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EVALUATION OF THE USE OF NON-PATHOGENIC PORCINE CIRCOVIRUS TYPE 1 AS A VACCINE DELIVERY VIRUS VECTOR TO EXPRESS ANTIGENIC DETERMINANTS OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

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Introduction

Two types of porcine circovirus (PCV), PCV1 and PCV2, have been identified thus far. PCV1, first identified as a contaminant of the PK-15 cell line1, is non-pathogenic and has a low prevalence in swine herds 2. PCV2 is highly prevalent in most swine-producing countries and is associated with clinical porcine circovirus associated disease (PCVAD). The non-pathogenic PCV1 shares similar genomic organization with PCV2. Genetically modified infectious PCV2 can tolerate up to a 27 aa insertion in the C-terminus of the ORF2 capsid gene and generate a dual immune response against PCV2cap and the inserted epitope tag 3. The aim of this study was 1) to generate chimeric viruses containing neutralizing epitopes of PRRSV using the backbone of the non-pathogenic PCV1, and 2) to evaluate infectivity and immunogenicity of PCV1-PRRSVEPI chimeric viruses in vivo.

Materials and methods

Four different B-cell linear epitopes derived from PRRSV strain VR2385, including GP2 II (aa 40–51, ASPSHVGWWSFA), GP3 I (aa 61–72, QAAAEAYEPGRS), GP5 I (aa 35–46, SSSNLQILYNLT), and GP5 IV (aa 187–200, TPVTRVSAEQWGRP), were cloned individually in frame into the C-terminus of the PCV1 capsid gene. In vitro infectivity and co-expression of the PCV1-capsid protein and PRRSV epitopes were evaluated by IFA. Infectivity and immunogenicity in vivo was evaluated by inoculation of a total of 21 specific-pathogen-free (SPF) pigs, randomly assigned into seven groups of three pigs each, including two positive control groups (PCV1 and PRRSV), a negative control (MEM-treated group), and four groups, one for each of the PCV1-PRRSVEPI chimeric viruses. Serum samples were collected from each pig prior to inoculation and weekly thereafter for a period of 7 weeks. Laboratory procedures performed included Taqman® qPCR for quantification of viral DNA loads in sera and tissues, serological evaluation of IgG anti-PCV1 specific antibodies and anti-PRRSVEPI antibodies by IFA and ELISA respectively, and serum virus neutralization assay to evaluate the neutralizing activity against PRRSV-VR2385.

Results

Four PCV1-PRRSVEPI were infectious in vitro and co-expressed PCV1cap as well as the respective PRRSV epitopes. Animal studies showed three PCV1-PRRSVEPI chimeric viruses produced viremia and replicated in lung and tracheobronchial lymph nodes. IgG anti-PCV1 antibodies were first detected in serum at 14 dpi in parental PCV1-infected pigs and remained seropositive at 42 dpi. Anti-PCV1 IgG antibodies were detected in PCV1-PRRSVEPIGP3I and PCV1-PRRSVEPIGP5IV infected groups at 14, 21, and 28 dpi, followed by a titer reduction compared to the parental PCV1 at 35 and 42 dpi. Anti-PRRSV-VR2385 neutralizing antibodies were detected in the PCV1-PRRSVEPIGP3I, PCV1-PRRSVEPIGP5I, and PCV1-PRRSVEPIGP5IV chimeric viruses-infected groups at 28 dpi.

Discussion

Our results demonstrated that PCV1-PRRSVEPI chimeric viruses were infectious in vitro and in vivo. More importantly, we found that three chimeric viruses elicited neutralizing antibodies against PRRSV-VR2385. Overall, the results from the present study provided a proof of concept for further exploring the use of the non-pathogenic PCV1 as a live virus vector for vaccine delivery.

References

EFFECTS OF TWO DIFFERENT CIRCOVIRUS TYPE 2 AND MYCOPLASMA HYOPNEUMONIAE VACCINE COMBINATIONS ON ACUTE PHASE PROTEINS IN PIGLETS

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Introduction
The age at vaccination against circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae (M.hyo) coincides with weaning, which is one of the most stressful events in the pig’s life. This can cause immune system dysfunctions affecting pig health, growth and feed intake. Therefore, vaccination should not contribute to compromised well-being and hinder the adaptation to this new situation. Acute phase proteins (APPs) have been proposed as suitable biomarkers to monitor stress, for detection of inflammation and for monitoring the well-being of pigs. In addition, it has been reported that weaning stress increases serum level of APPs and that the Hp level and the average daily weight gain (ADWG) may be inversely related post-weaning. The aims of this study was to evaluate the development of haptoglobin (Hp) and C-reactive protein (CRP), rectal temperature and the ADWG obtained after application of two different vaccination protocols against PCV2 and M. hyo in piglets, during the nursery phase.

Materials and methods
The study was conducted in a 1000 sow farm located in Southeast Spain. Two groups of 20 piglets (10 males and 10 females per group) were vaccinated, 7 days after weaning, with 1 mL of CircoFLEX® and with 1 mL of MycoFLEX® in a single injection of 2 mL (A, FLEXcombo®; Boehringer Ingelheim, Spain, SA) or with a single injection (2 mL) of (B) Porcilis® PCV-M Hyo (Intervet International B.V., The Netherlands). Blood samples and weight of each animal were taken before vaccination (basal levels; Fig 1.), 24h after vaccination (24h Post-V) and 48h after vaccination (48h Post-V). Also, the weight at 39 days after vaccination was taken (39d Post-V). The rectal temperature was recorded before and 7h after immunization. The Hp and CRP concentrations in serum were determined using an automatic biochemical analyzer (Olympus 2700 automatic chemistry analyzer, Germany). The statistical analyses were performed using GraphPad Prism 6 (Graph Pad, Software, USA). A two-ways ANOVA test was performed and a value of p < 0.01 was used to indicate significance.

Results
The administration of both vaccines increased concentrations of Hp (Fig 1a) and CRP (Fig 1b) respect to basal level of each group. However, this increase is only significant in group B. In addition, 24h Post-V, Hp and CRP concentrations were significantly higher group A. 7h post immunization, the rectal temperature was significantly higher (p < 0.01) in animals vaccinated with B (40.9 °C) compared to A (39.9 °C). Moreover, in relation to baseline, the ADWG were higher in animals vaccinated with A compared to the animals vaccinated with B at 24h (173g vs -29g) and at 48h (193g vs 48g) Post-V.

Discussion and Conclusions
Based on the data obtained in this study, the production of both APPs has been significantly higher in animals vaccinated with Porcilis® PCV-M Hyo. Furthermore, after immunization with this vaccine a significant increase of rectal temperature and lower ADWG was observed. As described in this and in other studies vaccination with FLEXcombo® had minor effect on production parameters and less stress than other products. This well-being is important for adaptation and growth performance during the nursery period.

References
7. Hernández-Caravaca et al: 2015 (Nantes), European Sympos...
Introduction
Co-infection with porcine circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae (M. hyo) plays a primary role in the porcine respiratory disease complex (PRDC). PRDC is one of the major causes of economic losses in the swine industry and its control is mainly based on management strategies and vaccination, against these diseases, in post-weaning period. The post-weaning is a critical period and for this reason is very important applying vaccines that do not hinder adaptation to this new situation. The acute phase proteins (APPs) in serum have been proposed as suitable veterinary biomarkers to monitor the stress and the inflammatory response, which makes APPs notable parameters for the global assessment of pig welfare. The aim of this study was to evaluate the welfare to vaccination after the application of two different vaccination protocols, against PCV2 and M.Hyo, through of measurement of rectal temperature and two APPs; haptoglobin (Hp) and C-reactive protein (CRP).

Materials and methods
In this study forty piglets Pietrain x (Landrace x Large White) crossbred, of 3.5 weeks of age, from a farm located in South-Eastern Spain, were used. The animals were divided in two groups of 20 animals (10 females and 10 males). The group A was vaccinated with 1 mL of CircoFLEX® and with 1 mL of MycoFLEX® in a single injection of 2 mL (FLEXcombo®; Boehringer Ingelheim, Spain, SA). The group B was vaccinated with Porcilis PCV® (Intervet International, The Netherlands) and Stellamune Mycoplasma® (Elanco Animal Heath, Spain) in two injections of 2mls each. Blood samples were taken before vaccination (baseline), 24 h post-vaccination (Post-V) and 48 h Post-V. Body temperature was measured before vaccination (baseline) and 8 h Post-V. The levels of Hp and CRP were measured using an automated biochemistry analyser (Olympus 2700, Germany). The statistical analyses were performed using GraphPad Prism 6 (Graph Pad, Sowftware, USA). A two-ways ANOVA test was performed and a value of P<0.05 was used to indicate significance.

Results
After 8 h Post-V, group B showed evidence of pyrexia relative to baseline (P<0.001) and rectal temperature levels in group B (40.6 ºC) were significantly greater compared to group A (39.7 ºC). The interaction between type of vaccination and day of sampling was significant for serum Hp (Fig 1a) and CRP (Fig 1b). Group B had elevated concentration of Hp relative to baseline at 24 h Post-V and 48 h Post-V (P<0.001). Relative to baseline CRP concentration in group B were greater 24 h Post-V (Fig 1b). Group B had significantly greater serum CRP concentrations compared to group A (P<0.001).

Discussion and conclusions
According to the results obtained, the immunization with Porcilis PCV® and Stellamune Mycoplasma® produces significant increases in concentrations of both APPs compared with basal levels. Also, with this combination has been observed a higher increase of rectal temperature. Therefore, the lesser body temperature and production of APPs with FLEXcombo® contribute to welfare and may facilitate the adaptation of piglets in this critical period. This enhanced adaptability of piglets vaccinated with CircoFLEX® and MycoFLEX® also have been showed in other trials versus these competitors.

References
INTRODUCTION
The purpose of this study was to evaluate the effect of an experimental serial of a modified live PRRS virus (Formulated in accordance with the outline of production for Fostera® PRRS) on the reduction of post challenge viremia when piglets were vaccinated at 3 weeks of age and challenged at 7 weeks of age with the virulent PRRSV isolate NADC20.

MATERIALS/METHODS
Twenty (20) healthy, 3 week old piglets were vaccinated IM with an experimental serial of PRRSV-MLV (T02). A second group of 20 piglets was inoculated with 2 mL of PBS (T01). Treatment groups were housed in separate rooms during the vaccination phase. Prior to the challenge phase pigs were rehoused into one room. Pigs were challenged intranasally and intramuscularly with PRRSV NADC20 (3.0 log10 TCID50 per dose). Serum samples were collected 3 times each week post challenge to detect PRRS virus (VI on PAM cells). The study concluded when ≥80% of the controls were determined to be virus negative (Day 55 post vaccination, day 27 post challenge).

Duration of viremia post-challenge was determined for each animal as {[(last day of virus detected + ½ the number of study days to the next sampling day) – (first day of virus detected - ½ the number of study days to the previous sampling day)] + 1} if the animal had virus isolated, or zero (0) for animals that did not have virus isolated.

RESULTS
The primary variable in determining reduction of post challenge viremia was duration of viremia in T02 vaccinates versus T01 controls. Duration of viremia was significantly lower (P = 0.0327) for T02 pigs versus T01 pigs (Table 1).

Table 1. Mean Duration of PRRS Viremia (Days) Post Challenge

<table>
<thead>
<tr>
<th>TX</th>
<th>N</th>
<th>Least Squares Mean</th>
<th>Standard Error</th>
<th>95% Confidence Limits</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>T01: PBS</td>
<td>20</td>
<td>19.8</td>
<td>1.27</td>
<td>17.2 - 22.4</td>
<td>7.0 – 28.0</td>
</tr>
<tr>
<td>T02: PRRS</td>
<td>20</td>
<td>15.8</td>
<td>1.27</td>
<td>13.3 - 18.4</td>
<td>7.0 – 25.5</td>
</tr>
</tbody>
</table>

In addition, post challenge VI titers were significantly lower (P ≤ 0.0053) in T02 vaccinates versus T01 controls for Day 36 through Day 50 (Figure 1).

Figure 1: Least Squares Mean PRRS VI Titers (Log10 TCID50/mL) Post Challenge with P values

CONCLUSIONS
Use of PRRSV-MLV at 3 weeks of age significantly reduced both the level and duration of viremia in piglets challenged at 7 weeks of age with a virulent PRRSV isolate. Reducing the level and duration of viremia resulted in a reduction over time of positive pigs in the vaccinated group.

IMPLICATION
Vaccination of a herd prior to exposure to field strains can reduce numbers of positive pigs and lower levels of viremia in a herd and result in decreased spread of infectious virus.
EFFECT OF ADDITIVES ON THE SURVIVAL OF PORCINE DELTA CORONAVIRUS AND PORCINE EPIDEMIC DIARRHEA VIRUS IN SWINE FEED

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Introduction
Contaminated feed can be a source of swine enteric viruses including porcine epidemic diarrhea virus (PEDv) and porcine delta coronavirus (PDCoV). The survival of these viruses in feed may be affected by storage time and chemical composition. Organic acids (OA) and feed additives are often included in swine diets to improve nutritional value and control pathogens. However, no studies have been conducted to determine the inactivation kinetics of organic acids on PEDv or PDCoV in feed. The objective of this study was to determine if OA or additives are effective in reducing the survival of PEDv and PDCoV in feed.

Material and methods
Aliquots of complete feed (5g) were placed in plastic vials followed by addition of one of 8 additives at recommended concentrations: 150 mg Ultracid P (orthophosphoric, citric, fumaric, and malic acid; Nutriad), 20 mg Activate DA (organic acids and 2-hydroxy-4-methylthiobutanoic acid; Novus Intl.), 10 mg KEM-GEST (phosphoric, fumaric, lactic, and citric acid; Kemin Agrifoods), 10 mg Acid Booster (phosphoric, citric, and lactic acid; Agri-Nutrition), 20 mg salt, 20 mg sugar, 56 µL Luprosil (propionic acid; BASF), and 46 µL formic acid (formic acid; BASF). Double concentrations of certain additives were also tested for PDCoV survival. Luprosil, formic acid and the double concentration of acidifiers were not tested for PEDv. Next, 1 mL of the specific virus was added to vials, followed by incubation at room temperature for up to 5 weeks. Double the recommended concentration of additives was added to certain aliquots and experimental conditions were identical to those used in previous experiment except that virus survival was evaluated after 0, 1, 3, 7, and 10 d of storage at 25°C. Samples were eluted with 3% beef extract-0.05 M glycine solution and inoculated into the appropriate cell cultures to calculate TCID_{50}/mL for each virus treatment sample.

Results
Data were analyzed by GInaFiT software to determine the best fitting kinetic model. The Weibull model, and its kinetic parameter delta (time needed to reduce initial virus titer by 1 log), was used to compare the virus inactivation kinetics among treatments. PEDv delta values were the lowest in samples with Activate DA (0.4 days) and KEMGEST (1.8 days), indicating faster inactivation kinetics. None of the additives at the recommended doses increased the inactivation kinetics of PDCoV (delta value = 0.86 day). However, adding double the recommended concentration reduced the delta values for all additives (delta values ≤ 0.28 days), except for sugar (4.94 days) and formic acid (4.95 days), compared with the control (0.35 days).

Conclusion and discussion
Addition of some organic acids can reduce the survival time of PEDv in feed, and complete inactivation is achieved after 14 days. Using double the recommended dosage of some additives may increase PDCoV inactivation. The inactivation kinetics of PEDV and PDCoV differed with the use of additives. This is an important finding because there have been at least 13 states that have reported co-infections of PEDv and PDCoV in the United States.

Acknowledgements
This work was supported by a grant from the National Pork Board and CHS Fellowship.

References
MEASURING PROGRESS ON PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) CONTROL AT A REGIONAL LEVEL: THE MINNESOTA N212 REGIONAL CONTROL PROJECT (RCP) AS A WORKING EXAMPLE

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Body of Abstract
Introduction: The porcine reproductive and respiratory syndrome (PRRS) is a highly transmissible viral disease that causes substantial losses to swine producers in North America and other endemically infected regions[1-3]. Because PRRS is a non-reportable disease in the US, voluntary regional control projects (RCPs), led and funded by the swine industry, have been established in an effort to mitigate the impact of the disease [3, 4].

Materials and methods: Using information from the Minnesota Voluntary Regional PRRS Elimination Project (RCP-N212), collected from June 2012 to July 2014, this paper outlines a protocol for evaluation of RCPs progress towards the intended goal of controlling the disease at a regional level. Demographic fluctuation was assessed through the composition of site types (sow sites=SS, non-sow sites=NSS) enrolled in the RCP, and using a repeated analysis of variance measure. The degree of active participation, defined as the proportion of sites that share PRRS status, was evaluated using a general linear mixed-effects model (GLMEM). By using site attitudes toward sharing PRRS status, the short-term trend of PRRS incidence was evaluated by a GLMEM. Spatio-temporal clustering was measured through a pair correlation function.

Results: RCP-N212 has increased geographical coverage and enrolled a growing number of sites, but the proportion of site types (NSS=77% and SS=23%) did not vary significantly through time. A rising percentage of sites shared information on PRRS status, however, NSSs were less prone to report than SSs. PRRS incidence decreased over time (p<0.001), without any difference between site types. Clusters were identified at small spatial and temporal windows of <3km and <3wks respectively. Results are consistent with an improvement in the epidemiological situation of PRRS in the RCP-N212.

Conclusions and discussion: These results provide information of PRRS dynamics that may be incorporated into epidemiological and economics models, with the ultimate objective of designing strategies to its control PRRS in the US and North America. The methods developed and presented here may also be applied to other RCPs to facilitate comparisons between and among RCPs in North America.

Acknowledgements
Funding for this project has been partially provided by the University of Minnesota MnDrive program and Boehringer Ingelheim®. We thank also swine producers from the RCP-N212, who shared data, as well as the Swine Disease Eradication Center (SDEC) of University of Minnesota, that provided the server in which data was collected, stored and protected. No other conflicts of interest exist.

References
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 IMPACT OF FOSTERA® PRRS VACCINATION ON LINEAGE 1 PRRSV CHALLENGE

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¹Zoetis Inc. Florham Park NJ ²Iowa State University, Ames IA

Introduction
This study evaluated the efficacy of Fostera PRRS vaccine in pigs challenged with a 2014 heterologous lineage 1 PRRS virus.

Materials and Methods
A field virus, ISU 14-4099, was selected and found to be 84.4% identical to Fostera PRRS vaccine virus at the ORF 5 nucleotide level and to cluster with lineage 1 reference viruses. The virus had a RFLP cut pattern of 1-18-2. Forty eight three-week-old mixed breed castrated male swine were sorted by weight and randomized into two experimental groups: vaccinated and challenged (V/C) and non-vaccinated and challenged (NV/C). V/C pigs were administered one 2 mL dose of Fostera PRRS vaccine intramuscularly at 4 weeks of age (study day 0 dpv) and the NV/C pigs were administered one 2 mL dose of sterile saline intramuscularly at the same time. 10 non-vaccinated/non-challenged (NV/NC) pigs were maintained in a separate room to serve as negative controls. All groups were maintained in separate rooms for the vaccination phase. On study day 26, half of the pens of pigs from the NV/C and the V/C rooms were swapped between the two rooms. On study day 28 (0 dpi), all pigs in the NV/C and V/C groups were challenged with one 2 mL dose of isolate ISU-14-4099 at 10^5 TCID50/mL by the intranasal route and one 2 mL dose of sterile saline intramuscularly. Pigs in the NV/NC group were inoculated similarly using sterile cell culture media. Pigs were necropsied on 12 dpi and the percentage of the surface area of each lung lobe affected with pneumonia was visually estimated by a single observer blinded to treatment group. Five sections of each lung were collected, fixed and scored for severity of interstitial pneumonia by a single histopathologist blinded to treatment group. Each section was scored on a 0-6 scale and the five scores were averaged for each lung. The pigs were weighed on days -7 dpv, -2 dpi and 12 dpi. Sera were collected at 0 dpv, 14 dpv, 3 dpi, 7 dpi, and 12 dpi. The pen was the experimental unit with two pigs per pen. Percent pneumonia was analyzed using the Wilcoxon rank-sum test and weight data was analyzed using analysis of variance. A p-value ≤ 0.05 was considered significant. Variables associated with the NC/NV group were not statistically analyzed.

Results
All pigs in the V/C group were PRRSV ELISA positive by 14 dpv. All pigs in the NV/C group were ELISA and PCR negative for PRRSV in serum at 14 dpv and 28 dpv. NV/NC pigs remained ELISA and PCR negative throughout the study. On days 3, 7 and 12 post-challenge, both V/C and NV/C groups were viremic, with virus levels significantly lower in V/C vs NV/C on all days. Mean percent pneumonia was reduced by 32% in the V/C group compared to the NV/C group (mean 15.38% vs 10.47%; p=0.1487). Microscopic lung lesion scores were not significantly lower in the V/C group compared to the NV/C group. Mean average daily gain lacked a statistical difference for the time period from -7 dpv to -2 dpi. Post-challenge average daily gain was significantly higher in the V/C group compared to the NV/C group (p=0.0112).

