks and was 71% (NVC), 19% (CF) and 20% (PCV). Viremia between the vaccinated groups was not different (P>0.10) at any time points measured in either trial (Tab. 2 and Fig. 1).

Table 2: Trial A average serum quantitative PCV2 PCR by group at specified weeks of age

	Treatment			P-value	
Age, wks	NVC	FLEX	PCVM	Vx vs. NVx	FLEX vs. PCVM
3	0.00	0.00	0.00	-	-
10	0.68	0.07	0.36	0.01	0.19
13	1.27	0.75	0.62	0.02	0.68
16	1.74	1.26	1.42	0.19	0.64
19	2.86	1.41	1.26	<.0001	0.63
22	2.60	1.03	0.99	<.0001	0.90

Figure 1: Trial B average serum quantitative PCV2 PCR, log level, by group at specified weeks of age



Discussion and Conclusions
Both studies represent low PCV2 field challenges (<4 logs average). However, commercial PCV2 vaccines continue to significantly reduce viremia compared to non-vaccinate controls and reduce viremia by the same magnitude. These viremia studies also indicate that vaccines do not completely eliminate viremia. More sensitive quantitative PCV2 tests (< 4 logs detection limit) should be considered in field trials in order to more accurately assess PCV2 status of pigs. This study continues to add to the body of evidence the industry requests on PCV2 viremia.

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References
1. Holck et al. (2009), Proc. Leman Conf. 168.
2. Mass et al. (2009), Proc. Leman Conf. 171.
3. Diaz et al. (2010), Proc. Leman Conf. 166.

RELATIVE REDUCTION IN PCV2 VIREMIA IN VIREMIC REPLACEMENT FEMALES, COMPARING TWO VACCINE PROTOCOLS. A PILOT STUDY.

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[2]*Boehringer Ingelheim Vetmedica, Inc, St. Joseph, USA*

Introduction and Objectives

The objective of this study was to observe the potential efficacy of PCV2 vaccines in reducing or eliminating PCV2 viremia in replacement gilts when vaccinated at weaning and boostered at a typical selection age and weight of 22 weeks of age and 200-275 pounds.

Materials and Methods

Pigs were weaned from a commercial sow farm and randomly allocated to one of three treatment groups at weaning. Viremic animals (n=81) weighing between 200-270 lbs (22 weeks of age) were selected from the commercial finishing facility and administered the appropriate booster vaccination (Table 1). All pigs were commingled and serially bled at 22, 27, 28, 29, 30 and 31 weeks of age. PCV2 qPCR viremia levels were analyzed. The positive/negative cut-off was 2 logs, 100 viral equivalents (HMC, Ames, IA).[1] Lung and lymphoid tissue were collected at marketing for PCV2 tissue burden assessment (ISU VDL, Ames, IA).

Table 1: Trial group description

Group	N	Treatment	Timing (Wk of age)	Dose
A	27	Non-Vac Controls[a]	3, 6, 22	3 x2 ml
B	27	FLEX[b] CF[c]	3 22	1 x 2ml 1x 1 ml
C	27	PCVM[d] PCV[e]	3, 6 22	2x 2 ml 1 x 2 ml

[a]Sterile Saline, [b]Ingelvac® CircoFLEX-MycoFLEX®, [c]Ingelvac CircoFLEX® (Boehringer Ingelheim Vetmedica, Inc., St Joseph, MO); [d]Circumvent® PCVM, [e]Circumvent® PCV (Merck Animal Health, Summit, NJ)

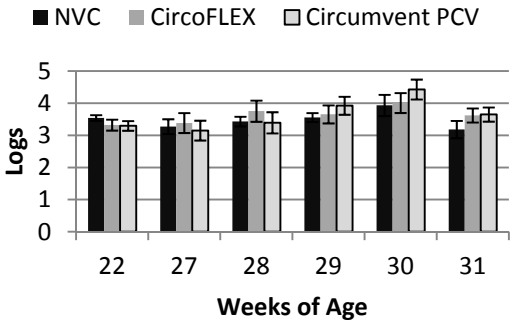
Results

Total and percent of positive pigs are displayed in Table 2. Figure 1 shows the average of log level for positive pigs. Histopathology and IHC results were negative on all tissues from all animals.

Table 2: Total and percent positive PCV2 qPCR

	Treatment			Contrast P-value	
Age wks	**A**	**B**	**C**	**A vs. B/C**	**B vs. C**
22	27/27 (100%)	27/27 (100%)	27/27 (100%)	-	-
27	14/27 (51.9%)	11/27 (40.7%)	6/27 (22.2%)	0.09	0.24
28	17/27 (63.0%)	8/27 (29.6%)	4/27 (14.8%)	**0.0005**	0.33
29	16/27 (59.3%)	9/27 (33.3%)	6/27 (22.2%)	**0.008**	0.54
30	22/27 (81.5%)	9/27 (33.3%)	13/27 (48.2%)	**0.008**	0.41
31	13/27 (48.2%)	6/27 (22.2%)	3/27 (11.1%)	**0.004**	0.47

Figure 1: Average log qPCR by weeks of age (only positive animals included)



Discussion and Conclusions

Even with lower PCV2 field challenge (<4.5 logs maximum average in any one treatment group) commercial PCV2 vaccines significantly reduced the percent of PCV2 qPCR positives compared to non-vaccinated controls. While both vaccines reduced viremia by the same magnitude, neither vaccine eliminated viremia. In this study, viremia was not a predictor of tissue PCV2 burden. Further field trials are underway to understand PCV2 vaccine use in replacement animals and the role of PCV2 in the breeding herd.

References

1) Chittick, W. 2013. Submitted Leman Conference.

UTILIZATION OF TAQMAN REAL-TIME PCR TO INCREASE SENSITIVITY IN PCV2 SAMPLING

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Introduction and Background

Prior to vaccine availability, viremia levels of ≥7 log genomic equivalents/ml were commonly observed in herds experiencing PMWS.[1] However, these high viremia levels are rarely observed today in groups of properly vaccinated pigs. Some PCV2 qPCR tests have viral detection limits of 4 logs, meaning a "negative" result indicates a sample with 0-10,000 virus particles/ml). When using an assay with a 4 log detection limit, there is high potential for several false negative results. A more sensitive set of TaqMan real-time PCR reagents (Life Technologies Corp., Grand Island, NY) has recently been developed and can reliably detect down to 2 logs (100 genomic equivalents / ml) with 45 PCR cycles.[2] This more sensitive test decreases the chance of false negative results in samples that have virus levels between 2-4 logs. A comparison was conducted between 4 log detection limit PCV2 qPCR test and the 2 log detection limit PCV2 qPCR test to evaluate the potential reduction in false negatives when utilizing the more sensitive qPCR test.

Materials and Methods

Four studies that included serially bleeding of individual pigs were conducted and PCV2 qPCR utilizing the Life Technologies qPCR was utilized to test for virus particles in individual pigs. Results of samples were categorized into 0-2 logs (reported as negative), 2-4 logs (detectable but below less sensitive qPCR tests, and >4 logs (were detected and would have been detected by less sensitive qPCR tests).

Results

The majority (75.9%) of the samples would not have been detectable by either test and 6.3% would have been detected by both tests (Table 1). The test utilizing the new assay detected PCV2 virus in 17.8% of the samples that would have been undetectable by the older assay.

Table 1: Categorized results from Life Technologies PCV2 qPCR test in 4 trials.

	Total N	Number of samples (%) between log range:		
Trial		0-2	2-4 logs	>4
A	525	500 (95.2%)	15 (2.9%)	10 (1.9%)
B	1051	765 (72.8%)	206 (19.6%)	80 (7.6%)
C	486	248 (51.0%)	184 (37.9%)	54 (11.1%)
D	293	274 (93.5%)	15 (5.1%)	4 (1.4%)
All	2355	1787 (75.9%)	420 (17.8%)	148 (6.3%)

Discussion and Conclusions

In certain cases, the previous PCR test was dramatically underestimating the percent PCV2 virus positives in a barn. Of all the positives, 73.9% were between 2-4 logs, undetectable by the old PCR test. The new PCR test increased the number of positives by 37.9% in study C and increased the total positives from 11.1% to 49%. The more sensitive PCR test will be of use in diagnostic projects and trials where there is a lower level of PCV2 challenge, that would have otherwise been undetectable using a threshold for positives at 4 logs.[3] Additionally, more sensitive PCR tests may be applicable in the continued discovery of the impact of PCV2 in gilts entering the sow herd and sow herd stability monitoring for PCV2. The impact of PCV2 in unstable herds is not well understood and more sensitive PCV2 qPCR tests may be a tool to help understand better so that there are fewer false negatives.

References

1) Brunborg et al.,2004.Virol.Meth.122:171-178.
2) Zhao et al. 2010.Virology Journal.7:234.
3) Payne et.al. 2013 ESPHM, Edinburgh, UK.

CONTROL OF PORCINE EPIDEMIC DIARRHEA VIRUS THROUGH INTESTINAL FEEDBACK AND PARITY SEGREGATION SYSTEM: FIELD CASES IN THAILAND

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Introduction

Porcine epidemic diarrhea (PED) re-emerged in Thailand in late 2007[1]. Since then, the disease has become endemic causing sporadic outbreaks throughout Thai swine herds, especially herds located in high pig proximity areas and herds located in other regions in which previously experienced an outbreak. Attempts to control PED including vaccination whole herd exposure with minced intestinal of infected piglets have been implemented but gained varying degree of success. Furthermore, well vaccinated herds including quarterly mass sow herd vaccination and pre-farrow vaccination have experienced re-breaks. The timing of the periods of disease recurrence is variable. The study reports the establishment of PED control through management of gilts.

Materials and Methods

Herd selection and history: Four farrow-to-finish PRRSV positive herds designated herds S, N, R and M with inventory of 1,200 – 2,700 sows and no history of PED vaccine used were selected for PED control using this procedure. Herds S and N are located in the highest pigs proximity of Thailand in contrast to herds R and M. All 4 herds had history of epidemic PED outbreaks. Herds S and N experienced the epidemic outbreak in January 2008 while herds R and M broke with PED in January 2009. Farrowing and nursery facilities of all herds operate all-in/all-out by building on weekly basic. Parity segregation system has been implemented in all herds for the purpose of PED and PRRSV control. Following outbreaks, PED control in all herds was through mass exposure of sow herds and replacement gilts with minced intestine of affected piglets. The episodes of outbreak were last for 3 – 4 weeks of production. To prevent the PED re-occurrence, gilts acclimatization with minced piglet intestine and strict biosecurity of gilts isolation and acclimatization were implemented.

Intestinal feedback: Following the outbreak, pig intestine with lesions suggesting PED was collected from pigs and assayed for the presence of PEDV by PCR. PEDV was isolated and grown on cell culture. The virus stock was detected for any pathogen contamination prior to piglet administration and used to orally inoculate the piglets in isolation facilities. The process was done once and then the piglet intestines were used to re-infect the piglets to make the next batch.

Sources of replacement gilts, and isolation and acclimatization facilities: Replacement gilts of herds R and M are internally produced. Herds S and N introduce external gilt replacement from negative PED positive herds, respectively. The isolation and acclimatization facilities are located at least 150 meters from all production facilities. Replacement gilts were orally administered with minced piglet intestine at least 8 weeks prior to introduction to the P1 units. Sentinel program and strict biosecurity were implement to assure the shedding status of gilts prior to introduction and prevent personal movement.

Results and Conclusion

Sentinel pigs commingled with gilts at 4 weeks post intestine administration displayed clinical disease associated with PED in contrast to sentinel pigs introduced at 6 and 8 weeks that featured no clinical signs.

Herds S and N have never experienced the re-occurrence of PED since the implementation of the control methods. Herd M, however, experienced the re-occurrence in the P1 unit in January 2011. The source of the outbreak was unable to determine. Herd R experienced 2 episodes of the disease re-occurrence in one multi-parous and the P1 unit, a month apart. The sources of the reoccurrence were from premature introduction of replacement gilts into both units. In conclusion, the results suggests the oral administration with piglet intestine and parity segregation system can be used in the herd to control PED even through herds are located in the highest pig density.

References

1. Puranaveja S et al., 2009, Emerg Infect Dis 15, 1112-1115.

DETECTION AND TYPING OF INFLUENZA A VIRUS IN PIG FARMS IN CHILE DURING 2012

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Introduction

Since 1918, there are reports for the presence of influenza A virus in pig populations[2]. At the present, it is described that the virus has cosmopolitan distribution and subtypes most frequently detected in pigs are H1N1, H1N2 and H3N2[3], however the background of the situation in Latin America has not published in deep. The primary objective of this study was to detect the virus in nasal swab samples from pigs, and therefore to generate the first report of detection and subtyping of influenza A in swine farms in Chile.

Materials and Methods

During 2012, 635 nasal swabs samples were obtained from pigs ranging 3 to 10 weeks of age, which showed clinical signs of respiratory disease such as coughing, sneezing and nasal discharge. All samples were taken under field conditions and involved about 42% of the country's commercial farms. Once obtained, the nasal swabs were transported in cooled (4°C) nutrient medium to the laboratory. Later, each sample was inoculated into MDCK cell culture, and those which shown cytopathic effect were analyzed by RT-PCR looking for swine influenza virus subtypes, using specific primers for H1/H3 or N1/N2[1].

Results

In all farms analyzed was possible to detect the presence of genetic material consistent with type A influenza virus. The most common subtype detected corresponded to H1N2 with over 60% of samples. However, also was possible to detect subtypes H1N1, H3N2 and H3N1 (Table 1).

Table 1. Detection of influenza virus subtypes from nasal swabs from pigs during 2012 in commercial farms in Chile.

Subtypes of virus influenza	Percentage detected during the 2012
H1N1	11%
H1N2	63%
H3N2	19%
H3N1	7%

Conclusions and Discussion

The results obtained in this study confirmed the presence of influenza virus in pigs which showed signs of respiratory disease in Chile, whose as far as we known to constitute the first report of the influenza virus subtypes circulating in pig farms in our country. In this way, in previous reports similar percentages were described for H1N2 subtype in Spanish farms[2]. Additionally during the last years, detections of H1N1, H1N2 and H3N2 subtypes were made in Brazil and Argentina, however the analysis performed in those countries indicate that these subtypes genetically differ from those present in North America[3], so it's probable that influenza viruses detected in Chile also differ from circulating in other countries, however further studies are needed to corroborate it.

References

1. Chan et al., 2006. J Virol Methods. 136(1-2):38-43
2. Torremorell et al., 2012. Transbound Emerg. Dis. 59: 68-84
3. Vicent et al., 2013. Zoonoses and Public Heatlh. 1-1

DETECTION OF PORCINE EPIDEMIC DIARRHEA VIRUS IN AIR SAMPLES AT VARYING DISTANCES TO EPIDEMIC FARMS IN OKLAHOMA

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Introduction

Porcine Epidemic Diarrhea (PED) was first detected in the United States in 2013. The disease is financially devastating for a farm due to high preweaning mortality.[3] A cluster of farms in the Oklahoma panhandle were all infected within such a short period of time and with no common sources that aerosol spread was suspected. Coronaviruses similar to PEDv are known to be relatively stable in arid conditions[1] that may also increase potential for aerosol spread at long distances.[4] There is a need to determine if PEDv can be detected in air samples.

Materials and Methods

Air collection was performed with a high-volume air sampler (Midwest Micro-Tek, Brookings, SD, USA). It was run for 30 minutes starting with 11 ml of transport media (MEM, anti-anti, BSA, trypsin, & gentamycin) in the reservoir at each sampling location. Air was sampled at varying distances from farms experiencing a PED outbreak. The remaining transport media was poured off into a 15 ml Falcon tube and kept on dry ice until submission to the U of MN VDL for PED PCR. Post-sampling residue was rinsed off with distilled water. Disinfection was with a 70% ethanol or 10% chlorine solution liberally sprayed on every surface and within the reservoir of the collectors and equipment was allowed to sit for >30 minutes between samples. Prior to sampling again, disinfectant residue was rinsed off with distilled water and dried with paper towel. The collector reservoir and spinning mechanism was then sampled with a sterile swab (liquid Stuart media) and submitted for PED PCR. Consecutive samples were taken down wind of the infected farms starting furthest away.[2]

ResultsPED PCR-positive air samples were detected outside of multiple mechanically-ventilated sow units and naturally-ventilated finishing units. Distances of 60 ft, 300 ft, ¼ mile, ½ mile, 1 mile, 2 miles, 3 miles, 5 miles, 10 miles, and 15 miles downwind of known positive farms were sampled yielding 64 samples in total with 11 positives. Positive samples were found at 60 ft (3/6), ½ mile (1/5), 1 mile (3/13), 3 miles (3/7), and 10 miles (1/3). Average CT values of positive S Gene PCR results positively correlated with distance sampled from the farm r=0.7892 (33.025 at 60 ft, 39.71 at 1 mile, and 38.72 at 10 miles).

Conclusions and Discussion

These results have confirmed that PEDv can be detected in the air as much as 10 miles from a herd. The analysis of CT value versus distance indicates a higher concentration of viral RNA in the air closer to the farm. This preliminary study was not extensive enough to fully characterize risk factors of aerosol spread of PED virus. Preliminary results did not show live virus as testing was performed with PCR.

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References

1. Casanova LM et al.: 2010, Appl Environ Microbiol 76(9):2712.
2. Cho JG, Deen J, Dee SA: 2007, Can J of Vet Res 71:23-27.
3. Pensaert MB, Yeo S: 2006, Diseases of Swine 9th Ed. 22:369
4. Verreault et al.: 2008, Microbiol Mol Biol Rev 72(3):413

DEVELOPMENT AND VALIDATION OF ELISA TESTING FOR SEROLOGICAL MONITORING AND SURVEILLANCE OF PORCINE EPIDEMIC DIARRHEA VIRUS

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Introduction
Porcine epidemic diarrhea virus (PEDv), a member of the *Coronaviridae* family, causes acute outbreaks of severe diarrhea and vomiting in suckling and growing pigs, leading to significant mortality. PEDv was first observed in Europe in 1971 and became endemic in Asia by 1982. Its emergence in the United States was first confirmed in May 2013 and has been identified in 15 states as of mid-July. Due to this emergence, the US swine industry has an immediate need for rapid, high-throughput diagnostic tests for serological monitoring and surveillance of PEDv. To that end, we have created a PEDv ELISA assay to detect the presence of antibodies to PEDv in swine serum.

