2021 Allen D. Leman
Swine Conference Research Abstracts

18 – 21 September, 2021

Saint Paul, Minnesota, USA

The Allen D. Leman Swine Conference is an annual educational event for the global swine industry. It is internationally acclaimed for bringing science-driven solutions to the complex challenges facing theindustry. Each year hundreds of participants from over 20 countries attend the Leman Swine Conference held in Saint Paul, Minnesota.

Organized by: The University of Minnesota College of Veterinary Medicine and University of Minnesota Extension

Poster Number: 1
Topic Area: Other
Title: Comparative field efficacy study between ready to mix and ready-to-use vaccine in Korea
Author(s): Carlo Maala, Boehringer Ingelheim; Yusik Oh, Boehringer Ingelheim Animal Health Korea; JaeUn Ryu, DongAn Animal Clinic

Introduction
Porcine circovirus type 2(PCV2) and Mycoplasma hyopneumoniae (M. hyo) are two major pathogens causing Porcine respiratory disease complex (PRDC). These two pathogens result in serious economic losses in Korean swine industry (1). To mitigate economic losses, two different kinds of commercial combination vaccines are available requiring fewer injections and providing better efficacy. Acute phase proteins (APPs) have been proposed as suitable veterinary biomarkers to monitor stress, for detection of inflammation and infections (2). In this study, the efficacy like mortality, average daily gain and APPs were evaluated for the better decision.

Material & Method
The field trial was conducted on farrow to finish farm with 216 sows. On weaning day, 102 pigs were randomly divided into 2 groups equally and weighed, ear-tagged and vaccinated individually. Group 1 (n = 51) was vaccinated with 2 ml freshly prepared mixture of Ingelvac CircoFLEX® and Ingelvac MycoFLEX® (Boehringer Ingelheim Vetmedica GmbH). Group 2 (n = 51) was vaccinated with 2 ml Porcilis® PCV M hyo (Intervet international B.V., Netherlands). Body temperature was measured prior to vaccination and 6, 24 and 48 hours post vaccination in all study animals from behind the ear using contactless infrared thermometer. A subset of 15 piglets of each groups was subjected to blood sampling prior to vaccination as well as 24 and 48 hours post vaccination for determination of the acute phase proteins haptoglobin and C-reactive protein using Life Diagnostics ELISA kit. To evaluate the performance of the pigs in the two different treatment groups, mortality rate and weight gain were recorded for each group. Statistical analyses were performed by two-way ANOVA.

Results
Group 1(3.9%) showed lower mortality than group 2(5.8%). Both treatment groups showed an increase of acute phase proteins in serum as well as body temperature compared to basal levels. However, this increase in acute phase proteins was more pronounced in the group 2 vaccinated with Porcilis® PCV M hyo and thereby significantly higher compared to the group 1 vaccinated with mixture of Ingelvac CircoFLEX® and Ingelvac MycoFLEX®. Final weight and average daily weight gain did not show statistically significant differences.

Conclusions and discussion
Evaluated parameters pointed in favor of the mixture of Ingelvac CircoFLEX® and Ingelvac MycoFLEX® vaccinated group. The statistical significant increase of APPs resulted from higher stress and acute inflammation due to vaccination. Uniformity of weight variation in fattener period resulted in better profits due to high turnover of space. The findings of this study are consistent with other studies in that the selection of vaccines should certainly be based on efficacy as well as on their effect on piglets' well-being (3).

References
1. National swine disease surveillance 2016. KPPA
2. Piñeiro et al., (2007) The Veterinary Journal, 173, 669-674
3. E. Streckel et al. (2018). Proceedings of the 25nd IPVS, Chongqing, China

Poster Number: 2
Topic Area: Bacterial Disease
Title: An Evaluation of Enterisol Salmonella T/C® Vaccine in Conferring Protection Against Salmonella enterica serovar I 4,[5],12:i:-
Author(s): Fernando Leite, Boehringer Ingelheim Animal Health USA Inc.; Paulo Arruda, VRI – AMVC; Dianna Jordan, Boehringer Ingelheim Animal Health USA Inc.; Shawn Bearson, NADC, ARS – USDA

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

Materials and Methods:
A randomized, blinded controlled study was conducted evaluating pigs in three different treatment groups. Each treatment group consisted of 20 pigs, blocked for the effects of litter, sex and weight. The treatment groups were: 1) non-vaccinated challenged (NVC); 2) non-vaccinated non-challenged (NVNC); 3) vaccinated with Enterisol Salmonella T/C® and challenged (EVC). Animals were challenged four weeks post vaccination at approximately 7 weeks of age with a dose of 2x109 S. I 4,[5],12:i:-. Animals were monitored for clinical signs, macroscopic enteric lesions and evaluated for weight gain for a period of 14 days following challenge.