Figure 1. Least Squares Mean Ave. Daily Gain

Discussion
This study demonstrated the ability of Fostera PRRS vaccine to provide partial protection against a 2014 PRRSV lineage 1 field strain in that ADG was higher and virus titers were lower after challenge in the V/C group versus the NV/C group.

Fostera is a registered trademark of Zoetis Services LLC
Introduction
Proper disease diagnosis is necessary to develop a comprehensive prevention protocol. Improper diagnosis could delay treatment, lead to unsuitable medications or development of inadequate vaccine protocols. Identifying porcine respiratory disease complex (PRDC) antigens or antibodies via serology or oral fluid testing is useful to identify exposure to pathogens. However, most often this does not indicate whether the interventions put in place properly address antigen exposure. Proper tissue diagnosis allows practitioners to identify the pathogen and histologic damage in the target tissue indicative of disease from the etiologic agent.

Materials and methods
Six production flows in North Carolina had finishing pigs exhibiting clinical signs of coughing±sneezing, nasal discharge and labored breathing. M. hyopneumoniae (M.hyo) was listed as a top differential. All pigs were vaccinated for PCV2 and M.hyo, but not for PRRS. Diagnostic investigations were performed at two time points in finishing (early and close to market) for each flow (n=12) due to increased finishing mortality and clinical disease presence. The study was conducted in both summer and winter to account for seasonality. A minimum of three acutely PRDC-clinical, non-treated pigs were selected from each farm and euthanized. Fresh and 10% formalin-fixed samples included four lung sections, lymph nodes and tonsil. Samples were screened on PCR, culture, histopathology, and immunohistochemistry (HMC and ISU-VDL, Ames, IA). Farms were considered diseased when one or more pigs tested positive for both pathology and antigen.

Results
All six flows were PRRS infected, five were IAV infected and five were associated with M.hyo. No bacteria were identified as primary pathogens. In both seasons, Farm C was not diagnosed with disease. In the summer, two of eight farms were diagnosed with a single disease, PRRS or Influenza Type A virus (IAV). Four farms were diagnosed with dual diseases: three with PRRS+IAV and one with IAV+M.hyo. In the winter, four of eleven farms were diagnosed with a single disease (PRRS or M. hyo). Five farms were diagnosed with dual diseases: four with PRRS+M.hyo and one with PRRS+IAV. In both seasons, one farm was diagnosed with M.hyo, PCV2, and PRRS (Table 1).

Discussion
M.hyo was the least common finding during the summer even with all flows considered M.hyo positive and presented with clinical signs suggestive of M.hyo. In the majority of M.hyo cases, there were viral co-infections. It appears that in these flows IAV was more prevalent in summer, M. hyo in winter and PRRS in both seasons. All six flows have added modified-live PRRS vaccination of wean-age pigs with three flows using a commercial trivalent PCV2, M.hyo, PRRS vaccine. It is necessary to conduct proper tissue diagnostics in order to appropriately diagnose disease in the system.

Table 1. Individual Farm Disease Diagnosis

<table>
<thead>
<tr>
<th>Farm</th>
<th>M.hyo</th>
<th>PRRS (Type II)</th>
<th>IAV Secondary (H1N1 or H1N2) Secondary Bacteria</th>
<th>PCV2 A</th>
<th>PRRS (Type II)</th>
<th>IAV</th>
<th>Secondary Bacteria</th>
<th>PCV2 A</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>B</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>C</td>
<td></td>
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<td>X</td>
<td></td>
<td>X</td>
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<td>D</td>
<td>X</td>
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<td>E</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
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<tr>
<td>F</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<td>X</td>
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<td>X</td>
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<td></td>
</tr>
</tbody>
</table>

2015 Allen D. Leman Swine Conference
FIELD OBSERVATION ON THE EFFICACY AND ECONOMIC IMPROVEMENT OF THE FLEXCOMBO IN AN INTEGRATED SWINE FARM IN CHINA

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Introduction
Porcine circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae (M. hyo) are two major contributors to the Porcine Respiratory Disease Complex (PRDC). Vaccination is an efficient tool to control both pathogens. FLEXcombo is the combination of the two commercial vaccines Ingelvac CircoFLEX® and Ingelvac MycoFLEX®. The aim of this field observation was to determine the effect and economic impact of FLEXcombo under China conditions.

Materials and methods
The study was performed in a 900-sow farrow-to-finish one-site production system in Henan province of China. Piglets had been vaccinated against M. hyo with a commercial M. hyo vaccine (Ingelvac M.hyo®) from 2009 to February 2013 at 2 weeks of age. PCV2 vaccination with Ingelvac CircoFLEX® was implemented from January of 2012. In March 2013 Ingelvac M. hyo was replaced by Ingelvac MycoFLEX® and from then on Ingelvac CircoFLEX® and Ingelvac MycoFLEX® were used freshly mixed as vaccine combination (FLEXcombo®). Also in 2012 mass vaccination twice per year with Ingelvac CircoFLEX® was implemented for the breeding. The production data and economic benefit were collected and compared from 2010 to 2014

Result
A clear improvement in marketed pigs per sow per year (M.S.Y.) was seen after implementation of PCV2 vaccination in 2012. (Figure1). Wean-to-finish FCR was improved from 2.92 to 2.52 at year 2010 and 2014. Farrow-to-finish mortality and culling rate was reduced from 26.99% in year 2010 to 8.21 % in year 2013; in 2014 an PEDV outbreak occurred in the farm and the mortality rate increase again to 13.51% (Figure 2). Furthermore, the costs of disinfectants and antibiotics were significantly reduced in the farms (Table 1).

Discussion
This survey demonstrated that FLEXcombo was efficacious as a combination shot application. It could improve growth performance of pigs especially in M.S.Y. mortality rate and FCR. FLEXcombo immunization can alleviate clinical symptoms of swine PRDC, which leads to a reduction in the amount of antibiotics used in pigs, resulting in clear economic benefits to the farmer. As PCV2 and M.hyo are widely distributed in swine farms, routine application of the FLEXcombo ensures the productivity of farms.

Table 1.Annual cost of Vaccines, disinfectants and antibiotics

<table>
<thead>
<tr>
<th>Year</th>
<th>Vaccines cost ( RMB )</th>
<th>Disinfectants cost ( RMB )</th>
<th>Antibiotics cost ( RMB )</th>
<th>cost ration of Vaccines: disinfectants: antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>381463</td>
<td>104973</td>
<td>923701</td>
<td>100 : 27 : 242</td>
</tr>
<tr>
<td>2011</td>
<td>609160</td>
<td>125334</td>
<td>789371</td>
<td>100 : 21 : 130</td>
</tr>
<tr>
<td>2012</td>
<td>1113641</td>
<td>74889</td>
<td>927158</td>
<td>100 : 7 : 83</td>
</tr>
<tr>
<td>2013</td>
<td>1205286</td>
<td>63795</td>
<td>604434</td>
<td>100 : 5 : 50</td>
</tr>
<tr>
<td>2014</td>
<td>1635116</td>
<td>69135</td>
<td>601594</td>
<td>100 : 4 : 32</td>
</tr>
</tbody>
</table>

Reference
2. Park JH (2012). IPVS. P. 107
EFFICACY OF A PRRSV MLV VACCINE AGAINST A GENETICALLY DIVERSE RANGE OF PRRSV ISOLATES

J Angulo1, JRD Allison1, JG Calvert2, B O’Brien1, RG Ankenbauer2

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Introduction

Modified live virus (MLV) vaccines are widely used in PRRSV control strategies, where they have been shown to reduce clinical signs and production losses. PRRSV is characterized by a high level of genetic diversity, and there is no simple relationship between genetic similarity and cross-protection. The performance of a vaccine against highly divergent PRRSV strains is difficult to predict. This paper summarizes the available cross-protection data for a MLV vaccine (Fostera PRRS) based on strain P129 (lineage 8).

Materials and methods

Data are summarized for 6 experimental challenge studies, each using a different challenge strain. All are further described in peer-reviewed publications or conference abstracts1-5, although some additional details are included here. Information on the challenge viruses is presented in Table 1. Isolates 1 and 2 were from the USA, 3 from Canada, 4 from Thailand and 5 and 6 from Korea. Several trials have been conducted using NADC20 and study 1 was selected based on protocol similarity to the other studies shown.

Table 1. Challenge strains and relationship to vaccine strain (ORF5 nucleotide % distance)

<table>
<thead>
<tr>
<th>Study</th>
<th>Isolate</th>
<th>Lineage</th>
<th>RFLP</th>
<th>Genetic Distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NADC 20</td>
<td>8/9</td>
<td>1-4-2</td>
<td>3.8%</td>
</tr>
<tr>
<td>2</td>
<td>ISU-12-39404</td>
<td>9</td>
<td>1-4-2</td>
<td>7.8%</td>
</tr>
<tr>
<td>3</td>
<td>FMV12-1425619</td>
<td>1</td>
<td>1-8-4</td>
<td>15.4%*</td>
</tr>
<tr>
<td>4</td>
<td>10PLI(HP-PRRS)</td>
<td>8</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>SNUVR090851</td>
<td>1</td>
<td>1-6-4</td>
<td>13.4%</td>
</tr>
<tr>
<td>6</td>
<td>SNUVR090485</td>
<td>NA†</td>
<td>NA</td>
<td>35.5%</td>
</tr>
</tbody>
</table>

* amino acid comparison. NA=Not Available.
† Genotype 1, subtype 1 (European PRRS)

All studies describe respiratory challenges in growing pigs vaccinated at 3 to 4 weeks of age. Challenge was 3 (study 3), 4 (studies 2 & 4), 5 (studies 5 & 6) or 24 (study 1) weeks later. There are between-study differences in the details of the experimental procedures, but post-challenge lung lesion scores from control and vaccinated groups are reported for all studies and viremia for four studies.

Results

Comparative lung lesion scores and viremia post challenge by study are summarized in Table 2. Because of the trial design study 2 was not subject to statistical analysis. All other differences (except study 3 lung scores where p=0.071) were significant at the 0.05 level or better. The HP-PRRS isolate in study 4 was highly pathogenic, with only 20% of control pigs surviving to the planned slaughter date, compared to 80% of vaccines.

Table 2. Lung lesion scores and viremia levels

<table>
<thead>
<tr>
<th>Challenge Studies</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung Score(%)</td>
<td>12%</td>
<td>1%*</td>
<td>16.9%</td>
</tr>
<tr>
<td>Viremia (7-13 dpc)</td>
<td>NA</td>
<td>NA</td>
<td>6.92</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Challenge Studies</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung Score(%)</td>
<td>69.9%</td>
<td>46.8%*</td>
<td>25.3%</td>
</tr>
<tr>
<td>Viremia (7-13 dpc)</td>
<td>NA</td>
<td>NA</td>
<td>8.1</td>
</tr>
</tbody>
</table>

Viremia expressed as log10 of the actual viral load  
*P<0.05  **P<0.07  † Descriptive statistics  
NA=Not Available

Conclusions

The studies differed in important aspects and comparisons between studies are not recommended. However, within-study comparisons are valid and demonstrate significant protection across a highly diverse range of PRRSV isolates, including strains currently circulating in North America as well as Highly Pathogenic PRRS from Asia and European (Genotype 1) strains.

References

3. Charoenchanikran P et al. 2015, CUV C
IMPACT ON NURSERY PERFORMANCE USING ONE OR TWO DOSE VACCINES AGAINST PCV2 AND MYCOPLASMA HYOPNEUMONIAE

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2Boehringer Ingelheim Vetmedica Inc., St. Joseph, USA

Introduction
The use of vaccines to prevent diseases is well established in pig industry worldwide in particular for PCV2 and Mycoplasma hyopneumonia. This study was performed to evaluate the effects of two vaccination protocols for PCV2 and Mycoplasma hyopneumoniae on nursery pig performance.

Materials and methods
The study was performed in a commercial sow herd with 6,000 sows, located in southern Brazil. A total of 150 barrows at 22 days of age were included into the study one day after weaning (study day 0), individually marked and randomly assigned to one of three treatment groups (50 animals per treatment group). Pigs of treatment group 1 (T1) were vaccinated with a 2-dose vaccine Circumvent PCVM (MSD Animal Health), treatment 2 (T2) consisted in 1-dose vaccines CircoFLEX and MycoFLEX (Boehringer Ingelheim, Vetmedica, Inc., St. Joseph, MO) and treatment 3 (T3) consisted in 1 dose of 2 ml (D0) of saline solution 0.9%. All vaccines were administered as separate intramuscular injections according to label directions (Circumvent PCVM: 2 ml/dose given on d 0 and D21; CircoFLEX: 1 ml/dose given on d 0; Mycoflex: 1 ml/dose given on D 0). Pig were weighted weekly from D0 to D34.

Results
The mean body weight for each treatment group and different time points is shown on table 1. On day 0 there was no significant difference in body weight between the three treatment groups. On day 7 pigs of treatment group 1 had a significant lower average body weight compared to treatment group 3. One and two weeks after the administration of the second dose in treatment group 1 pigs of this treatment group had a significant lower average body weight compared to treatment group 2 and 3 and no significant difference was seen between treatment group 2 and 3.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>22d - D0</td>
<td>6,87</td>
<td>6,9</td>
<td>6,89</td>
</tr>
<tr>
<td>29d - D7</td>
<td>6,95b</td>
<td>7,33ab</td>
<td>7,45a</td>
</tr>
<tr>
<td>36d - D14</td>
<td>9,21</td>
<td>9,44</td>
<td>9,43</td>
</tr>
<tr>
<td>43d - D21</td>
<td>12,06</td>
<td>12,54</td>
<td>12,77</td>
</tr>
<tr>
<td>50d - D28</td>
<td>14,88b</td>
<td>15,96a</td>
<td>15,86a</td>
</tr>
<tr>
<td>56d - D34</td>
<td>16,67b</td>
<td>18,72a</td>
<td>18,43a</td>
</tr>
</tbody>
</table>

T1= (Circumvent PCVM, 2-doses, D0 and D21),
T2= (CircoFLEX + MycoFLEX, 1 dose, D0)
T3= (saline solutions 0,9%, 1 dose, D0):
a,b: P < 0.05

Conclusions and discussion
The significant difference in body weight on study day 34 of treatment group 1 compared to treatment group 3 might be related to the reactivity of the vaccine and in particular to the adjuvant. The findings of this study are in line with other studies.1,2,3.

In addition to vaccine efficacy, convenience and safety, implications for animal welfare, pork quality assurance and the economic impact of side effects should be considered when selecting products for immunization of swine.

References
2. Villaca K et al. 2010. AASV. 245-246
PCV2 NEUTRALIZING ANTIBODY LEVELS IN COLOSTRUM FROM FARMS WITH HIGH OR LOW LEVELS OF VIREMIA

CMT Dvorak1, BJ Payne2, JL Seate2 and MP Murtaugh1

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2Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO, USA

Introduction
Porcine circovirus type 2 (PCV2) is the causative agent of porcine circovirus-associated disease (PCVAD). Vaccination against PCV2 at or around weaning effectively controls disease in finishers. Nearly all pigs in the US are vaccinated and develop PCV2-specific antibodies, but virus often is not eliminated, even in the presence of PCV2-specific antibodies. It is thought that antibody titers may not be indicative of a protective immune response, but that anti-PCV2 neutralizing antibodies (NA) are a more effective indicator of a controlled infection. Four sow herds were evaluated on vertical transmission of PCV2, sow PCV2 ELISA and colostrum PCV2 NA to gain information on antibody control of infection.

Methods and materials
Pre-farrow sow serum samples (30-44/farm) were tested on PCV2 ELISA (IA State VDL, Ames, IA, 0.3 S/P ratio cut-off). Placental umbilical cord serum (PUCS, 52-111/farm) and colostrum (53-91/farm) samples were collected and tested for antigen (PCV2 PCR, detection limit below 3.5 genomic equivalents/reaction, Life Technologies Corp., NY at HMC, Ames, IA). For the study, a PCV2 NA assay using VR1BL cells was developed. This assay was optimized for use with both serum and colostrum samples. PCV2 NA titers were determined for colostrum samples (32-68/farm).

Results
Significant differences in PUCS and colostrum PCV2 PCR were seen between Farms A&B and Farms C&D (p<0.05, Figure 1). No significant differences were observed in sow PCV2 ELISA (p>0.05, Figure 1). There was a broad range of 50% neutralizing antibody titers, from 1:159 to 1:1x10^6. A significant difference in average neutralizing antibody titers was observed between Farms A&B and Farms C&D (Table 1).

Figure 1: PCV2 PCR (PUCS and colostrum) percent positive & sow ELISA, avg. S/P ratio

Table 1: Avg colostrum NA between farms

<table>
<thead>
<tr>
<th>Farm</th>
<th>Avg. colostrum NA titers</th>
</tr>
</thead>
<tbody>
<tr>
<td>A&amp;B</td>
<td>1:74,000^a</td>
</tr>
<tr>
<td>C&amp;D</td>
<td>1:27,000^b</td>
</tr>
</tbody>
</table>

Discussion
PUCS is more sensitive for PCV2 detection than colostrum in paired samples1. PUCS sample cannot be saved and resampled therefore colostrum was used for the NA testing. Sow ELISA results do not indicate differences in sow herd stability2. However, in this study, NA titers differed significantly between stable (A&B) and unstable farms (C&D); higher NA titers observed in farms with no to low virus levels compared to farms with high levels of vertical transmission. This suggests that sows on farms with higher vertical transmission may develop less protective NA and may be more susceptible to disease due to amounts of replicating virus present. Likely, protective colostrum antibodies need to be produced and passed to piglets in order to protect against disease. Further studies on the effect of vaccination on viral levels and NA levels are being conducted, but high viral levels and low NA titers might suggest additional vaccinations may be useful for those farms.

References
1. Seate et al, 2015, ISERPD, p70.
2. Seate et al, 2015, ISERPD, p220.
Introduction
Infection with PEDV and PDCoV causes diarrhea, vomiting, and mortality in piglets. The PEDV is excreted in high amounts in feces making it highly contagious and a serious problem for the swine industry (Pensaert and De Bouck, 1978). The PDCoV has similarities to PEDV, but clinical signs are less severe. Infected feed may be a vehicle of transmission for these viruses and their survivability in feed may vary based on feed processing and composition. The objectives of this experiment were to: 1) obtain virus survival kinetics of PDCoV and PEDV in various feed ingredients; and 2) determine if heat treatment of ingredients at low temperatures can effectively reduce the infectivity PEDV.

Materials and methods
In Exp. 1, feed ingredients including dried distillers grains with solubles, corn, soybean meal, blood meal, meat meal, meat and bone meal, spray dried porcine plasma, and complete feed were inoculated with 1 mL of cell-culture adapted PDCoV (3.2x10^5 TCID50/mL) and incubated for 0, 1, 3, 7, 14, and 21 days. In Exp 2, the same feed ingredients were inoculated with 1 mL of cell-culture adapted PEDV (3.2x10^4 TCID50/mL). The samples were incubated at 60°, 70°, 80°, or 90°C for 0, 5, 10, 15, or 30 min. After the incubation period, the virus was eluted and inoculated into Vero-81 cells for PEDV, or swine testicular cells for PDCoV to determine the titer of surviving virus. After completion of both Exp, data were analyzed using the Weibull model through glnafit software (GlnaFiT) and the delta values were estimated to determine the amount of time necessary to reduce initial virus titer (TCID50) by one log10 (90%). Delta values were compared with by the t-test and considered different when P < 0.05.

Results
There were differences in survival of PDCoV among feed ingredients (P < 0.01). Survival time of PDCoV was least in blood meal (0.12 ± 0.05 days) and complete feed (0.22 ± 0.26 days), and longest in dried distillers grains with solubles (11.08 ± 1.99 days) and meat meal (10.05 ± 4.21 days). There were no differences in survival of PEDV among the 8 ingredients that were heated for the 30 min period. When these ingredients were heated, the delta value decreased as the temperature increased at 60°C (4.72 ± 2.84 min), 70°C (3.05 ± 1.93 min), 80°C (2.72 ± 1.60 min), and 90°C (2.64 ± 1.84 min). After 30 min of heating at 90°C, there was no detectable virus in any of the ingredient samples (> 3 log reduction).

Conclusion and discussion
Survival of PDCoV varies among feed ingredients. Some ingredients such as meat meal and dried distillers grains may need to be more carefully monitored for PDCoV contamination, due to greater survivability in these matrices. Although there were no differences in survivability of PEDV among ingredients, this response was likely due to the shorter incubation times used in the PEDV Exp. However, despite the lack of differences among ingredients, heating each feed ingredient for 30 min at temperatures above 90°C can successfully reduce PEDV concentration to below detectable levels.

References
WITHIN-PEN PREVALENCE PERFORMANCE OF THE IDEXX PRRS OF TEST FOR DETECTION OF ANTIBODIES IN SWINE ORAL FLUIDS

Introduction: Recently, we developed IDEXX PRRS OF (IDEXX Laboratories), a same-day protocol ELISA to detect antibodies to Porcine Reproductive and Respiratory Syndrome virus (PRRSV) in swine oral fluids. In pen-based samples of oral fluids, the pen represents the infectious unit, and the collected sample is a composite of oral fluids from most pigs in the pen. The probability of detection of PRRS antibodies in these composite samples has been previously evaluated as a function of within-pen prevalence of PRRS antibody-positive pigs using a non-commercial adaptation of our serum test, PRRS X3 (IDEXX Laboratories) based on an overnight incubation protocol (1, 2). We used samples from this study to evaluate the performance of the commercial IDEXX PRRS OF test as a function of within-pen prevalence.