Materials and Methods
PCR primers were designed to amplify nucleocapsid (N) and spike protein domains 1 (S1) and 2 (S2). PEDv RNA was extracted from infected pig feces, generously provided by D. Marthaler, MN Veterinary Diagnostic Laboratory, cDNA was synthesized, and PCR-amplified. The PCR products were cloned into two plasmid vectors, pET-25b and pMAL-p5X, and gene sequences were confirmed by sequencing. Recombinant plasmids were then transformed into E. coli Rosetta cells for induction of protein expression. Expressed proteins were purified using a 6-his tag and immobilized metal affinity chromatography (pET-25b), or maltose binding protein fusion and amylose affinity chromatography (pMAL-p5x). Purity and concentration of the recovered proteins was confirmed by gel electrophoresis and spectrophotometry. Purified proteins were then used for the development of a direct ELISA for the detection of anti-PEDV antibodies.

Results
Multiple US PEDv isolates were sequenced and found to be >99% similar, thus recombinant proteins from any isolate should be representative of US PEDv isolates. In an ELISA assay all isolates will be expected to cross-react due to the high sequence similarity.

PEDv nucleocapsid (N) and spike protein domains 1 (S1) and 2 (S2) amplification was achieved from pig fecal samples. Cloning success was variable using the Gibson strategy and standard ligation methods. Protein expression is underway at the time of abstract preparation and results will be presented at the conference.

Conclusions and Discussion
Nucleocapsid and spike proteins in coronaviruses are highly immunogenic and have been used previously for screening pig serum for serological evidence of exposure and previous infection by coronaviruses, including PEDv. The S1 domain of PEDv spike protein contains the neutralization epitope. Thus, this recombinant protein might be useful for evaluation of neutralization and cross-neutralization activity. ELISA development will facilitate screening and monitoring of PEDv status of US swine herds and are expected to facilitate control and eradication of PEDv.

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DYNAMICS OF INFLUENZA A VIRUS IN AEROSOLS FROM SWINE FARMS.

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Introduction

Influenza A virus (IAV) causes lethargy, sneezing and coughing in pigs; however, subclinical infection might have occurred. The virus is transmitted mainly by direct contact and aerosols[1]. IAV has been detected and isolated in air samples from experimentally infected pigs with and without immunity[2]. IAV has also been identified in aerosols under field conditions; however, the duration of IAV bioaerosols over time has not been reported.

The main objective of this study is to characterize the presence of IAV aerosols from swine commercial facilities during influenza outbreaks over time. An additional objective is to estimate the relationship between severity of clinical signs and detection of IAV in aerosols.

Material and Methods

Five swine herds experiencing acute influenza like clinical signs were identified based on local veterinarian communication. Upon confirmation of IAV infection by diagnostics, each herd was visited every 3 to 7 days until the virus could not be detected in oral fluids testing. During the first visit, 2 pens of pigs were conveniently selected and evaluated in the follow up visits. At each visit, one oral fluid and one air sample were collected in each pen, and 2 upwind and 2 downwind air samples were collected at approximately 50m from the barn. Cyclonic collectors were used to collect the air samples[1] (Midwest MicroTek). IAV RT-PCR was performed on all samples, and virus isolation was attempted in positive and suspect samples. Clinical measures of swine health including coughing and sneezing scores were recording during each visit[3]. Farms were visited between day 0 and day 42 after the reported onset of clinical signs in the herd.

Results and Discussion

Sampling took place between October 2012 and May 2013. Overall 35 farm visits were conducted. In 33 out of 35 visits, IAV was detected in at least one sample. Thirty three (47%) air samples collected inside the barns were positive for IAV by PCR and 8 (11%) were suspect. In 22 visits (63%) IAV RNA was detected in at least one air sample and IAV was detected in all the farms of the study.

All upwind and downwind samples were negative, except in one sample where IAV RNA was detected downwind.

Among 59 oral fluid samples, 49 (70%) were positive for IAV and 10 (14%) were suspect. There was a positive association between detection of IAV in air samples and oral fluids.

IAV was generally detected in the air for 10 to 13 days following the reported onset of clinical signs in pigs, except for one herd, where IAV was only detected during the first visit. In two herds, air samples tested negative 16 days after the onset of clinical signs but tested positive again at 22 days. At one farm, IAV was detected at 42 days from the onset of signs. Further testing is needed to determine whether the later detection was due to the same virus strain detected earlier. IAV was isolated from air and oral fluid samples in all farms, except for one farm, where IAV could not be recovered from the air.

Preliminary analysis did not show a relationship between presence of IAV in aerosols and severity of clinical signs in pigs. Our results indicate that IAV can be present in aerosols in swine production facilities for an extended period of time.

References

1 Tellier, R. 2006. Emerg Infect Dis.12:1657-62.

2 Corzo C, et al., 2012. Trans and Emerg Dis Doi:10,1111/j.1865-1682.2012.01367.x

3 Sobestiansky J and Barcellos D. 2007. Doenças dos suínos. Goiânia: Cânone Ed

EVALUATING SAMPLING STRATEGIES TO DIAGNOSE INFLUENZA VIRUS IN PRE-WEANED PIGS

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Introduction: Influenza A virus (IAV) is endemic in pigs and despite its widespread appearance it may be difficult to diagnose. Specifically, IAV is difficult to be reliably diagnosed in pre-weaned pigs due to low infection prevalence, the short course of clinical signs and infection, and variable levels of maternal antibodies. Both nasal swabs and tissues are used for diagnosis, but it is unclear whether one method is superior to the other when diagnosing IAV in pre-weaned pigs. The objective of this study was to compare two sampling strategies, nasal swabs and tissues from dead pigs, in their ability to diagnose IAV in pre-weaned pigs and consider a farm infected with IAV.

Materials and methods: Two farms in Southern Minnesota, known to be IAV positive before the study, were selected and monitored over a 4 week period. In sampling method 1, nasal swabs were collected 4 weeks apart from pre-weaned pigs at 21 days of age (n=151). In sampling method 2, necropsies were performed weekly on dead piglets 7 to 21 days of age (n=79); deads were due to natural mortality or euthanasia. Swabs were collected from the upper respiratory tract (nasal cavity) and lower respiratory tract (trachea, and main stem bronchi). All samples were tested by a real time RT-PCR targeting the IAV matrix gene, and positive swabs from RT-PCR were confirmed positive using virus isolation (n=11). Statistical analyses compared two proportions, i.e. proportions of positive pigs from sampling methods 1 and 2, using Microsoft Office Excel. Results were considered statistically significant if p-values were < 0.05. Cohen's kappa coefficient for statistical agreement was also calculated between the nasal and tissue swabs from dead piglets.

Results: IAV prevalence was 8.9% (7/79) and 13.9% (21/151) based on samples from dead or live pigs, respectively (p=0.27). Seven nasal swabs and 4 lung swabs tested RT-PCR positive from samples within a dead pig, and 5 of these swabs were positive for IAV using virus isolation. There were no statistically significant differences between the following parameters: nasal to tissue swabs from dead piglets (p=0.35), farm 1 to farm 2 (p=0.74), and swabs from live piglets to dead piglets (p=0.27). There was substantial within-pig agreement between using nasal swabs and lung swabs from dead piglets (Cohen's $\kappa = 70.7$). Discordant results were obtained when comparing nasal swabs from live pigs and swabs from dead pigs, due to positive swabs from live piglets and negative swabs from dead piglets during the same sampling event. Weekly detection of IAV throughout the study was irregular, which may illustrate the intermittent shedding pattern of IAV in infected herds.

Discussion: Detecting IAV in pre-weaned pigs can be frustrating. In this study, prevalence levels were higher in samples collected from live pigs compared to natural mortality. In this study, live pigs were older than the naturally occurring dead pigs, which may explain why tissue swabs did not offer an advantage over nasal swabs from live pigs. Overall, this study showed the difficulty of diagnosing IAV in pre-weaned pigs due to low prevalence levels. In conclusion, ongoing diagnostic submissions are necessary in order to characterize IAV infections in breeding herds.

Acknowledgements: Swine Disease Eradication Center, Holden Farms Inc., and Shetek Farms.

References:

1. Lorusso A et al.: 2010, J Virol Methods 164(1-2):83-7.
2. Meguro H et al.: 1979, J Clin Microbiol 9(2):175-9.
3. Villegas P and Alvarado I: 2008, Am Assoc Avian Path 5th ed:218.

IDENTIFICATION, GENETIC DIVERISTY, AND CLASSIFICATION OF PORCINE ROTAVIRUS C

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Introduction

Rotaviruses (RVs) possess a genome composed of 11 dsRNA segments and are classified into eight species (RVA-RVH). RVA, RVB, RVC, and RVH have been detected in both humans and pigs. While swine rotavirus C (RVC) was identified in 1980's, more recently, RVC has been identified as a major cause of gastroenteritis in swine, causing clinical disease subclinical infections. The objective of this study was to identify RVC in porcine tissues and investigate the genetic diversity of the VP7 gene segment.

Material and Methods

Porcine samples (n=7,520) were submitted to the University of Minnesota Veterinary Diagnostic Laboratory between December 2009 and October 2011 from United States and Canadian swineherds and were screened for RVA, RVB, and RVC by RT-PCR. Additionally, lung tissues with histological lesions, but negative for common porcine respiratory pathogens were also screened for RVA, RVB, and RVC by RT-PCR. From conveniently selected RVC positive samples, the VP7 gene segment was sequenced using Sanger technology. Phylogenetic analysis was calculated using the Kimura 2-parameter correction at the nucleotide level while phylogenetic trees were constructed utilizing the Neighbor-joining.

Results

RVC was detected in 3,447 samples (46%) tested. These porcine intestinal tissues samples also had RV-like lesions including villus blunting and fusion, atrophy, and necrosis. From the 3,447 positive RVC samples, 1156 samples were negative for RVA and RVB, with the majority (78%) of single RVC infections in the ≤3 day old piglets. A high prevalence of RVA (50%), followed by RVC (10%) and RVB (5%) was detected in 635 porcine lung tissues. The RVC VP7 gene segment was sequenced from 65 samples, yielding 70 sequences. An 85% nucleotide percent identity cut-off value based on pairwise identity frequency graphs was proposed, dividing the phylogenetic tree into 9 G genotypes.

Conclusion and Discussion

While RVC is an important cause of diarrhea in all swine age groups, piglets (≤3 and 4-20day age groups) contained the highest percentage of single RVC infections. RVC's viremia capability and subsequently pneumonia needs further investigating. Additionally, 9 VP7 G-genotypes were proposed based on an 85% nucleotide percent identity cut-off value.

IN VITRO PROPAGATION OF U.S. STRAINS OF PORCINE KOBUVIRUS

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Introduction

Enteric disease is an important cause of morbidity and mortality among domestic pigs. The porcine kobuvirus was first identified in Hungary in 2007. Since then, the virus has been reported from both healthy and diarrheic pigs in Hungary, China, Thailand, Japan, Korea, and USA (Reuter et al., 2011; Verma et al., 2013). The cultivation of porcine kobuvirus in vitro has not been yet reported. The aim of this study was to determine if porcine kobuvirus can be grown in vitro using three different cell types. Another objective was to develop a serum neutralization test for detecting antibodies against this virus in infected pigs.

Material and Methods

In a previous study, porcine kobuvirus was detected by RT-PCR in intestinal contents from diarrheic piglets submitted to the Minnesota Veterinary Diagnostic Laboratory (MVDL) from 15 different states. The virus was also found in apparently healthy pigs from Minnesota. In the current study, four samples from healthy pigs and six from diseased pigs (that were positive for porcine kobuvirus by RT-PCR) were grown in three different cell lines e.g., PK-15 (porcine kidney), porcine alveolar macrophages (PAM), and Vero (African green monkey kidney) cells.

Results and Discussion

Porcine kobuvirus was successfully isolated from five samples using PK-15 cells. Two samples were from apparently healthy pigs and three from diarrheic pigs. The presence of virus was confirmed by RT-PCR and electron microscopy. Using the newly isolated virus, we developed a serum neutralization test to detect antibody against porcine kobuvirus. This study shows that PK-15 cells are most susceptible for the propagation of porcine kobuvirus. The newly developed serum neutralization should be helpful in conducting seroprevalence studies in swine.

References

Reuter G, Boros A, and Pankovics P. Kobuviruses - a comprehensive review. Rev Med Virol. 2011; 21:32-41.

Verma H, Mor SK, Abdel-Glil MY, and Goyal SM. Identification and molecular characterization of porcine kobuvirus in U. S. swine. Virus Genes. 2013; 46:551-553.

MODELING INFLUENZA DYNAMICS IN SWINE HERDS

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Introduction

Influenza virus infections are widespread in swine herds across the world. This negatively affects swine health and production, and represents a significant threat to public health due to the risk of zoonotic infections, with swine herds acting as reservoirs for potentially pandemic strains. By using mathematical modeling based on empirical data, we aim to analyze the dynamics of influenza in swine farms, and to compare the efficiency of different vaccination strategies.

Materials and Methods

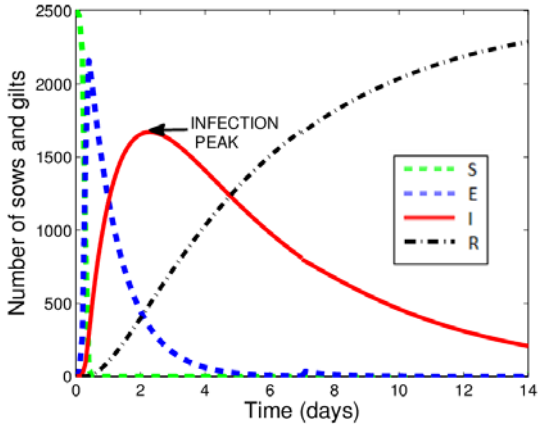
We have constructed a metapopulation model of a typical Midwestern swine breeding farm, incorporating realistic demographic and transmission parameters. Numbers of Susceptible, Exposed, Infectious and Recovered animals are tracked through time using a SEIR model framework. Empirical studies[1,2] were used to inform and parameterize the model.

To make this model representative of a breeding farm, we defined separate SEIR equations for each class of swine (e.g. gilts, sows and piglets) in each location on the farm (e.g. gilt development units, farrowing rooms and gestation areas). The model incorporates movement between these at appropriate times and frequencies.

Results

Our model predicts the impact and duration of influenza outbreaks in a breeding farm (see figure). We used this model to compare the effectiveness of different vaccination strategies that are currently employed. We tested (i) mass vaccination of sows and (ii) 3-5 week pre-farrow vaccination (targeting piglet immunity), each with either a heterologous or homologous vaccine.

Figure: *Influenza dynamics in a swine breeding farm, resulting from one infected gilt entering into a naïve population, with no vaccination.*



We find that vaccination delays the onset of an influenza outbreak. Both regimes with heterologous vaccines have little effect on the magnitude of an outbreak, whereas homologous vaccines significantly reduce this. Homologous mass vaccination prevents the spread of influenza in sows, but not in piglets. Homologous pre-farrow vaccination reduces the number of infectious sows and gilts at the infection peak, yet results in more infectious individuals after the initial outbreak.

Conclusions and Discussion

Our results highlight the need for an understanding of the effects of intervention strategies such as vaccination on the dynamics of influenza *at the herd level*. Our findings also raise an important question: is (a) a high level of infection over a short time, or (b) a moderate level of persistent infection, of the most concern?

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References:
1. Allerson M et al.: 2013, Vaccine 31: 500-505.
2. Romagosa A et al.: 2011, Vet Res 42: 120-13

CROSS-PROTECTION OF FLUSURE® XP IN PIGS CHALLENGED WITH A GAMMA CLUSTER H1N1/pH1N1 REASSORTANT SWINE INFLUENZA VIRUS

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Introduction

Characterization of swine influenza virus (SIV) from the US since the introduction of pH1N1 influenza virus has demonstrated multiple reassortant events between pH1N1 and endemic SIV, resulting in H1 and H3 variant viruses with internal genes from pH1N1, most notably the pH1N1 M gene that was of Eurasian swine origin.[1,2,3] The objective of this study was to evaluate the efficacy of a Swine Influenza Vaccine, Killed Virus, H1N1, H1N2 & H3N2 (FluSure® XP) in pigs challenged with a heterologous contemporary field isolate that is a reassortant between SIV H1N1 (gamma-cluster) and pH1N1.[4]

Materials and Methods

Forty-five weaned, 3-week-old SIV-negative pigs were randomly assigned to treatment using a generalized block design. Pigs were vaccinated twice, 3 weeks apart, with either an Amphigen® placebo (T01, n=20), FluSure XP (T02, n=20), or held as non-vaccinated, non-challenged controls (NTX, n=5). Pigs were challenged 8 days after re-vaccination with A/Swine/Minnesota/PAH-618/ 2012 (H1N1), a gamma-cluster field isolate that contains the M gene of pH1N1. Based on amino acid similarity the HA gene of the virus is ~ 92.8% similar to the FluSure XP gamma vaccine strain. Pigs were necropsied 5 days post-challenge. The primary variable analyzed was lung lesions at necropsy. Virus isolation from nasal swabs and bronchial aveolar lavage fluids (BALF), clinical observations, rectal temperatures, and HI antibody titers were analyzed as secondary variables. The level of significance was set at 0.05. This study was conducted in accordance with the guidelines of the Veterinary Resources Inc. IACUC.