Results:
NVC animals had a significant increase in the incidence of diarrhea (68/266), compared NVNC pigs (0/280) and EVC pigs (12/280) ($p<0.05$). One pig in the NVC group died following challenge and had intestinal lesions compatible with enterocolitis due to salmonellosis. At 14 days post challenge, four pigs in the NVC group had intestinal lesions while no pigs had lesions in the NVNC and EVC groups ($p<0.05$). Average daily weight gain was significantly decreased in the NVC group (1.197 lbs) compared to NVNC group (1.693 lbs) and EVC group (1.536 lbs) ($p<0.05$).

Discussion and Conclusions:
This study confirms the impact that S. I 4,[5],12:i:- can have in swine both clinically and in production performance. Enterisol Salmonella T/C® is the only commercially available vaccine in the U.S. that contains a live Typhimurium serovar vaccine strain, a serovar of the same serogroup as S. I 4,[5],12:i:-.[3] The results suggest that the Enterisol Salmonella T/C® vaccine conferred protection to S. I 4,[5],12:i:- under the conditions of this study, as measured by clinical signs, intestinal lesions and improved average daily weight gain.

References:
1. Naberhaus SA et. al., Emergence of Salmonella enterica serovar 4,[5],12:i:- as the primary serovar identified from swine clinical samples and development of a multiplex real-time PCR for improved Salmonella serovar-level identification. J Vet Diagn Invest. 2019. 31(6):818-827.
2. Shippy DC, et al., Porcine Response to a Multidrug-Resistant Salmonella enterica serovar I 4,[5],12:i:- Outbreak Isolate. Foodborne Pathog Dis. 2018. 15(5):253-261.
3. Leite F., Salmonella enterica serovar I 4,[5],12:i:- update on risk factors and control. 52nd Annual Meeting of the American Association of Swine Veterinarians, 2021: 161-163.

Poster Number: 3
Topic Area: Viral Disease
Title: Similar protection with a single dose of Fostera® PRRS administered via mucosal, parental, or a combination of routes
Author(s): Kimberly Vonnahme, Zoetis; Micah Jansen, Zoetis; Deb Amodie, Zoetis; Manuel A. Vasquez-Hidalgo, Zoetis; Marnie Mellencamp, Zoetis; Lucina Galina Pantoja, Zoetis

Introduction
Heterologous prime-boost is a relatively new approach to vaccination that has been shown to improve immune response and disease protection in human and animal diseases[1,2]. In heterologous prime-boost protocols, the first (prime) and second (boost) doses may contain different antigens or are administered by different routes. The objective of this study was to determine the effects of different routes of vaccine administration on protection against PRRSV 1-7-4.

Materials and Methods
Three-week old weaned PRRSv-naïve pigs (n = 100; 20 per treatment) were randomly allotted by weight to 5 Fostera® PRRS (experimental serial L0817LW02; 4.36 Log_{10} $TCID_{50}$ per 2 mL) vaccination groups: 1) Saline-Saline; 2) IM-Saline; 3) IN-Saline; 4) IM-IM; and 5) IN-IM, where IM denotes intramuscular injection and IN denotes intranasal administration. Saline was administered IM. Treatment groups were housed in individual rooms so virus shedding (nasal swab), and circulating vaccine virus (serum) could be determined on study days 0, 21 and 49. On day 49, pigs were comingled and challenged with PRRSv 1-7-4 [$1x10^4$ $TCID_{50}$/4 mL; 2 mL IN (1 mL/nostril) and 2 mL IM]. Blood and nasal swabs were collected prior to inoculation and on days 3, 6, 9, and 12 post-challenge. Surviving pigs were necropsied 12 days post challenge and macroscopic lung lesions were assessed.

Results
All vaccination protocols reduced lung lesions ($P<0.01$) compared to controls [Saline-Saline 13.69 ± 2.84% vs. IM-Saline (1.38 ± 0.97%); IN-Saline (0.77 ± 0.75%). IM-IM (0.93 ± 0.79%), and IN-IM (1.00 ± 0.82%)]. Saline-Saline pigs had more ($P \leq 0.05$) viremia compared to all vaccinated groups. While all vaccinated pigs had similar levels of viremia by 12 days post-challenge, day 9 results showed lower ($P \leq 0.05$) Ct values in IN-IM pigs compared to IM-IM pigs, with IM-Saline, and IN-Saline pigs being intermediate. Nasal shedding was greater ($P \leq 0.05$) in Saline-Saline pigs compared to all vaccinated pigs by 6 days post challenge and remained higher through the end of the study. By 9 days post challenge, IM-IM pigs had greater ($P \leq 0.05$) Ct values than IM-Saline and IN-IM pigs; IN-Saline pigs had greater ($P \leq 0.05$) Ct values than IN-IM pigs. By 12 days post challenge, IM-IM pigs were similar in their Ct values as IM-Saline, but greater ($P \leq 0.05$) than IN-Saline and IN-IM vaccinated pigs.