Materials and methods: The design of the prevalence study has been published (1). Briefly, pigs vaccinated with modified live vaccine (Ingelvac® PRRS MLV, Boehringer Ingelheim Vetmedica Inc.) were introduced into pens of pigs sero-negative for PRRS antibodies at different prevalence of vaccinated (sero-converted) pigs (0%, 4%, 12%, 20%, and 36%); 5 pens were studied for each prevalence level. Blood samples were drawn from all pigs to confirm the presence of serum antibodies. Oral fluids were collected from each pen by hanging a cotton rope in the pen for 30 minutes; 5 successive 30-minute replicate collections were done per pen by hanging a new rope per collection event. Serum was tested with PRRS X3 to verify PRRS serological status of each pig, while the oral fluid samples were tested using the IDEXX PRRS OF test as well as the published adaptation of PRRS X3 (2). The probability of detecting a positive oral fluid sample was modeled as a function of within-pen prevalence using a logistic regression model on SAS, with both the pen and the rope sample within a pen considered as random effects.

Results: Table 1 shows the percentage rate of positive rope samples of oral fluids from pens within a range of within-pen prevalence of PRRS-antibody positive pigs of 0-36%. The IDEXX PRRS OF test detected anti-PRRS antibodies in ≥96% of all collection events in pens of at least 20% prevalence, compared to 88% for the PRRS X3 overnight adaptation for oral fluids. Even in circumstances of low within-pen prevalence (1 positive pig in 25, or 4%), the rate of detection of PRRS antibodies in pen rope samples was 4-fold higher than with the overnight test. The probability of detecting a positive sample at a minimum of 20% within-pen prevalence using the SAS regression model is 91% or higher.

Conclusions: The same-day IDEXX PRRS OF test shows increased sensitivity as a function of within-pen prevalence compared to an overnight protocol employing PRRS X3. Such enhanced sensitivity is designed to facilitate the use of pen-based oral fluid sampling for PRRS surveillance in commercial pig populations.

References

Table 1. Detection of anti-PRRS antibodies in pen oral fluids at different levels of within-pen prevalence of seroconverted pigs.

<table>
<thead>
<tr>
<th>Within-pen prevalence (%)</th>
<th>Rate of positive oral fluids*</th>
<th>Detection probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IDEXX PRRS OF</td>
<td>Standard procedure (2)</td>
</tr>
<tr>
<td>0</td>
<td>0/25 (0%)</td>
<td>0/25 (0%)</td>
</tr>
<tr>
<td>4</td>
<td>8/25 (32%)</td>
<td>2/25 (8%)</td>
</tr>
<tr>
<td>8-16</td>
<td>17/25 (68%)</td>
<td>12/25 (48%)</td>
</tr>
<tr>
<td>20</td>
<td>24/25 (96%)</td>
<td>22/25 (88%)</td>
</tr>
<tr>
<td>32-36</td>
<td>24/25 (96%)</td>
<td>22/25 (88%)</td>
</tr>
</tbody>
</table>

*# of positive samples per 25 rope collections (5 pens per level X 5 collections per pen)
**0.39 at 8% prevalence, 0.62 at 12% prevalence, 0.80 at 16% prevalence
USE OF AUTOGENOUS VACCINES TO PROTECT AGAINST A VIRULENT HETEROLOGOUS INFLUENZA CHALLENGE

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Swine origin influenza viruses are among the most important viral pathogens affecting the health of the U.S. swine herd. Genetic drift in the virus population has allowed the virus to evolve a significant degree of antigenic diversity. The major subtypes that affect swine can be classified into several sub-clusters based on the sequence of the HA segment (Vincent et al. 2006). The virus is often able to evade host immunity to the strains that are present in commercially available vaccines. Autogenous vaccines can be used to provide protection against viruses that evade immunity provided by commercial vaccines. However, recent work has shown that inactivated vaccines can cause vaccine associated enhancement of respiratory disease (VAERD) (Vincent et al., 2008). Furthermore, maternal antibodies have been shown to interfere with inactivated vaccines when they are delivered early after weaning (Kitikoon et al., 2006). A recent study has demonstrated that by using an intranasal (IN) delivery platform in combination with poly I:C adjuvant, maternal antibody interference can be circumvented (Thomas et al., 2015). In the work presented here, we performed a similar experiment demonstrating that vaccines delivered via both IN and intramuscular (IM) routes can provide protection against a virulent heterologous challenge strain.

Vaccine strains were selected for use in the experiment based on their susceptibility to neutralization with a panel of antisera described previously (Hause et al, 2011). In addition, the HA sequence of each strain was central to the sub-cluster from which it was selected. Pigs were divided into seven treatment groups as follows: A) Monovalent H1N1 vaccine delivered IN, B) Bivalent H1N1 and H3N2 delivered IN, C) Trivalent H1N1, H3N2, and H1N2 delivered IN, D) Trivalent H1N1, H3N2, and H1N2 delivered IM, E) Mock-vaccine delivered IN, F) Mock-vaccine delivered IM, G) Non-vaccinated/Non-challenged controls. The vaccine was delivered on days 0 and 21 of the study. Poly I:C was used as the adjuvant for the vaccines delivered IN. The IM group received a proprietary oil-in-water adjuvant.

Animals were challenged 14 days after their last vaccination with an H1N2 delta cluster strain that was antigenically distinct from the strains included in the vaccine. Following challenge, animals were humanely euthanized and necropsied. Gross lung lesions were evaluated for each animal and group results are summarized below in Figure 1.

Serum was drawn from all animals at day 34 of the study immediately prior to challenge and tested in an HI assay against all of the strains included in the vaccines and the challenge virus (Figure 2).

The results of the study demonstrate that both the trivalent vaccines delivered were able to confer protection against a virulent heterologous challenge with a delta sub-cluster H1N2 isolate regardless of adjuvant or route of delivery. Our results also demonstrate the ability of the vaccine delivered IM to induce protection in the presence of maternal antibody in weanling aged pigs.

References

Safety of Fostera® PCV MH vs Other Combination Vaccines.
G. Nitzel MS¹, J. Bubolz MS¹, M. Smutzer BS¹, P. Runnels DVM, PhD¹, L. Taylor MS¹
¹Zoetis VMRD, Kalamazoo MI

Introduction
A research study was conducted to evaluate vaccination regimens using 1 or 2 doses of Fostera PCV MH compared to other competitive vaccines in their ability to limit reaction due to vaccination.

Materials and methods
The trial involved 248 healthy baby piglets (mixed gender) serologically negative for M. hyo and PCV2. Piglets were weaned at approximately 3 weeks of age (study day 0) and randomly allocated to 5 treatment groups by blocks based on body weight. The vaccination involved administration of 1 or 2 mL of the following products at approximately 3 weeks of age (right neck) with a second dose administered at 5 to 6 weeks of age to 2 groups (left neck): Fostera PCV MH (one 2-mL dose), n=48; Fostera PCV MH (two 1-mL doses, 2nd dose on day 14), n=48; Ingelvac® CircoFLEX-MycoFLEX® (one 2-mL dose, mixed into a single bottle before vaccination), Boehringer IngelheimVetmedica, n=48; Circumvent® PCV-M (two 2-mL doses, 2nd dose on day 21), Intervet/Merck Animal Health, n=48. Pigs were observed throughout the study for general health and clinical signs of respiratory distress, lethargy, wasting, etc. The primary variable was injection site reactions. All right-neck injection sites were observed and palpated for adverse reactions on days 1, 4, and 7, and sites on both the left and right neck were assessed on days 15, 18, 22, 23, 28, and 35. Reaction severity was scored using a numerical system ranging from 0 to 3 (0=normal, 3=severe). Any injection site reactions were monitored until resolution. Data were statistically analyzed by appropriate methods using each pig as the experimental unit. Statistical significance recognized at P ≤ 0.05

Results
The incidences of injection site reactions are summarized in Figure 1. With the exception of the Circumvent PCV-M group, all other vaccines and controls had low incidences of injection reactions for both the first and second injections. However, pigs vaccinated with Circumvent PCV-M exhibited significantly more site reactions, especially when the second vaccination was administered. Notably, the incidence of second-vaccination site lesions was reduced 86.5% (P ≤ 0.0001) for pigs vaccinated with Fostera PCV MH compared to those vaccinated with Circumvent PCV-M. The duration of site reactions was also much longer in the Circumvent PCV-M group compared to other vaccines, particularly with the second injection (5.38 days vs 0.27-0.68 days, P ≤ 0.05). Outcomes were further confirmed by results of reaction severity scoring. Over 40% of pigs receiving the second Circumvent PCV-M vaccination demonstrated a ‘severe’ injection site reaction score (3: over 5-cm-diameter swelling, evidence of irritation and pain such as persistent rubbing or withdrawal and vocalization upon palpation, and/or an abscess). These various site data indicate that the Circumvent PCV-M formulation triggered much more site reactivity than other test vaccines (which were not different than saline controls).

Discussion
This study confirms that Fostera PCV MH is a safe combination vaccine with reaction rates not different than saline.
EFFICACY OF FOSTERA® PCV MH VS OTHER COMBINATION VACCINES FOLLOWING DUAL CHALLENGE WITH MYCOPLASMA HYOPNEUMONIAE AND PCV2

G. Nitzel MS1, J. Bubolz MS1, M. Smutzer BS1, P. Runnels DVM, PhD1, L. Taylor MS1
1Zoetis VMRD, Kalamazoo MI

Introduction
A research study was conducted to evaluate vaccination regimens using 1 or 2 doses of Fostera PCV MH compared to other competitive vaccines in their ability to limit M. hyo lung lesions and PCV2 viremia in swine challenged with both virulent M. hyo and PCV2b.

Materials and methods
The trial involved 248 healthy piglets (mixed gender) serologically negative for M. hyo and PCV2. Piglets were weaned at approximately 3 weeks of age (study day 0) and randomly allocated to 5 treatment groups by blocks based on body weight. The ‘vaccination phase’ of the study (3 to 9 weeks of age) involved administration of 1 or 2 mL of the following products: Fostera PCV MH (one 2-mL dose), n=48; Fostera PCV MH (two 1-mL doses, 2nd dose on day 14), n=48; Ingelvac® CircoFLEX-MycoFLEX® (one 2-mL dose, mixed into a single bottle before vaccination), Boehringer IngelheimVetmedica, n=48; Circumvent® PCV-M (two 2-mL doses, 2nd dose on day 21), Intervet/Merck Animal Health, n=48. The ‘challenge phase’ of the study (9 weeks of age and following) involved 2 separate events: M. hyo challenge: at approximately 9 weeks of age (6 weeks after initial vaccination), each pig was challenged intratracheally; PCV2b challenge: at approximately 10 weeks of age (7 weeks after initial vaccination), each pig was challenged via both the IM and intranasal routes. The primary variables of interest were the severity of M. hyo lung lesions and PCV2 viremia. Individual serum samples were collected at study days 0, 28, 41, 48, 56, 63, and 70. Samples were analyzed by quantitative polymerase chain reaction (qPCR) for detection of PCV2 viremia, and serological testing using enzyme-linked immunosorbent assay (ELISA) assessed M. hyo and PCV2 antibody titer. At necropsy, sections of 3 lymph nodes (tracheobronchial, mesenteric, inguinal) and tonsil were collected from each pig and submitted for histopathological examination for lymphoid depletion (associated with PCVAD) and histiocytic replacement, as well as testing for PCV2 antigen by immunohistochemistry (IHC). Data were statistically analyzed by appropriate methods using each pig as the experimental unit. Statistical significance recognized at P ≤ 0.05

Results
The percentages of total lung with M. hyo lesions for the various treatment groups are summarized in Figure 1. Only 2.8% to 3.5% lesion severity was observed for pigs vaccinated with Fostera PCV MH. In contrast, pigs vaccinated with Ingelvac CircoFLEX-MycoFLEX demonstrated no significant lesion reduction compared to controls (P > 0.05). Furthermore, both Fostera PCV MH groups experienced significantly less M. hyo lesion pathology than Ingelvac CircoFLEX-MycoFLEX vaccinates (69-75% reduction with Fostera PCV MH, P ≤ 0.05). Improvements in M. hyo protection relative to controls and Ingelvac CircoFLEX-MycoFLEX were also provided by Circumvent PCV-M. All vaccinated groups significantly reduced viremia incidence relative to controls (70-91% reduction, P ≤ 0.05).

Discussion
This study further confirms that Fostera PCV MH is an effective combination vaccine that helps provide protection from both PCVAD and mycoplasmal pneumonia.
NETWORK ANALYSIS OF MAIN SERVICE PROVIDERS FOR SWINE HERDS PARTICIPATING IN REGIONAL PRRS CONTROL PROGRAMS

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¹Department of Population Medicine, University of Guelph, Guelph, ON, Canada, ²Woodstock, ON, Canada, ³Strategic Solution Group, Puslinch, Canada

Introduction
The importance of networks in infectious disease epidemiology has been recognized in the last decade for several animal diseases. In Ontario, a link was established between the introduction of porcine epidemic diarrhea and the exposure to contaminated feed (Pasick et al., 2014); and truck networks have been linked to the occurrence of specific porcine reproductive and respiratory syndrome (PRRS) virus genotypes in a defined area of the province (Arruda et al., 2015). The current North American swine industry is characterized by a high degree of connectedness of swine sites clustered within production systems, and a limited number of specialized service providers focusing on specific parts of the system, such as animal transporters and feed suppliers. This requires the use of new approaches to evaluate and account for important relationships that would otherwise go unnoticed. The objective of the current study was to describe static relationships between swine sites and their service providers (including transportation, feed, semen, gilt and boar companies) and extract parameters to be used in risk factor analysis for PRRS virus positivity.

Materials and methods
The source of data was a SQL Server 2008 database containing data from PRRS area control and elimination projects in Ontario, Canada. Demographics, biosecurity and network information was collected using a standardized questionnaire. Network analysis was conducted in Gephi 0.8.2 and UCINET 6. Edges were undirected, and defined as a connection between a site and a service provider. The five above-mentioned networks were combined and transformed to a one-mode network for analysis, from which the number of degrees (indirect connections) was extracted for each swine site. Risk factor analysis was conducted using a generalized mixed model in SAS 9.3, and included number of neighbors within three km and number of degrees as main predictors. Clustering of sites within production system was taken into account using a random effect.

Results
A total of eight hundred and sixteen sites were enrolled in the study. These sites were connected to a total of 56 feed companies and 93 truck companies. The two hundred and fifty-four breeding herds included in the study reported to receive semen from 23, gilts from 54 and boars from 37 genetic companies. A representation of the two-mode network is showed on Figure 1. A preliminary statistical model showed a significant positive association between being PRRS positive and number of indirect connections with other swine sites. However, the statistical significance of such association disappeared when production system was included. Detailed description and analysis of networks separately is pending.

Conclusions and discussion
Indirect relationships between swine sites are important for control of infectious disease spread in a timely matter, and network analysis is a promising approach to accomplish this.

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References
GENETIC DIVERSITY OF SWINE ORIGIN INFLUENZA A VIRUS IN CHILE

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Introduction
Influenza A virus (IAV) is a major pathogen in pig production. IAV causes pneumonia and predisposes pigs to secondary infections which results in economic losses to producers. Additionally, pigs can act as reservoirs of IAV strains with zoonotic potential.

In Chile IAV was reported in 2009 in clinical cases of pigs with respiratory disease. However, there is limited information on IAV genetic diversity across the Chilean swine industry. In this study we conducted active surveillance for IAV in swine herds representative of commercial swine production in Chile.

Materials and methods
Twenty seven commercial swine herds were sampled from December 2013 to June 2015. These herds are representative of modern swine pig production, and are located between Valparaiso and Araucania administrative regions, with most of them located in high-density areas of pig production.

Nasal swabs (NS) or oral fluids (OF) were obtained in each visit and tested by real-time RT-PCR (RRT-PCR) and virus isolation. Selected positive RRT-PCR samples were subtyped using PCR. Subsequently, positive samples were sequenced by Sanger (hemaglutinin (HA)) or using full genome sequencing by Illumina.

Diagnostic tests were performed in the Virology Lab, FAVET, University of Chile and at the Molecular Virology Lab, PUC. HA sequencing was performed at the Veterinary Diagnostic Laboratory, University of Minnesota and full genome sequencing at Icahn School of Medicine at Mount Sinai, USA.

Bayesian Evolutionary Analysis Sampling Trees approach was used to reconstruct the phylogenies using HA sequences. Additionally, 167 H1 reference sequences published in GenBank were included in the analysis. The relaxed uncorrelated exponential clock model and coalescent Bayesian skyline tree prior were used. Each tree was run for $5 \times 10^8$ iterations. Trees generated before reaching a stationary state were burned, and trees results were annotated.

Results
A total of 1500 samples were collected during 50 visits (83% NS and 17% OF). From the total of samples tested, 347 (23%) were positive. Of these, 20% of nasal swabs and 34% of oral fluids were positive. Twenty one out 27 farms (78%) were positive at least in one visit. Subtypes H1N1, H1N2, H3N2 were identified.

Finally were generated a total of 69 HA sequences and a total of 50 new IAV whole genomes. Phylogenetic analysis identified the circulation of 6 distinct IAV genotypes in Chile, which included pH1N1-like, SwH1N2, SwH3N2, human like H3N2, and a reassortant H1N1 containing a swine HA gene (SwH1) and NA gene derived from the pH1N1 strain. SwH1N2 (45%) and pH1N1-like (45%) were the most frequently identified.

Discussion and conclusions
SwH1N2 virus is frequently detected in Chilean pig production. This virus is genetically different from H1 clusters seen in North America and not closely related to any other reported IAV. The phylogenetic trees constructed suggest at least 3 human-to-swine introductions of the pH1N1 strain.

IAV is endemic with multiple strains co-circulating in Chilean intensive swine production. This is the first study of the diversity and origin of swine IAV in Chile based on active surveillance.

Acknowledgements
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FONDEF Grant number ID1410201
ZOETIS-FAVET Identificación, aislamiento y caracterización de Influenza 1-2
MOLECULAR, PATHOLOGICAL, AND IMMUNOHISTOCHEMICAL EVIDENCE
OF SENECAVIRUS A-INDUCED INFECTIONS IN PIGS OF DIFFERENT AGE GROUPS
WITH VESICULAR DISEASE FROM BRAZIL

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1,2Laboratories of Virology and Animal Pathology, Department of Veterinary Preventive Medicine, Universidade Estadual de Londrina, Paraná, Brazil, alfieri@uel.br; 3Department of Immunology, University of Manitoba, Winnipeg, Manitoba, Canada; 4National Centre for Foreign Animal Disease, Winnipeg, Manitoba, Canada; 5MSD Saúde Animal, Cotia, São Paulo, Brazil

Introduction
Senecavirus A is the single representative species of the Senecavirus genus, Picornaviridae family (1). Studies conducted in North America (2,3) have suggested that Senecavirus A-induced infection might be associated with a vesicular disease of pigs known as porcine idiopathic vesicular disease (PIVD). Outbreaks of PIVD were reported from pigs of Indiana, USA (3,4) and Manitoba, Canada (2). This study investigated the participation of Senecavirus A in PIVD-affected pigs of different ages from distinct geographical regions of Brazil.

Materials and methods
Two adult and 3 weaned pigs from 2 pig farms with PIVD from distinct cities of Paraná state, southern Brazil were investigated. Vesicular fluids (n=2) and scrapings of ruptured vesicles and ulcerative lesions (n=5) were collected for molecular diagnostics. Additionally, 10 1-4 day-old piglets that died spontaneously from 5 pig farms located in the states of Mato Grosso do Sul (Midwest Brazil, n=1), and Santa Catarina (n=2) and Paraná (n=2), Southern Brazil, were received for diagnostic investigations. A total of 15 animals and 7 pig farms were evaluated during this study.

Routine necropsy was performed on the 10 piglets; duplicate tissue sections were selected for molecular, histopathological, and immunohistochemical, IHC (5) evaluations. RT-PCR assays were performed to detect specific amplicons of viral agents associated with vesicular and/or cutaneous diseases such as foot and mouth disease, vesicular stomatitis, swine vesicular disease (6), Teschovirus A, Sapelovirus A, Enterovirus G (7), Porcine circovirus-2 (8), and Porcine parvovirus (9). A set of primers were designed to amplify a 542 bp product of the VP3/VP1 region of the Senecavirus A genome.

Results
Significant gross pathological findings included diphtheric glossitis (n=6), and ulcerative lesions at the coronary band (n=3). Histopathology revealed necrotizing glossitis with ballooning degeneration of epithelial cells, interstitial pneumonia, myocarditis, and lymphoplasmacytic encephalitis. IHC revealed positive immunoreactivity at the degenerated epithelium of the ulcerative lesions of the tongue of all piglets. RT-PCR assays identified Senecavirus A from the vesicular fluids, scrapings of the ruptured vesicles, and ulcerative lesions, as well as from multiple tissues of all piglets; all other RT-PCR assays were negative. Sequence analyses confirmed the specificity of the RT-PCR assay; phylogenetic evaluation revealed that the isolates from Brazil clustered with similar strains of Senecavirus A identified in North America.