Results

Vaccinated pigs had significantly lower percentage of lung with lesions at necropsy, significantly fewer pigs ever with fever, significantly lower virus titers in nasal swabs collected on 4 of 5 post-challenge days ($P \leq 0.05$, data not shown), a significantly lower percentage of pigs ever shedding virus, and a significantly lower percentage of pigs positive for virus isolation from BALF at necropsy (Table 1).

Table 1. Results

Group	% Lung Lesions*	% Pigs Ever Postive		
		Fever	Nasal Shedding	BALF
Placebo	12.1%	60%	100%	100%
FluSure XP	1.1%	10%	20%	0%
P value	0.0001	0.0050	0.0001	0.0001

*Back transformed LS Mean.

Conclusions

- Under the conditions of this study, pigs vaccinated with FluSure XP were protected against challenge with a contemporary gamma-cluster H1N1/pH1N1 reassortant SIV.
- It has been previous reported that FluSure XP helped to protected pigs challenged with A/swine/Ohio/ 02973 /2010, a contemporary gamma-cluster H1N1 virus that does not contain the pH1N1 M gene.[5]
- These studies demonstrated that FluSure XP can help protect pigs experimentally challenged with contemporary gamma-cluster viruses representative of the field isolates currently circulating in the US swine population.

References

1. Ducatez MF et al.: 2011. Emerg Infect Dis 17: 1624.
2. Nelson MI et al.: 2012. J Virol 86: 8872.
3. Kitikoon P et al.: 2012. J Virol 86: 6804.
4. Data on file, Study Report No. B820R-US-12-056, Zoetis Inc.
5. Data on file, Study Report No. 3121R-60-908, Zoetis Inc.

COMBINED AND SEPARATED PCV2 AND *Mycoplasma hyopneumoniae* ONE SHOT VACCINATION UNDER FIELD CONDITIONS

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Introduction

Mycoplasma hyopneumoniae (M. hyo) is the main component of PRDC and enzootic pneumonia (1). PCV2 is considered the necessary factor to trigger PCVAD (2). This study describes under field conditions the growth performance of animals vaccinated either with a mixture of one shot M. hyo and PCV2 vaccines or with both vaccines applied separately.

Materials and Methods

The study was performed in a 3 site farm with 7000 sows under intensive production in the Metropolitan Region of Chile. 510 fourteen days old piglets were randomly allocated into 3 study groups: Group A (n = 30) was injected in the neck with 2 ml IM of sterile saline solution. Group B (n = 240) was injected with 2 ml of a mixture of 1 mL of an subunitary one shot PCV2 vaccine and 1 mL of an one shot M.hyo aqueous adjuvanted vaccine. Group C (n = 240) was vaccinated with the traditional farm protocol, injecting 1 mL of the same vaccines separately on the right and left side of the neck. Animals were monitored during 7 days post injection in search of local or systemic adverse reactions. The experimental unit was the pig. Individual weights were registered after each stage of production and estimated average daily gain weight (ADGW) was calculated for each study group. ANOVA and Tukey post hoc tests were utilized to establish statistical significant differences among groups.

Results

No adverse local or systemic reactions were documented after vaccination. Figure 1 describes the average liveweight in the different treatment groups. At 140 days of age there is statistically significant difference between the weight of group A with groups B and C ($P<0.05$). Similarly ADGW in groups B and C were statistically higher than group A during finishing and wean to finish intervals (Table 1).

Figure 1. Average liveweight (kg) of pigs in each study group at different production ages.

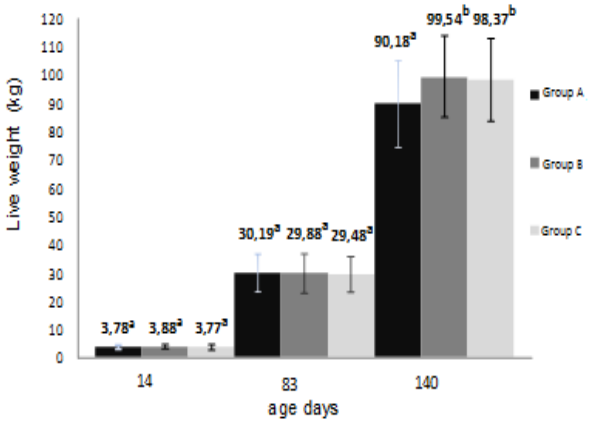

Table 1. Average daily gain weight (grams (SD)) at different weight intervals in each study group.

Period	Group A	Group B	Group C
14-70 days	0.382[a] (0.098)	0.376[a] (0.095)	0.373[a] (0.088)
70-140 days	0.849[a] (0.157)	0.978[b] (0.151)	0.969[b] (0.163)
14-140 days	0.617[a] (0.107)	0.683[b] (0.101)	0.676[b] (0.103)

Conclusions and Discussion

Under the conditions of this study, one shot vaccination against M. hyo and PCV2 improved liveweight and ADGW regardless of the administration procedure. Vaccinated groups (B and C) were similar throughout the study and superior to the control non vaccinated group in the evaluated parameters ($P>0.05$).

References

1. Maes et al., 2008. Vet Microbiol. 126: 297 – 309.
2. Segalés et al., 2005. Anim Health Res Rev. 6 (2): 119 – 142.

NEEDLE AND NEEDLE FREE INJECTION WITH A MIX OF PCV2 AND *Mycoplasma hyopneumoniae* ONE SHOT VACCINES UNDER FIELD CONDITIONS

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Introduction

Vaccination is a key point of sanitary programs in contemporary swine production. In many systems vaccine administration is being held by needle injection. Over the last decades new needleless devices have provided an alternative to traditional methods. This study describes under field conditions the effect on average daily gain weight (ADGW) and feed conversion rate (FCR) of needle and needleless injection for *Mycoplasma hyopneumoniae* (M. hyo) and PCV2 vaccines.

Materials and Methods

The study was performed in a wean to finish farm with 1500 sows under intensive production in Maule Region of Chile. 420 twenty one days old piglets were randomly allocated into 2 study groups: Group A (n= 210) was needleless injected with 2 ml IM of a mixture of 1 mL of an subunitary one shot PCV2 vaccine and 1 mL of an one shot M.hyo aqueous adjuvanted vaccine. Group B (n= 210) was vaccinated IM by needle injection with the same mixture of vaccines. Animals were monitored during 7 days post injection in search of local or systemic adverse reactions. Feed was delivered manually on *ad libitum* basis. Consumption was daily monitored by registering the amount of feed in kg placed in each feeder - 30 pigs capacity – pen. Each pig was individually weighted at 21, 70 and 160 days of age and ADGW between these periods was calculated, statistical significant differences between groups were assessed by means of ANOVA and Student T test. FCR was obtained per pen and was statistically analyzed using Wilcoxon Test.

Results

No adverse local or systemic reactions were documented after vaccination. Tables 1 and 2 describe ADGW and FCR through different production periods. There were no statistically significant differences ($P < 0.05$) between treatments groups in these parameters.

Table 1. ADGW (g) for both treatment groups in different productive periods.

Groups	21-70 days	70-160 days	21-160 days
A	447,7 g	945,7 g	780,6 g
B	451,3 g	939,1 g	777,9 g

Table 2. FCR (kg/kg) for both treatment groups in different productive periods.

Group	21-70 days	70-160 days	21-160 days
A	1,47	2,67	2,44
B	1,48	2,71	2,45

Conclusions and Discussion

In line with existing evidence that sustains the efficacy in the use of needle free injection devices (1,2,3,4), under the conditions of this study, needleless administration of PCV2 and M.hyo vaccines was as safe and efficacious as the traditional needle injection in terms of ADG and FCR performance.

References

1. Chuo et al., 2008. 18th IPVS Congress Proceedings. Hamburg, Germany. 2. 15
2. Jones et al., 2005. J Swine Health Prod. 13 (1): 19 – 27.
3. Martelli et al., 2007. Vaccine. 25: 3400 – 3408.
4. Phillips et al., 2010. 21st IPVS Congress Proceedings. Vancouver, Canada. 531.

COMPARATIVE EVALUATION OF ONE AND TWO DOSE PCV/ *Mycoplasma hyopneumoniae* VACCINATION PROTOCOLS IN SWINE CHALLENGED WITH PCV AND *Mycoplasma hyopneumoniae*

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Introduction

Vaccination is considered an essential strategy for prevention of porcine circovirus associated disease (PCVAD) caused by porcine circovirus type 2 (PCV2), a swine pathogen present in herds worldwide. The study evaluated the post-challenge serologic response, viremia, and productivity performance in pigs vaccinated with various PCV2/*Mycoplasma hyopneumoniae* vaccines.[1]

Materials and Methods

Healthy pre-weaned pigs were negative by PCR for PRRSV and PCV and the herd was *Mycoplasma hyopneumoniae* stable. Animals were randomly assigned to one of five test groups: nonvaccinated controls (T1, n = 84), vaccination with Respisure One® at 3-7 days of age and Fostera™ PCV2 at weaning (T2, n = 86), vaccination with Circumvent® PCV M at weaning and 3 weeks later (T3, n = 77), vaccination with Ingelvac® CircoFLEX-MycoFLEX® at weaning (T4, n = 80), vaccination with Respisure One® at 3-7 days of age and Fostera™ PCV2 at weaning and 3 weeks later (T5, n = 86) and a one bottle PCV2/*M. hyopneumoniae* combination vaccine (PCV2/M Hyo combo), one dose at weaning (T6, n=85). All groups were challenged with *M. hyopneumoniae* at eight weeks of age and PCV2b at ten weeks of age.

Results and Discussion

Four PCVAD fatalities occurred, all in group T1. Compared to groups given a single PCV2 vaccine dose (T2, T4, and T6), groups given two PCV2 vaccine doses (T3 and T5) had significantly ($p \leq 0.05$) higher least squares means (LSM) PCV2 serologic responses and significantly ($p \leq 0.05$) lower levels of post-challenge (PC) PCV2b viremia and fecal shedding as determined by qPCR assay. Nonvaccinated controls (T1) had significantly ($p \leq 0.00001$) higher levels of PCV viremia compared to all vaccinated groups, and 100% of control pigs tested for fecal shedding was PCV2b-positive for up to 80 days following PCV2 challenge. Average daily gain (ADG) on Day 146 was significantly greater ($P \leq 0.05$) for T2, T3, T4 and T6 vaccinated groups compared to nonvaccinated controls (≥1.61 lbs vs. 1.56 lbs), but ADG among the vaccinated groups was not significantly different. (Table 1)

This dual-challenge study demonstrated that a 1- or 2-dose regimen of Fostera PCV and the PCV2/*M. Hyo* combination vaccine effectively helped control PCVAD and helped reduce PCV2 viremia and fecal shedding, which helped vaccinated pigs sustain favorable growth performance. No differences ($P > 0.05$) in viremia, shedding, or ADG outcomes were detected between the Fostera PCV regiments, PCV2/*M. Hyo* combination vaccine and competitor vaccines/regimens. In addition, the *M. hyopneumoniae* challenge infection was successfully controlled by early vaccination with RespiSure-ONE. Fostera PCV (one or two dose) and a one dose PCV2/*M. Hyo* combination vaccine represents an effective and versatile vaccines that helps provide excellent control of PCV2 viremia, which is a critical feature for helping reduce fecal shedding (exposure risk for other pigs) and for helping sustain profitable growth performance.

1. Data on file, Study Report No 12ORBIOPORK01, Zoetis Inc.

Table 1

Treatment	Weeks 6-15 ADG	Weeks 1-22 ADG
Control	1.29[a]	1.56[a]
RSO and Fostera PCV (1 dose)	1.38[b]	1.61[b]
Circumvent PCV M	1.43[b]	1.62[b]
Ingelvac CircoFlex + Mycoflex	1.40[b]	1.61[b]
RSO and Fostera PCV (2 dose)	1.43[b]	1.60[b]
PCV2/M Hyo combo (1 dose)	1.40[b]	1.63[b]

[a,b] LS means in columns with different superscripts are significantly different ($P \leq 0.05$)

EFFICACY AND SAFETY OF A ONE DOSE ONE BOTTLE PORCINE CIRCOVIRUS/MYCOPLASMA HYOPNEUMONIAE COMBINATION VACCINE

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Introduction

PCV2 associated disease (PCVAD) and *M. hyopneumoniae* (*M. hyo*) are two of the most economically important swine diseases worldwide. Three studies were conducted to demonstrate efficacy against PCV2 and *M. hyo* challenges and safety in the field of a bivalent product containing both cPCV1-2 and *M hyo* antigen fractions.

Materials and Methods

Commercially available pigs were vaccinated intramuscularly with 2 mL at approximately three weeks of age in all three studies. Individual PCV2 and *M. hyo* efficacy studies were designed with four treatment groups each and a control group containing the monovalent version of the non-test fraction and tested against their respective challenge pathogens at approximately six weeks of age. For purposes of this presentation, only one treatment and the control group are shown. The primary variable for PCV2 efficacy was viremia and secondary variables were serology, shedding and lymphoid lesions and colonization. The primary variable for *M. hyo* was lung lesions and the secondary variable was serology. A field safety study was conducted at three separate commercial farms. A total of 632 animals were vaccinated with two different vaccine lots and an additional 158 pigs were vaccinated with sterile saline as controls. All pigs were observed for injection site reactions and adverse clinical reactions at weekly intervals following vaccination for three weeks and then returned to herd. These studies were conducted in accordance with the guidelines of Zoetis' IACUC or Ethical Review Board.

Results

In the PCV2 efficacy study, PCV2 vaccinated pigs compared to control pigs had a significant reduction in the percent of PCV2 ever viremic pigs (P<0.0001; Table 1), significantly lower PCV2 DNA copies/mL in the serum (P<0.0001), significantly higher PCV2 antibody titers post-challenge (P<0.0001), as well as significant reductions in the percent of pigs shedding PCV2 (P=0.0002), the number of PCV2 DNA copies/mL in the feces (P<0.0001), the total amount of PCV2 antigen detected in tissues (P<0.0003), and lymphoid depletion (P<0.0053). In the *M. hyo* efficacy study, *M. hyo* vaccinated pigs had significantly lower percentage of lung with lesions at necropsy (P<0.0001; Table 2), and significantly higher *M. hyo* antibody titers post-challenge (P<0.0001). In the field safety study, 7 pigs out of 632 (0.01%) were observed with mild injection site reactions a day after administration, which all resolved within three days. There were no adverse events related to the vaccines.

Table 1. PCV2 Viremia

Group	N	Percent Ever Viremia Positive	P-value vs T01
T01	24	95.8	
T02	24	8.3	<0.0001

Table 2. *M. hyo* Percent Lung with Lesion

Group	N	Percent Lung Lesions*	P-value vs T01
T01	30	7.01	
T02	30	1.66	<0.0001

*Back transformed LS Mean.

Conclusions

These studies demonstrated that this one bottle PCV2/*M. hyo* combination vaccine is safe and efficacious against PCV2 and *M. hyo* experimental challenges when administered intramuscularly as a single 2 mL dose.

References

Data on file, Study Report Nos. B822R-US-12-046, B823R-US-13-136, and B921R-US-12-009, Zoetis Inc.

EFFICACY AND SAFETY OF A TWO DOSE ONE BOTTLE PORCINE CIRCOVIRUS/*Mycoplasma hyopneumoniae* COMBINATION VACCINE

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Introduction

PCV2 associated disease (PCVAD) and *M. hyopneumoniae* (*M. hyo*) are responsible for significant production and economic losses worldwide. Two studies were conducted to demonstrate vaccine efficacy against PCV2 and *M. hyo* challenges following vaccination with a bivalent vaccine containing both cPCV1-2 and *M hyo* antigen fractions.

Materials and Methods

Commercially available pigs were intramuscularly vaccinated with 1 mL at approximately three weeks of age and again at five weeks of age in the two studies. Individual PCV2 and *M. hyo* efficacy studies were designed with one treatment group (T02) and a control group (T01) containing the monovalent version of the non-test fraction and tested against their respective challenge pathogens at approximately six weeks of age. The primary variable for PCV2 efficacy was PCV2 viremia and secondary variables were serology, fecal shedding and lymphoid lesions and colonization. The primary variable for *M. hyo* was lung lesions and the secondary variable was serology. These studies were conducted in accordance with the guidelines of the Zoetis IACUC.

Results

In the PCV2 efficacy study, all PCV2 vaccinated pigs compared to control pigs had a significant reduction in the percent of PCV2 ever viremic pigs (P<0.0001; Table 1), significantly lower PCV2 DNA copies/mL in the serum (P=0.0001), significantly higher PCV2 antibody titers seven days post-second vaccination until necropsy ($P \leq 0.0014$), as well as significant reductions in the percent of pigs shedding PCV2 ($P \leq 0.0061$), significant reduction in the number of PCV2 DNA copies/mL in the feces ($P \leq 0.0001$), and significant reduction of microscopic lesions (Lymphoid Depletion) in lymphoid tissues ($P \leq 0.0235$). In the *M. hyo* efficacy study, all *M. hyo* vaccinated pigs had significantly lower percentage of lungs with lesions at necropsy (P<0.0001; Table 2), and significantly higher *M. hyo* antibody titers prior to challenge and at necropsy ($P \leq 0.0001$).

Table 1. PCV2 Viremia

Group	N	Percent Ever Viremic	P-value vs T01
T01	28	92.9	
T02	29	0	<0.0001

Table 2. *M. hyo* Percent Lung with Lesion

Group	N	Percent Lung Lesions*	P-value vs T01
T01	31	8.70	
T02	32	1.59	<0.0001

*Back transformed LS Mean.

Conclusions

These studies demonstrated that this one bottle PCV2/*M. hyo* combination vaccine is efficacious against PCV2 and *M. hyo* experimental challenges when administered intramuscularly in a two – 1 mL dose regimen.

References

Data on file, Study Report Nos. B822R-US-12-111, B822R-US-12-112, Zoetis Inc.