Conclusions and Discussion
Overall, heterologous prime-boost and conventional vaccination showed similar efficacy, demonstrated by reductions in lung lesions. However, the route of administration or second booster dose differ in their ability to control viremia and virus shedding. A different route of administration may be of value to practitioners for developing mass vaccination protocols as IN administration was efficacious. For pigs naïve to PRRSV, there appears to be no efficacy advantages for priming via one administration route and boosting using a different one. Future studies are underway to determine if prime-boost vaccination protocols will help prevent disease in PRRSV positive flows.

References
1. Woodland D. (2004) Jump-starting the immune system: prime-boosting comes of age. Trends Immunol. 25:98-104.
2. Lu S (2009) Heterologous prime–boost vaccination. Curr Opin Immunol. 21:346–351

Poster Number: 4
Topic Area: Viral Disease
Title: Development of an Updated Porcine Reproductive and Respiratory Syndrome Virus Assay
Author(s): Mazen Ismail, Thermo Fisher Scientific; Angela Burrell, Thermo Fisher Scientific; Rohan Shah, Thermo Fisher Scientific; Michelle Swimley, Thermo Fisher Scientific; Bob Qiu, Thermo Fisher Scientific

Porcine reproductive and respiratory syndrome (PRRS) is a viral disease that leads to lower reproductive performance in breeding animals and respiratory disease in pigs. Due to the highly mutating nature of PRRSV and newly circulating strains, the original ThermoFisher Scientific™ PRRSV assay recently started exhibiting lower sensitivity with an occasional false negative test results when encountering a specific novel mutant sequence. A project was launched to update this assay based on the molecular design of the VetMAX™ PRRSV EU & NA 2.0™ assay which is available in Europe. A new assay design was constructed after bioinformatically analyzing nearly 1600 sequences from public and private databases as well as testing nearly 400 positive field samples in vitro. Testing results displayed the ability of the new assay to generate more accurate calls for NA PRRSV strains compared to the original PRRSV assay. The new assay has been externally validated by multiple collaborator labs and it generated concordant calls with the original assay in 94.6% of samples and generated more accurate call for 5.15% of samples previously tested by the PRRSV assay. The new assay significantly simplifies the testing setup process by using a 2-step master mix that includes all the reaction components compared to the 4-step protocol used by the original PRRSV assay. The updated assay runs in FAST mode and drops the run time down from 1 hour and 45 minutes to 49 minutes per run. This assay is for research use only and not to be used in diagnostic procedures.

Poster Number: 5
Topic Area: Reproduction
Title: Five risk factors and their interactions of probability for a sow in breeding herds having a piglet death during days 0-1, 2-8 and 9-22 days of lactation
Author(s): Yuzo Koketsu, Meiji University; Ryosuke Iida, Meiji University; Carlos Piñeiro, PigCHAMP Pro Europa

Introduction
An increased number of piglet deaths during lactation is one of the biggest concerns of veterinarians and producers related to sow performance and piglet welfare [1, 2]. Possible factors for pre-weaning piglet mortality risk for sows (PWM) as probabilities of a sow having a piglet death could be separately examined during each of three lactational phases. Our idea is to analyze PWM as a binary outcome during early, mid- and late lactation, because unlike sow deaths, piglet death events can occur multiple times at the sow level, with several piglets in a litter possibly dying at different times during a single lactation. Our objectives were 1) to characterize PWM during early (0-1 days), mid- (2-8 days) and late (9-22 days) lactation and 2) to quantify the following five factors and their interactions, parity, the number of piglets born alive (PBA), the number of stillborn piglets (SB), gestation length (GL) and season for PWM during the three lactation phases.

Methods
Data contained in 264,333 parity records of 55,635 sows farrowed in 2015 and 2016 from 74 Spanish herds. Three multi-level mixed-effects models were separately applied for PWM during three lactation phases. The PWM was analyzed as whether or not a sow had a piglet death (i.e. probability of a sow having a piglet) in each lactation phase.

Results
Mean PWMs during early, mid- and late lactation were 36.9, 27.0 and 14.7%, respectively. Four of the factors: parity, PBA, SB and GL were significant for PWM during all three lactation phases ($P < 0.01$). As PBA increased from 11 or less to 16 or more pigs, PWM during early, mid- and late lactation increased by 15.8, 6.0 and 0.8%, respectively. Also, as GL decreased from 117-120 to 110-113 days, PWM during early, mid- and late lactation increased by 7.5, 6.8 and 1.5%, respectively. Additionally, PWM during the respective lactation phases increased by 8.3, 5.2 and 1.0%, as SB increased from 0 to 3 or more pigs. Also, in early lactation parity 1 sows had 2.1% lower PWM than parity 5 or higher sows, but in mid- and late lactation they had 3.9-4.2% higher PWM ($P < 0.05$). However, there were no differences in PWM between summer and winter during any lactation phase ($P > 0.26$).