Conclusions and discussion
The molecular, pathological, and IHC findings confirmed the participation of Senecavirus A in the lesions observed in these pigs. These results suggest that Senecavirus A was the etiological agent associated with the vesicular disease outbreaks in the 7 pig farms from 3 distinct states of Brazil. This is the first study to report Senecavirus A-induced infection in clinically affected pigs outside of North America; the novel primer set reported in this study was suitable for the rapid and specific molecular detection of Senecavirus A.

References
A STOCHASTIC, MATHEMATICAL MODEL OF INFLUENZA A VIRUS WITHIN SWINE BREEDING HERDS: IMPLICATIONS OF POSSIBLE MANAGEMENT INTERVENTIONS

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Introduction: Influenza A virus (IAV) is a globally endemic infection in swine herds that causes significant morbidity in the swine industry and poses a substantial public health risk (1, 2). Initial modeling work suggests that vaccination (especially heterologous) does not effectively remove influenza from breeding herds (3), and recent empirical findings suggest that IAV is most likely maintained endemically through gilt introductions and in piglet subpopulations (4). The goal of developing this model is to test intervention strategies that swine producers could feasibly employ to control IAV in their herds.

Materials and methods: Building on the work of Reynolds et al. (2014), we have developed a stochastic Susceptible-Exposed-Infectious-Recovered simulation of IAV dynamics in a swine breeding herd. Using Gillespie’s Direct Method (5), this model allows for stochastic births, deaths, direct and indirect infection, and maternally derived immunity in piglets. The construction of this model reflects the spatial organization of a standard breeding herd and accounts for the different classes of pigs therein including gilts, sows, and piglets in various production and/or immune stages. The interventions tested include: serial routine heterologous mass vaccination with waning immunity, pre-farrow vaccination with and without piglet early weaning, and varying the timing of gilt purchases.

Results: Our preliminary results suggest that none of the interventions can effectively eliminate infection from a medium sized herd (~1500 sows) when implemented individually. In concert, pre-farrow vaccination, early weaning of piglets (removal 7-14 days after birth), and longer periods between introductions of gilts (intervals greater than one month) were the most effective at reducing prevalence, but did not result in elimination of IAV.

Conclusions and discussion: Our findings support other modeling and empirical studies that suggest that piglets maintain IAV in breeding herds (3,4,6). Our next steps will be to evaluate the sensitivity of results to epidemiological parameters, vaccine efficacy, and farm size. We will also test the potential role of seasonality in transmission rates of IAV (4). We hypothesize that one or more of these interventions will prove effective at reducing the incidence and prevalence of IAV when timed appropriately with seasonality in flu transmission.

References:
Introduction
Immunity to Porcine Epidemic Diarrhea virus (PEDV), whether active or transferred, is poorly understood. The objectives of this study were to 1) characterize the immune response in naïve sows when infected with PEDV utilizing different serological assays, and to 2) assess lactogenic immunity and protection in piglets subsequently challenged with the homologous strain of PEDV.

Materials and methods
In April 2014, a commercial sow farm became infected with a prototype stain of PEDV. At infection, 30 sows were randomly selected and monitored for PEDV antibodies and fecal shedding by PCR for five months. Piglets weaned immediately after herd infection were also monitored for fecal shedding for 20 weeks.

Sows from the original group of 30 that were bred following PEDV exposure were relocated to Iowa State University in September 2014 for homologous PEDV re-challenge at farrowing. Eight sows were randomly allocated into one of two groups: negative control (NC) (n=3) and treatment (T) (n=5). Three additional sows were sourced from a PEDV-naïve farm for a positive control (PC) comparison (n=3). Groups were housed separately by room and farrowing crate.

At 110-111 days of gestation, sows in groups T and PC were challenged via gavage with a 1 mL dose of 1x10³ TCID₅₀/mL of PEDV. The PEDV used for challenge was grown in vero cells from an isolate obtained from the source farm at initial infection. At 24-48 hours post parturition, all piglets in groups T and PC were inoculated with the same dose as the sows by gavage. Sample collection days post inoculation (DPI) included: 1) sow fecal and serum samples on 0, 3-6, 11, 14, 18, 21, and 25 DPI; 2) sow colostrum or milk at farrowing 11, 14, 18, 21, and 25 DPI; 3) piglet serum at birth, 7, 14, and 18 days post inoculation; and 4) piglet fecal swab at birth, 4, 7, 11, 14, and 18 days post inoculation. Individual piglet weights were determined at birth 7, 14, and 18 days post inoculation.

All serum, colostrum, and milk samples were assayed using the Iowa State University IFA test, IgA and IgG ELISAs, and the South Dakota State FFN test.

Results
All sow fecal samples were PCR positive for PEDV at the time of the initial out-break; however, serum antibodies were not detected, all assays. All sow fecal samples were PCR negative at 6 weeks post-break. Serum antibodies were detected by all assays at 14 days post-break and throughout the sampling period.

All litters of the PC group displayed clinical signs consistent with PEDV infection, with 100% mortality by 4 DPI. Sows in this group also had diarrhea. All litters in group T had PCR positive fecal samples; however, only one of the five litters demonstrated clinical disease consistent with PEDV infection with minimal mortality in the affected litter.

Positive antibody titers were measured in previously exposed sows and their piglets across IFA, FFN, and ELISA. Statistical analysis is pending on final results.

Conclusion and discussion
PEDV antibodies were detected for at least a 5 month duration in previously infected sows using the IFA test, FFN test, and both the IgG and IgA ELISA assays. Lactogenic immunity was present and protective to piglets born to previously exposed sows in the face of strong PEDV challenge. Clinical disease was only seen in 1/5 litters; viral shedding occurred in all litters. Additional conclusions are pending statistical analysis.
INFLUENCE OF POOLING ON *MYCOPLASMA HYOPNEUMONIAE* POLYMERASE-CHAIN REACTION DIAGNOSTIC ASSAY RESULTS IN EXPECTED LOW PREVALENCE SCENARIOS

A Sponheim¹; E Fano¹; D Polson¹; K Doolittle¹, M Pieters²

¹Boehringer Ingelheim Vetmedica, Inc., St. Joseph, USA; ²University of Minnesota, CVM, St. Paul, USA

Introduction: A recent study compared ante-mortem and post-mortem samples by individual and pooled samples for detection of *Mycoplasma hyopneumoniae* (Mhp) by polymerase-chain reaction (PCR)¹. The authors demonstrated with the use of stochastic modeling and the diagnostic sensitivities (DxSe) obtained during the study that increased sample size resulting from ante-mortem sampling combined with pooling allowed for a higher herd detection rate while pursuing the most economical approach in a high prevalence population. To further test this model in a Mhp low prevalence population scenario, the following study was designed. The objective of this study was to determine the extent pooling samples from an artificially created Mhp low prevalence ante-mortem population scenario lowers test DxSe.

Materials and methods: Artificial positive and negative samples comparable to those found in low Mhp prevalence ante-mortem testing scenarios were created due to the desired power required for the study. An artificial Mhp positive stock solution for each treatment group was created from a Mhp strain AP 414 (Ct 20) stored at the University of Minnesota, PBS, and known Mhp negative oral fluids. Dilutions were made to create the desired Ct value for each treatment group: Low (L=26), middle (M=31), and high (H=36) Ct value treatment groups were chosen for the study based off of a histogram crated from previously collected ante mortem field samples. Artificial negative samples were made using PBS and known Mhp negative oral fluids. The L, M, and H treatment groups were tested in 3:1 and 5:1 pools. One L, M, or H known positive sample was placed in each 3:1 and 5:1 pool. The remaining samples in each pool were composed of the known negative samples. Artificial negative samples were made using PBS and known Mhp negative oral fluids. The L, M, and H treatment groups were tested in 3:1 and 5:1 pools. One L, M, or H known positive sample was placed in each 3:1 and 5:1 pool. The remaining samples in each pool were composed of the known negative samples. Ninety PCR tests (VetMAX-Plus qPCR Master Mix) were run for each pool and Ct value for a total of 540 PCR tests. Six PCR plates were used to test all of the samples. Fifteen PCRs for each Ct value and pool were run per plate. To reduce potential variation, one technician was responsible for all extractions (MagMAX-96 Viral RNA Isolation Kit) and one technician was responsible for the amplification process. All PCRs were tested on one day using 3 machines (2 plates/machine).

Results: DxSe results are reported in Table 1.

### Table 1 Diagnostic sensitivity results

<table>
<thead>
<tr>
<th></th>
<th>High (H) Ct (36)</th>
<th>Middle (M) Ct (31)</th>
<th>Low (L) Ct (26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pools of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>DxSe (positive/expected positive)</td>
<td>90/81/90</td>
<td>72.6/5/90</td>
<td>100/90/90</td>
</tr>
<tr>
<td>95% Confidence Interval</td>
<td>81.9-95.3</td>
<td>61.8-81.1</td>
<td></td>
</tr>
<tr>
<td>99% Confidence Interval</td>
<td>79.1-96.4</td>
<td>58.5-83.5</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions: As expected in a Mhp low prevalence scenario (low prevalence and high Ct value), there is a risk of not detecting one positive animal in a pool when the rest of the pool is negative. DxSe and confidence intervals obtained from this artificial ante-mortem sample pooling study will be used in a stochastic model to give veterinarians guidance in determining number of animals along with number of times to sample herds to most economically detect Mhp in low prevalence scenarios. The model will also help to better understand the financial implications associated with missing one or a few positive animals in Mhp low prevalence scenarios.

References:
EFFICACY OF KAVAULT™ (AVILAMYCIN) IN PIGS CHALLENGED WITH ESCHERICHIA COLI IN AN INDUCED POST-WEANING DIARRHEA MODEL

1Thomas Marsteller DVM, Brandon Carter DVM, Kevin Eggers DVM, Robert Evelsizer DVM, James Mark Hammer DVM
1Elanco Animal Health, Greenfield Indiana, USA

A. Objective
This study was conducted to determine Kavault (avilamycin) efficacy in pigs following oral Escherichia coli challenge in an induced post-weaning diarrhea model. Previously reported studies1,2,3 have reported efficacy of avilamycin in controlling post-weaning E. coli disease with a unique mode of action.

B. Study design
Healthy weaned pigs were randomly assigned to one of four treatments: non-medicated feed (negative control), carbadox 55ppm (positive control), or one of two avilamycin feed concentrations (40 or 80ppm). Treatment feed administration was initiated on study day 0 and pigs were challenged with hemolytic Escherichia coli on study days four, five, and six. Treatment feeds were administered for the duration of the 32-day treatment phase. Each animal was observed daily from study day 0 through study day 32 for diarrhea, perianal inflammation, depression, and appetite scoring. Fecal samples were collected throughout the study for quantitative assessment of haemolytic E. coli shedding. Treatment efficacy was assessed based on relative differences between treatment groups for diarrhea scores to define prevalence, incidence, and severity of disease during the treatment phase. Secondary effects of colibacillosis were evaluated by measurement of growth and feed conversion parameters.

C. Results
Significant findings in these data include reduced diarrhea incidence and severity in pigs administered either avilamycin 40ppm, avilamycin 80ppm, or carbadox, relative to negative controls (P<0.05). Diarrhea incidence in avilamycin 80ppm and carbadox treatments were significantly improved relative to avilamycin 40ppm (P<0.05). Additionally, pigs in the carbadox treatment group had a higher rate of average daily gain and feed intake relative to negative controls and pigs administered avilamycin 40 ppm (P<0.05). Average daily gain in the avilamycin 80ppm treatment group was not significantly different from any treatment. No differences were observed for feed conversion or E. coli fecal counts.

D. Conclusions
Avilamycin administered in feed at a dose of 80ppm (73 gm/ton) effectively controlled the incidence and severity of colibacillosis in nursery pigs challenged with Eschericia coli, characterized by diarrhea and perianal inflammation. Supported by diarrhea incidence and severity scores, efficacy associated with the avilamycin 80ppm treatment group was consistently superior to negative controls and comparable to positive controls fed carbadox. These results demonstrated avilamycin administered at 80ppm is an effective dose to both prevent and control incidence and severity of diarrhea associated with nursery pig colibacillosis.

Please follow the label directions when using Kavault™ which is: Feed at 73 grams avilamycin per ton of Type C medicated feed (80 ppm) as the sole ration for 21 consecutive days. A VFD from the herd veterinarian is required to implement Kavault™.

E. References
Haemophilus parasuis is an important pathogen of swine and the cause of Glässer’s disease, one of the most common causes of mortality in swine operations worldwide. Glässer’s disease is primarily characterized by fibrinous polyserositis, meningitis, and polyserositis.(Amano et al., 1994). Fifteen serovars of *H. parasuis* have been identified to date, however, many isolates remain non-typable (Kielstein et al, 1992). Of these serovars, serovar 5 is most commonly associated with virulence in pigs (Rapp-Gabrielson et al, 1992).

Newport Laboratories, Inc. manufactures a vaccine under the ParaSail® brand against *H. parasuis* that includes a serovar 5 strain. This vaccine is effective in protecting against *H. parasuis* infection. Recently, two farms that use ParaSail® vaccine had reported outbreaks of severe pneumonia. Tissue samples from infected animals yielded highly virulent *H. parasuis* serovar 4 isolates.

In order to elucidate the potential causes for the outbreak, we performed a comparative genomic analysis of two virulent serovar 4 strains against three avirulent serovar 4 strains, and the serovar 5 strain included in the ParaSail® vaccine (Lawrence et al, 2014). The results of the analysis showed that both of the virulent serovar 4 outbreak strains lacked a functional gene for sialyltransferase, an enzyme responsible for adding sialic acid groups to the lipooligosaccharide portion of the *H. parasuis* capsule. However, avirulent serovar 4, and serovar 5 strains encoded a functional sialyltransferase. Strains lacking this enzyme might be expected to induce a more potent immune response resulting in a “cytokine storm” manifesting as enhanced lung lesions in infected pigs.

In order to develop a solution for farms experiencing infection with these strains, we manufactured an inactivated vaccine that could be used alongside ParaSail® vaccine to enhance protection against disease caused by the serovar 4 strains. This vaccine was tested in susceptible pigs. The pigs were divided into four treatment groups as follows: A) ParaSail® vaccine + Inactivated serovar 4, as a one shot vaccine, B) ParaSail® vaccine as prime followed by inactivated serovar 4 as a booster dose, C) ParaSail® vaccine alone and D) mock vaccinated control. Animals were challenged with the highly virulent serovar 4 strain. Following challenge, surviving animals were humanely euthanized and necropsied. Animals that exhibited gross lesions indicative of *H. parasuis* infection or that died as a result of challenge were recorded. The results of this experiment are briefly outlined in Figure 1. Results were analyzed using a Student’s t-test to determine whether significant differences were present between the groups. The analysis showed that all treatment groups were significantly better protected than the control group.

**Figure 1:**

Furthermore, when comparing the results from group A and group C, the data demonstrates an increased benefit of using ParaSail® vaccine in combination with an inactivated NPL vaccine to protect pigs from disease caused by highly virulent serovar 4 strains.

**References**

4. Lawrence et al. 2014, Genome Announc. 4:e00884.

*ParaSail is a registered trademark of Newport Laboratories, Inc.*
CHARACTERIZATION OF NURSERY PIG COLIBACILLOSIS IN A NATURAL INFECTION STUDY

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Elanco Animal Health, Greenfield Indiana, USA

A. Objective
This study was completed to create a natural exposure post-weaning colibacillosis model and to characterize the clinical signs and duration of post-weaning E. coli disease. Enterotoxigenic E. coli disease is a common nursery pig disease. Post-weaning colibacillosis has been noted to be a co-infection in weaned pigs complicating nursery performance in positive porcine epidemic diarrhea virus herds. Conditions in this study were intended to simulate a commercial swine environment void of colibacillosis preventatives or treatments such as vaccination, oral inoculum, individual pig antibiotic treatment, and nursery feed additives or antibiotics.

B. Study Design
The nursery pigs were not treated during the study. Sixty pre-study pigs from a farm with a history of colibacillosis were housed in the 12 experimental study pens for 7-14 days. On Study Day 0, pre-study pigs were removed and pens were scrapped and cleaned, but not disinfected. Subsequently, sixty (60) weaned pigs, 3-4 weeks of age, from a commercial sow farm with a history of post-weaning colibacillosis were placed into the 12 experimental pens (5 pigs per pen). Fecal consistency was evaluated on days 0-28 via rectal probe and daily diarrhea scores (0-3 scoring system) were recorded for each pig. Fecal samples were collected on the first day a pig received a daily diarrhea score ≥2 for microbiological culture isolation and identification. Neither body weight nor feed intake was recorded during the study.

Clinical endpoints evaluated were colibacillosis prevalence, percent diarrhea days, mean diarrhea severity score, distribution of disease onset, and mortality attributable to colibacillosis. Mean day of onset, range, and tolerance interval was calculated for disease onset. Mortality is reported as the number of animals dead or euthanized prior to day 28, study completion.

C. Results
Microbiological isolation and identification confirmed the presence of hemolytic E. coli in the study population. Colibacillosis prevalence in the study population was found in 45/60 pigs (75%), and the percent diarrhea days was 10.3%. The mean diarrhea severity score during Days 0-28 was 0.45. The mean day of disease onset was 6.76 days. The range of the disease onset was from 1-22 days. Two moribund animals were euthanized on study day 16 and were confirmed to have chronic colibacillosis via necropsy (3.3% mortality). These pigs were PEDV negative, as the study was completed prior to PEDV diagnosis in the US.

D. Conclusions
These data indicate the colibacillosis at-risk period in newly weaned nursery pigs is up to 22 days. No new cases were observed after 22 days. The disease course and severity observed in the study demonstrates a natural exposure model can recreate clinical colibacillosis. These data describing clinical endpoints of post-weaning colibacillosis parameters, in the absence of interventions, yields a reliable understanding of nursery pig susceptibility to colibacillosis and associated clinical signs.

E. References
DEVELOPMENT OF A MULTIPLE LOCUS VARIABLE NUMBER OF TANDEM REPEAT ANALYSIS (MLVA) FOR TYPING OF MYCOPLASMA HYORHINIS.

L.F. Dos Santos1,2, M. Clavijo3, S. Sreevatsan1, A. Rovira1, M.A.S. Moreira2, M. Pieters1

1Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, United States. 2Departamento de Veterinaria, Universidade Federal de Vícosa, Campus Universitário, Viçosa, MG, Brazil. 3 PIC North America, 100 Bluegrass Commons Blvd. Ste. 2200, Hendersonville, TN, 37075

Introduction

Mycoplasma hyorhinis (M. hyorhinis) is a swine pathogen that causes polyserositis in animals from 3 to 10 weeks of age when it becomes systemic and the mechanism that M. hyorhinis uses to become systemic is unclear1. The heterogeneity of M. hyorhinis has been the focus of a few studies in the past years. Restriction endonuclease analysis2, sequencing of p37 gene and a Multilocus Sequencing Typing (MLST)3 were used to study the genetic diversity in this pathogen. The present study aimed to develop a new epidemiological typing tool based on the variable tandem repeats loci present in M. hyorhinis.

Materials and methods

A total of 116 M. hyorhinis isolates were used in this study, including one reference strain of M. hyorhinis (ATCC 17981). The 115 clinical isolates were obtained from diseased pigs from cases submitted to the Veterinary Diagnostic Laboratory (VDL) of the University of Minnesota during 2010-2012. VNTR markers were identified in the genome of M. hyorhinis strain (HUB-1 NC_014448) using the Tandem Repeat Finder software (www.tandem.bu.edu/trf/trf.html). A set of 6 selected VNTRs were tested with specific primers to amplify DNA from a set of 20 M. hyorhinis isolates and the reference strain as a preliminary selection. Each locus was amplified individually, the PCR products were purified with a QIAquick PCR Purification Kit (Qiagen, California, United States) and were sequenced to confirm the presence of tandem repeats. By this approach, two VNTR loci were selected and taken forward for full assessment. The two loci selected for MLVA were multiplexed and the amplifications were performed in an Eppendorf thermal cycler (Eppendorf, Hamburg, Germany) in a final volume of 25 µl.

Results

The two loci were clearly amplified by PCR. Variations in the first loci yielded 8 different types while variations in the second loci yielded 3 types. Analysis of the combination of 2 loci revealed 14 MLVA types for all samples in the study. The Simpson’s diversity index for the assay was D=0.870. The most frequent MLVA types were: 8-4 (25%) followed by 8-3 (14.6%); 9-4 (12.9%) and 10-4 (12.9%) (Figure 1).

Conclusion and discussion

The developed MLVA assay for typing M. hyorhinis showed an efficient discriminatory power to differentiate M. hyorhinis isolates. High genetic heterogeneity between isolates of the same herd were found suggesting that more than one strain is circulating in the production system. The typing method developed in this study could be useful to perform epidemiological investigations in herds affected by M. hyorhinis.