ANTIBIOTIC RESISTANCE OF K88+*ESCHERICHIA COLI* STRAINS ISOLATED IN 1997 AND 2012 FROM DIARRHEA IN POST-WEANED PIGS IN BRAZIL

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Introduction

E. coli K88 is an important agent of diarrhea in post-weaned pigs[2]. The disease is difficult to control due to the capacity of the bacteria to develop and disseminate mechanisms of resistance to antibiotics used to treat the disease[1]. The aim of this work was to compare the resistance to 14 antibiotics of *E. coli* K88 + strains from post-weaned piglets with diarrhea collected in two periods in southern Brazil.

Materials and Methods

Fecal samples were collected in 1997 and in 2012 from post-weaning piglets with diarrhea in two states of southern Brazil. The samples were cultured in Blood Agar and Mac Conkey Agar. After *E. coli* isolation, the genes for fimbrial adhesins (K88, K99, F18 and F41) were studied by Multiplex PCR using the NewGene Amp kit. Forty three K88 + strains isolated in 1997 and 119 from 2012 were selected and tested to 14 antibiotics commonly used in the pig industry, using the disk diffusion method. The antibiotic sensitivity was determined by the inhibition zone diameter and classified as sensitive and resistant using CLSI recommendations.

Results

The results of antibiograms are shown in Table 1. Resistance was not found against 8 out of 14 antibiotics tested to strains collected in 1997. Resistance developed to the same antibiotics in strains collected in 2012. Five other antibiotics presented less than 10% resistance to strains collected in 1997, and only AMO presented more than 25% resistance. From strains collected in 2012, there was a high resistance (>90%) to FLO, TET and OXY. This high frequency of resistance to OXY and TET was also found in other studies[1,3]. COL resistance remained stable (9.3%-10.1%). The occurrence of COL resistance is uncommon[2,5] and the disk diffusion method is not recommended to detect its resistance[4]. Despite the common usage of CEF[5], there was no resistance in samples of 1997 and little resistance was found (1,7%) in strains of 2012. Other drug that presented less than 25% resistance in 2012 were NEO and LIN.

Table 1. Frequency of resistant strains of *E. coli* K88 + collected in 1997 and 2012

Antibiotic	Year	
	1997	2012
Ciprofloxacin (CIP)	0%*	65.5%
Enrofloxacin (ENR)	0%	75.6%
Norfloxacin (NOR)	0%	58.0%
Doxycycline (DOX)	0%	83.2%
Trimethoprim-sulfa (TRI)	0%	86.5%
Ceftiofur (CEF)	0%	1.7%
Neomycin (NEO)	0%	23.5%
Lincospectin (LIN)	0%	10.9%
Oxytetracycline (OXY)	2.3%	96.6%
Tetracycline (TET)	4.7%	98.3%
Florfenicol (FLO)	4.7%	96.6%
Ampicillin (AMP)	9.3%	58.8%
Colistin (COL)	9.3%	10.1%
Amoxicillin (AMO)	27.9%	59.7%

* % of resistant strains

Conclusions and Discussion

All tested *E. coli* strains presented increase in resistance between the two tested periods. Five antibiotics with no resistance in 1997 presented in 2012 high percentage of resistant strains (>57%) and three had resistance below 24%. Three antibiotics presented very high level of resistance (>90%, OXY, TET, FLO), all were widely used in the farms of origin of the strains. The most effective antibiotic was CEF, with only 1.7% resistant strains in 2012. The results show a significant increase in resistance to all tested products with time and suggest a need for alternative measures for disease control and a prudent use of antibiotics in pig industry.

References

1.Baccaro MR et al.:2002, Arq Inst Biol 69:15. 2.Dosen R et al.:2011, Biotech Ani Husbandry 27:861. 3.Menin A et al.:2008, Ciênc Rural 38:1687. 4.Morales AS et al.:2012, Scientific World Journal 2012:1. 5.Mota RA et al.:2005, Braz J Vet Res Anim Sci 42:465.

PRE-INOCULATION FECAL MICROBIOTA OF PIGS WITH DIFFERENT CLINICAL OUTCOMES FOLLOWING CHALLENE WITH *Brachyspira hampsonii* CLADE II (STRAIN 30446)

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Introduction and objective

Brachyspira species can cause colitis and diarrhea in grow-finish pigs, resulting in economic losses worldwide. Recently, a novel species provisionally designated "*Brachyspira hampsonii"* has been found to cause mucohaemorrhagic diarrhea in pigs[1]. During herd outbreaks, a proportion of pigs remain unaffected despite likely exposure. Similarly, in a recent inoculation trial with *"B. hampsonii"* clade II strain 30446, the pathogen was detected by culture and/or species-specific qPCR in 12/12 inoculated pigs following inoculation, although mucohaemorrhagic diarrhea was observed in only 8/12 inoculated pigs[1]. This prompted us to investigate why some animals do not develop diarrhea.

In this study we characterized and compared the pre-inoculation fecal microbiota of pigs that did or did not develop mucohaemorrhagic diarrhea following inoculation with *"B. hampsonii"* clade II to determine if host intestinal microbiota composition might be a contributing factor to susceptibility.

Material and methods

Pigs (n=12) were obtained from a commercial facility and housed at the Animal Care Unit (University of Saskatchewan). During an acclimation period of 8 days, fecal samples were collected from all pigs 8, 5, 3 and 0 days pre-inoculation. Total DNA was extracted from feces using a commercial kit. The total bacterial population was estimated using a qPCR targeting the bacterial 16S rRNA gene. For microbiome profiling, samples (n=47) were used as template for a PCR protocol targeting the universal *cpn*60 gene, and purified PCR products were subjected to pyrosequencing. Resulting data was analyzed using the mPUMA bioinformatics pipeline[3].

Results and discussion

Out of the 12 pigs inoculated with pure cultures of *"B. hampsonii"* clade II, 8 broke with mucohaemorrhagic diarrhea (MH) and 4 did not (Non-MH)[2] following inoculation with 10^8 genome equivalents on three consecutive days. Over 600,000 high-quality sequence reads were obtained from the *cpn*60 amplicon libraries, with a mean length of 451 bp and an average of 40.000 reads per sample. *cpn*60 sequences were assembled into operational taxonomic units (OTU) and sequences were compared to the cpnDB reference database for identification. Fecal microbiomes of non-MH and MH pigs were dominated by Bacteroidetes, followed by Firmicutes, Proteobacteria, Actinobacteria and other phyla. The proportion of Proteobacteria was significantly different ($p<0.05$) between groups at 8 days pre-inoculation, with pigs in the Non-MH group having higher counts. However, this difference was not observed on subsequent days. There were no statistical differences observed in richness and diversity measurements (Chao1, Shannon index, Simpson's reciprocal index) between the MH and non-MH groups.

Interestingly, a sequence similar to *Flavonifractor plautii* was detected only in MH pigs on day 0 (6/8 MH, 0/4 non-MH). Whether the significant difference in the abundance of this anaerobic species is indicative of a general difference between the groups remains to be investigated.

No statistical differences were observed between groups in terms of total bacterial population estimated by 16S rRNA gene copies at 8, 5 or 3 days pre-inoculation. However, on day 0, a significant difference was observed ($p<0.05$). The mean for Non-MH pigs was $9.7x10^{10}$ 16S rRNA copies, while the mean for MH was $2.82x10^{11}$ copies.

This study including a small number of samples identified some interesting differences in the fecal microbiota of MH and non-MH-groups that warrant further investigation. Identification of susceptibility factors in the intestinal microbiota could lead to novel strategies for *Brachyspira* disease reduction through manipulation of the intestinal microbial community.

References

1. Rubin JE, et al. 2013. *PLoS One.* 8:e57146.
2. Chaban et al. 2013. 8(7): e69559

THE EVALUATION OF MOLECULAR EPIDEMIOLOGICAL RELATEDNESS WITHIN ST5 *Staphylococcus aureus* ISOLATED FROM SWINE VETERINARIANS IN THE USA.

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The livestock associated *Staphylococcus aureus* (LA-MRSA) ST 398 has been isolated frequently from livestock, especially pigs, worldwide since first detected from pigs and pig farmers in the Netherland in 2005[1]. Subsequent studies are revealed greater genetic diversity in LA-MRSA using molecular epidemiologic characteristics. Several studies reported that ST9 isolates are predominate among MRSA isolates from pigs in Asian countries, while both ST398 and ST5 lineages have been found in pigs and livestock workers in North America[3,4]. Unlike ST398, which is rarely involved in significant human infections, the occurrence of ST5sequence type in pig industry is of some public health concern as this ST5 lineage has long been associated with human MRSA infections related the USA100 group (defined by sma1 PFGE)[2,5]. In a pilot study of pig farms in Minnesota, the predominant spa types found were t034 *(ST398*), and t002 (ST5), comprising 37% and 29% of isolates respectively (all MSSA). The aim of this study was to investigate the prevalence of ST5 *S. aureus* nasal swabs in US swine veterinarians, and to evaluate the diversity of spa type t002 isolates using PFGE with *Sma*I.

Similar to our findings in pigs, the most prevalent spa types found in US swine veterinarians were t034-ST398 and t002-ST5. ST9 spa types (t337, t3446, t2498) were also common. Sixty (19%) of 308 MSSA isolates and 6 (19%) of 32 MRSA isolates detected were *spa* type t002 (ST5). Other ST5 MSSA spa types found included t045 (14), t062 (6), t570 (3) and t2049 (1). Sma1 PFGE analysis of 23 isolates indicated that t002 isolates from the swine veterinarians were not clonal but heterogeneous between isolates. Fifteen distinct pulsotypes were seen, and eight isolates were classified as USA100. Given previous reports of t002-ST5 MRSA in North American pigs, our findings of diverse ST5 spa types as well as genetic diversity within spa type t002, provide improved understanding of ST5 lineage, which may have long association with pigs overtime after introduction from human. Further investigation of ST5 lineage is warranted to better understand their potential implications for human health.

References

1. Voss, A. et al. : 2005, Emerg. Infect. Dis. 11, 1965-1966.
2. Khanna, T. et al. : 2008, Vet. Microbiol. 128, 298-303.
3. Smith, T.C. et al. : 2009, PLoS One 4, e4258.
4. Molla, B. et al. : 2012, J. Clin. Microbiol. 50, 3687-3693
5. Frana, T.S. et al. : 2013, PLoS One 8, e53738.

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If selected, I would prefer to present the paper as poster presentation.

A SERIAL STUDY TO DETERMINE PIGLET VACCINATION AGE BASED ON MATERNAL ANTIBODY DECAY, TIME OF INFECTION AND POTENTIAL AEROSOLIZATION OF *Mycoplasma hyorhinis*

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Introduction

Mycoplasma hyorhinis is a bacterium commonly found in the upper respiratory tract of swine. This bacterium primarily causes polyserositis and arthritis in post weaning pigs.[1,2] In addition to these lesions, it is considered a common cause of condemnations due to lung peel outs at slaughter.[3] The objectives of the study were (1) to determine the age at which *M. hyorhinis* maternal antibodies decay and nasal colonization occurs; (2) determine if *M. hyorhinis* is capable of aerosolization.

Materials and methods

The sampling was initiated at a 6000 (flow A) and a 4000 (flow B) head sow unit. Both herds were experiencing polyserositis and lameness cases in nursery pigs, where *M. hyorhinis* was consistently detected in lesions by PCR. Piglets were followed through the nursery stage. Forty piglets (1 barrow and 1 gilt/litter) were selected at each sow unit from 20 mixed parity sows. Nasal swabs and blood samples were collected. The sows were sampled at day 0 and piglets were sampled weekly starting at day 0 (parturition) for 10 weeks. Serum samples were tested using a research *M. hyorhinis* ELISA. ELISA testing was performed at days 0, 7, 14 then biweekly until the end of the nursery stage (Flow A day 70 and Flow B day 56). Nasal swabs were tested using an *M. hyorhinis* gel based PCR. PCR target testing dates were determined by the history of the flow's clinical signs. Target testing was performed for Flow A at days 0, 7, 14, 28, 63 and 70 and for flow B at days 0, 7, 14, 21 and 28.. A cyclonic air collector with Hayflick's media was placed outside the exhaust fans during the nursery stage once a week for 30 minutes. Samples were tested using *M. hyorhinis* PCR and Mycoplasma culture.

Results and Discussion

The ELISA values of both flows had similar decays over time (Figure 1). At day 70, 100% (35/35) animals were PCR positive in flow A. In flow B, 89% (31/35) were PCR positive at day 28. Flow A had no positive PCR samples or cultures from the air collectors. In flow B, 3/10 (day 35, 49, 56) aerosol samples were PCR positive and 1/10 (day 35) aerosol samples was culture positive. This indicates that *M. hyorhinis* is capable of aerosolization and is a potential form of transmission. The positive air samples collected from flow B occurred after the majority of animals tested *M. hyorhinis* PCR positive by nasal swab. This study demonstrated that maternal antibodies for *M. hyorhinis* have a gradual decay over the nursery stage and may allow for an increase in nasal colonization. Due to the drastic differences in colonization between flow A and B, vaccine timing should be flow specific.

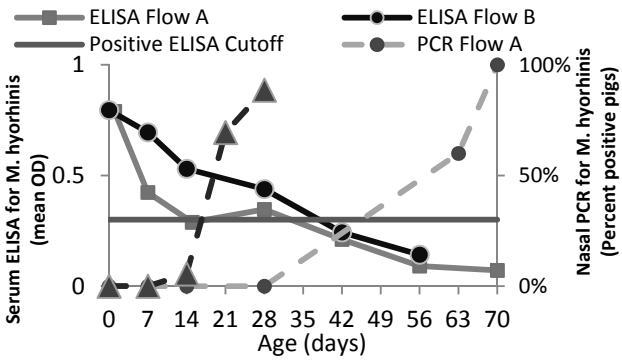

Figure 1: Average optical density (OD) and the percent PCR positive animals for flow A and B.

References

1. Duncan J.R. et al: 1973. *Am J Vet Res.* 34(3):363-6.
2. Gois M. et al.: 1974. *Zentralbl Veterinarmed B.* 21(5): 352-361.
3. Murray D. 2012. In: Proceedings of the 43rd AASV Annual Meeting (Denver, Colorado). pp 457-459.

A NOVEL MYCOPLAMSA HYOPNEUMONIAE ELISA, SPECIALLY DESIGNED FOR HIGH SPECIFICITY

Eric van Esch[1]

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Introduction

Mycoplasma hyopneumoniae (M.hyo) diagnostics are considered to be a dilemma[a]. For serology there is no true Gold Standard available and until now serological assays lacked specificity. On this basis there is a strong need for a highly specific assay with good sensitivity . This paper describes the first set of results with a high specificity M.hyo ELISA.

Materials and Methods

Sera sensitivity panel

This panel contains 108 samples from experimental infected animals (CReSa, Spain). Testing was done at CReSa with the BioChek ELISA.

Sera specificity panel

In total, 1695 samples from SPF herds from different geographical regions were tested. Samples were taken from animals of different ages, different breeds and different farm management systems. Samples were send by veterinary practioners to BioChek B.V., Reeuwijk, Netherlands for testing.

ELISA

The BioChek Mycoplasma hyopneumoniae Antibody Test Kits was used. This ELISA is based on a recombinant antigen for M.hyo and is supposed to be specific for M.hyo.

Statistical analysis

For test evaluation, WinEpiscope 2.0 was used to calculate Sensitivity and Specificity with a 95% confidence level.

Results and discussion

Table 1. Results on Mycoplasma hyopneumoniae antibody positive and negative samples

	Mycoplasma hyopneumoniae positive and negative samples (n=1803)	
	Sample True Positive	Sample True Negative
ELISA positive	92	13
ELISA negative	16	1682

To serologically confirm the M.hyo free status of herds, it is imperative that the test has high specificity combined with good sensitivity to be sure that recent outbreaks are not missed. In the study described, the specificity of the ELISA is high, 99.2%, [95%CI: 98.8; 99.6] while at 21 days post infection the sensitivity is 85.2% [95%CI: 78.5; 91.6]. This is considerably sufficient in monitoring programs and therefore, based on the overall results, the ELISA is suitable for this purpose.

Conclusion

The evaluated ELISA demonstrates high specificity and very good sensitivity which makes this ELISA suitable for monitoring M.hyo free herds.

Reference

[a]Buddle, J.R, O'Hara, A.J., 2005, Aus.Vet.Journal, vol.83, no.3, 134-139

EFFICACY OF DRAXXIN® 25 INJECTABLE SOLUTION (TULATHROMYCIN 25 MG/ML) FOR TREATMENT AND CONTROL OF SWINE RESPIRATORY DISEASE

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Introduction: DRAXXIN® 25 Injectable Solution (25 mg tulathromycin/mL) was recently approved in the US for the treatment and control of swine respiratory disease (SRD). DRAXXIN 25 is administered at 2.5 mg/kg BW, the same dose of DRAXXIN Injectable Solution (DRAXXIN 100; 100 mg tulathromycin/mL) used in pigs for SRD, but in a larger injection volume of 1.0 mL/22 lb that makes it easier to accurately dose younger, light weight pigs. DRAXXIN 25 Injectable Solution is indicated for the treatment of (SRD) associated with *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Bordetella bronchiseptica*, *Haemophilus parasuis*, and *Mycoplasma hyopneumoniae*; and for the control of SRD associated with *A. pleuropneumoniae, P. multocida,* and *M. hyopneumoniae*. This abstract presents data supporting the efficacy of this new lower concentration formulation of tulathromycin.

Materials and Methods: Effectiveness of DRAXXIN 25 was established using a plasma bioequivalence approach. A PK study was conducted using gilts and barrows (n=32 each sex) comparing DRAXXIN 25 to DRAXXIN 100. Pigs weighed 17 – 24.7 kg at start of study. There were 4 animals of each sex within each of 8 pens. Within pen and sex, pigs were randomly assigned to receive one of the 2 products administered IM in the neck at 2.5 mg/kg BW. Blood samples were collected at 24 h prior to dosing and after dosing at 20 and 40 min and 1, 1.5, 2, 3, 4, 7, 10, 24, 48, 96, 144, 192, 240, 288 and 336 h. Plasma samples were assayed for tulathromycin using a validated LC-MS/MS assay.