Conclusion
Management practices to reduce PWM need to take account of these factors, and be modified for different phases. For example, during early lactation special care should be given to piglets born to parity 5 or higher sows farrowing 16 or more PBA, having 3 or more SB or GL 110-113 days, whereas during mid- and late lactation more care should be given to piglets born to parity 1 sows with the same PBA, GL and SB conditions.

Keywords: farm data, piglet death, pre-weaning mortality, sow data

References
1. Muns R, Nuntapaitoon M, Tummaruk P. Non-infectious causes of preweaning mortality in piglets. Livest Sci. 2016; 84:46–57.
2. Koketsu Y, Tani S, Iida R. Factors for improving reproductive performance of sows and herd productivity in commercial breeding herds. Porcine Health Manag. 2017, 3: 1.

Poster Number: 6
Topic Area: Feed/Nutrition
Title: Use of a natural additive based on pronutrients to replace ractopamine and improve performance and meat leanness in grow-finishing pigs
Author(s): Narkie Nartey, IFTA USA, INC.; Julia Pie, Biovet, S.A.; José Omar Arratea Cama, Universidad Católica de Santa María, Arequipa (Peru); David Díez Arias, Biovet S.A., Tarragona (Spain); Carlos Domenech Rates, Biovet S.A., Tarragona (Spain); Alexander Daniel Obando Sánchez, Universidad Católica de Santa María, Arequipa (Peru)

Ractopamine is a β-agonist used as a growth promoter to obtain leaner meat [1] that has been banned in more than 160 countries. Many others, like the USA, interrupted its use since pork meat exports can be limited if this product is used. Pronutrients, commercialized as Alquernat® Nebsui, are active molecules from plant extracts such as Allium sativum that represent a natural alternative to the use of ractopamine as they allow to improve growth, feed conversion (FCR) and meat quality in a completely natural way [2].

Methods:
A trial was conducted to evaluate whether pronutrients could replace ractopamine in grow-finishing pigs. 42 fattening pigs from two different farms with an average weight of 48.19 kg were reared for the 28 days prior to slaughter. Feed administration was restricted according to weight. Pigs were distributed into three groups receiving different diets, each of the farms having the three treatment groups: (T1) diet without any growth promoter; (T2) diet supplemented with ractopamine hydrochloride at 10 ppm of the active principle; and (T3) diet supplemented with pronutrients at 0.5 kg/t. The two supplements were fed during all the trial. Each animal was considered as an experimental unit and the trial design was completely randomized with 3 treatments and 14 replicates each. Duncan's test was used to determine the differences between treatments. P values below 0.05 were considered significant. Performance parameters (weight, average daily gain and FCR) as well as scours were evaluated weekly. A scale from 1 (mild) to 5 (severe) was used to evaluate scours' severity. Carcass yield and backfat thickness were evaluated at the end of the trial.

Results:
The average weekly weight of pigs was higher in T3 (pronutrients) during the four weeks. At the end of the trial, T3 pigs weighted an average of 2.1 more kg per pig, compared to T1 (control diet), despite this, no significant differences were observed between groups. Average daily gain got numerically improved in T3, with a final improvement of 6.8%, compared to T2. All the supplemented groups obtained higher average daily gains than T1 (+5.3% and +12.4% respectively for T2 and T3). Similar results were obtained regarding the FCR, as it got improved in the two supplemented groups ($P<0.05$) compared to T1. Pigs during the trial did not suffer from diarrhea. Despite this, the average scours' severity was significantly lower in T3 ($P<0.05$), compared to the other two groups. At the end of the trial, carcass yield got increased in T3 ($P>0.05$) compared to T1 and T3; and backfat thickness was significantly smaller in T3 and T2, compared to T1.

Conclusions:
Results in this trial show a trend of pronutrients to improve weight gain and carcass yield. Additionally, pronutrients and ractopamine significantly improve FCR and reduce backfat thickness to obtain leaner meat. Certainly, these parameters point out that pronutrients are an effective alternative to ractopamine in grow-finishing pigs. Besides, pronutrients are natural molecules that do not leave residues and are do not limit pork meat imports and exports.

Citations:
[1] M. J. Ritter et al., "Effects of Ractopamine Hydrochloride (Paylean) on welfare indicators for market weight pigs," Am. Soc. Anim. Sci., 2017.
[2] J. Borrell, "Uso de pronutrientes de origen natural en veterinaria," RACVE (Real Acad. Ciencias Vet. España), 2005.