Acknowledgements

Student supported by Capes Foundation, Ministry of Education of Brazil (proc. No: BEX17617/12-0).

References

**Lawsonia Intracellularis Serological Profile and Seroprevalence in Swine Herds from Minas Gerais, Brazil**

TP Resende¹; MPGabardo¹; CER Pereira¹; RMC Guedes²

*Animal Pathology Laboratory, Department of Veterinary Clinic and Surgery, Veterinary School – Universidade Federal de Minas Gerais*

**Introduction**

*Lawsonia intracellularis* is the causative agent of porcine proliferative enteropathy (4), a widespread and economic relevant disease in swine herds (6). The immunoperoxidase monolayer assay (IPMA) has demonstrated high specificity and sensitivity (2) in detecting serum antibodies against *L. intracellularis*. This is the first Brazilian serological study using IPMA as a diagnostic method and the first evaluating *L. intracellularis* serological profile in herds in Minas Gerais (MG) state.

**Materials and methods**

All serum samples were collected between May and July 2012 in 30 farrow-to-finish commercial swine herds located in the four major pig production regions of MG, Brazil. Serum samples were collected from five age categories of pig, 20 animals per category (pregnant and lactating sows, nursing piglets, nursery pigs, growing and finishing pigs), totaling 100 samples per herd. Samples were tested by IPMA (2). A herd was considered positive if at least one sample was positive. Data was analyzed by “survey” command in Stata®. Data about herd management was collected to verify the presence of risk factors associated to the infection.

**Results**

All herds were positive for *L. intracellularis* antibodies. Seroprevalence was 34.7%, considering all collected samples, regardless of age. There was no difference between results among regions and, in general, finishing pigs had the highest seroprevalence (Fig.1). Herds that adopted the practice of “cleaning before disinfection” had lower *L. intracellularis* antibody seroprevalence.

**Discussion and conclusion**

The only previous seroprevalence study in Brazil revealed that 96.3% of 109 investigated in MG herds were exposed to *L. intracellularis* (8), with 22.1% of seropositivity in finishing pigs, which was the only sampled category. They used the IFAT test in glass-slides. The increased seroprevalence observed in the present study might be explained by the wider spread of the disease over the years. The higher seroprevalence among finishing pigs might be explained by the reduction of medication used in this period, allowing higher dissemination of the infection. Our findings are similar to those observed in Canada, where farrow-to-finish herds were sampled (7). In USA, a lower seropositivity were found (1,5) among farrow-to-finish, breeding and finishing herds. Adequate cleaning and disinfection of the facilities reduces environmental *L. intracellularis* maintenance (9), preventing the infection of naïve pigs. *L. intracellularis* antibodies are present in all investigated herds, indicating high circulation of this bacterium in swine herds in MG.

**Acknowledgements:** CNPq, CAPES and Fapemig for financial support.

**References**


![Figure 1 - Serological profile for *L. intracellularis* antibodies. The bars indicate the standard error.](image-url)
EVALUATING THE USE OF AN ENVIROBOOTIE™ TO DETECT LAWSONIA AND SALMONELLA FROM KNOWN POSITIVE CONCRETE SURFACES

T Fangman, G Cline
Boehringer Ingelheim Vetmedica, Inc., St. Joseph, USA

Introduction: An EnviroBootie™ (Hardy Diagnostics) has been described in the poultry industry as a surveillance tool to identify the presence of Salmonella enteritidis following an FDA mandate for egg-layers. It is our intention to demonstrate the feasibility of a cotton mesh booty soaked in neutralizing broth as a surveillance tool for demonstrating the presence of enteric organisms in the growing pig environment. The objective of this pilot study was to evaluate the ability to detect Lawsonia intracellularis, Salmonella choleraesuis and Salmonella Typhimurium in the environment utilizing the environmental booty.

Materials and methods: Utilizing a 20ft x 60ft solid concrete slab (drive way), 3 blocks of 3 collection patterns (9 blocks total) were created. Each treatment block measured 10ft x 12ft. A 1 pint spray bottle was utilized to mist the contents of two 100 dose bottles of vaccine over the concrete surfaces (1x100 dose bottle of Enterisol® Ileitis and 1x100 dose bottle of Enterisol Salmonella T/C®; Boehringer Ingelheim Vetmedica, Inc.). The surface of each treatment square was sprayed with vaccine just prior to sample collection (entire surface was observed as saturated following application and prior to sample collection). The technician intentionally walked each of nine 10ft x 12ft squares so that 3 squares were walked in a cross pattern (squares: 1x, 2x, 3x) and 3 squares in a circle pattern (squares: 1c, 2c, 3c) and 3 squares by shuffling feet (squares 1s, 2s, 3s). *Note: walking pattern consisted of Heel-to-Toe steps across treated surface. The surveillance socks were removed from the technician and placed into individual plastic bags (2/bag) containing 50ml of DE neutral broth and identified by treatment number. All samples were submitted to BI HMC for Lawsonia PCR and ISU-VDL for Salmonella culture. Two socks were placed into a plastic bag with neutralizing broth without exposure to concrete surface to serve as a negative control.

Results: Table 1 shows all concrete samples were positive for Salmonella culture at ISU-VDL and all concrete samples were PCR positive for Lawsonia except Rep 3 when shuffling feet. The negative control sample was negative for Lawsonia PCR but positive via Salmonella culture.

Table 1 Detection of Salmonella and Lawsonia.

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Rep 1</th>
<th>Rep 2</th>
<th>Rep 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross Pattern</td>
<td>Law+, Sal+</td>
<td>Law+, Sal+</td>
<td>Law+, Sal+</td>
</tr>
<tr>
<td>Circle Pattern</td>
<td>Law+, Sal+</td>
<td>Law+, Sal+</td>
<td>Law+, Sal+</td>
</tr>
<tr>
<td>Shuffling feet</td>
<td>Law+, Sal+</td>
<td>Law+, Sal+</td>
<td>Law-, Sal+</td>
</tr>
<tr>
<td>Control</td>
<td>Law-, Sal+</td>
<td></td>
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</table>

Conclusions: The environmental bootie appears to be a valid and highly sensitive method for detecting Lawsonia via PCR and Salmonella via culture on a concrete surface independent of walking pattern utilized. However, walking in a circle or cross pattern across the concrete surface was 100% effective in detecting the known Salmonella or Lawsonia applied to the concrete surface. A positive Salmonella culture of the control sample (no concrete contact) suggests strict attention will need to be given to handling and packing of samples for shipping.

References:
A CASE STUDY OF MYCOPLASMA HYOPNEUMONIAE
GILT MANAGEMENT: ASSESSMENT AND INTERVENTION

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Introduction
Improper gilt management may result in replacement gilts that are either naïve or Mycoplasma hyopneumoniae (Mhp) shedders entering in a positive sow herd. This could result in sub-populations within the sow herds, leading to Mhp colonization of the suckling pigs, which has been shown to have a negative impact on pig performance. For this reason gilt management is a critical process and constitutes an important element of the systematic/whole herd approach of Mhp control. This case study documents the use of a previously proposed gilt diagnostic assessment protocol¹ leading to an intervention program with the final goal of minimizing exposure and maximizing immunity during the first gestation.

Materials and Methods
This case study was documented in a 800 sows farrow to finish farm located in southern Brazil. The farm is Mhp positive and receives Mhp positive replacement. The gilt assessment consisted in serum collection from 30 gilts with 150 days of age (doa). In addition 45 non selected gilts (same age) were selected and sent to the slaughterhouse for bronchial swabbing, using a technique previously described². On the sow herd the assessment consisted in serum collection from 30 P0 sows. Serologic assessment was performed by a Mycoplasma hyopneumoniae ELISA kit (IDEXX Laboratories, Inc., Westbrook, ME, USA). This assessment was performed before and after intervention. Before intervention, gilt program consisted in Mhp re vaccination at 150 doa and intense use of antibiotics during the complete gilt development process. After the diagnostic assessment the implemented/adjusted protocol (intervention) consisted in Mhp re vaccination at 90 doa and strategic use of antibiotics (targeting Mhp) at 140 doa using the parenteral route (IM). The second diagnostic assessment was performed 6 months later.

Results
Serologic assessment of 150 doa gilts and P0 sows before and after intervention is shown in Figure 1. Percentage of PCR positive gilts is show in Figure 2.

Conclusions and Discussion
Before intervention, the serological assessment, suggests an irregular exposure pattern of the agent during the gilt development process, and the agent identification assessment by PCR showed a late exposure and high level of shedding just prior entering the sow herd. This could cause the development of subpopulations in the sow herd, as seen in previous studies³,⁴. One of the reasons of irregular exposure could be the intense use of antibiotics targeting Mhp during the acclimation stage.

After intervention (adjustment of the protocol), seroconversion pattern suggests a homogenous and early exposure to the agent during the gilt management process. Recovery of the infection was documented at 150 doa, just prior gilt introduction to the sow herd by PCR (bronchial swabbing).

The introduction of new diseases and its perpetuation in the reproductive herd, is strongly associated with the introduction of gilts, leading to the presence of subpopulations³⁵. Adopted measures to reduce the infection pressure and maximize immunity of replacement gilts are a strategy to be used to better control Mhp, ensuring comprehensive control of this agent.

Figure 1: Mycoplasma hyopneumoniae seroprevalence results on serum collected from gilts with 150 days of age, P0 sows before and after intervention

Figure 2: Mycoplasma hyopneumoniae positive PCR results on bronchial swabs from gilts with 150 days of age.

References
DRAXXIN® 25 AT WEANING FOR CONTROL OF SWINE RESPIRATORY DISEASE

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¹Swine Services Unlimited, Inc., Rice MN USA and ²Zoetis Inc, Florham Park NJ USA

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 25 per label at weaning.

Materials and methods
One thousand and five weaned pigs from a sow farm experiencing a break with PRRS and Pasteurella multocida in the pre- and post-weaning phase of production were blocked by gender and weaning weight and were randomly enrolled to either Draxxin 25 or saline control groups. The Draxxin 25 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 20, and study day 148. From study days 1 to 20, the pigs were observed daily by a person blinded to treatment group. If any pig demonstrated a respiratory or depression score of ≥1 out of a 0-3 score range, the pigs were treated with Excede for Swine (ceftiofur crystalline free acid) per label and pigs 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 enrofloxacin per label. 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. All mortalities were necropsied and cause of death was determined by a licensed veterinarian who was blinded to treatment group. Enrollment body weight and ADG were analyzed by a linear mixed model approach. Percent retreatments, moves to hospital pen and mortality were defined as binary variables and analyzed using a generalized linear mixed model.

Results

Pasteurella multocida, Hemophilus parasuis and Streptococcus suis were isolated from the pneumonic lung tissue samples of pigs that died or were euthanized. Six pools of five serum samples were tested with PRRS PCR and all six pools were positive at enrollment.

Table 1: Results Summary Day 0-20

<table>
<thead>
<tr>
<th></th>
<th>Draxxin 25</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>N enrolled</td>
<td>502</td>
<td>503</td>
</tr>
<tr>
<td>% pulled to hospital pen</td>
<td>1.45ᵇ</td>
<td>3.28ᵃ</td>
</tr>
<tr>
<td>% treated once</td>
<td>9.78ᵇ</td>
<td>15.14ᵃ</td>
</tr>
<tr>
<td>% treated twice</td>
<td>1.28ᵇ</td>
<td>3.87ᵃ</td>
</tr>
<tr>
<td>% mortality</td>
<td>2.82ᵇ</td>
<td>6.69ᵃ</td>
</tr>
<tr>
<td>% mortality due to SRD</td>
<td>0.60ᵇ</td>
<td>2.38ᵃ</td>
</tr>
</tbody>
</table>

All percents are LSMeans. LSMeans with different superscripts within a row are significantly different at p≤0.05

Table 2: Results Summary Weights

<table>
<thead>
<tr>
<th></th>
<th>Draxxin 25</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrollment weight</td>
<td>12.60</td>
<td>12.60</td>
</tr>
<tr>
<td>ADG day 0-20</td>
<td>10.93ᵃ</td>
<td>9.9ᵇ</td>
</tr>
<tr>
<td>ADG day 0-148</td>
<td>223.52ᵃ</td>
<td>218.43ᵇ</td>
</tr>
</tbody>
</table>

All numbers are LSMeans. LSMeans with different superscripts within a row are significantly different at p≤0.05

Pigs treated with Draxxin 25 at weaning for control of SRD showed increased weight gain from both days 0-20 as well as days 0-148. This was a derived benefit of controlling SRD, which was evidenced by fewer deaths due to SRD as well as reduced rates of antibiotic treatment between days 0 and 20. This study demonstrates that controlling SRD in pigs at weaning with Draxxin 25 brings value by reducing mortality, morbidity and labor as well as by increasing pounds of pork available for market.

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A CLINICAL EFFICACY STUDY OF BAYTRIL 100 INJECTABLE SOLUTION FOR THE CONTROL OF COLIBACILLOSIS IN WEANED PIGS

AB Smith1, JS Nickell1, TL Settje1, DJ Keil1
1Bayer Animal Health; Shawnee, KS; U.S.A

Introduction
Diarrhea associated with Escherichia coli (E. coli; colibacillosis) is prevalent in populations of both neonatal and weaned pigs. In the nursery, colibacillosis is commonly observed within the first 1-3 weeks post-weaning and is generally associated with stressors encountered during this transition period. Given the rapid spread of the bacteria, disease control is most successful when instituted upon initial observation of clinical signs among a subset of the population. The objective of this study was to evaluate the clinical efficacy of Baytril®100 Injectable Solution for the control of colibacillosis in groups or pens of weaned pigs where colibacillosis associated with Escherichia coli has been diagnosed.

Materials and methods
This study was conducted according to VICH GL9 GCP quality standards. Five study sites located in four different states within the USA (IA, IN, NE, MD) served as test systems for the study. For each study site, candidate pigs sourced from commercial confinement swine production facilities arrived at each study site either the day of or day after weaning. The average weaning age and Study Day 0 body weight of candidate pigs across all five sites were 25.5 days and 13.5 lbs, respectively. Prior to arrival of candidate pigs, each study site procured a group of recently weaned pigs referred to as beta-hemolytic E. coli positive pigs (bHEC+ pigs) from which a minimum of 10 were required to be diagnosed as having colibacillosis. Laboratory isolation of beta-hemolytic E. coli from a rectal swab collected at the study site and clinical signs of diarrhea consisting of depression and gauntness scores confirmed colibacillosis in the bHEC+ pigs at each study site. Candidate pigs were commingled with the cohort of bHEC+ pigs and were observed daily for colibacillosis. Once a minimum of 5% of the candidate population was observed with clinical signs of colibacillosis in one day (which ranged from Day 2-7 post-commingling across the 5 sites), pigs were randomly allocated to one of two treatment groups and respective pens. Each study site administered a single intramuscular (IM) dose of either Baytril®100 (7.5 mg/kg) or saline to 300 pigs stratified into two groups, each consisting of 30 non-commingled pens of 5 pigs, resulting in a total of 60 pens of 5 pigs at each study site. The pen was considered the experimental unit. Enrolled pigs were observed for general health observations for 7 days and clinically scored for diarrhea on Study Day 7.

Results
Administration of Baytril®100 to weaned pigs either not yet clinically affected or clinically affected was effective for the control of colibacillosis. In this study, the percentage of clinically normal Baytril®100-treated pigs (61.5%, [95% confidence interval {95% CI}; 56.5% - 66.4%]) was significantly greater (p=0.0350) than saline-treated pigs (44.7%, [95% CI; 39.7% - 49.6%]) across all sites. No investigational product-related adverse events (AEs) were observed during this study.

Conclusions and discussion
Colibacillosis is a significant factor of production loss among recently weaned nursery pigs. A single IM dose of Baytril®100 (7.5 mg/kg) was shown to be efficacious in controlling the disease and is now indicated for the control of colibacillosis in groups or pens of weaned pigs where colibacillosis associated with Escherichia coli has been diagnosed.
**Introduction**

One of the most common problems in swine production is diarrhea in nursery pigs, which causes significant losses by weight loss, mortality, dehydration and cost of medication. Enterotoxigenic *E. coli* (ETEC) is the main agent of post-weaning diarrhea. Transmission occurs by fecal-oral route, and water may be an important contamination source. The aim of this study was to investigate the possibility of enterotoxigenic *E. coli* transmission through water, by isolating and typifying *E. coli* from nursery water samples and from rectal swabs from nursery pigs presenting post-weaning diarrhea.

**Materials and methods**

Fifteen rectal swabs from piglets with post-weaning diarrhea and one water sample were collected from each of ten nurseries in a State from southern Brazil. Piglet samples were cultured in Blood Agar and Mac Conkey Agar, and identified by biochemical tests. The same techniques were applied to water samples enriched using Colilert (Idexx). Following *E. coli* isolation, the genes for fimbrial adhesins (K88, K99, 987P, F18 and F41) and toxins (LT, Stb, StaP e Stx2e) were assessed by Multiplex PCR using QIAGEN® Multiplex PCR Kit.

**Results**

The virulence factors found by PCR are shown in Table 1. *E. coli* was isolated in four (40%) of the water samples out of 10 herds, and none expressed ETEC virulence factors. *E. coli* was isolated from 21/60 rectal swabs from diarrheic pigs from four nurseries, seven (11.66%) strains from three nurseries expressed ETEC virulence factors (at least 1 fimbria and 1 toxin simultaneously). From one of the herds ETEC was not detected neither in piglets or water. The most frequent fimbria was F18 (62.5%) and F4 (25%) and the toxins were STb (100%) and STA-P (75%).

**Conclusions and discussion**

ETEC was not found in the water of nursery barns, but was present in 11.66% diarrheic feces assessed. The low number of piglets infected with ETEC indicated that other aetiologies were involved. ETEC is an important agent causing post-weaning diarrhea, even though it seemed incapable to develop its virulence factors in the water. It is possible that this could depend on conditions present exclusively in the animal intestine. The results show that contamination of drinking water with ETEC was not an important cause of infection of the nursery piglets in the experiment. However, considering the low number of samples analyzed, these results must be repeated in larger scale to get a better understanding of the present results.

**References**


**Table 1: Fimbria and toxins found by Multiplex PCR on samples from piglets from 3 nurseries.**

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>Herd 1</th>
<th>Herd 2</th>
<th>Herd 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Stb, StaP, K88, LtB</td>
<td>StaP, Stb e F18</td>
<td>---</td>
</tr>
<tr>
<td>3</td>
<td>Stb, StaP e F18</td>
<td>StaP, Stb e F18</td>
<td>---</td>
</tr>
<tr>
<td>4</td>
<td>---</td>
<td>StaP, Stb e F18</td>
<td>---</td>
</tr>
</tbody>
</table>
EVALUATION OF MICE (Mus musculus) INVOLVEMENT IN THE EPIDEMIOLOGY OF PORCINE PROLIFERATIVE ENTEROPATHY

MP Gabardo, TP Rezende, J.P Sato, AGS Daniel, CER Pereira, MR Andrade, RMC Guedes
Animal Pathology Laboratory, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Introduction
Proliferative enteropathy (PE) is enteric infectious disease caused by Lawsonia intracellularis that affects different animal species. Interspecies transmission and the role of rodents in the epidemiology of disease are not fully understood. L. intracellularis DNA was detected in rodent feces trapped in horse (2) and hog farms known to be positive for bacteria (1). Collins et al (1) demonstrated that rats can shed about $10^3$–$10^5$ L. intracellularis per gram of feces and may be an important reservoir of this bacterium on pig farms (1). The aim of this study was to evaluate the fecal-oral transmission of L. intracellularis between mice and pigs.

Materials and methods
The study was divided in two parts. The first part aimed to check if mice could be infected by feces from L. intracellularis experimentally infected pigs. Thirty-four Swiss mice were allocated in 4 boxes, and received 20g/box of feces of experimentally infected pigs PCR positive for L. intracellularis (M1), for four consecutive days. Twelve other mice received swine feces negative for L. intracellularis (M2). Pool of mice feces were collected from each box on 0, 7, 14, 21, 28 days post exposition (dpe). All mice were euthanized on day 28 dpe and intestinal samples were collected for immunohistochemistry for L. intracellularis. The second part of the study aimed to test if pigs could be infected when exposed to PCR positive feces of L. intracellularis experimentally infected mice. In this second study, twelve 5-week-old pigs received feed mixed with PCR positive mice feces (S1), while other two pigs received feed with feces from negative mice feces (S2), for four consecutive days. All mice were inoculated with $9.35 \times 10^7$/gr of pure culture of L. intracellularis (PHE/MN-01). Pig feces and serum samples were individually collected on 0, 7, 14, 21, 28 dpe. All pigs were euthanized on day 30 dpe and intestinal samples were collected for immunohistochemistry for L. intracellularis. Serum samples were tested by IPMA for IgG anti-L. intracellularis, according the protocol described by Guedes et al. (3). Pig and mice feces were tested by PCR and nested-PCR according to Jones et al. (4), respectively.