Results: Summary PK statistics are presented in Table 1. Table 2 shows the assessment of bioequivalence for $AUC_{0\text{-}t(last)}$ and C_{max}. These confirm that bioequivalence criteria were met for both variables. Demonstration of bioequivalence of plasma concentrations of tulathromycin between both products provides substantial evidence of effectiveness of DRAXXIN 25 for treatment and control of swine respiratory disease. Plots of the geometric mean plasma concentration of tulathromycin are presented in Figure 1.

Table 1. Comparative tulathromycin pharmacokinetics in swine (LS mean ± SD) after IM administration of DRAXXIN 25 or DRAXXIN 100.

PK Variable	DRAXXIN 25	DRAXXIN 100
C_{max} (ng/mL)	437 ± 122	509 ± 154
$AUC_{0\text{-}t(last)}$ (h·ng/mL)	8200 ± 1190	7710 ± 1220
t_{max} (h)	0.68 ± 0.64	0.39 ± 0.12
$t_{1/2}$ (h)	71.7 ± 14.5	72.9± 16.7

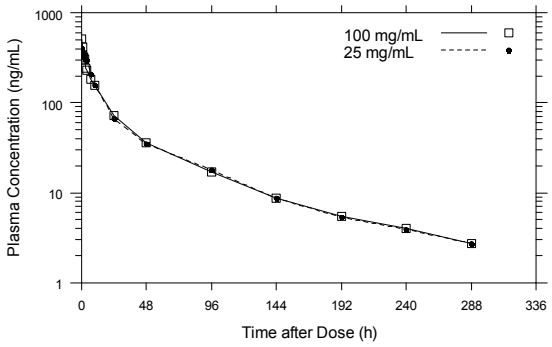
C_{max} – observed maximum plasma concentration
$AUC_{0\text{-}t(last)}$ - the area under the plasma concentration - time curve from time of dosing to the last observed concentration above the limit of quantification of the assay (3 ng/mL)
T_{max} – the time from dosing to C_{max}
$T_{1/2}$ – plasma elimination half life

Table 2. Assessment of bioequivalence: Ratio of DRAXXIN 100 to DRAXXIN 25 with 90 % confidence limits (CL) for $AUC_{0-t(last)}$ and C_{max}

Variable	Ratio	90% CL
$AUC_{0\text{-}t(last)}$ (h·ng/mL)	0.94	0.89, 1.0*
C_{max} (ng/mL)	1.17	1.04, 1.31†

* Must be completely contained within 0.80, 1.25 to conclude bioequivalence.
† Must be completely contained within 0.70, 1.43 to conclude bioequivalence.

Figure 1. Tulathromycin geometric mean plasma concentration after a single 2.5 mg/kg BW dose DRAXXIN 25 or DRAXXIN 100.



Conclusion: This study supports the efficacy of DRAXXIN 25 for treatment and control of SRD including *M. hyopneumoniae*.

DRAXXIN AT WEANING FOR CONTROL OF SWINE RESPIRATORY DISEASE IN A NATURAL INFECTION

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Introduction

Swine respiratory disease (SRD) is commonly found in weaned pigs and is attributable to both viral and bacterial pathogens. DRAXXIN (tulathromycin) is labeled for the control of SRD associated with *Pasteurella multocida*, *Actinobacillus pleuropneumoniae* and *Mycoplasma hyopneumoniae* (*M.hyo*). The purpose of this study was to evaluate the derived production benefits of controlling 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 with a documented history of *M. hyo*, *Pasteurella multocida* and PRRS associated SRD in the post weaning phase of production were blocked by gender and sow farm source 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, the pigs were treated with EXCEDE for swine per label. The pigs were ear notched and could not be retreated for seven days. If the pigs continued to exhibit qualifying respiratory and depression scores after seven days, they were treated with Baytril per label and another seven days were allowed to elapse. The Baytril 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, moved to hospital pen and 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, an analysis was preformed for that parameter separately for each gender.

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. Unvaccinated, sentinel pigs seroconverted to *M. hyo* by day 152, confirming exposure to this pathogen.

Table 1. Results Summary

	DRAXXIN	Saline
ADG day 0-54	0.79[a]	0.75[b]
ADG day 0-152	1.44[a]	1.41[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 significantly different at $p<0.05$

Twenty nine extra pigs and 8,137 extra pounds of pork were produced in DRAXXIN group. This study demonstrates that controlling SRD in pigs at weaning with DRAXXIN brings value by reducing mortality, morbidity and labor for retreatments as well as by increasing pounds of pork available for market.

COMPARISON OF TWO SAMPLE TYPES FOR DETECTION OF *Mycoplasma hyopneumoniae* IN NATURALLY INFECTED PIGS

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[2] *Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO*

Introduction

Nasal swabs are a common and rapid sample type used to detect *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) using molecular diagnostic tools[1]. Laryngeal swabs can also be used to detect *M. hyopneumoniae* genetic material in live pigs. However, a comparison of both sample types for *M. hyopneumoniae* detection in naturally infected pigs has not been performed. A study was conducted to compare nasal vs. laryngeal swabs for detection of *M. hyopneumoniae* in naturally colonized and infected pigs at different ages.

Materials and methods

Paired nasal and laryngeal swabs were collected randomly from 108 piglets at weaning age in sow farm A. Also, paired nasal and laryngeal swabs were collected randomly from 60 pigs (>20 weeks of age) in two finishing sites sourced from sow farm B. Nasal swabs were collected by introducing a swab into each nostril at 45^{o} angle latero-medial towards the nasal septum, then straightened and inserted carefully as rostral as possible, meanwhile rotating the swab clockwise and counterclockwise upon entry and exit. Laryngeal swabs were collected by opening the mouth with a mouth gag, depressing the tongue using a laryngoscope to expose the larynx, and introducing a swab guided by the laryngoscope blade to swab the laryngeal area. In older pigs, a sanitized BIC® pen fits snug in the cap of a double swab (BBL™), and acts as an extension to reach the laryngeal area. Samples were refrigerated and transported immediately after collection and were submitted to the U of MN VDL for real time PCR (rt-PCR) testing[2].

Results

The percentage of positive pigs by rt-PCR testing of all nasal and laryngeal swabs is shown in Fig.1. At weaning age, 33/108 pigs (31%) were positive by nasal swabs, 13/108 (12%) were positive by laryngeal swabs, and 41/108 (38%) by combining the result of both sample types (Na+La). At market age, 10/60 pigs (17%) were positive by nasal swabs, 18/60 (30%) were positive by laryngeal swabs, and 18/60 (30%) by combining the result of both sample types.

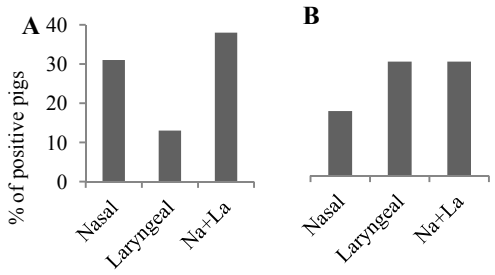

Figure 1. Detection of *M. hyopneumoniae* by RT-PCR in nasal, laryngeal and nasal laryngeal swabs. A) Samples collected at weaning age. B) Samples collected at market age.

Discussion and conclusions

Sensitive, fast and practical samples are needed for *in vivo* diagnosis and further control of *M. hyopneumoniae*[4]. The most common sample type used to detect *M. hyopneumoniae* in the field is the nasal swab. On the other hand, experimental trials and field studies indicate that more sensitive sampling sites are located in the lower part of the respiratory tract, specifically the trachea and the bronchi[3,4]. Results from this investigation showed that at weaning age more nasal than laryngeal swabs tested positive, and the combination of both sample types increased the level of detection by 7%. At market age, more positive pigs were detected using laryngeal swabs. Results from this study suggest that the detection rate of *M. hyopneumoniae* could be increased by selecting a sample type based on the estimated time interval between pathogen exposure and sampling time point.

Acknowledgements
This project was funded by Boehringer Ingelheim Vetmedica, Inc.

References

[1]Calsamiglia M, et al. 1999. J Vet Diagn Invest 11:246-251.
[2]Strait EL, et al. 2008. Aug, J Clin Microbiol 46(8):2491-8.
[3]Kurth , et al. 2002. J Vet Diagn Invest 14, 463-469.
[4]Fablet, et al. 2010. Vet Microbiol 143:238-245.

INVESTIGATING RISK FACTORS FOR *Mycoplasma hyopneumoniae* PIGLET COLONIZATION DURING THE LACTATION PERIOD

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[1] *College of Veterinary Medicine, University of Minnesota, St. Paul, MN*. [2] *Boehringer Ingelheim Vet Medica, St. Joseph, MO*.

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.

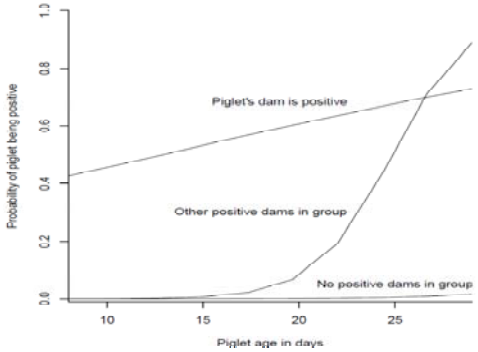

Figure 1. Piglet probability of *M. hyopneumoniae* colonization as a result of dam PCR status and piglet age.

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.

Acknowledgements

The authors would like to thank Cesar Prado, Jon Ertl, and Jason Daniels for their help in sample/data collection. Also, thanks to the owners, crew members and practitioners attending the participating farms. This study was funded by Boehringer Ingelheim Vetmedica, Inc.

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
3. Nathues et al. 2013. Acta Vet Scandinavica. 55:30

RATIONALE FOR THE USE OF TILMICOSIN FOR THE CONTROL OF BACTERIAL PNEUMONIA COMPLICATED BY COINFECTION WITH *Mycoplasma hyopneumoniae*

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Introduction

Mortality and production losses caused by bacterial pneumonia complicated by *Mycoplasma hyopneumoniae* (MH) coinfection is a concern of swine industry stakeholders. Significant advances in animal husbandry practices and new MH vaccines have improved the welfare of swine at risk for MH infection. Antibiotics also serve an essential role in swine welfare once infection with MH has occurred. A rationale for the selection of tilmicosin when attempting to control bacterial pneumonia complicated by MH coinfection is presented here.

Materials and Methods

Minimum inhibitory concentration (MIC) results (n = 132) for tilmicosin against MH were reviewed from published and unpublished data (1970-2011) worldwide.[1,2,4-6,8-11] Datum was excluded if a result was not for a wild type strain or individual results were not reported. MIC values were rank ordered and a modal MIC determined. Tilmicosin concentration was determined in secretions and at cellular and tissue levels in 6 male and 6 female healthy swine fed tilmicosin *ad libitum* at 363 g/ton for 7 days.[3] Tilmicosin concentrations were then compared to the modal MIC.

Two clinical experiments that compared various antibiotics provided to 5- to 6-week old pigs challenged with *Pasteurella multocida* (PM), *Actinobacillus pleuropneumoniae* (AP), and MH were reviewed.[7] Pigs were inoculated with MH, PM, and AP on days 1, 8, and 15, respectively. Treatment was initiated on day 9 and continued for 12 days in each experiment. Results associated with the feeding of tilmicosin at 0 (control) and 363 g/ton (medicated-1) or 273 g/ton (medicated-2) only were further reviewed.

Results

The modal MIC (n = 106) was determined to be ≤ 2 µg/mL. Mean tilmicosin concentration was 2.5, 1.4, 1.7, 1.9, 5.3, and 4.9 µg/mL for nasal secretions, nasal turbinate mucosa, tonsils, tracheal mucosa, pulmonary alveolar macrophages, and peripheral neutrophils, respectively. Tilmicosin concentration in nasal secretions, pulmonary alveolar macrophages, and peripheral neutrophils exceeded the modal MIC.

Decreases in clinical score, occurrence of pneumonia, lung lesion score, and MH re-isolation in medicated-1 and medicated-2 pigs differed significantly from that of control pigs. Body weight gain was significantly greater in medicated-1 pigs compared to that of control pigs.

Conclusions and Discussion

A rationale for the use of tilmicosin for the control of bacterial pneumonia complicated by coinfection with MH is supported by historical MIC data, in vivo tilmicosin concentration, and clinical study results. Tilmicosin has the ability to accumulate at the microenvironment of infection in nasal secretions, pulmonary alveolar macrophages, and peripheral neutrophils at concentrations exceeding the modal MIC determined here. In clinical challenge studies, tilmicosin-fed pigs had decreases in occurrence of pneumonia, clinical score, lung lesion score, and MH re-isolation compared with control pigs.

References

1. Archambault M et al. 2010. *Proc 21st IPVS,* Paper 335.
2. Elanco T5CAM0709. 2011 (Unpublished).
3. Elanco T5C769712. 2010 (Unpublished).
4. Inamoto T et al. 1994: *J Vet Med Sci* 56:393-394.
5. McLaren I et al. 1995 (Unpublished Elanco Study).
6. Ose EE. 1987: *J Antibiot (Tokyo)* 40:190-194.
7. Stipkovits L et al. 2001: *Can J Vet Res* 65:213-222.
8. ter Laak E. 1995 (Unpublished Elanco Study).
9. Thongkamkoon P et al. 2002: Proc *17th IVPS,* Paper 219.
10. Vicca J et al. 2004: *Antimicrob Agents Chemother* 48:4470-4472.
11. Wu C et al. 1997: *Swine Health and Production* 5:227-230.

SINGLE FIXED-TIME INSEMINATION IN GILTS USING pLH AT ESTRUS ONSET

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Introduction

In addition to the reduction in labor time, a fixed-time artificial insemination (FTAI) protocol is a strategy to optimize the use of genetically superior boars. As the control of ovulation time is critical to define the time of artificial insemination (AI), hormones such as porcine luteinizing hormone (pLH) have been used to induce ovulation for the implementation of FTAI protocols. This study aimed to evaluate the reproductive performance of gilts submitted to single FTAI using pLH at estrus onset.

Materials and Methods

Estrous detection was carried out twice a day (7a.m and 7 p.m) in 318 Agroceres PIC gilts using backpressure test in front of mature male. Gilts were uniformly distributed according to number of estrus (2 or 3), time of flushing and average daily gain of weight during flushing period in two treatments: Control - without hormonal application and AI at 12, 36 and 60 h after estrus detection and FTAI - 2.5 mg pLH injection (Lutropin-V®Bioniche Animal Health, Belleville, Ontario, Canada) by vulvar submucosal route at estrus onset and a single FTAI 12 h after pLH. The application route, pLH dose and the moment of FTAI were defined in previous experiments (unpublished data). Split-samples AI doses were produced and contained 2 billion of motile sperm cells. Farrowing rate (FR), adjusted farrowing rate (AFR) and total born piglets (TBP) were analyzed using the software Statistical Analysis System (SAS, 9.1) through Chi-square test for FR and AFR and MIXED procedure for TBP.

Results

Gilts of control group received 2.3 ± 0.6 AI doses whereas one dose was used in FTAI group. Gilts that received a single FTAI had lower FR (P=0.084) and AFR (P=0.028) compared to Control gilts but TBP was not different (P=0.512) between treatments (Table 1). The number of total born piglets per AI dose was 12.3 ± 0.3 for FTAI gilts and 6.0 ± 0.3 for control group (P<0.05).

Conclusions and Discussion

Single insemination has been evaluated in multiparous sows [1, 4] with induced ovulation. However, more than one fixed-time AI has been used in gilts after estrus synchronization with progestagens followed by follicular growth and ovulation induced by hormones [2, 3]. The observed reduction in FR contrasts with results of studies performed in sows, since higher [1] or similar [4] FR were observed with single fixed-time AI compared to control AI protocols. The fact that a single FTAI did not affect the TBP corroborates the similar number of embryos and percentage of recovered embryos observed in gilts receiving two fixed-time AI in comparison to those traditionally inseminated [2]. Similarly, litter size was not affected by single insemination in sows [1, 4]. This study shows the possibility of using a single FTAI in gilts whose ovulation was induced by pLH at estrus onset. Considering the moderate reduction in FR and no impairment of litter size, this protocol might be suggested mainly for contingency plans.

Table 1 – Reproductive performance of gilts submitted to a single fixed-time AI (FTAI) or a traditional AI protocol (Control)

Treatment	FR (%)	AFR (%)	TBP
Control	145/159 (91.2)	145/155 (93.6)	12.5 ± 0.2
FTAI	135/159 (84.9)	135/157 (86.0)	12.3 ± 0.2
P-value	0.084	0.028	0.512

FR-farrowing rate; AFR-adjusted farrowing rate; TBP-total born piglets

References

1. Cassar, G. et al.: 2005. J. Swine Health Prod. 13: 254-258. 2. Degenstein, K.L. et al.: 2008. Theriogenology 70: 1075–1085. 3. Martinat-Botté et al.: 2010. Theriogenology 73: 332-342. 4. Zak, L.J.; et al.: 2011. Proc. Amer. Assoc. Swine Vet. pp. 197-200.

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THE IMPACT OF PIG HEALTH ON PUBLIC HEALTH: QUANTITATIVE DATA FOR RISK ASSESSMENTS

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Introduction

While clinically ill animals will not pass ante-mortem inspection, it is possible that animals with subclinical illness or lesions could pass inspection and be harvested. These animals could be more likely to harbor pathogens that could cause food-borne illness, such as *Salmonella*.