Poster Number: 7
Topic Area: Viral Disease
Title: Heterologous prime-boost vaccination with commercial swine influenza virus vaccines: revising the traditional vaccination strategy
Author(s): Anna Parys, Ghent University, Faculty of Veterinary Medicine, Laboratory of Virology; Elien Vandoorn, Ghent University, Faculty of Veterinary Medicine, Laboratory of Virology; Koen Chiers, Ghent University, Faculty of Veterinary Medicine, Laboratory of Veterinary Pathology; Kristien Van Reeth, Ghent University, Faculty of Veterinary Medicine, Laboratory of Virology

Introduction
Respiporc® FLU3, GRIPORK® and Respiporc® FLUpan H1N1 are commercial swine influenza A virus (swIAV) vaccines available in Europe. These are whole inactivated, adjuvanted trivalent (TIV), bivalent (BIV) and monovalent (MOV) vaccines respectively, including different H1N1, H1N2 and H3N2 IAV vaccine strains. The antigenic diversity of swIAVs continues to increase and the current vaccines protect only against virus strains that closely match the vaccine strains. Alternative strategies that may broaden the protection induced by commercial swIAV vaccines are therefore needed. We have previously performed pig vaccination experiments with experimental monovalent, whole inactivated and adjuvanted vaccines based on various H1N1 and H3N2 IAV strains. These experiments showed that the use of different vaccine strains for the first and subsequent vaccinations is a valuable strategy to broaden the immune response. Three sequential administrations of heterologous H1N1 vaccine resulted in seroprotective (≥40) hemagglutination inhibition (HI) titers against 75% of the H1N1 strains examined, compared to 21% for a matched homologous prime-boost vaccination regimen. It remains unknown whether this heterologous prime-boost strategy is also advantageous with different European commercial swIAV vaccines. Here we explored the antibody response and protection against challenge after vaccination with commercial swIAV vaccines in a homologous or heterologous prime-boost vaccination regimen.

Methods
Thirty-six influenza-negative pigs were allocated to six groups that were vaccinated three times intramuscularly at four- to six-week intervals. Three groups received three sequential administrations of the same vaccine (homologous prime-boost), two groups received three different vaccines (heterologous prime-boost) in a different order. The sixth group was mock-vaccinated and served as a naïve challenge control group. Four weeks after the last vaccination, all pigs were challenged intranasally with a recent European avian-like H1N1 swIAV antigenically different from all vaccine strains. Serum was collected four weeks post each vaccination to determine HI and neuraminidase inhibition (NI) antibody titers against sixteen antigenically divergent swine and human IAV, including vaccine and challenge viruses. Three days after challenge, all pigs were euthanized and tissues of the respiratory tract were collected for virus titrations and (histo)pathology.

Results
One heterologous prime-boost group (TIV-BIV-MOV) had enhanced HI and NI antibody responses compared to the other heterologous prime-boost group (BIV-TIV-MOV) and the homologous prime-boost groups (3xTIV; 3xBIV; 3xMOV). TIV-BIV-MOV had seroprotective HI titers against 50% of the tested viruses compared to 38% in BIV-TIV-MOV and 13-38% in the homologous prime-boost groups. In addition, the same heterologous prime-boost group (TIV-BIV-MOV) had significantly reduced virus titers in the respiratory tract compared to the challenge control group and, except for one pig, complete protection in the lungs. The other vaccinated groups had significantly reduced titers in the trachea only (BIV-TIV-MOV and 3xBIV) or no significant reduction at all (3xTIV and 3xMOV).

Conclusions
Our results suggest that the use of different commercial swIAV vaccines for successive vaccinations may result in broader antibody responses and protection than the traditional, homologous prime-boost vaccination regimens. In addition, the order in which the different vaccines are administered seems to have an impact on the breadth of the antibody response and protection.

Poster Number: 8
Topic Area: Viral Disease
Title: Heterologous Prime-Boost Vaccination Expands Cell-Mediated Immunity Targets for Influenza
Author(s): Lucina Galina Pantoja, Zoetis; M. Jansen, Zoetis; C. Li, University of Minnesota, St. Paul ; M. Torremorell, University of Minnesota, St. Paul ; M. Culhane, University of Minnesota, St. Paul; A. Gutierrez, EpiVax Inc, Providence, RI

We recently explored heterologous prime-boost vaccination approaches to enhance influenza control in pigs[1]. By priming with one vaccine (trivalent autogenous -AUT) and boosting with a different vaccine (quadrivalent commercial - COM), we observed decreased viral shedding, increased hemagglutination inhibition (HI) serological responses, and fewer reassortants compared to pigs that were primed and boosted with the same vaccine (AUT or COM). All pigs in the study received a dual H1N1 gamma and human-like H3N2 influenza challenge using a seeder pig model.[1,2] These improved clinical outcomes were observed with a heterologous prime boost even though the AUT vaccine had a better aminoacid match to the challenge strains, suggesting that the benefits to heterologous prime boost were not uniquely related to genetic homology. This study explored cell mediated immunity aspects and addressed whether heterologous prime-boost vaccination broadened T-cell epitope targets against two prevalent IAV strains using influenza strain sequence information from Li's study[1].