Results and discussion
All pigs and mice used in the present study were negative for L. intracellularis at beginning of the studies. Animals in the negative groups (M2; S2) remained negative until the end of the experiments. In the first study, at least one box of mice of M1 were PCR positive for L. intracellularis on 7, 14 and 21 dpe. Using immunohistochemistry, three mice from the exposed group were positive at the end of the study. In the second study, 10 pigs (S1) were infected by L. intracellularis, based on PCR and/or immunohistochemistry positive results. PCR positive animals were detected from 7 to 30 dpe. Eleven pigs seroconvert between 21 and 30 dpe. Using immunohistochemistry, 50% of the pigs were positive. Despite the host specificity of L. intracellularis described by Sampieri et al (5), Pusterla et al. (6), bacterial fecal shedding was detected in foals that received feces from experimentally infected rabbits. Our study demonstrates that L. intracellularis experimentally infected mice and pigs can infected each other, therefore, rodent have to be considered as player in the epidemiology of the disease in hog farms.

Acknowledgements:
FAPEMIG for financial support.

References
EFFECT OF ENROFLOXACIN ON HAEMOPHILUS PARASUIS INFECTION, DISEASE AND IMMUNE RESPONSE

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¹Department of Veterinary Clinic and Surgery, Veterinary School, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
²University of Minnesota, College of Veterinary Medicine, USA

Introduction
Antimicrobial treatment is widely used to treat Haemophilus parasuis infections¹, but the effects of early elimination of H. parasuis before immunity is activated are unknown. We characterized the antibody and IFN-γ responses to H. parasuis in pigs treated with enrofloxacin before or after inoculation with a pathogenic H. parasuis strain to better understand the effect of enrofloxacin on immunity against H. parasuis.

Materials and methods
Sixty 3-week-old conventional pigs were divided into six groups. Pigs were either treated with enrofloxacin (Baytril® 100) 3 days prior (ABT/EXP) or 3 days after (EXP/ABT) intranasal inoculation with 10⁶ CFU/ml of virulent live H. parasuis strain at day 0. Control groups were either inoculated only (EXP), treated only (ABT), challenged only (CHA) or received no intervention (NEG). Nasal swabs were collected on days -3, 0, 2, 3, 5, 7 and 18 for H. parasuis isolation. ELISA was used to measure IgG in serum collected before and after inoculation and at necropsy. All groups, except the NEG, were challenged on day 21 with a higher dose (10⁸ CFU/mL) of the same H. parasuis strain, intranasally. Pigs were monitored for clinical signs daily. After challenge, surviving pigs were euthanized and necropsied on days 25 or 35.

Results
Pigs that were inoculated only (EXP group) and pigs that were treated with enrofloxacin and then inoculated (ABT/EXP group) developed signs of disease starting at 4 days post inoculation (DPI). Pigs from these groups also presented a significant increase on levels of serum IgG (Figure 1). This seroconversion was associated with protection against challenge. In contrast, pigs treated after inoculation (EXP/ABT group) did not have signs of disease or seroconverted after inoculation (Figure 1). EXP/ABT pigs as well as naïve control pigs [enrofloxacin only (ABT) and challenge only (CHA)] were susceptible to challenge. Variable levels of serum IgA antibodies, IgG and IgA in bronchoalveolar fluid (BALF) and IFN-γ responses were observed after H. parasuis inoculation in the different groups, but the values were not associated with protection.

Figure 1. Average levels of serum IgG antibodies optical density (OD) prior to start the study (day -3), prior to challenge (day 17) and at necropsy (days 25 or 35). Different letters indicate significant differences between groups (p<0.05).

Conclusions and discussion
In summary, enrofloxacin treatment before H. parasuis inoculation did not interfere with H. parasuis infection and subsequent seroconversion and protection against challenge. However, pigs treated with enrofloxacin after H. parasuis inoculation did not seroconvert and were susceptible to challenge. Therefore, timing of enrofloxacin administration in relation to H. parasuis infection is important to the immune response.

Reference
LOW PREVALENCE OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) BUT HIGH PREVALENCE OF MULTIDRUG RESISTANT STAPHYLOCOCCUS AUREUS (MDRSA) IN PIGS IN THE USA

Sun Jisun, Yang My, Davies R. Peter*
Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota, USA

It has been suggested that the emergence of livestock associated MRSA in swine may be a consequence of feeding antibiotics and/or zinc to control swine diseases or promote growth. During the past decade, numerous publications have reported about the prevalence of methicillin resistant S. aureus (MRSA) since the pigs have been deemed as a reservoir of livestock associated MRSA of human colonization or infection. Although there is clear evidence of a higher risk of colonization by MRSA in people working with pigs, the association between colonization and infection is still unclear. The predominant genotypes of MRSA in pigs vary geographically, with ST398 and ST9 being the predominant lineages in Europe and Asia respectively. Studies of MRSA in pigs in North America have variably reported predominance of ST398 or ST5, and also detected ST9 MRSA. In terms of risk factors of MRSA in pig farm, larger herds have an impact on higher probability of introduction of SA from pigs and a greater risk of being potential shedders to other susceptible animal. Other factors – type of farm, animal trade and environment hygiene etc., - are suggested but there was lack of strong epidemiological information of S. aureus at national level of swine population in the USA.

This study was conducted to estimate the prevalence and diversity of Staphylococcus aureus (SA) on 36 swine farms across 11 states in the USA and to characterize the antimicrobial and zinc susceptibility profiles in a purposive sample of 130 isolates selected by spa type and farm origin.

MRSA was not detected on any of the 36 farms, but 100% of pigs from a positive control farm yielded MRSA. SA was detected on 35 of the 36 farms, and from 76% of pigs sampled. A total of 33 spa types were detected, with the most prevalent being t337 (ST9), t034 (ST398) and t002 (ST5). 98% of isolates belonged to these 3 MLST lineages. Since we have extremely low MRSA positive farm, a risk factor analysis was not able to perform. However, based on SA prevalence, we found herd size has a positive factor for being SA positive on farm. Antimicrobial resistance testing for 17 antibiotics showed resistance was most common to spectinomycin (100%), tetracycline (94%), clindamycin (75%) and penicillin (72%). 89% (116/130) of isolates were resistant to 5 or more antibiotics (multidrug resistance SA, MDRSA). MRSA isolates from the positive control farm were positive for the czrC gene, which encodes zinc resistant related protein, but no other SA isolates tested were positive. However, 14% of SA (18/130) tested were phenotypically zinc resistance based on a break point of 4mM zinc. These results support earlier studies indicating a relatively low prevalence of MRSA in pigs in the USA, but a high prevalence of MDRSA. The data also concur with the hypothesis that zinc resistance is more strongly associated with MRSA than is resistance of antibiotics such as tetracyclines.

References
SALMONELLA ELISA SEROLOGY FROM WEANED PIGS AS AN INDICATOR OF SALMONELLA CIRCULATION IN BREED TO WEAN SITES

T Fangman\textsuperscript{1}, D Baumert\textsuperscript{2}, T Painter\textsuperscript{2}, B Whitt\textsuperscript{2}, M Ptaschinski\textsuperscript{2}, G Cline\textsuperscript{1}  
\textsuperscript{1}Boehringer Ingelheim Vetmedica, Inc., St. Joseph, USA, \textsuperscript{2}Cargill Pork, USA

\textbf{Introduction:} Understanding prevalence of Salmonella in market pigs has become a priority within USDA. With this priority pork production systems have a greater interest in understanding Salmonella prevalence in market pigs and the relationship of this prevalence to breed-to-wean sites. In a European study, a 90\% reduction in breeding herd Salmonella prevalence reduced market pig Salmonella prevalence in the lymph-node by 66\%.\textsuperscript{1} The objective of this study was to evaluate the ability of an indirect Salmonella ELISA antibody test (Vetsign\textsuperscript{TM}, Svanova) to detect Salmonella positive weaned pigs (5 weeks of age) and to determine if Salmonella antibodies at 5 and 9 weeks of age could be a predictor of Salmonella circulation at breed-to-wean sites.

\textbf{Materials and methods:} At risk breed-to-wean sites within a large pork production system were identified. These sites ranged in inventory from 300 to 800 sows. Salmonella risk factors identified at the selected sites included: 1) Open gutter flush 2) Rodent feces observed in barn 3) Sanitation practices below expectations. Weaned pigs from these sites were generally of good health and meeting company expectations only occasional post weaning diarrhea was observed. Twenty, 5-week-old pigs from 12 breed to wean sites were tagged and bled after placement into the nursery facility. If \( \geq 6 \) (30\%) of the pigs demonstrated Salmonella ELISA antibodies the prevalence was considered high. These same 20 pigs were then retested 4 weeks later. Decreasing Salmonella ELISA antibodies from these paired samples could then be interpreted as decreasing maternal antibodies. For this study, if \( \leq 5 \) weaned pigs were ELISA positive to Salmonella then the Salmonella prevalence of the breed-to-wean site was considered to be low.

\textbf{Results:} Table 1 demonstrates that sites A,B,C,E and F had \( \geq 30\% \) Salmonella ELISA positive pigs and that these ELISA titers decreased 4 weeks later in serial bled pigs (suggesting these antibodies were maternally derived). The ELISA titer rose at site D four weeks later in serial bled pigs (suggesting active exposure). At sites G-L the Salmonella ELISA positive pigs were <30\%. Pigs at sites G-L were not retested.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
\textbf{Site} & \textbf{5 wks old} & \textbf{9 wks old} \\
& \%(\%) & \%(\%) \\
\hline
A & 35 & 10 \\
B & 40 & 5 \\
C & 45 & 25 \\
D & 30 & 42 \\
E & 30 & 0 \\
F & 65 & 10 \\
G & 15 & N/A \\
H & 15 & N/A \\
I & 5 & N/A \\
J & 20 & N/A \\
K & 10 & N/A \\
L & 15 & N/A \\
\hline
\end{tabular}
\caption{\% Positive Salmonella ELISA (20 pigs serial bled/site)}
\end{table}

\textbf{Conclusions:} Utilizing Salmonella ELISA titers in 5 and 9-week-old pigs appears to be a predictor of Salmonella circulation of breed-to-wean sites. Salmonella serological assays demonstrate the variation of maternal antibodies between the sites. Sites where high maternal antibody circulation has been identified are a concern and suggestive of increased Salmonella pressure at the breed-to-wean site. Further investigation is planned at the source sites from which the 5-week-old pigs demonstrating \( \geq 30\% \) positive findings originated.

\textbf{References:}
**EFFECT OF SPRAY DRIED BOVINE PLASMA ON PERFORMANCE OF NURSERY PIGS WITH PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS**  
JD Crenshaw¹, D Bussières², J Polo¹, and JM Campbell¹  
¹APC, Inc., Ankeny, IA, USA and Groupe Ceres, Inc., St-Nicolas, Québec, Canada²

**Introduction:** Porcine reproductive and respiratory syndrome virus (PRRSV) is responsible for an estimated economic loss of $45 million in Québec [1]. Spray dried plasma is commonly used in nursery pig diets for its well documented superior effects on reducing post-weaning growth associated with weaning stress compared to other alternative protein sources [2]. Recent research indicates similar performance of pigs fed diets with either spray dried porcine or bovine plasma [3]. The objective of the current study was to determine the effect of diets with spray dried bovine plasma (SDBP) compared to a combination of alternative specialty proteins and additives on performance and mortality of PRRSV positive weaned pigs.

**Materials and Methods:** A total of 960 PRRSV positive pigs weaned at 21 d of age were allotted into 40 pens with 24 pigs per pen and fed a 3-phase nursery feed regimen (phase 1, d 0-14; phase 2, d 14-21; phase 3, d 21-48 post-weaning) either with 5%, 2.5%, and 0% dietary SDBP per respective phase, or a combination of alternative specialty proteins plus other feed additives (ALT). Phase 1 ALT diet consisted of a combination of egg and fish concentrate, peptones, highly digestible poultry protein, yeast culture and extract of brewer’s yeast, along with other additives including probiotics, prebiotics, proteases, sweeteners, organic acids, Na butyrate, and betaine, all of which were excluded from the SDBP phase 1 diet. Phase 2 ALT diet contained the combination of egg and fish concentrate and peptones along with Na butyrate. Phase 3 diet was common for both feed regimens and did not contain specialty protein sources.

**Results:** Serology revealed 4 pools of 10 samples as strongly positive from PRRSV by PCR, while ELISA indicated 31/40 samples with high level of PRRS antibodies. The PRRS strain had a homology of 99.34% with the strain found at the sow farm. Pigs fed SDBP diets had improved BW, ADG, ADFI and FG during d 0-21 of the study compared to the ALT diets. Over the entire study (d 0-48) pigs fed SDBP had improved ADG, ADFI, final BW and a strong tendency for reduced mortality and removals compared to the ALT feed regimen.

<table>
<thead>
<tr>
<th>Feed Regimen</th>
<th>SDBP</th>
<th>ALT</th>
<th>SEM</th>
<th>P =</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1-2 (d 0-21)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial BW, kg</td>
<td>5.98</td>
<td>6.00</td>
<td>0.04</td>
<td>0.74</td>
</tr>
<tr>
<td>ADG, g</td>
<td>320</td>
<td>294</td>
<td>4.31</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>ADFI, g</td>
<td>377</td>
<td>355</td>
<td>4.46</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>FG</td>
<td>1.178</td>
<td>1.207</td>
<td>0.006</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>BW d 21, kg</td>
<td>12.92</td>
<td>12.37</td>
<td>0.10</td>
<td>&lt;.01</td>
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<tr>
<td>Phase 1-3 (d 0-48)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG, g</td>
<td>463</td>
<td>444</td>
<td>3.79</td>
<td>0.02</td>
</tr>
<tr>
<td>ADFI, g</td>
<td>667</td>
<td>643</td>
<td>6.04</td>
<td>0.01</td>
</tr>
<tr>
<td>FG</td>
<td>1.442</td>
<td>1.448</td>
<td>0.005</td>
<td>0.33</td>
</tr>
<tr>
<td>BW d 48, kg</td>
<td>28.52</td>
<td>27.74</td>
<td>0.19</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Mortality, %</td>
<td>4.38</td>
<td>7.29</td>
<td>1.00</td>
<td>0.06</td>
</tr>
</tbody>
</table>

¹Values are least squares treatment means of 20 pens per feed regimen.

**Conclusions:** Nutrition provided by SDBP at 5% of diet for the initial 14 d and 2.5% of diet for d 14-21 after weaning resulted in 14 more pigs by the end of the study that were 0.78 kg heavier than pigs fed the ALT diets. The economic return over feed and medication costs for SDBP resulted in about a $2 per pig advantage. The SDBP feed regimen was more effective for reducing the negative effects of PRRSV on nursery pigs.

**References:**
ALBAC® AND SKYCIS® IMPROVE FINISHING PIG PERFORMANCE
R. Fleck1, D.A. Nelson1, C. Fralick2, D. Amodie1
1Zoetis Inc, Florham Park NJ USA and 2Swine Tek Research and Consulting, Convoy OH USA

Introduction
Albac (bacitracin zinc) is labeled for the improvement of both average daily gain (ADG) and feed efficiency (F/G) in swine when fed from 10-50 grams per ton. Skycis (narasin) is labeled for the improvement of both ADG and F/G in swine when fed from 18.1 to 27.2 grams per ton for at least four weeks. This study was conducted to compare the ADG and F/G of finishing pigs fed Albac (25 grams per ton) or Skycis (18.1 grams per ton) or a non-medicated control diet.

Materials and Methods
A total of 1222 PRRS negative, single source, mixed-sex pigs with an average initial weight of 72 lbs were utilized in this study. Pigs were individually weighed on study day -3, blocked by weight and sex and randomly assigned to three treatment groups; 1) Albac (25 grams/ton) 2) Narasin (18.1 grams/ton), and 3) non-medicated control. A total of 41 pens per treatment were enrolled. Blocks of six contiguous pens were randomly assigned to treatment (Albac males, Albac females, Narasin males, Narasin females, Control males and Control females). Treatments were initiated on day 0 and continued until day 84. All pigs were weighed on study days -3, 10, 60 and 84. Any pig removed from the study for any reason was weighed and the date of the removal was recorded. Feed weigh-backs were performed on the days that pigs were weighed. ADG and F/G were analyzed by a linear mixed model for repeated measures. Comparisons of LSMeans were performed by the two-sided Student’s t-test. A P value ≤ 0.05 was considered significant.

Results
Because there was a significant treatment by sex by period interaction for both ADG and F/G, data was analyzed by sex. The ADG of barrows and gilts consuming Albac or Skycis was significantly greater (p≤0.05) than control pigs during day 10-60 (Table 1.). From day 10 to 60, the F/G of Albac gilts was significantly lower (p≤0.05) than control gilts. There were no other significant differences among treatments for F/G. The final body weights (Table 2.) of pigs on day 84 reflected the improvement in ADG exhibited by pigs consuming Albac or Skycis.

Table 1. LSMean Production Parameters

<table>
<thead>
<tr>
<th>Day</th>
<th>Barrows</th>
<th>Gilts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Albac</td>
<td>Skycis</td>
</tr>
<tr>
<td>-3-10</td>
<td>2.50a</td>
<td>2.59a</td>
</tr>
<tr>
<td>10-60</td>
<td>2.38a</td>
<td>2.41a</td>
</tr>
<tr>
<td>60-84</td>
<td>2.29a</td>
<td>2.24a</td>
</tr>
<tr>
<td>FE</td>
<td>A</td>
<td>S</td>
</tr>
<tr>
<td>0-10</td>
<td>1.59a</td>
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</tr>
<tr>
<td>A=Albac, S=Skycis, C=Controls</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
a,b,cValues within sex and row with different superscripts differ significantly (p≤0.05)

Table 2. Mean Start and Final Weights (lbs)

<table>
<thead>
<tr>
<th>Study Day</th>
<th>Albac</th>
<th>Skycis</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>-3</td>
<td>72.0</td>
<td>71.71</td>
<td>71.98</td>
</tr>
<tr>
<td>84</td>
<td>261.40</td>
<td>261.75</td>
<td>257.43</td>
</tr>
</tbody>
</table>

Discussion
This study demonstrated that both Albac and Skycis significantly improve ADG over non-medicated diets from study day 10 to 60. Albac significantly improved the F/G of gilts compared to non-medicated control from day 10-60. There were no significant differences in the ADG or F/G of pigs consuming Albac or Skycis during any period. Pigs consuming either Albac or Skycis had numerically heavier mean live weights on study day 84.

Albac is a registered trademark of Zoetis Inc
Skycis is a registered trademark of Elanco
USE OF MIAKICK TO IMPROVE THE ENERGY AND NUTRITIONAL SUPPLY TO NEW-BORN PIGLETS

Laura Klatte¹, Alfons Heseker², C. Wecke¹
¹University of Göttingen, Göttingen, Germany, ²MIAVIT GmbH, Essen (Oldb.), Germany

Introduction: Pig farming has consistently recorded large financial losses due to mortalities in the suckling and rearing periods. Piglet losses of up to 15% of live birth piglets are no rarity. The highest number of piglet losses occurs within the first 72 hours after birth. Common causes of mortality include crushing by the sow and chilling and starving of the piglet due to inadequate colostrum intake. The increasing fertility of breeding sows is exacerbating this problem, as larger litters generally lead to falling birth weights. This ultimately results in a larger number of underweight and non-viable piglets. The objective of this experiment was to test the oral administration of the complementary feedstuff MiaKick under production conditions. The trials focused on the question of what effect MiaKick has on raising the survival chances of weak and underweight piglets and thereby reducing the overall mortality rate in the first days of life.

Materials and Methods: For the current study, 159 pregnant sows of the YOUNA breed, which had mated with Piétrain boars, were selected and randomly divided up according to their litter numbers into control and trial groups. The study was carried out in two successive trial stages with approximately 40 sows each in the trial and control groups per trial. The feeding regime and the housing of sows in farrowing pens was identical for both groups. Overall, 2,188 piglets born alive were included in the groups. The average birth weight of all piglets was 1.45 kg. The median litter size was 13.8 piglets per sow. Newborn piglets in the trial groups were orally administered with two doses each of a 2 ml of MiaKick. The first dose was given within the first 6 to 18 hours of life, the second followed within 12 hours of the first. The number of piglets born alive and piglet losses were recorded during the first five days after birth.

Results: Piglet losses up until the 5th day of life were reduced in both trial groups. In the first trial, there were 13% fewer piglet losses compared with the control group, while in the second trial group there were 8% fewer. The higher overall losses in the second trial are mainly attributable to the high percentage of gilts having their first litter. If one takes the relative deviation between the control and trial groups, only gilts, then piglets losses are 19% lower in the first trial and 27% in the second.