One possible source of contamination is what is referred to as a peel-out, or a pleural adhesion which does not allow for complete removal of the viscera, requiring extra trimming. A previous study found that approximately 7% or 1 in 15 carcasses had some degree of pleural adhesions, and carcasses with peel-outs were 90% more likely to be contaminated with *Salmonella*.[1]

The objectives of this study are threefold: 1) estimate a national prevalence of peel-outs, 2) determine what respiratory pathogens are more likely to be associated with peel-outs, and 3) determine if peel-outs are associated with an increase in *Salmonella*.

Materials and Methods

Six different slaughtering facilities were selected. Lung samples and pleural swabs were collected. Cases and controls were identified in random order. The total number of carcasses was counted to determine peel-out frequency.

A piece of lung was taken right after evisceration. After the final trimming and before the final carcass wash, both sides of the carcass were swabbed using Whirl-Pak bags with Speci-Sponges hydrated with buffered peptone water.

Lung and pleural swabs were incubated on Tergitol 7 as well as 5% sheep blood agar and 4% bovine blood agar with Staph nurse. Typical *Haemophilus parasuis, Actinobacillus pleuropneumoniae, Pasteurella multocida*, *Streptococcus suis*, and *Actinobacillus suis* isolates were identified with biochemical testing, gram stain, and matrix-assisted laser desorption time of flight mass spectrometry.

Buffered peptone water was transferred to Rappaport-Vassiliadis broth, incubated, and then streaked onto XLT4 and Brilliant Green with Novobiocin agars. Suspect colonies were confirmed as *Salmonella* with biochemical analysis (lysine-iron agar, motility-indole-lysine agar) and slide agglutination with polyvalent anti-O sera.

Results

Data was analyzed using logistic regression. The prevalence of peel-outs varied significantly between plants, with a range of 2.64%-28.39%, with an average of 8.11%. *Actinobacillus pleuropneumoniae, Haemophilus parasuis,* and *Actinobacillus suis* were not found in a high enough frequency to run a statistical analysis. Lesioned carcasses were 1.44 times more likely to be contaminated with *Salmonella*, 1.164 likely to be contaminated with *Streptococcus suis*, and 0.768 more likely to be contaminated with *Pasteurella multocida*. However, these data did not have significant p-values.

Discussion and Conclusions

Based on previous research, it was hypothesized that carcasses exhibiting peel-outs would be more likely to be contaminated with *Salmonella*, and that common respiratory pathogens would cause peel-outs. However, the data gathered does not support these hypotheses. While this study did have a significantly larger sample size than the previous study, we were only able to spend one day at each plant for each sampling period. Because bacteria counts and peel-out prevalence could vary from day to day, it would be beneficial if sampling was taken over multiple days. Also, it is possible that there are other respiratory pathogens that are more associated with peel-outs. Additional microbial testing could help answer this research question.

References

[1]Hurd, H.S., et al: 2012 *Am J Vet Res.* 73:91-7.

UNDERSTANDING GENOME-ENABLED SELECTION

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Introduction

The common method to estimate breeding values and rank animals based on genetic merit is known as traditional BLUP (best linear unbiased prediction) selection. This method relies on phenotypic information on animals and their relatives to determine the expected genetic potential for all animals. Researchers have established ways to incorporate information of the genetic make-up of individuals into breeding programs. The process of integrating this genomic information into a selection strategy is known as genome-enabled selection.

For genome-enabled selection, a tissue or blood sample is collected and used to obtain genetic information; the genetic information from an individual animal's sample is decoded or sequenced to determine the specific genetic code at certain points along the entire DNA sequence. This information, specifically the areas where the sequence is different at a single location (called a single nucleotide polymorphism, or SNP, which simply means at a single point there are differences in the DNA code) can be used to enhance traditional breeding value estimation. There are two approaches to genome-enabled selection: genome-enhanced BLUP and SNP-effect models.

Genome-enhanced BLUP utilizes genomic information by modifying the relationship among animals involved in the analyses (relationship matrix) that is used in traditional BLUP selection. Typically, the relationship between animals used in genetic evaluations is based on pedigree relationships. Genome-enhanced BLUP utilizes the actual genomic relationship among relatives rather than just using the average relationship based on pedigree. This approach can be extended to incorporate phenotypes from individuals that are not genotyped in what is called single-step genome-enhanced BLUP.

In SNP effect models, the effect of each position (or SNP) on phenotype is simultaneously estimated for all genotyped SNPs in what is called a training population (Erbe et al., 2010). The resulting SNP-key is then used to predict the breeding value of animals that are genotyped but have no phenotype by summing the estimated effects at each genotyped SNP. To incorporate information from individuals that are not genotyped, the resulting genomic prediction is 'blended' with an estimate of the breeding value derived using traditional BLUP. This blended estimate is used as the final genetic index value for each animal, combining both traditional BLUP and genomic information.

The expected benefit from genome-enabled selection is improved accuracy of EBV and obtaining more accurate EBV at a younger age, since there is no need to wait for phenotypes (Meuwissen et al., 2001). Increasing EBV accuracy will proportionally increase the rate of genetic gain expected. Having more accurate EBV at a younger age allows selection decisions to made earlier (if possible given reproductive age), which reduces the generation interval and increases genetic gain per unit of time.

Impact

Genome-enabled selection has been implemented in some pig breeding programs (e.g. PIC). Because generation intervals are already low in pigs, the greatest impact is on increasing accuracy of selection. The greatest impact of genome-enabled selection is expected for lowly heritable traits that are hard to measure or measured late in life such as disease resistance, feed efficiency, and longevity. Disease resistance is not easily defined and systematically measured. Feed efficiency is expensive to measure directly and sow longevity is a sex limited trait that is not recorded until the sow is culled from the herd.

References

1. Erbe M et al.: 2010, J Anim Breed Genet 128:3.
2. Meuwissen, THE et al.: 2001, Genet 157:1819.

EFFECTS OF FEEDING DRIED DISTILLERS GRAINS WITH SOLUBLES (DDGS) TO IMMUNOLOGICALLY CASTRASTED PIGS

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Introduction

Current swine production practices in the US have included physically castrating (PC) male pigs within days following birth. This prevents unpalatable off-odors in pork that occur with the onset of puberty in intact males. Reducing growth promoting androgens by PC early in life results in pigs with slower lean gain, increased fat deposition, and reduced gain efficiency compared with intact males. Temporary immunological castration (IC) with Improvest® (*gonadotropin releasing factor analog - diphtheria toxoid conjugate*; Zoetis, Inc., Florham Park, NJ) during the finishing period allows male pigs to remain intact for a longer period thus capturing the advantages in lean growth efficiency and carcass lean while eliminating off-odors in pork prior to harvest. Information is limited regarding growth and carcass characteristics of IC pigs fed high dietary levels (40%) DDGS and marketed at different harvest time points.

Materials and Methods

Crossbred boar pigs (n=863) were assigned randomly to one of 12 treatments in a completely randomized design using a 4 x 3 factorial arrangement (BW = 21.5 ± 0.2 kg). Dietary treatments were fed in four phases (Table 1).

Table 1. Dietary treatments of DDGS inclusion fed in 4 phases for 3, 4, 4, and 5 weeks, respectively

	Dietary Treatment (DDGS, %)[a]			
Phase	PCon	SD	WD	NCon
1	0%	40%	40%	40%
2	0%	30%	40%	40%
3	0%	20%	40%	40%
4	0%	10%	0%	40%

[a]PCon=Positive Control; SD=DDGS Stepdown; WD=DDGS Withdrawal; NCon=Negative Control

All pigs received the first dose of Improvest at 11 weeks of age and the second dose at either 15, 17, or 19 weeks of age to correspond with 9 (IC-9), 7 (IC-7), or 5 (IC-5) weeks prior to harvest, respectively. Pigs were weighed individually and pen feed disappearance was determined. Pen ADG, ADFI, and G:F were calculated. Prior to harvest, 10th rib backfat (BF) and longissimus muscle area (LMA) were measured using real-time ultrasound. Pigs were harvested at a commercial abattoir, where hot carcass weight (HCW) was collected and used to calculate fat-free lean (FFL). Data were analyzed using PROC MIXED in SAS.

Results

Overall ADFI was greater ($P \leq 0.05$) in pigs fed WD-IC9 compared with NC-IC5 and NC-IC9 pigs, and tended ($P = 0.09$) to be greater in WD-IC9 compared with SD-IC5 pig.Pigs fed PCon had greater ($P \leq 0.05$) ADG compared with pigs fed WD, or NCon. Gain efficiency was poorer ($P \leq 0.05$) in pigs fed WD and NCon compared with pigs fed PCon or SD. Gain efficiency was improved ($P \leq 0.05$) in IC-5 pigs compared with IC-7 and IC-9 pigs. Pigs fed NCon had lighter ($P \leq 0.05$) ending live weight compared to PCon fed pigs, while ending live weight of pigs fed SD and WD was not different compared with NCon and PCon pigs. Hot carcass weight of SD, WD, and NCon fed pigs was lighter ($P \leq 0.05$) compared with pigs fed PCon. Carcass yield was greater ($P \leq 0.05$) in pigs fed PCon, compared to pigs fed NCon. Percentage FFL was not affected ($P \geq 0.35$) by diet or IC treatment.

Conclusion

Use of a DDGS SD feeding strategy mitigated the negative effects on overall ADG, G:F, HCW, and carcass yield that occurred when feeding 40% DDGS to IC pigs. Feeding a WD diet mitigated the negative effects of feeding 40% DDGS on carcass traits but not the negative effect on growth response. Time of marketing after the second Improvest dose did not influence carcass yield or FFL percentage, but marketing IC pigs 5 weeks post-second dose of Improvest resulted in the highest overall gain efficiency.

INTESTINAL BARRIER DYSFUNCTION DURING CONSTANT AND CYCLICAL HEAT STRESS IN PIGS

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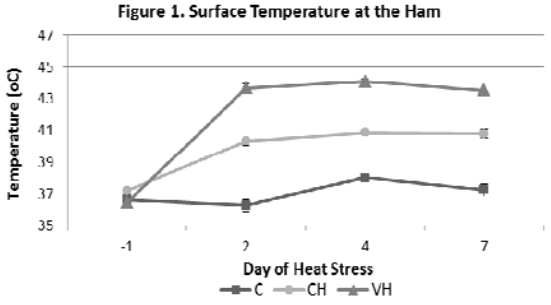
Introduction: Economic losses to the livestock industry due to heat stress (HS) are estimated to be greater than $2.0 billion annually.[1] HS is a chronic burden in tropical/arid environments and, along with global climate change, HS represents an evolving challenge to livestock producers worldwide. HS morbidity and mortality in pigs is linked to the disruption of normal intestinal tract (IT) absorptive and barrier functions and is often manifested as decreased performance. Extreme HS may result in severe IT dysfunction, reproductive failure, multi-organ failure, and death. HS models often consist of constant exposure to high heat, unlike the cyclical heat pattern pigs are exposed to during production. The goal of this study was to compare two HS models, constant heat (CH) versus variable heat (VH), in terms of health statistics, performance, and immune endpoints.

Materials and Methods: Pigs were divided into three treatment groups (n=12/group) at weaning (21 d old): CH, VH, and control (C). All pigs were allowed free access to water and a standard nursery diet. The HS period started 4 weeks later and lasted for 7 d. CH pigs were subjected to 33°C during the entire HS period. VH pigs were subjected to a repeated cycle of 40°C for 5 hrs then 25°C for 19 hrs[2]. C pigs were maintained at 25°C. Endpoints included performance, health, and immune measurements. Samples/data were collected prior to and during the HS period.

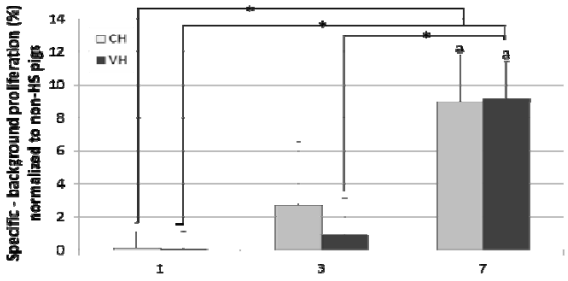
Results: Both CH and VH animals responded to HS as evidenced by lower ADG and ADFI and higher respiratory rates and skin and rectal temperatures (during HS) compared to controls (Fig. 1).

Figure 1. Surface Temperature at the Ham



Immune function was altered during HS. Non-specific immune function was lower in both CH and VH animals compared to control. Only heat-stressed pigs had lymphoproliferative responses (indicator of barrier dysfunction) to a sonicate of 12 bacteria isolated by our lab from the IT mucosa of healthy pigs (Fig. 2).

Lymph node lymphocytes proliferate to IT mucosal bacteria



a: p<0.01 compared to non-HS pigs; *: p<0.05 within treatment over time

Conclusions and Discussion: Pigs in both HS groups were stressed by our criteria. In general, pigs were more affected by variable HS than by constant HS. A healthy IT permits absorption of nutrients and protects from penetration of toxic compounds and pathogenic microbes; however, heat-stressed pigs experienced IT dysfunction as evidenced by lymphoproliferative responses to IT mucosal bacteria.

The results indicate that both the constant and variable temperature stresses are acceptable models of HS in this age pig. The VH design is more representative of what the commercial pig experiences. Future studies will apply the VH design to optimize HS mitigation at the level of the IT in order to strategically reduce the burden of HS and to quicken recovery following a HS event.

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References

1. St-Pierre et al: 2003, J Dairy Sci, 86: E52
2. Yu et al: 2010, Comp Biochem Phys, 156: 119

EFFECT OF SKYCIS, DIET NUTRIENT DENSITY AND FEEDER TYPE ON GROW-FINISH PIG PERFORMANCE

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Introduction

Skycis is approved for addition to grow-finish diets for improvement of weight gain and feed conversion (18.1-27.2 g/t) and weight gain (13.6-27.2 g/t) when fed for at least 4 weeks. There is no published information regarding possible interactions between Skycis addition, diet ingredients or feeder type on the response to this ionophore.

Materials and Methods

At weaning, 1728 pigs (Danbred 241 x PIC 359, 13.3 lb [SE = 0.01 lb]) were assigned to experimental treatments within sex to double stocked (54 pigs/pen) fully slatted pens in a tunnel ventilated facility in a 2x2x2 factorial arrangement of treatments in a randomized complete block design with 32 pens. Treatments were wean-finish feeder type (shelf wet/dry vs dry feeder – 4-15" holes per pen), grow-finish diet (25% DDGS reduced to 20% prior to slaughter with no added fat vs 10% DDGS with 3% added fat reduced to 5% DDGS prior to slaughter) and Skycis in grow-finish diets (13.6 vs 0 g/t). Pigs were individually weighed at 57d post-wean and split into 2 pens of 25 pigs/pen within sex (7.2 ft^2/pig) with pigs randomly identified prior to weighing for removal to attain this reduction. Sales of pigs on the dry feeder were delayed one week to attain sale weights similar to pigs on the wet/dry feeder. Pigs were individually weighed prior to the sale of the first pig to slaughter within sex by feeder type combinations. The randomized complete block ANOVA included treatment block, Skycis level, diet, feeder type and all 2 and 3-way interactions for pig performance. The pen of pigs was the experimental unit. Removals and death loss was examined using the ChiSquared statistic.

Results

There were no 2 or 3-way interactions between experimental treatments. Pigs fed diets containing 13.6 g/t Skycis from 65 lb to slaughter had a 4.3 lb heavier sale weight to the primary slaughter plant (286.3 vs 282.0 lb, P=0.002)and 4.6 lb heavier overall sale weight including cull pigs (285.8 vs 281.0 lb, P=0.001) compared to pigs fed diets with no added Skycis. Grow-finish feed conversion was improved by 0.04 units (2.53 vs 2.57, P<0.0001) with the addition of Skycis to the meal diets. The difference in live weight at slaughter resulted in a heavier carcass for pigs fed Skycis (212.7 lb vs 209.0 lb, P=0.022). There was no effect of Skycis addition on carcass yield (P=0.16) or lean % (P=0.912). Pigs fed grow-finish diets with lower levels of DDGS plus added fat and amino acids had a heavier sale weight in the winter trial (284.2 vs 282.1 lb, P=0.049), an improvement in feed conversion (2.46 vs 2.64, P<0.0001) and no difference in carcass lean percentage or yield (P>0.50). The number of pigs removed during the grow-finish phase (deads plus pulled pigs that lived) was reduced for pigs fed the high DDGS diets (1.76% vs 3.75%, P=0.016) with the reduction in deaths identified as fewer intestinal torsions or ulcers. Pigs on the wet/dry feeder were 1.4 lb heavier on D52 post-wean (P=0.010) with no difference in feed conversion during the double stock period (P=0.621). While sale weights were heavier for pigs on the dry feeder due to the differences in sale dates (284.6 vs 282.2 lb, P=0.077), daily gain during the grow-finish period was faster for pigs fed on the wet/dry feeder (2.15 vs 2.05 lb/d, P<0.0001) with a reduction in within pen variation for daily gain (8.9 vs 9.9% CV, P=0.042) and no difference in grow-finish feed conversion (2.55 vs 2.54, P=0.288).

Conclusion

The 2.4% and 1.6% improvements in ADG and F/G with 13.6 g/t Skycis additions to grow-finish diets were independent of diet and feeder type.

EFFECTS OF FEEDING RACTOPAMINE TO PHYSICALLY AND IMMUNOLOGICALLY CASTRATED PIGS ON GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS

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Introduction

Immunological castration (IC) with Improvest® (*gonadotropin releasing factor analog - diphtheria toxoid conjugate*; Zoetis, Inc., Florham Park, NJ) allows male pigs to capture the advantages in lean growth efficiency and carcass lean while eliminating off-odors in pork. Information is limited regarding growth and carcass characteristics of IC pigs fed ractopamine (Paylean®, Elanco, Greenwood, IN).