Materials and Methods: Hemagglutinin sequences of FluSureXP (COM), Ingelvac Provenza (LAIV) and autogenous (AUT) vaccines, and two challenge strains (H1N1 gamma and human-like H3N2) were screened for potential class I and II T-cell epitopes. T-cell epitopes of vaccines and challenge strains predicted to bind common SLA (Swine Leukocyte Antigens) alleles were compared to assess T-cell epitope coverage (i.e., percentage of challenge strain epitopes matched by the vaccine), as described by EpiVax.[3]

Results: For H1, ~19.7% increased coverage (to 82.47%) was found when using LAIV and COM compared to LAIV alone (68.9%). For H3, a 24.9% increase of epitope coverage (to 60.34%) was found compared to using only LAIV (48.30%). For the COM/AUT combination, there was a 13.2% increase coverage (to 93.96%) for H1 compared to using the COM (81.63%) or AUT (82.98%) alone. Some H3 coverage (54.36%) was observed between H3N2 cluster IV vaccine strains (COM) and the human-like H3N2 strain, but it may confer partial or no protection. No significant improvement was observed with the COM/AUT combination for H3, but this was expected as AUT was the only vaccine containing an H3N2 human-like strain of the same clade and the highest similarity to the challenge strain.

Conclusion: New influenza strains infect pig farms frequently. A robust strategy using a vaccination approach that offers the broadest antibody and cell-mediated coverage against new strains is desirable. A heterologous prime-boost protocol can increase cell-mediated recognition, in addition to the other benefits previously reported, such as better protection against influenza infection and decreased economic losses. Ready to use commercial vaccines with USDA approval should be part of the heterologous prime-boost vaccination protocol. Swine influenza is complicated, and selecting vaccination protocols supported by sound science can improve swine health and performance.

References:
1. Li et al., Vet Res (2020) 51:89.
2. Li et al., Proceedings Leman Conference (2020) 17.
3. Gutierrez et al., BMC bioinformatics (2015) 16:290.

Poster Number: 9
Topic Area: Reproduction
Title: Determination of puberty using salivary progesterone in gilts
Author(s): Kimberly Vonnahme, Zoetis; Manuel A. Vasquez-Hidalgo, Zoetis; George Perry, Texas A&M University AgriLife Research; Deb Amodie, Zoetis; Marnie Mellencamp, Zoetis; Lucina Galina Pantoja, Zoetis

Attainment of puberty in gilts can be determined by behavioral estrus, or experimentally by assessing circulating levels of progesterone, as the first ovulation is often silent (i.e., no exhibited estrus). It is labor intensive and stressful to both gilts and people to obtain blood samples for analysis. Our hypothesis was that salivary progesterone could be a marker for cyclicity in gilts, and that gilts treated with Improvest® (IMV), a gonadotropin releasing factor (GnRF) analog-diphtheria toxoid conjugate, which immunologically blocks GnRF, would have decreased salivary progesterone compared to untreated controls (CON). The objective was to compare salivary progesterone concentrations collected weekly from IMV and CON gilts and their ovarian structures at harvest in market gilts.

Material and Methods
This investigation was part of a larger study (i.e., 120 pens; 21 gilts/pen). At 8 weeks of age (WOA), pens were randomized by weight to treatment. Pens of gilts assigned to the IMV treatment received their doses at 9 and 19 WOA. Weekly saliva samples (19 to 28 WOA) were collected via a cotton rope hung (20 min) in 12 IMV and 12 CON pens. Saliva was frozen at -20°C until centrifugation. Progesterone was validated and analyzed via radioimmunoassay. The intra- and inter-assay CV were 5.1% and 6.0%, respectively. At 25 WOA, pens of gilts (n=30 IMV; n=30 CON), not being sampled for progesterone, were harvested. Reproductive tracts were collected (n = 109 IMV; n = 120 CON) and ovaries that had corpora hemmorhagica, corpora lutea, and/or corpora albicans were considered cycling. Progesterone area under the curve was analyzed by a generalized linear mixed model approach (GLMM) with fixed effect of treatment and random effects of room, block-within-room and the residual error. Cyclicity determined by ovarian score was defined as a binary variable and analyzed by a GLMM approach with the same model as stated above but utilized a binomial error and logit link.

Results
There was more ($P<0.01$) progesterone secreted in CON (285.64 ± 14.91) vs IMV (166.16 ± 14.91) gilts. There were more ($P<0.01$) cycling CON vs. IMV gilts (52.5 ± 0.05 vs. 0.92 ± 0.01%).