Table 1: Absolute and relative piglet losses on the fifth day of life

<table>
<thead>
<tr>
<th>Trials</th>
<th>1st</th>
<th>2nd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>c</td>
<td>t</td>
</tr>
<tr>
<td>All sows (n)</td>
<td>40</td>
<td>39</td>
</tr>
<tr>
<td>Piglets born alive</td>
<td>576</td>
<td>501</td>
</tr>
<tr>
<td>Piglet losses (5d)</td>
<td>49</td>
<td>37</td>
</tr>
<tr>
<td>Piglet losses (%)</td>
<td>8.5</td>
<td>7.4</td>
</tr>
<tr>
<td>Δ absolute (%)</td>
<td>-1.1</td>
<td>-0.9</td>
</tr>
<tr>
<td>Piglet losses (rel.) (100)</td>
<td>(100)</td>
<td>(87)</td>
</tr>
<tr>
<td>Δ relative (%)</td>
<td>-13</td>
<td>-8</td>
</tr>
<tr>
<td>Gilts only (n)</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Piglets born alive</td>
<td>104</td>
<td>89</td>
</tr>
<tr>
<td>Piglet losses (5d)</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Piglet losses (%)</td>
<td>12.5</td>
<td>10.1</td>
</tr>
<tr>
<td>Δ absolute (%)</td>
<td>-2.4</td>
<td>-3.7</td>
</tr>
<tr>
<td>Piglet losses (rel.) (100)</td>
<td>(100)</td>
<td>(81)</td>
</tr>
<tr>
<td>Δ relative (%)</td>
<td>-19</td>
<td>-27</td>
</tr>
</tbody>
</table>

Conclusion: Early stage oral administration of MiaKick aims to provide nutritional support to new-born piglets. In two successive trials, its positive effects were proven by lower piglet losses up until the 5th day of life. In particular, new-born piglets from gilt litters are frequently prone to energy deficits and higher rates of mortality. For these litters, the positive effect of MiaKick is especially high.
THE EFFECT OF ZINC SUPPLEMENTATION ON PERFORMANCE PARAMETERS IN PIGLETS

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Introduction: Post-weaning diarrhea is commonly caused by the pathogen *Escherichia coli* and causes considerable economic losses in rearing piglets. After adhesion to the intestinal mucosa *E. coli* produces toxins which induces the typical form of diarrhea. The latest concept to minimize the use of antibiotics in feed in Germany, as well as the occurring formation of resistance to these substances urged to rethink. The therapeutic use of zinc oxide in high doses (e.g. 2,500 ppm Zn) has yet demonstrated beneficial effects on growth performance in piglets as well as the prevention of post-weaning diarrhea. The precise mode of action is still not known, but recent results indicate that the zinc-ions affect the gastrointestinal milieu and thus prevent the adhesion of pathogens. While zinc is not only known as essential micronutrient but also as heavy metal, the supply of high amounts of Zn-oxide in diets is banned throughout the EU (limit: 150 mg / kg diet).

The objective of the current study was to investigate the effects of supplementing *MiaTrace Zn* (120 ppm Zn), which provides a high gastro-stability compared to a conventional zinc supplementation, on health and performance parameters in 7-30 kg piglets. Due to a special physicochemical fabrication process the zinc ions in *MiaTrace Zn* can be released targeted into the small-intestine. Therein the adhesion of coliform bacteria into the intestinal mucosa to be reduced.

Materials and Methods: For the current study 80 weaned pigs (21 d of age, 7.6 ± 0.6 kg BW) were randomly allocated into two treatment groups (10 pens per group with 4 piglets per pen). Both treatments received the same basal diet, equal at energy level, crude protein content, and Zn concentration (Treatment A: 120 ppm Zn from zinc oxide Treatment B: 120 ppm Zn from *MiaTrace Zn*). The animals were fed ad libitum with a two phase diet (starter: from 7.6 to 14 kg BW, grower: from 14 kg to approx. 30 kg BW). Data were collected individually for body weight and on pen average for feed intake. Fecal scoring was detected on day 14.

Results: ADG was not different in starter period, but pigs fed the diet supplemented with *MiaTrace Zn* had significantly higher ADG in the grower period (p = 0.019) as well as over the total trial period (p = 0.041). ADFI and FCR were not affected by the different types of Zn supplementation, still the amendment with *MiaTrace Zn* tended to a higher feed intake for pigs in grower period (Table 1). Fecal scoring showed no differences between treatment groups – overall no abnormalities or diarrhea appeared.

Table 1: Effects of Zn-supplementation on average daily gain (ADG), feed intake (FI), and feed conversion ratio (FCR) in 7-30 kg piglets

<table>
<thead>
<tr>
<th></th>
<th>A zinc oxide</th>
<th>B MiaTrace Zn</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADG in g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>starter</td>
<td>276</td>
<td>280</td>
<td>0.737</td>
</tr>
<tr>
<td>grower</td>
<td>545&lt;sup&gt;a&lt;/sup&gt;</td>
<td>585&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.019</td>
</tr>
<tr>
<td>total</td>
<td>435&lt;sup&gt;a&lt;/sup&gt;</td>
<td>460&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.041</td>
</tr>
<tr>
<td>ADFI, in g per d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>starter</td>
<td>390</td>
<td>392</td>
<td>0.911</td>
</tr>
<tr>
<td>grower</td>
<td>824</td>
<td>866</td>
<td>0.053</td>
</tr>
<tr>
<td>total</td>
<td>662</td>
<td>688</td>
<td>0.108</td>
</tr>
<tr>
<td>FCR in kg per kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>starter</td>
<td>1.42</td>
<td>1.40</td>
<td>0.549</td>
</tr>
<tr>
<td>grower</td>
<td>1.56</td>
<td>1.53</td>
<td>0.131</td>
</tr>
<tr>
<td>total</td>
<td>1.52</td>
<td>1.49</td>
<td>0.125</td>
</tr>
</tbody>
</table>

Conclusion: Due to a special physico-chemical fabrication process, free zinc ions can be released targeted from *MiaTrace Zn* to the small intestine. Our results indicate, that the supplementation with *MiaTrace Zn* could significant improve growth (ADG), while FCR was generally not affected. Furthermore, pigs fed the diet supplemented with *MiaTrace Zn* tended show higher FI and BW within the total period. Supplementing a diet with *MiaTrace Zn* can meet legal regulations of Zn supplementations in the diet but also improve performance parameters in piglets. Additionally, the environmental impact of high zinc oxide dosages can be reduced.
EFFECT OF HOT TEMPERATURE AND DRINKER TYPE ON GROWTH PERFORMANCE OF AND WATER DISAPPEARANCE BY GROWING-FINISHING PIGS

KD Vande Pol1, NS Grohmann1, TE Weber2, MJ Ritter2, and M Ellis1
1Department of Animal Sciences, University of Illinois, Urbana, IL, USA
2Elanco Animal Health, Greenfield, IN, USA

Introduction:
There is increased interest in delivering pharmaceutical compounds via water rather than in feed, a commonly used administration route. Accurate delivery of compounds via water requires a reliable estimate of water disappearance (intake + wastage) levels in pigs. There is little information in the scientific literature on water disappearance levels in growing-finishing pigs under commercial conditions. Also, there is limited information on the effect of factors such as drinker type and environmental temperature on water disappearance levels. The objective was to quantify water disappearance levels in grow-finish pigs under commercial conditions and determine the effect of hot conditions and of drinker type on water disappearance.

Materials and Methods:
A split-plot design was used with a 2 x 2 factorial arrangement of treatments: 1). Drinker Type (Nipple vs. Cup); 2). Temperature Regime (Thermoneutral (TN) vs. Hot). Temperature Regime was the main plot, Drinker Type was the sub-plot. 320 pigs were used, in 4 rooms with 8 pens/room and 10 pigs/pen (mixed sex). The study was carried out for 17 weeks from 10 weeks of age. Two rooms were kept at a TN temperature (decreasing from 29 to 18°C from start to end of the study period) and 2 rooms at the Hot temperature (30°C in the daytime and 20°C in the nighttime throughout the study period). Pens had fully slatted concrete floors with 1 feeder and 1 drinker/pen; floor space was 0.67m²/pig. Half of the pens had a nipple-type drinker, and the other half had a cup-type drinker. Pigs were phase fed industry standard corn-soy diets to meet or exceed nutrient requirements proposed by NRC (2012). Pigs were weighed at the start and end of the study period, and all feed additions to the feeder were recorded. Water disappearance was measured using a meter fitted to the water line supplying each pen.

Results:
Results are summarized in the Table. There were no (P > 0.05) Drinker Type by Temperature Regime interactions. Drinker Type did not affect (P > 0.05) growth performance. Water disappearance was greater (P < 0.01) for nipple than cup drinkers. Pigs on the Hot treatment were lighter (P < 0.05), grew more slowly (P < 0.001) and had a numerically greater water disappearance rate (P = 0.20) than those on the Thermoneutral treatment.

Conclusions and Discussion:
Drinker type did not affect growth performance, but water disappearance was greater for nipple than cup drinkers. This was most likely due to lower water wastage for the cup drinkers. Pigs kept under hot conditions grew more slowly and had numerically higher water disappearance rates. These results suggest that both drinker type and environmental temperature can have effects on water disappearance rates; further research is needed to quantify the amount of water wastage from pigs under commercial conditions.

References:
Porcine epidemic diarrhea virus (PEDV) is an *Alphacoronavirus* of the family *Coronaviridae* and was first identified in Iowa pigs in April 2013 (Stevenson et al., 2013). Transmissible gastroenteritis virus (TGEV) is also an *Alphacoronavirus* that shows clinical signs similar to PEDV. Porcine deltacoronavirus (PDCoV) of the *Coronaviridae* family was identified in Ohio in January 2014 (Wang et al., 2014). These enteric diseases mainly affect the small intestine and produce clinical signs of diarrhea and dehydration in pigs. The impact of these agents on growth performance has been described, there are no studies that have compared these three viruses simultaneously. Our objective was to determine and effect PEDV, TGEV, and PDCoV challenges on growth performance and protein accretion in growing pigs.

One hundred Choice Genetic gilts and barrows (BW = 9.81 ± 1.77) naïve for PEDV, TGEV and PDCoV were selected. Prior to allotment, pigs were weighed and a subset of 32 pigs were scanned using dual-energy x-ray absorptiometry (DXA) to determine starting whole body composition. Pigs were allotted into 1 of 4 treatments based on BW for a 42 d period. Treatments were: 1) control (n = 25); 2) PEDV inoculated (n = 25); 3) TGEV inoculated (n = 25); and 4) PDCoV inoculated (n = 25). On 0 dpi, PEDV, TGEV, and PDCoV challenged pigs were inoculated with respective virus at a rate of $10^3$ TCID$_{50}$/ml via gastric gavage. Infection was confirmed by PCR testing of feces. Pen was the experimental unit. All pigs were allowed free access to a standard corn-soybean meal diet and water. After 42 d, pigs were euthanized and DXA scanned to determine final whole body composition and protein accretion rates were calculated.

Pigs inoculated with PEDV had the greatest reduction ($P < 0.05$) in growth performance compared with controls (Table 1) with approximately 18% and 22% reductions in average daily gain (ADG) and average daily feed intake (ADFI), respectively, although, gain-to-feed ratio (G:F) was not different. Pigs inoculated with PDCoV had approximately 12% greater ($P < 0.05$) ADFI compared with controls and TGEV infected pigs had the same ADFI as controls. Pigs inoculated with TGEV had reduced ($P < 0.05$) G:F compared with all other treatments. Compared with controls, PEDV infection reduced ($P < 0.05$) protein accretion (g/d) by 20%. However, protein accretion (g/d) was not different in PDCoV or TGEV infected pigs compared with controls.

In summary, pigs inoculated with PEDV had the greatest reduction in ADG, ADFI, and protein accretion compared with controls. These findings suggest that PEDV infection has a greater impact on growth performance compared with controls than do PDCoV and TGEV infections.


### Table 1. Growth performance and tissue accretion of pigs inoculated with porcine epidemic diarrhea virus (PEDV), porcine deltacoronavirus (PDCoV), transmissible gastroenteritis virus (TGEV), and Control pigs over a 42 day period.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>PEDV</th>
<th>PDCoV</th>
<th>TGEV</th>
<th>SEM</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start BW, kg</td>
<td>11.06</td>
<td>9.71</td>
<td>11.65</td>
<td>11.54</td>
<td>0.567</td>
<td>0.084</td>
</tr>
<tr>
<td>End BW, kg</td>
<td>41.89</td>
<td>35.39</td>
<td>43.39</td>
<td>41.06</td>
<td>1.762</td>
<td>0.018</td>
</tr>
<tr>
<td>BW gain, kg</td>
<td>30.82</td>
<td>25.68</td>
<td>31.74</td>
<td>29.52</td>
<td>0.085</td>
<td>0.001</td>
</tr>
<tr>
<td>ADG, kg</td>
<td>0.72</td>
<td>0.59</td>
<td>0.73</td>
<td>0.65</td>
<td>0.039</td>
<td>0.032</td>
</tr>
<tr>
<td>ADFI, kg</td>
<td>1.11</td>
<td>0.87</td>
<td>1.27</td>
<td>1.14</td>
<td>0.047</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>G:F</td>
<td>0.70</td>
<td>0.62</td>
<td>0.60</td>
<td>0.53</td>
<td>0.026</td>
<td>0.001</td>
</tr>
<tr>
<td>Protein accretion, g/d</td>
<td>105</td>
<td>83</td>
<td>105</td>
<td>99</td>
<td>5.289</td>
<td>0.020</td>
</tr>
</tbody>
</table>

*Different letters represent significant differences within row ($P < 0.05$).

1Each parameter is represented by at least 8 observations per treatment.

2Estimated protein accretion obtained from dual-energy x-ray absorptiometry.
REPETITION PATTERNS OF REGULAR, IRREGULAR AND LATE RETURNS TO SERVICE OF FEMALE PIGS ON SOUTHERN EUROPEAN COMMERCIAL FARMS, AND THEIR LONGEVITY

S Tani¹, C Piñeiro² and Y Koketsu¹

¹School of Agriculture, Meiji University, Kawasaki, Japan, ²PigCHAMP Pro Europa, Segovia, Spain

Introduction and objective
Return to service increases nonproductive sow days, and consequently decreases herd productivity. The objectives of the present study were 1) to characterize 3 types of return to service occurrences and re-service intervals, 2) to examine factors associated with the returns to service, 3) to determine a repetition pattern of the returns to service and 4) to assess longevity of returned females using data from commercial farms in southern EU.

Materials and methods
This study analyzed 120,938 lifetime records and 653,483 service records of female pigs entered into 125 farms between 2008 and 2010. The re-service intervals were categorized into three groups: regular returns (RR: 18-24 days), irregular returns (IR: 25-38 days) and late returns (LL: 39 days or later). Multilevel generalized linear models with random intercept were applied to the data.

Results
Mean risks of RR, IR and LR per service were 3.6 ± 0.06, 2.5 ± 0.05 and 3.0 ± 0.06%, respectively. Of the 43,931 females having a first return, 32.7% had a second return in the same or later parity (Table 1). A chi-square test showed that the frequency distributions of increasing re-service intervals for serviced females differed depending on the number of services (P < 0.05; Fig 1). The proportion of RR females that had a third service was greater than those that only had a first service. In contrast, the proportion of IR females that had a third service was lower than those only had a first service.

For serviced gilts, higher RR, IR and LR risks were associated with being serviced in July to September (P < 0.01). Also, higher gilt age at first-servicing was associated with increased LR risk (P < 0.01), but not with that of RR or IR. For serviced sows, factors associated with higher RR, IR or LR risks were summer servicing, lower parity, farrowing more stillborn piglets, and having a weaning-to-first-servicing interval of 7 days or later (P < 0.01). In first serviced female pigs, the removal parity of no-return females was 0.6-1.2 higher than that of returned females (P < 0.01).

Conclusion
We recommended that producers take more measures to reduce return to service in high risk groups, such as ensuring a weaning-to-servicing interval of less than 7 days for re-serviced sows, or having a lower gilt age at first-servicing.

Table 1: Subsequent return occurrences (%) in 43,931 first-returned females at parity 0 to 6

<table>
<thead>
<tr>
<th>Parity at second return</th>
<th>First type of return occurrence, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>First return</td>
<td>N</td>
</tr>
<tr>
<td>0</td>
<td>15,957</td>
</tr>
<tr>
<td>1</td>
<td>10,511</td>
</tr>
<tr>
<td>2</td>
<td>5,950</td>
</tr>
<tr>
<td>3</td>
<td>4,596</td>
</tr>
<tr>
<td>4</td>
<td>3,320</td>
</tr>
<tr>
<td>5</td>
<td>2,280</td>
</tr>
<tr>
<td>6</td>
<td>1,317</td>
</tr>
<tr>
<td>Total</td>
<td>43,931</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Second type of return occurrence, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>1.0</td>
</tr>
<tr>
<td>2.0</td>
</tr>
<tr>
<td>3.0</td>
</tr>
<tr>
<td>4.0</td>
</tr>
<tr>
<td>5.0</td>
</tr>
<tr>
<td>6.0</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Figure 1: Relative frequency distribution (%) of re-service intervals by the number of services.

Reference
SPERM CELL SEDIMENTATION DOES NOT AFFECT THE QUALITY OF LIQUID-STORED BOAR SEMEN

MB Menegat1, APG Mellagi1, ML Bernardi2, I Wentz1 and FP Bortolozzo1

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Introduction
It has been suggested that sedimentation of sperm cells creates a harmful microenvironment, with unequal distribution of the extender components and exposure of spermatozoa to toxic metabolites1. Although rotation in liquid stored semen resulted in an improvement of sperm quality2, this beneficial effect has been recently contested3. The present study investigated the influence of rotation frequency on sperm quality during storage.

Materials and methods
Ejaculates from 28 boars were extended in split sample with BTS and Androstar® Plus (Minitüb GmbH) to 1.5 billion sperm in 45 ml total volume and conditioned in Flexitubes® (60 mL, Minitüb GmbH). Insemination doses were stored at 17.8 ± 0.49°C and assigned to one of the storage groups: NONE, non-rotated tubes; ONE, manual rotation once a day; TWO, manual rotation twice a day. Motility and kinetics parameters were assessed at 24, 72, 120 and 168 h after semen processing, using a CASA system (AndroVision®, Minitüb GmbH). Acrosome integrity was analyzed in a formalin-fixed sample using phase contrast microscopy (1000x) and sperm membrane integrity by double-staining with SYBR14/PI (LIVE/DEAD kit, Invitrogen™) and fluorescence microscopy (400x). Both analyses were performed at 72 and 168 h. Variables were analyzed as repeated measures (PROC GLIMMIX – SAS 9.4) considering fixed effects of group, extender, storage time and their interactions.

Results
Total motility (Figure 1) and progressive motility were negatively affected compared to NONE group, in BTS extender, at 168 h. Negative effect of rotation was evidenced in DCL, DAP, VCL and VAP (Table 1). DSL was reduced (P<0.05) in group TWO compared to group NONE at 168 h (6.95 vs. 7.79 µm). ALH of group NONE was superior to TWO at 72 h and to ONE and TWO at 120 and 168 h (P<0.05). Sperm membrane integrity was not influenced by rotation (P>0.05). There was no difference in damaged acrosome among the groups, except between ONE and TWO at 72 h in BTS extender (3.38% vs. 5.01% P<0.05).

Conclusions and discussion
Rotation of semen doses once or twice a day affects sperm motility after a long time period of storage, as observed in BTS at 168 h, probably due to cell ageing. Still, some sperm kinetic parameters were affected by rotation regardless the storage time. It has been suggested that rotation could increase the oxidative stress, decreasing cell motility3. Rotation process seems not to be necessary since sperm sedimentation had no adverse effects in sperm motion traits, membrane and acrosome integrity.

References

Table 1. Means of sperm kinetics parameters for none, once or twice rotation a day.

<table>
<thead>
<tr>
<th>Kinetics</th>
<th>NONE</th>
<th>ONE</th>
<th>TWO</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCL, µm</td>
<td>31.97a</td>
<td>30.51b</td>
<td>30.11b</td>
<td>1.34</td>
</tr>
<tr>
<td>DAP, µm</td>
<td>13.42a</td>
<td>12.83ab</td>
<td>12.66b</td>
<td>0.61</td>
</tr>
<tr>
<td>VCL, µm/s</td>
<td>86.57a</td>
<td>82.70ab</td>
<td>81.11b</td>
<td>3.94</td>
</tr>
<tr>
<td>VAP, µm/s</td>
<td>37.48a</td>
<td>35.87ab</td>
<td>35.13b</td>
<td>1.81</td>
</tr>
</tbody>
</table>

DCL: curved lined distance; DAP: average path distance; VCL: curvilinear velocity; VAP: average path velocity.
SEM: standard error of mean; a,b in the row (P<0.05).
USE OF PEDIGREED COMMERCIAL FEMALE DATA IN MATERNAL LINE NUCLEUS GENETIC IMPROVEMENT PROGRAMS

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¹ PureTek Genetics, Gentryville, IN
² National Swine Registry, West Lafayette, IN
³ Iowa State University, Ames IA

Maternal performance is one of several key profitability drivers in commercial swine units. Purebred breeders and commercial breeding stock companies strive to improve reproductive performance in their maternal lines through selection programs implemented in their nucleus herds. Genes of these genetically superior animals are then passed to the multiplication level and ultimately the commercial level as quickly as possible.

However, genetic selection programs are implemented at the nucleus level in high health, intensely managed herds. The genetic improvement at the nucleus level does not always translate into improved performance at the commercial level. The genetic correlation between reproduction at the nucleus level and the same traits at the commercial level has been estimated between 0.25 and 0.75. These results indicate a need to make nucleus selections utilizing a genetic evaluation system that includes reproductive records from pedigreed females at the commercial level.