Materials and Methods

Sixty-four pens with 22 pigs per pen were used to evaluate effects of feeding ractopamine (RAC; 5 mg/kg) to physically castrated (PC) and immunologically castrated (IC) pigs. Male pigs were randomly assigned to sex treatments at birth and fed the same nursery diets prior to allotment in a grow-finish barn. Pigs in the PC treatment were castrated at 5d of age and pigs in the IC treatment were immunized at 11& 18 wk of age with Improvest. Diet treatments (control or RAC) were initiated on d87. Final treatment was a 2x2 factorial of sex and diet. Body weights were collected at d0 (allotment), d21, d42, d65 (2nd Improvest injection), d87 (start of diet treatment), d99 (1st marketing), d106 (2nd marketing), and d120 (final marketing). Pens were standardized to 17 pigs per pen for marketing group 1, 9 pigs per pen for marketing group 2, and all remaining pigs were marketed in group 3. Growth performance data were analyzed using PROC MIXED in SAS with fixed effects of sex, diet, and their interaction. Carcass characteristics were analyzed as a split-plot with treatment combination serving as the whole plot and market group serving as the split-plot. Pen was the experimental unit.

Results and Discussion

From d 21-65, IC pigs had 12% greater ($P<0.01$) G:F and 11% less ($P<0.01$) ADFI than PC pigs while having similar ($P=0.38$) ADG. From d 65-87, IC pigs had 7% greater ($P<0.01$) ADG and 12% greater ($P<0.01$) G:F than PC pigs while having similar ($P=0.16$) ADFI. Neither sex ($P=0.32$) nor diet ($P=0.65$) had an effect on diet treatment starting (d 87) BW. From d 87-120, IC pigs had 10% greater ($P<0.01$) ADG and 10% greater ($P<0.01$) ADFI than PC pigs while having similar ($P=0.64$) G:F. Additionally, RAC-fed pigs had 17% greater ($P<0.01$) ADG and 18% greater ($P<0.01$) G:F than control-fed pigs while having similar ($P=0.42$) ADFI from d 87-120. There were no significant interactions between sex and diet on growth performance from d 87-120. For the entire study (d 0-120), IC pigs had 2% greater ($P<0.01$) ADG, 4% decreased ($P<0.01$) ADFI, and 7% greater ($P<0.01$) G:F than PC pigs. For carcass characteristics, IC pigs were 2.5 kg heavier ($P<0.01$) at slaughter, had similar ($P=0.10$) carcass weights, and a 1.8 percentage unit less ($P<0.01$) dressing yield than PC pigs. Additionally, PC pigs had 1.3 mm more ($P<0.01$) carcass fat and 1.7 mm deeper ($P<0.01$) loins than IC pigs. Also, RAC-fed pigs were 2.9 kg heavier ($P<0.01$) at slaughter and had 2.3 kg heavier ($P<0.01$) carcasses than control-fed pigs while having similar ($P=0.21$) dressing yields. Carcasses from RAC-fed pigs had 2.2 mm deeper ($P<0.01$) loins and tended ($P<0.10$) to have 0.4 mm less fat than control-fed pigs. Market group 3 pigs were the heaviest ($P<0.01$) at slaughter, had the heaviest ($P<0.01$) carcasses, greatest ($P<0.01$) dressing yields, and the most ($P<0.01$) carcass fat of the market groups. For leanness, RAC-fed IC pigs were estimated to be the leanest ($P<0.05$) of pigs in the first market group; control-fed PC pigs were estimated to have the least amount of lean ($P<0.05$) of pigs in the second market group; and RAC-fed pigs were estimated to be the leanest ($P<0.05$) of pigs in the third.

These results show that the effects of immunological castration and feeding RAC are additive in terms of improving growth performance and carcass characteristics.

AGGREGATE ECONOMIC IMPACTS OF IMMUNOLOGICAL CASTRATION ADOPTION IN THE U.S.

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Introduction

Improvest® (*gonadotropin releasing factor analog-diphtheria toxoid conjugate*) is an FDA-approved veterinary prescription product to manage boar taint and allows producers to replace physical castration with immunological castration. Direct economic impacts identify the benefit of Improvest® adoption to a single producer and associated entities that slaughter, process, and sell the meat. However, they do not measure the overall market impact of adopting Improvest industry wide. When multiple adjustments are introduced into the vertically-related pork chain, the net impacts on quantities and prices are important to estimate.

Materials and Methods

An equilibrium displacement model (EDM) was used to estimate changes in quantities and prices that drive the overall industry-wide market impacts on segments of the supply chain. The EDM is a structural supply and demand model of the vertically linked livestock and meat marketing chain composed of three sectors in the hog and pork chain: 1) retail pork (consumer), 2) wholesale pork (processor/packer), and 3) slaughter hogs (producer) as well as four beef and two poultry chain sectors. Considering Improvest adoption over 10 years, changes in market prices and quantities were estimated.

Results and Discussion

Quantities at all three market levels in the hog and pork marketing chain increase and prices decline at the retail and wholesale pork levels for all 10 years. Hog prices initially increase but subsequently decline beginning in year 6 as the marketing chain adjusts to the production expansion triggered by Improvest adoption. Consumer and producer surplus are used to quantify the net economic impact of Improvest adoption following changes in market prices and quantities. Consumer surplus is the difference between what consumers are willing to pay and the price they actually pay. Producer surplus quantifies the difference between what producers are willing to sell product for and what they actually receive from the market. In aggregate, pork industry producers and consumers benefit from the introduction of Improvest. Combining all surplus measures of producers and consumers across beef, pork, and poultry the cumulative ten-year present value is a societal surplus gain of $3.66 billion in the base model considered. The cumulative ten-year present value of Improvest introduction is $1.48 billion for hog producers, $104.39 million for packers at the wholesale pork level, and $362.63 million for pork retailers. Total meat consumer surplus also increases by $1.81 billion.

Across several scenarios, numerous key findings emerge including hog producers, packers, and retailers each stand to benefit from Improvest adoption and all segments are significantly better off when higher and faster adoption occurs. Since the U.S. is not the first country to have Improvest available for adoption, "no adoption" or "slow adoption" scenarios place the US in a declining comparative advantage to other pork exporting countries which have adopted Improvest and this result would be detrimental to all protein sources in the U.S. This underscores the economic importance of the various levels of the industry working together for successful product introduction. Finally, several aspects of this EDM based analysis were conservatively conducted indicating actual benefits to adopters likely exceed those inferred from estimated market-level impacts.

BEHAVIOR AND WELLBEING OF IMMUNOLOGICALLY CASTRATED PIGS

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Introduction
Physical castration (PC) is a common management practice on commercial pig farms that reduces boar taint in pork, aggressive behavior in post-pubertal male pigs and undesirable pregnancy at slaughter. However, production losses associated with PC are substantial and include increased preweaning mortality (1.6%), reduced feed efficiency (6-10%), and loss of carcass lean (4-8%). Attempts to reduce the pain and distress of PC with local or general anesthetics have been unsuccessful. Immunological castration (IC) using Improvest® (*gonadotrophin releasing factor analog – diphtheria toxoid conjugate,* Zoetis, Inc., Florham Park, NJ) is an alternative to PC that yields a carcass without boar taint and may improve pig welfare by reducing the stress of physical castration. We present two studies investigating behavior associated with IC. Study 1 investigated the behavior of Improvest managed pigs on a commercial farm. Study 2 compared the stress of handling and injections to the pain and stress of PC.

Study 1: Commercial farm behavior
The objectives of this study were to evaluate the effects of IC on human-pig interactions, aggressiveness, feeding, social, mounting, and lying behaviors on the home farm, as well as behavior during loading and unloading. Pigs were in two adjacent barns containing 48 pens per barn initially stocked with 2,304 pigs. Pigs were a cross of widely used commercial lines. Observers used a scan sampling method to record behavior in each pen every 12 min for 24 h. A modified fear test[4] was used to assess pig fear. Handling during loading and unloading of trailers going to market were also quantified. Data were converted to a percentage of pigs expressing each behavior over time. Data were summarized by hour over the 24 h observation period. Data were then converted to percentages of time each hour that pigs expressed each behavior. Prior to analysis, percentage data were subjected to square root-arcsine transformation. Statistical Analysis Systems (SAS) General Linear Models (GLM) was used for all analyses.

The percentage of time each pig showed a specific behavior throughout the study was recorded (Phases 1-3). Prior to the first injection, intact males showed increased aggression ($P = 0.014$) and mounting ($P = 0.048$) while the PC barrows spent more time feeding ($P = 0.003$) than boars. The treatment by time interaction were significant for lying ($P = 0.018$), aggression ($P = 0.0001$) and standing ($P = 0.009$) behaviors. Few differences were observed in pig-human interactions between PC barrows and IC barrows. IC approached people in the same amount of time as PC barrows but were more aggressive in their chewing and rubbing on the test person's pant leg and boots. When handling and loading for processing in the home barn, PC barrows were more vocal than IC barrows ($P < 0.05$). Fewer dead and down pigs were observed among IC (zero) compared with PC barrows (1.17%). IC may result in similar or improved animal welfare compared to physical castration without pain relief.

Study 2: Stress associated with injections
The stress of the handling associated with IC (or other immunizations) has not been assessed. Our objectives were to determine if subcutaneous (SC) or intramuscular (IM) injections were more painful or stressful than PC for piglets as well as measure the pain and stress associated with receiving a SC or IM injection in finishing pigs.

We conducted two experiments in which litter served as blocks in this randomized complete block design. After farrowing, 3-5d old male piglets were randomly assigned to nothing (NO), SHAM handling (SHAM), IM, SC or PC with no pain relief. Piglets were videotaped and behavior was sampled for 1h prior and 1h post treatment. Behavior monitored included standing, walking, lying, nursing, lying with sow contact and signs of pain (standing hunched over, shaking). Finishing pigs received SC, IM, SHAM or NO injection. Finishing pigs were also monitored for 1 h pre and post treatment, and monitored for eating, drinking, lying, standing, walking, vocalization, open mouth breathing, blotchy skin and signs of irritation (rubbing the injection site). Blood was collected from all pigs for cortisol analysis 60 min post treatments so that the handling of blood collection did not impact the pig behavior. Analysis was performed in SAS using GLM procedure.

PC piglets showed less ($P < 0.05$) lying in contact with sows and more ($P < 0.05$) pain-like behaviors than IM, NO, or SHAM treatments, but SC did not differ from PC. SHAM finishing pigs spent more time lying than the pigs in other treatment groups ($P < 0.05$). Cortisol did not differ among treatments for neither piglets nor finishing pigs. IM injections do not change piglet behaviors relative to no handling or sham handling. SC injections caused a small change in piglet and finishing pig behaviors. PC caused measureable pain-like behaviors and general behavioral dysregulation whereas SC or IM caused barely measurable behavioral effects.

IMMUNOLOGICAL CASTRATION SIGNIFICANTLY IMPROVES FEED EFFICIENCY BEFORE AND AFTER IMMUNIZATION

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Introduction

Physical castration (PC) early in life is effective in controlling boar taint. However, production losses associated with its use are substantial and include increased preweaning mortality (1.6%), reduced feed efficiency (6-10%), and loss of carcass lean (4-8%). Immunological castration (IC) offers producers an alternative to PC that uses the animal's own immune system to suppress testicular function close to the time of slaughter. Immunized animals temporarily become like PC barrows with similar control of boar taint and aggressive behavior. The difference in timing allows pigs that are IC to grow as intact males for most of their life, benefitting from the natural improvements in feed conversion and carcass composition.

Improvest® (*gonadotropin releasing factor analog-diphtheria toxoid conjugate*) is an FDA-approved veterinary prescription product that eliminates the need for PC. The 1st dose is given after 9 wk of age to prime the immune system. The 2nd dose is given at least 4 wk after the first and 3-10 wk before harvest. This approach allows the IC barrow to express its naturally efficient growth potential resulting in substantial feed savings per pound of gain compared to PC barrows. After the 2nd dose, the pig transitions to castrate-like metabolism. Feed intake increases and fat in the carcass increases to a desirable level for primal cut characteristics. The objective of this trial was to compare the performance of IC harvested at the early (3 wk) and later (9&10 wk) limits of the Improvest marketing window with contemporary PC and intact males (IM).

Materials and Methods

Male pigs (n=978) were blocked by body weight at 7±3 d of age, allotted to pen and allotted to 4 treatment groups: IC1, IC2, PC or IM. Pigs were weaned at 20±3 d and moved to a wean-to-finish facility with 19 pigs/pen and 12 pens per treatment. IC1 received dose 1 at 9 wk of age. IC2 received dose 1 at 16 wk of age. Both groups received dose 2 at 20 wk of age. IC were harvested at 3, 9 or 10 wk after dose 2, along with a similar number of PC and IM.

Results

There were no statistical differences between IC1 and IC2 at each processing date so data were pooled. Wean-to-finish results are presented. IC had significantly ($P<0.05$) higher ADG and significantly ($P<0.05$) lower F:G than PC for all marketing periods (Table 1). No significant ($P>0.05$) differences in ADG or F:G were observed between IC and IM. IC feed intake was significantly ($P<0.05$) less than PC before the 2nd immunization and no different from ($P>0.05$) PC after the 2nd immunization.

Table 1. Wean-to-finish performance of IC and PC barrows.

Age (wk)	Marketing Period IC (wk)[a]	ADG % Change[b]	F:G % Change[b]
24	3	6.76	-13.69
29	9	9.74	-8.08
30	10	10.14	-7.95

[a]IC barrows were harvested at 3 time periods after the 2nd Improvest immunization. A similar number of age-matched PC barrows and intact males were harvested at the same time.
[b]% Change = (IC-PC)-1

Discussion

These results demonstrate that the improved feed efficiency advantage associated with Improvest is retained when harvest occurs later in the Improvest marketing window. Later harvest gives the producer flexibility in management, allows for higher market weights and increased revenue, and achieves desired packer primal and belly characteristics.

LIFE-CYCLE ENVIRONMENTAL BENEFITS DERIVED FROM IMMUNOLOGICAL CASTRATION OF PIGS AS COMPARED TO PHYSICAL CASTRATION: FROM A GLOBAL PERSPECTIVE TO A UNITED STATES SPECIFIC MODEL

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Introduction

As the world's population grows, global meat consumption will also increase. There is pressure from all sectors of society to produce food more sustainably. This will mean further intensification and industrialization of livestock production and adoption of technology that improves production efficiencies while also accounting for animal welfare issues. Improvest® (*gonadotropin releasing factor analog-diphtheria toxoid conjugate, Z*oetis, Florham Park, NJ) reduces boar taint and eliminates the need for physical castration and is approved for use in 63 countries. This product works with the pig's immune system. Boars grow to their full potential with all the inherent advantages of intact males; improved feed conversion, less manure, and carcasses with a greater percentage of lean meat than barrows. These efficiencies and resource savings provide significant life cycle environmental benefits (1).

Objective

This life cycle assessment (LCA) quantified the potential environmental benefits of using Improvest in US pork production.

Materials and Methods

During 2009-2011, a global study was conducted using life cycle burden data collected from modern farms with intensive pig production where pigs were physically castrated (PC) and compared to data collected from the same/similar farms in the same countries where pigs were immunologically castrated (IC). The study was conducted using LCA ISO compliant guidelines. Data were collected by direct interviews in modern farms and abattoirs in many countries and an Environmental Product Declaration was published in early 2012 (2). When Improvest was introduced in the US in 2011, the global LCA model was adapted to the US specific inputs according to the University of Arkansas LCA model (3).

Results and Discussion

The main contributions to the global warming potential (GWP) are related to the production of feed given to pigs and pig manure management. The US LCA key input was feed conversion from 8 US product approval trials. Feed conversion for IC barrows was improved by 8.4%, which resulted in feed savings of 26 kg/pig. Based on USDA crop yields during 2009 –2011 (2012 yield data was excluded due to drought conditions), a land savings (devoted to crop production) of 31 m^2/pig was realized. Reduction in pig manure was assumed to be directly proportional to the reduction in feed intake. IC pigs had a 6.1% lower GWP than PC pigs when comparing live weight results and 3.8% for carcass weight results. With an average of 124 kg of pig live weight at harvest, the use of Improvest reduces GWP by ~28.6 kg CO_2 equivalents per pig. If only 33% of the 53.3M male pigs (2011 data) raised annually in the US were IC, that is equivalent to approximately 508,000 mt of avoided GHG emissions per year, equivalent to removing emissions of 99,579 passenger vehicles/year or the carbon sequestered by 164,850 hectares of pine forests.

Table 1. Carbon footprint of immunological castration compared with physical castration.

Global warming potential	PC kg CO_2e	IC kg CO_2e
Live wt, per kg	3.71	3.48
Carcass wt, per kg	4.95	4.76

References

1. Moraes P., et al. 2013. J. Env. Assmt. Pol. Mgmt. 15:1-26.
2. www.environdec.com
3. Frank J. and East C., 2011. Univ. Arkansas.

OPTIMAL FINISH WEIGHTS AND GROSS MARGINS FOR IMMUNOLOGICALLY CASTRATED BARROWS

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Introduction

Improvest® (*gonadotropin releasing factor analog-diphtheria toxoid conjugate*) is an FDA-approved veterinary prescription product to manage unpleasant aromas that can occur when cooking pork from some male pigs. Improvest use in male pigs improves feed efficiency, changes lean composition and may reduce the variation in finished weights of pigs compared to physical castrate males. These impacts result in cost and revenue changes for Improvest managed pigs. Typical analyses of these changes simply simulate the change in cost impacts but do not consider the fact that as producers adopt Improvest they will optimize its use by altering finish weights to capture maximum benefits. Similarly, producers using physical castration will also optimize their pig weights. These optimal weights affect lean composition and cutout values which in turn affect prices paid by packers through their quality premium programs. Carcass weight, carcass cutout yields and cutout proportions in turn affect the returns packers receive for cutouts.