Discussion
Salivary progesterone concentrations are a non-invasive method to determine cyclicity in pens of gilts. While puberty in an individual gilt cannot be assessed with pen collections, it is well known that pens of gilt will have synchronized cycles. The use of IMV allowed for the investigation of progesterone concentrations in gilts that were similar in age, being fed similar diets, and within a similar environment. Salivary progesterone may prove to be a good biomarker for cyclicity in breeding females.

Poster Number: 10
Topic Area: Viral Disease
Title: Optimal time for boost in an influenza vaccination protocol
Author(s): Micah Jansen, Zoetis; Kimberly Vonnahme, Zoetis; Deb Amodie, Zoetis; Manuel A. Vasquez-Hidalgo, Zoetis; Marnie Mellencamp, Zoetis; Lucina Galina Pantoja, Zoetis

Introduction
Recent influenza A virus in swine (IAV-S) challenge studies have reported that vaccination boosting is advantageous for overall effectiveness of disease control. Authors noted that several aspects need to be considered including: time between doses, route of administration, adjuvant type, and type of vaccine. We hypothesized that there is an optimal interval for time between doses in a homologous prime boost protocol using a commercially available quadrivalent whole inactivated virus (WIV) influenza vaccine. Humoral immunity to the vaccine IAV-S strains was measured by hemagglutination inhibition (HI) titers. Cell mediated immunity (CMI), was measured by interferon (IFN)-γ release by ELISPOT.

Materials and Methods
At weaning (day 0), pigs (n = 120) received their priming dose of a WIV vaccine (containing H1 gamma, H1 delta 1, H3 Cluster IV-A, and H3 Cluster IV-B), and were randomly assigned to receive their boosting dose on days 28 (n = 24), 42 (n = 24), 56 (n = 24), 70 (n = 24), or 84 (n = 24). Blood samples were collected 7 and 14 days after the boost to determine IFN-γ release and HI titers, respectively. HI testing was run for H1N1 gamma (homologous to vaccine) and H3N2 human-like (non-vaccine related). All HI titers (log10 +1) and ELISPOT counts (log +1) were transformed. Variables were analyzed using a generalized linear mixed model approach with the fixed effect of treatment and the random effects of block and the residual error.

Results
There was no effect (P = 0.3812) of time of the boosting dose of vaccine for the H1N1 HI titer. The interval between the doses influenced (P = 0.0059) the HI titers for H3N2. Titers for H3N2 were increased (P ≤ 0.05) in pigs receiving their boost on days 42 (151.6 ± 30.5), 70 (156.1 ± 31.4), and 84 (192.9 ± 39.6) from the priming dose compared to pigs that were boosted at 28 days (70.6 ± 14.2). Pigs boosted at 56 days (117.1 ± 23.5) were intermediate. Cell mediated immunity differences were determined by IFN-γ secretion specific to the same strains that HI titers were determined. While there were no differences (P = 0.9263) in IFN-γ secretion specific to H3N2, H1N1 was reduced (P = 0.0152) in pigs with a 42- (32.2 ± 10.0 spots /106 cells; spc) or 84- (27.4 ± 8.6 spc) day interval between the priming and boosting dose having reduced IFN-γ compared to those pigs with a 28 (119.6 ± 37.4 spc)-day interval. Pigs with a 56- and 70-day interval were intermediate (56.9 ± 17.8 and 49.8 ± 15.5 spc, respectively).

Conclusions and Discussion
This study demonstrated that protective HI titers were observed with up to a 12-week interval between the priming and boosting doses of a commercially available influenza vaccine. This was true against both strains of IAV evaluated. The CMI responses did not follow HI titer patterns, and therefore the role that CMI plays to compliment humoral immunity needs to be delineated. Validating the 12-week interval between doses supports the use of quarterly vaccination against influenza, a common strategy in US swine herds.

Poster Number: 11
Topic Area: Viral Disease
Title: Comparison of T cell epitope content to predict coverage against circulating influenza A viruses by a commercially available influenza A virus vaccine
Author(s): Micah Jansen, Zoetis; Lucina Galina Pantoja, Zoetis; Andres Gutierrez, EpiVax

Introduction
As influenza A virus in swine (IAV-S) continues to evolve, vaccine selection has become increasingly difficult for swine producers and veterinarians alike. Often the only method available for comparison of field strains to vaccine strains (both commercial and autogenous) is comparison of the amino acid sequence of the hemagglutinin (HA) gene. While percent homology of amino acids and matchup of putative amino acids can be obtained through a diagnostic laboratory, little is known about correlations to vaccine efficacy.