Yorkshire and Landrace records from 2010 – 2015 from the National Swine Registry’s Swine Testing and Genetic Evaluation System (STAGES™) were utilized for this analysis. Pedigreed commercial female records from NSR members’ customers from the same time period were also included. Purebred records included were Number Born Alive (P_NBA), Number Weaned (P_NW), and Litter Weaning Weight (P_LWT). Each of these traits were pre-adjusted according to NSR breed specific guidelines (P_NBA for parity; P_NW for parity, age at breeding, and number after transfer; P_LWT for age at weaning, parity, and number after transfer). The same traits were utilized from purebred sows farrowing pedigreed F1 litters; these traits were pre-adjusted as well. Commercial records utilized were Number Born Alive (C_NBA), Number Weaned (C_NW), and Litter Weaning Weight (C_LWT) and were unadjusted.

All traits were evaluated in a six-trait evaluation using REMLF90. Parity, age at breeding, number after transfer and age at weaning were included in the six-trait evaluation as needed for the unadjusted commercial records.

Summary Statistics

<table>
<thead>
<tr>
<th>Litter Breed</th>
<th>Litter Records</th>
<th>NBA¹</th>
<th>NW¹</th>
<th>LWT, kg¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Landrace</td>
<td>11,199</td>
<td>11.28</td>
<td>11.37</td>
<td>66.3</td>
</tr>
<tr>
<td>Yorkshire</td>
<td>19,156</td>
<td>11.25</td>
<td>11.80</td>
<td>64.4</td>
</tr>
<tr>
<td>F1</td>
<td>20,304</td>
<td>11.59</td>
<td>11.66</td>
<td>69.2</td>
</tr>
<tr>
<td>Commercial</td>
<td>31,915</td>
<td>11.92</td>
<td>10.77</td>
<td>71.5</td>
</tr>
</tbody>
</table>

¹ Number born alive, number weaned, litter weaning weight raw means - pre-adjusted for pure and F1 litters, unadjusted for commercial litters

Heritability and Genetic Correlations

<table>
<thead>
<tr>
<th>Trait</th>
<th>Pure h²</th>
<th>Cross h²</th>
<th>r_g¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number Born Alive</td>
<td>0.08</td>
<td>0.11</td>
<td>0.54</td>
</tr>
<tr>
<td>Number Weaned</td>
<td>0.05</td>
<td>0.04</td>
<td>0.33</td>
</tr>
<tr>
<td>Litter Weaning Wt.</td>
<td>0.13</td>
<td>0.05</td>
<td>0.76</td>
</tr>
</tbody>
</table>

¹ Genetic correlation between pure and crossbred trait

Results show heritability is low for each trait, being consistent with previous results. The genetic correlation between purebred and crossbred traits is less than 1, indicating selection based on an index that includes only purebred data will not maximize the rate of genetic improvement in number born alive, number weaned, and litter weaning weight. Additional daughter records in the form of commercial females add significant accuracy to Breeding Value estimation, further improving genetic gain. Maternal genetic evaluation systems should be updated to include pedigreed commercial female records to enhance selection programs for reproductive traits.
EFFECTS OF ACETAMINOPHEN OR FLUNIXIN MEGLUMINE ANALGESIA PROTOCOLS ON HEPATIC AND RENAL SERUM PARAMETERS IN TWO TO FIVE-DAY-OLD PIGLETS

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1Triple-V services vétérinaires, Acton Vale, Canada
2Quebec, Canada

Introduction: In our modern society, there is growing concern regarding animal welfare. Some elective husbandry procedures such as castration and tail-docking can be painful. Consequently, all elective procedures and alternative options must be reviewed and evaluated regularly. In Canada, as of July 2016, providing analgesia to manage postsurgical pain will be mandatory1. Furthermore, as in humans, non-steroidal anti-inflammatory drugs’ (NSAIDs) pharmacodynamics and toxicological profiles may differ between neonates and adults due to physiological differences. The objective of this study was to investigate if different analgesic protocols could induce metabolic changes indicative of hepatic or renal toxicity in newborn piglets.

Materials and methods: 90 male piglets from 11 litters of a 600 sow barn were identified with a numerical ear tag and were subjectively categorized by weight (small, medium or large). The piglets were randomly assigned to one of the three treatment groups shown in table-1. Then, in chronological order, blood was drawn from the jugular vein, the treatment was administered and the castration and tail-docking procedures were applied. Blood was also drawn three and six days following processing. All frozen samples were sent to a commercial laboratory for analysis on the same day. The measured parameters were urea nitrogen, creatinine, phosphorus, alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT). Data was analysed using a linear model for repeated measures with litter as random effect. The Benjamini-Hochberg method was used to adjust P-values in multiple comparisons.

Table 1. Treatment groups

<table>
<thead>
<tr>
<th>Description</th>
<th>Dose</th>
<th>Route</th>
<th>#</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 Saline</td>
<td>1 ml</td>
<td>IM</td>
<td>29</td>
</tr>
<tr>
<td>T2 Pracetam (acetaminophen)</td>
<td>0.3 ml (60mg) diluted to 1mL</td>
<td>PO</td>
<td>30</td>
</tr>
<tr>
<td>T3 Banamine (flunixin meglumine)</td>
<td>0.05ml (2.5mg) diluted to 1mL</td>
<td>IM</td>
<td>31</td>
</tr>
</tbody>
</table>

Results: The serum parameters were very variable amongst individual piglets (Table 2). No statistical differences have been noticed in serum parameters when comparing the three treatment groups at days 0, 3 and 6.

Table 2. Least square mean parameters before (Day 0) and after (Day 6) administration of NSAID to piglets at processing (range).

<table>
<thead>
<tr>
<th></th>
<th>Urea mmol/L</th>
<th>Creat. µmol/L</th>
<th>Phosp. mmol/L</th>
<th>ALT U/L</th>
<th>ALP U/L</th>
<th>GGT U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>2.5-15.0</td>
<td>35-141</td>
<td>2.19-3.95</td>
<td>27-186</td>
<td>535-2243</td>
<td>132</td>
</tr>
<tr>
<td>Day 6</td>
<td>2.4-7.6</td>
<td>32-121</td>
<td>3.2-4.2</td>
<td>30-68</td>
<td>324-1367</td>
<td>121</td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>2.3-8.8</td>
<td>35-186</td>
<td>1.89-3.52</td>
<td>24-80</td>
<td>627-1869</td>
<td>83</td>
</tr>
<tr>
<td>Day 6</td>
<td>2.4-4.5</td>
<td>35-80</td>
<td>2.71-4.42</td>
<td>30-68</td>
<td>505-1572</td>
<td>98</td>
</tr>
<tr>
<td>T3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>2.2-4.7</td>
<td>35-80</td>
<td>2.16-4.56</td>
<td>31-142</td>
<td>776-2802</td>
<td>78</td>
</tr>
<tr>
<td>Day 6</td>
<td>2.3-4.5</td>
<td>35-80</td>
<td>2.88-4.59</td>
<td>39-1657</td>
<td>345-139</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion and discussion: The use of flunixin meglumine or acetaminophen before castration and tail-docking, as a mean to control pain, did not seem to influence the tested serum parameters. Consequently, it appears that these two NSAIDs do not induce any signs that could be related to hepatotoxicity or nephrotoxicity in young piglets.

References:
Modeling the probability of detecting PRRS over time
Ana Alba-Casals 1,*, Andres Perez 2, Robert B Morrison 1
Swine Disease Eradication Center, University of Minnesota, College of Veterinary Medicine, 385B Animal Science

Introduction
To control porcine reproductive and respiratory syndrome virus (PRRSv) it is important to determine whether the virus is present or has been eliminated. [1] The confidence in freedom from PRRSv infection in a pig herd is derived from sampling conducted over time. Different strategies to conduct consecutive samplings are used. The cost-efficiency differs according to the goal and the epidemiological circumstances of the herd. This study proposes a model to assess the probability of detecting PRRS and compares the cost-efficiency of different sampling strategies taking into account the context of the farm.

Material and methods
This model is based on the approach proposed by Martin el al. [2-4] to substantiate freedom of disease at population level and has been adapted according to the specific conditions of swine herds in multi-site production.

The model takes into account the sensitivity and specificity diagnostic tests, the minimum expected prevalence to be detected in the event that the herd is infected, the number of samples selected from the herd over the production cycle, the herd size, the initial probability that the herd is infected based on retrospective data, the probability of introduction of PRRSv during the elapse of time between consecutive samplings, the agreement between pig groups tested in different times, and the cost of each individual test.

As an example, we compared sampling approaches for two scenarios: (1) a multiplier that intends to become PRRSv negative (status 4), and (2) a commercial herd that intends to be stable (status 2vx or 2fvi). The multiplier herd has a very low risk of incursion (once every 10 years) and we compare sampling strategies A and B. The commercial herd has high incidence of lateral infection over the sampling period (once every 3 years) and we compare strategies C and D.

The model estimates the probability of having less than 5% prevalence based on negative outcomes after each consecutive sampling, computes the cost of each strategy, and computes the overall probability using the area under the curve (AUC) for a 12-month period.

Results
Table 1 compares the number of total samples, the sampling costs and the probability of being free over all a 12-month period for each scenario.

Table 1. Summary of inputs and outputs by each scenario

<table>
<thead>
<tr>
<th>SCENARIOS</th>
<th>INPUTS</th>
<th>SCENARIOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Herd size</td>
<td>3000</td>
<td>3000</td>
</tr>
<tr>
<td>Prevalence to detect</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Total # samples</td>
<td>610</td>
<td>340</td>
</tr>
<tr>
<td>Initial prob. Infection</td>
<td>0.49</td>
<td>0.47</td>
</tr>
<tr>
<td>Prob. of incursion between samplings</td>
<td>0.0083</td>
<td>0.0083</td>
</tr>
<tr>
<td>Cost of samplings</td>
<td>$3,050</td>
<td>$1,700</td>
</tr>
<tr>
<td>AUC (complete agreement among groups)</td>
<td>97%</td>
<td>97%</td>
</tr>
<tr>
<td>AUC (partial agreement among)</td>
<td>93%</td>
<td>85%</td>
</tr>
</tbody>
</table>

Discussion and conclusion
The model indicates important factors that influence the probability of been free at herd level over time and allows to determine the optimum sampling strategy.

References
PORCINE FACIAL INFERENCE TECHNOLOGY: COMPUTATIONAL ASSESSMENT OF FACIAL BIOMETRICS AS PREDICTORS OF CONSPECIFIC AGGRESSION

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North Carolina State University, Raleigh, USA

Introduction
Swine are an intelligent species that innately seek to establish and reinforce strict dominance hierarchies. When pigs are mixed with novel conspecifics, this drive to determine a peck order results in aggressive encounters that can cause an animal stress and put them at risk for injury. This is detrimental not only to the animal’s welfare, but also their productive potential. Research has shown post-mixing aggression to be a highly heritable trait, suggesting that fighting could be reduced through genetic selection. Like most behavioral indicators, however, individual variations in innate aggression can be difficult to quantify. Traditional ethological assessments, such as resident intruder or paired encounter tests, and even proxy measures like lesion scores, can be time and labor intensive to collect and difficult to interpret.

Within the equestrian community, there is a considerable amount of antiquated knowledge relating variations in the structural features of a horse’s face to aspects of innate personality. Facial Width-to-Height ratios have been correlated with aggression in capuchin monkeys, but an analogous relationship has not been explored in a porcine model. The purpose of this study was to assess the efficacy of facial biometrics in the prediction of individual differences in the innate aggressive tendencies of swine.

Materials and methods
Facial photographs were acquired from 120 piglets at 24-48 hours of age using an average quality 2D camera. Algorithms developed using the image processing toolbox in MATLAB for a previous project with horses were adapted to porcine facial structures, and a total of 38 facial biometrics were extracted from each piglet image. At two weeks of age, prior to weaning and mixing, 46 of these piglets were then randomly paired by gender and subjected to a paired encounter test with an unfamiliar conspecific in a novel environment. From behaviors observed during this encounter, piglets were assigned binary classifications for proactive and reactive aggression. For both these binary response variables, Wilcoxon ranked sum tests were used to screen for facial metrics with categorical potential, which were subsequently added to a series of increasingly complex logistic regression models optimized using the R software package.

Results
The current measures considered by the industry to inform grouping decisions - gender and weight - did not yield statistically significant models to predict aggressive outcomes of randomized pairings. Addition of facial biometrics significantly improved the predictive potential of both models (p<0.001), but only yielded a strong pseudo-R² value (0.50) for the reactive aggression model. Addition of interaction terms with fitness measures and facial metrics of the opponent were needed to achieve greater accuracy with the proactive aggression model. Final models for both proactive and reactive aggression yielded strong R² values of 0.64 and 0.69 respectively.

Conclusions
ANOVA analysis of nested models did confirm that facial biometrics accounted for a statistically significant portion of variability amongst the aggressive behaviors observed. Overall, facial metrics related to the eyes, forehead, and nose preformed the best, which agrees with traditional equestrian wisdom. Given the limited sample size, however, additional data would be needed to fully validate the robustness of model predictions. Results certainly substantiate further research into the potential use of facial biometrics as proxy measures for post-mixing aggression.

References
BEHAVIOUR AND PERFORMANCE EVALUATION IN PIGLETS AFTER MELOXICAM ADMINISTRATION IN PRE CASTRATION PROCEDURE

TS Gaggini¹, A Panzardi¹, TCF Silva², R Nunes¹, A Silva¹, MLG Rezende¹

¹Animal Health Technical Department (Ourofino Agronegócio Ltda.), Cravinhos-SP, Brasil
² Federal University of Uberlândia - UFU, Uberlândia - MG, Brazil.

Introduction
Castration procedure in male piglets is performed routinely in many countries to avoid boar-tainted meat. This procedure induces acute pain in piglets, and few studies have been shown that piglets may suffer pain for a prolonged period after the procedure which can lead to a productive performance reduction (2, 5). Some medications that reduce this pain have been studied, and one of them is meloxicam which have shown interesting results (1, 3, 6). This study aimed to evaluate behavior and performance of piglets with administration and with not meloxicam administration right before (30 minutes) castration procedure.

Materials and methods
This study was conducted in a commercial pig farm located at Minas Gerais State, in the Southeast region of Brazil. Forty-seven (47) piglets from 11 sows from different parity order (PO), average 3.58 ± 1.3 PO were utilized and divided in 2 groups. Group 1 was the control group (C) with 23 piglets and Group 2 the meloxicam group administration (M) with 24 piglets. All sows had similar body condition with average 3-3.5 and all teats viable. All piglets in the study were visibly healthy, randomly selected but matched by similar weight. Piglets from both groups were identified individually. At the moment of selection it was measured rectal temperature (RT) and each piglet was weighted (W) individually. Thirty minutes before castration procedure all piglets from M group were treated with 0.1 mL (0.4 mg/kgBW) of meloxicam by intramuscular injection. Two hours later after castration RT was measured and after two days RT and weight was taken again. All castration procedures from both groups were recorded to a further better behavior analyses. During the day and the day after castration all piglets were observed 10 times per day by 5 minutes in each observation. It were observed if some possible strange behavior (scratch) (S), some clinical signs (diarrhea) (D) and mortality occurred. The data obtained was analyzed by SAS Software (4). RT and ADG were analyzed by GLM model

and the behavior (scratch and diarrhea) analyzes were done by FREQ procedure.

Results
There were no statistical difference between C and M group in RT, W and ADG analyzes (Table 1). When analyzing behavior both S and D where better to piglets in M group, which showed lower percentage in both parameters when compared to C group.

Table 1. Performance and behavior parameters analysis between Control and Meloxicam group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Meloxicam*</th>
<th>P**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nº piglets</td>
<td>23</td>
<td>24</td>
<td>---</td>
</tr>
<tr>
<td>PCW, g</td>
<td>2678,26</td>
<td>2654,58</td>
<td>0,87</td>
</tr>
<tr>
<td>PCRT, ºC</td>
<td>39,25</td>
<td>39,23</td>
<td>0,92</td>
</tr>
<tr>
<td>PTCRT2h, ºC</td>
<td>39,04</td>
<td>38,96</td>
<td>0,46</td>
</tr>
<tr>
<td>PTCW, g</td>
<td>3050,25</td>
<td>3091,36</td>
<td>0,83</td>
</tr>
<tr>
<td>PTCRT2d, ºC</td>
<td>38,9</td>
<td>38,65</td>
<td>0,14</td>
</tr>
<tr>
<td>ADG, g</td>
<td>147,83</td>
<td>175,68</td>
<td>0,46</td>
</tr>
<tr>
<td>Scratch (%)</td>
<td>34,79</td>
<td>58,34</td>
<td>0,07</td>
</tr>
<tr>
<td>Diarrhea (%)</td>
<td>78,26</td>
<td>95,83</td>
<td>0,07</td>
</tr>
</tbody>
</table>

*Maxicam®injetável2%. Ourofino Animal health. Cravinhos-SP/Brazil. **No significant results (p>0,1). Significant results (p<0,1).

Conclusions and discussion
Although piglets in M group did not shown better performance when compared to C group it could be seen a better numerical ADG in the M group. Besides were found an association of better behavior in this group, especially in D parameter, which may lead that increasing the number of piglets per group it will be possible to find some important results. Further research needs to be done to better elucidate this previous results.

References
Introduction
Lameness is a welfare problem for sows and a common cause of economic loss for producers. An important first step in addressing lameness is quantifying the problem. There are few published studies addressing locomotion scoring in sows. We wished to understand whether one such scoring method was a reliable method of assessing lameness across multiple observers.

Materials and methods
Sows and gilts were housed at the Penn Vet Swine Teaching and Research Center. The facility is a 230 sow herd with large pre-implantation dynamic pens fed using an electronic sow feeder. The gilt pen contained approximately 65 animals (1.9 m$^2$) and the sow pen 230 animals (2.1 m$^2$). A variety of genetics are housed there including purebred show lines, PIC 1050 and DNA genetics line 241. Study sows varied in stage of gestation from 3 days post breeding to day 109 of gestation.

The scale used to assess sows was the Zinpro Feet First® Scale where 0= Moves easily, bears weight on all limbs; 1= Visible signs of lameness in at least one leg resulting in a change in weight bearing; 2=Lameness in one or more leg, compensatory gate changes like a head bob, hip hike or arched back; 3=Very obvious lameness with a reluctance to bear weight on one or more limbs.

All observers were trained using the training video provided by Zinpro. A scoring sheet was created using the wording from the training video. For the first session, where 20 sows were scored, all observers shared their scores for training purposes. After the first session, observers did not share their scores. Lameness assessment occurred over six sessions with 10-30 sows scored in each session. In all, 103 sows were assessed. Animals were scored by all 3 observers at the same time as they walked on a solid concrete hallway about 10 meters long.

Statistical analysis was done using Stata version 13.1. Cohen’s Kappa statistic was calculated as well as the percent agreement between observers. Strength of kappa values of 0.01 to 0.20 were considered slight agreement, 0.21 to 0.4 fair, 0.41 to 0.6 moderate, and 0.61 to 0.8 substantial.

Results
Analysis of kappa values found only “slight” to “moderate” agreement levels for the 4 possible scores (Table 1).

<table>
<thead>
<tr>
<th>Score</th>
<th>Kappa</th>
<th>Z</th>
<th>Prob&gt; Z</th>
<th>Strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.4306</td>
<td>7.57</td>
<td>0</td>
<td>Moderate</td>
</tr>
<tr>
<td>1</td>
<td>0.235</td>
<td>4.13</td>
<td>0</td>
<td>Slight</td>
</tr>
<tr>
<td>2</td>
<td>0.5913</td>
<td>10.39</td>
<td>0</td>
<td>Moderate</td>
</tr>
<tr>
<td>3</td>
<td>0.3268</td>
<td>5.74</td>
<td>0</td>
<td>Fair</td>
</tr>
</tbody>
</table>

Combined 0.3948 9.27 0 Fair

Percent agreement between rater 1 and rater 2 was 69.9%, between rater 1 and rater 3 was 62.1%, and between rater 2 and rater 3 was 74.8%.

Conclusions and discussion
Lameness in sows is a difficult problem to address. Reliable methods of assessing lameness are important in both research and production. Our study demonstrates the challenges in using a subjective scoring system to detect and quantify lameness when multiple observers are involved. Often the cause of lameness is unknown in the live animal, but a better understanding of the degree of lameness promises better targeting of management and therapeutic interventions.

Across this set of observers a score of 1 was least reliable as it was apparently the hardest degree of lameness for observers to consistently detect. Conversely a score of 2 was the most reliable, but still only exhibited moderate agreement between observers. Taken together these findings highlight the difficulties in assessing lameness with subjective scoring methods. The scale, as implemented here with these training methods, did not result in reliable scores by multiple observers. More work is needed to develop a subjective scale that is more reliable. This study also highlights the need for objective tools to measure sow lameness.

Acknowledgments
This work was funded by Zinpro Performance Minerals®.

References
2. Landi JR et al. 1977, Biometrics 33:159-74