Materials and Methods

We use partial budgeting techniques followed by a stochastic optimization method to evaluate the net return over feed costs to producers using Improvest® compared to those using physical castration with hogs sold at optimal weights based on market base prices and packer quality grids. This analysis included consideration of the variation of growth and performance of pigs to replicate pig marketing under real world conditions. Based on results of the producer optimized weights, an additional model was developed to analyze the impact of the change in pork cutout yields and proportions to estimate returns to packers.

Results and Discussion

Partial budgeting analysis prior to optimization reveals that stopping castration is expected to reduce direct costs (labor and materials), decrease pre-weaning mortality (an average of 1.6% for male pigs) and increase total gross margin due to increased livability. The sum of these effects is expected to total $1.61/head on average (Table 1). Using prices and costs from the period 2007-2011, we estimate that the Improvest® managed animal will be marketed from 10-12 lb heavier (5-6 lb in the carcass) compared to physically castrated animals at their profit optimal weight, resulting in an added revenue on average of $6.71/head. This revenue results from added weight, improved lean and less variation in finished weights (sort loss is lessened, all things being equal). In addition, the Improvest® managed males are expected to achieve this added weight with just over $2.00 in feed savings compared to physical castrates. Altogether, the Improvest® animal is expected to produce $10.32 in added benefits ($1.61 + $6.71 + $2.00) netted against a cost of purchasing and administering the product of $5.00 per head, resulting in an expected improved net return of $5.32 per head.

Table 1. Summary of Improvest producer benefits.

Producer Impacts	IC	PC
Carcass wt, lb	205.70	201.15
Live wt, lb	284.79	272.18
Net profit, $/hd	$75.64	$65.32
Improvest benefits, $/hd	$10.32	$0
From noncastration, $/hd	$1.61	$0
From feed savings, $/hd	$2.00	$0
From revenue, $/hd	$6.71	$0
Producer returns, % change	15.80	0

OPTIMIZING LONG-TERM FEEDING AND BUILDING DECISIONS ON FARMS USING IMMUNOLOGICAL CASTRATION

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Introduction

Improvest® (*gonadotropin releasing factor analog-diphtheria toxoid conjugate*) is an FDA-approved veterinary prescription product to manage unpleasant aromas that can occur when cooking pork from some male pigs, and allows pork producers to replace physical castration by immunological castration. Male pigs receiving Improvest® grow intact at substantial feed efficiency improvements compared to physical castrated barrows while maintaining the same meat quality characteristics. We wanted to understand the longer run optimization questions which face today's Improvest adopters. We gathered information necessary to examine how producers adopting Improvest might be incented to alter current building and feeding structures as well as pig flow to more fully realize the benefits of adoption over time. Four basic facilities and management options were examined over a 20 year period for a baseline 5,000 sow farrow-to-finish operation.

Materials and Methods

The four options included first, a mixed-sex barn with animals fed a mixed-sex diet. This was the baseline model without Improvest® as its use is disqualified when males are not separated for management of Improvest® administration. The second option was a mixed-sex barn with animals separated by sex on each side of the barn so that a split-sex diet could be utilized. The third option included separate-sex barns with animals fed separate-sex diets and lastly, a double-stocked wean-to-finish building flow with females (or males) removed at feeder pig stage to separate-sex finishing. Separate-sex diets were fed after the move. Each of the options (except as noted, the baseline mixed-sex option) was run assuming no Improvest® was utilized and then with Improvest® adoption leading to a total of seven separate scenarios. We evaluated which of these several proposed long run adaptations is likely to produce the greatest net return under conditions of risky input and output prices and whether one strategy dominates others when risk is accounted. In addition, the payback period or liquidity outcome of each choice was determined. Because building costs differ widely throughout the United States, each alternative was estimated without upfront costs of facilities or facility changes specified. This generates a breakeven type analysis which reveals how much a producer *could* spend under each option to reconfigure the farm for greater returns.

Results and Discussion

When Improvest® is adopted; the greatest present value of net returns accrues to split-sex feeding in separate barns. Producers who already have this configuration would have the greatest advantage in adopting Improvest®, all other things being equal. Split-sex, mixed-barn with sexes in separate space is second with split-sex, double stocking last. However, since fewer buildings are required to execute the double stocking strategy, producers who are changing from their current configuration to any of these choices should carefully weigh the total building cost of implementing each option, as the building cost savings of double stocking may, when added to the net present value of expected returns, may make this option become superior to the others.

EFFECTS OF CASTRATION ON PREWEANING MORTALITY

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Introduction

In the US, as in most countries, male pigs are physically castrated (PC) during the first week of life, primarily to reduce boar taint. Improvest® (*gonadotrophin releasing factor analog – diphtheria toxoid conjugate*, Zoetis) is a PC alternative which allows male pigs to remain intact for most of their life. Pigs are immunologically castrated (IC) later in life by immunization against endogenous gonadotrophin releasing factor (GnRF). The 2nd immunization (given 3-10 wk before slaughter) results in a temporary IC by suppressing testicular function and, consequently, reducing boar taint. Many anecdotal reports indicate that castration causes a small but significant level of preweaning mortality (PWM). A meta-analysis of 15 Improvac trials in Europe showed that PWM was 1.6% higher in physical castrates (PC) than intact males (IM)[1]. In 24 trials in China, the PWM difference was 2.8%[2].

Objective

The objective of this study was to determine pre-weaning death loss of PC and IM on 4 US farms with different types of management.

Materials and Methods

Four different commercial sow farms in the Midwest were enrolled in the study. The study began on the day of processing (age 4-7days). Male piglets needed to weigh at least 2.2 lb and be in good health. Study pigs were randomized to treatment by selecting every other pig for castration. Castration was performed by the farm's usual method. Records were kept of all animals that died.

Results

Records were available for 4,099 PC and 4,071 IM (farm A: 963, 958; farm B: 994, 995; farm C: 1110, 1110; farm D: 1000, 1000, respectively). PWM on all farms ranged from 1.0% to 4.98% (Table 1), which is less than the US industry average (12.9%)[3]. However, this is most probably due to starting the study on the day of processing, weight requirement, and health status of the animals. Three sites showed a reduction in PWM for IM compared with PC. This reduction ranged from 1.3% to 2.69%. Farm B showed 0.61% less mortality in PC than IM. Overall, there was a 1.3% difference in PWM between IM and PC.

Table 1. Preweaning mortality from day of processing to weaning of physical castrates (PC) and intact males (IM) on 4 US farms.[5]

Farm	% PWM		
	PC	IM	Difference[a]
A	4.98	3.13	1.85
B	1.0	1.61	-0.61
C	3.69	1.0	2.69
D	4.9	3.6	1.3
Com-bined	3.64±1.86	2.34±1.2	1.31±1.40

[a]Intact male – physical castrate

Conclusions

The design of the study was to determine the effects of castration on PWM on US farms under different management systems. This study confirms that, depending on the management system, most farms will have a reduction in PWM when castration is discontinued. On average for all farms in the study, PC increased PWM. Implementation of Improvest and eliminating castration can increase the number of animals that survive to weaning, which in the long run will impact the pounds of marketable product to the producer and the profitability of that operation. Economic modeling indicates this savings will be about $1.61 per pig.[4]

References

1. Allison, JR et al. (2010). Proc. 21st IPVS.
2. Liu, Z et al. (2012). Proc. 22nd IPVS.
3. USDA NAHMS (2006).
4. DiPietre, D et al (2013). Proc. 44th AASV.
5. Study on file at Zoetis LLC 12ORBIOPORK05

THE IMPACT OF IMMUNOLOGICAL CASTRATION ON WEAN TO FINISH BODY WEIGHT VARIATION

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Introduction

Physical castration (PC) early in life is effective in controlling boar taint. However, production losses associated with its use are substantial and include increased preweaning mortality (1.6%), reduced feed efficiency (6-10%), and loss of carcass lean (4-8%). Immunological castration (IC) offers producers an alternative to PC that uses the animal's own immune system to suppress testicular function close to the time of slaughter. Immunized animals temporarily become like PC barrows with similar control of boar taint and aggressive behavior. The difference in timing allows IC pigs to grow as intact males for most of their life, benefitting from the natural improvements in feed conversion and carcass composition.

Improvest® (*gonadotropin releasing factor analog - diphtheria toxoid conjugate,* Zoetis, Florham Park, NJ) is an FDA-approved veterinary prescription product that eliminates the need for PC. The 1st dose is given after 9 wk of age to prime the immune system. The 2nd dose is given at least 4 wk after the first and 3-10 wk before harvest. This approach allows the IC barrow to express its naturally efficient growth potential resulting in substantial feed savings per pound of gain compared to PC barrows. After the 2nd dose, the pig transitions to castrate-like metabolism. Feed intake increases and fat in the carcass increases to a desirable level for primal cut characteristics.

Objective

The objective of this study was to determine the impact of Improvest on reducing wean to finish body weight variation.

Materials and Methods

Twelve hundred PIC 359 X C29 male pigs were enrolled in the study. Male pigs were randomly allocated to one of two treatments (PC or IC) at 3 days of age. Pigs allocated to the PC group were physically castrated at 3-5 days of age. Improvest was administered on days 64 and 122 days of age. All pigs were individually weighed at weaning, immediately before first market, and at the time of market.

Results

At the time of first market, 1,164 pigs were on study (583 PC barrows and 581 IC barrows). The pigs that were remaining in the facility at the time of first market had a weaning standard deviation of 2.82 vs 2.91 lbs. respectively. The standard deviation at the time of market was 24.6 lbs for the PC barrow vs 22.6 lbs for the IC barrow. In addition, the coefficient of variation of the barn was 10.5 lbs for PC barrows vs 9.4 lbs for IC barrows. One hundred sixty-five pigs were removed from the facility for the first group marketed. Of those 165 pigs, 96 pigs were PC barrows and 69 pigs were IC barrows. Upon further evaluation, the average weight of first market group was 270.7 vs 276.2 lbs. with a 10.3 vs 7.8 lbs standard deviation, respectively. Pigs were marketed over a 5 week period in which the target weight for the PC barrows was 275 lbs. and the IC barrows 285 lbs. At the time of emptying the barn, 112 PC barrows averaged 267.2 lbs with a 15.0 lbs standard deviation compared to 65 IC barrows that averaged 279.3 lbs with a 13.6 lbs standard deviation. In addition, there were a total of 3 PC barrows and 2 IC barrows as culls.

Conclusions

In conclusion, the utilization of Improvest reduced the variation at the time of market compared to physical castration.

FLOCKED SWAB LITERATURE REVIEW AND CURRENT USE IN THE SWINE INDUSTRY

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Introduction and Background

Flocked swabs have gained popularity since their introduction (2006) due to their unique perpendicular arrangement of the Nylon® fibers resulting from flocking where fibers are sprayed onto the swab tip while in an electrostatic field. Unlike traditional swabs, flocked swabs have no internal absorbent core to entrap the specimen maintaining the sample close to the surface for fast and complete elution.

Current Application

Flocked swabs are popular in human medicine and forensic sciences. In human medicine, flocked swabs are a best practice for collecting respiratory viruses due to superior pathogen yields and ease of collection[1-7]. Research showed nylon flocked swabs were not significantly different[8-11] or were significantly superior for bacteria transport[12-14], storage[12], culture[15,16], PCR detection[15], and released more bacterium[13,14,17,18] compared to cotton swabs. Flocked swabs were not significantly different than polyester swabs[19], but were superior to sponge applicator swabs[20,21]. Flocked swabs have been shown to not be significantly different in influenza detection rates[22,23], but yielded significantly more cells for Herpes simplex virus[24] compared to cotton swabs. Pre-wetting flocked swabs resulted in significantly better bacterium carriage rates[25] and a higher bacterium yield with two repeat samples with the same swab[26].

There is relatively little flocked swab research for veterinary diagnostics. Cost is not the primary factor as flocked swabs are only slightly more expensive than cotton swabs ($0.03-0.04). Studies found no statistical differences in *M. gallisepticum* and *M. synoviae* rtPCR results by flocked, cotton, or polyester swabs[27]. Flocked swabs were more sensitive at lower dilutions and equivalent to cotton swabs at higher dilutions[28] for *Steptococcus equi* PCR testing.

Discussion and Conclusions

The application of flocked swabs in veterinary diagnostics is apparent for collection, transport and analysis of field sampling. Additional research utilizing flocked swabs for swine viral and bacteria infection is underway. More education on the benefits of flocked swabs should be completed.

References

1. Ginocchio C. et al. 2011. J Clin Micro v49.
2. Walsh P et al. 2007. Amer Col of Emerg Physic.
3. Walsh P.et al. 2007. Assoc Molec Path Ann Mtg
4. Abu-Diab A et al.2007. Clin Viro Symp.
5. Ng P et al. 2008. Clin Viro Symp.
6. Daley P et al. 2006. J Clin Micro. June.
7. Condon S et al.2007. Amer Soc Micro Mtg.
8. DeFields M et al. 2007. Can J Infect Dz Med Micro.v18.
9. Silva S et al. 2010. J Clin Micro.v48.
10. Goldfarb D et al.2009, J Clin Micro.v47.
11. Allibardi S et al.2010 Euroclone & Copan Italia.
12. Coleman S et al.2007. Amer Soc Micro mtg.
13. Dalmaso G et al.,2007. EU Conf and Exhib mtg.
14. Dalmaso G et al.2008, J Pharm Sci Tech.v62.
15. Davidson J et al.2007. Amer Soc Micro mtg.
16. Snyder J et al.2010. Amer Soc Micro mtg.
17. Horn K et al.2006. Amer Soc Micro mtg.
18. Sarina M et al.2010. Amer Soc Micro mtg.
19.Gage J et al.2011. Acta Cytologica, v55.
20. Issa A et al.2011. Amer Soc Micro mtg.
21. Castriciano S et al.2009. EU Soc Clin Virol mtg.
22. Esposito S et al.2009. EU Soc Clin Virol.
23. Curran T et al.2010. 4th EU Cong of Virol.
24. Väre P et al.2008 Clinical Virology Annual mtg.
25. Jones G et al.2010. Amer Soc Micro mtg.
26. Hedin G et al.2010, J Hosp Infection, v75.
27. Ferguson N et al.2012. Amer Assoc Avian Path. v56.
28. Boylea A et al..2012, Vet Micro, v159.

INFLUENCE OF A PHYTOGENIC FEED ADDITIVE ON THE PERFORMANCE OF SOWS

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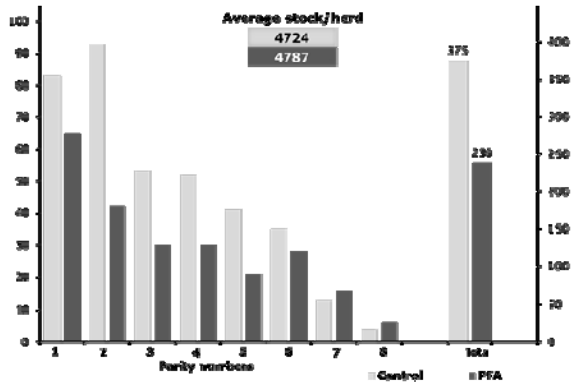
High culling rates, in early parities, reduce the average utilization time of sows, thus affecting lifetime performance.
Several studies have shown the positive influence of phytogenic feed additives on sow and litter performance when used in the lactation feed. Sows had higher feed intake during lactation, produced more milk, converted the ingested feed more efficiently, lost less body weight, and their litters grew better.
The objective of this study was to explore the influence of the continuous use of a Phytogenic Feed Additive (PFA) during lactation and gestation on production parameters. The study was carried out in a large scale sow unit maintaining two separate herds, each with an average inventory of more than 4,700 sows of Danish genetics.
Both herds were fed under the same feeding program, with the same basal diet. A four phase feeding concept was employed consisting of lactation-, insemination-, early gestation-, and gestation feeds. Early gestation feed was supplemented by stillage and gestation feed by spent grains and stillage. Digestarom® Sow, a blend of herbs, spices and essential oils, was applied at a concentration of 150 g/t complete feed (88% DM-base) in all diets of the trial herd.

Herd comparisons are based on production parameters commonly recorded at the farm (sow planner). Piglet Index (number of live born piglets/litter x farrowing rate x 100) was calculated additionally.

The study showed that continuous supplementation of the diets with the PFA Digestarom® Sow improved performance parameters in sows.
Overall sow fertility was enhanced, culling rates were reduced by more than 30% in the trial herd, especially in early parities (Figure 1).
In addition, production parameters such as farrowing rate, piglet index and number of piglets weaned at proper weight were enhanced over all parities.

Figure 1: *Sows culled for fertility issues (06.06.2011 – 16.06.2012)*



In the first production cycle, the PFA was applied, the trial herd showed higher and more stable piglet indexes. These differences were most apparent in the second production cycle when applying this Phytogenic Feed Additive (Table 1).

Table 1: *Piglet indexes of different parity numbers and their relative differences in the first and second reproduction cycle of PFA use*

Parity Number	1st Reprod. Cycle			2nd Reprod. Cycle		
	Contr.	PFA	Relat. Diff.	Contr.	PFA	Relat. Diff.
1	1005	1007	0.20	918	957	4.13
2	1212	1254	3.35	1147	1279	10.30
3	1286	1350	4.79	1212	1322	8.29
4	1271	1310	2.98	1134	1260	10.00
5	1193	1265	5.69	1159	1298	10.71
6	1091	1217	10.35	1050	1211	13.36
7	978	1138	14.06	1001	1213	17.48
≥ 8	1017	1112	8.54	950	1096	11.17
2 - 8	1150	1238	6.92	1093	1236	11.55

Finally the replacement rate was reduced in the trial herd (51.5%) compared to the control herd (54.9%), resulting in a longer utilization time and higher lifetime performance of the sows.
This field trial showed in a large scale sow unit that the continuous use of the Phytogenic Feed Additive Digestarom® Sow not only enhances the zoo-technical performance, but also contributes to a better lifetime performance of the sows.
Keywords: phytogenics, additives, sows