It has been previously suggested that comparing the T-cell epitope content of IAV-S field strains to vaccine strains might be able to predict vaccine protection in pigs[1]. However, this has not been demonstrated with contemporary IAV-S strains. This study compared the epitope content of 100 strains of IAV-S circulating in 2020 to strains included in a commercially available quadrivalent whole inactivated virus (WIV) influenza A vaccine.

Methods
One hundred IAV field strains, including H1 alpha, H1 gamma, H1 pandemic, H1 delta 2, H3 Cluster IV-A, and H3 human-like viruses, were compared to the WIV vaccine. The vaccine contains H1 gamma, H1 delta 1, H3 Cluster IV-A, and H3 Cluster IV-B strains. Utilizing PigMatrix[2], field strains and vaccine strains were screened to determine the presence of putative class I and class II T-cell epitopes restricted by Swine Leukocyte Antigen. Next, field strains were compared to vaccine strains to generate a relatedness score. These T cell epitope content comparison (EpiCC) scores look at the shared epitope content between field strains and vaccine strains. Each field strain is also compared to itself to determine the baseline score. The EpiCC score is then divided by the baseline score to determine the percent T cell epitope coverage of the field strain by the vaccine strains. Since the WIV vaccine contains four strains, epitope coverage was assessed both by individual vaccine strains as well as in combination within H1 and H3 subtypes.

Results
Individually, vaccine strains had the highest percent coverage for field strains that fell within the same clade. Overall coverage increased when evaluating vaccine strains in combination. The combination of the WIV vaccine H1 strains resulted in 54.16%, 71.46%, 69.45%, and 58.56% coverage of the H1 alpha, gamma, pandemic, and delta 2 field strains, respectively. The combination of the WIV vaccine H3 strains resulted in 87.19% and 52.49% coverage of the H3 cluster IV-A and human-like field strains, respectively.

Previously established thresholds for protection and partial protection were recalculated. Using those new thresholds, protection is predicted to occur at or above 68% coverage, and partial protection is predicted between 56.24-67.9% coverage. Utilizing those cutoffs, the WIV vaccine should provide protection against 44 field strains and partial protection against 15 field strains.

Discussion
By comparing the T-cell epitope content of contemporary IAV-S strains to the WIV vaccine, this study determined that the WIV vaccine could offer at least partial protection against over half of the contemporary circulating influenza strains that were evaluated.

References
1. Gutiérrez AH, Rapp-Gabrielson V, Terry FE, Loving CL, Moise L, Martin WD, De Groot AS. T-cell epitope content comparison (EpiCC) of swine H1 influenza A virus hemagglutinin. Influenza and Other Respiratory Viruses. 2017;11(6):531-42.
2. Gutiérrez AH, Martin WD, Bailey-Kellogg C, Terry F, Moise L, De Groot AS. Development and validation of an epitope prediction tool for swine (PigMatrix) based on the pocket profile method. BMC Bioinformatics. 2015;16:290.

Poster Number: 12
Topic Area: Bacterial Disease
Title: Efficacy of Suiseng® Diff/A vaccine and its association with Suiseng® Coli/C against *C. difficile* and *C. perfringens* type A
Author(s): Merce Canal, Hipra; Taberner E., Hipra; Gibert X., Hipra; Roca M., Hipra; Sitjà M., Hipra

Introduction
Clostridioides difficile (Cdiff) and *Clostridium perfringens* type A (CpA) are two gram-positive spore-forming anaerobic bacteria responsible for neonatal diarrhoea in suckling piglets[1,2]. Piglet diarrhoea is a relevant problem in the swine industry, since it can induce mortality and morbidity, reduce growth rates and increase antimicrobial usage. Suiseng® Diff/A (Diff/A) is an inactivated vaccine containing Cdiff and CpA toxoids. Suiseng® Coli/C (Coli/C) is a multivalent vaccine indicated against neonatal colibacillosis and necrotic enteritis in piglets and sudden death in sows. It contains subunits of *Escherichia coli* known as F4ab, F4ac, F5, F6 and LT, as well as β-toxoid of *Clostridium perfringens* Type C and α-toxoid of *Clostridium novyi.*

The aim of this study was to demonstrate the efficacy of the Diff/A vaccine administered alone or associated with Coli/C in pregnant sows to protect their progeny against Cdiff and CpA.

Methods
Fifteen pregnant sows, seronegative against these pathogens, were used for the study. The sows were vaccinated intramuscularly (IM) before farrowing (6 and 3 weeks before farrowing) according to the manufacturer's instructions. Group A (n=5) was vaccinated with Diff/A, Group B (n=5) was vaccinated with Diff/A associated with Coli/C and Group C (n=5), the control group, was injected with PBS (Phosphate Buffered Solution) according to the same schedule. 24h-colostrum-fed piglets were challenged with CpA or Cdiff and monitored for clinical signs and mortality for 5 days.

Results
Cdiff challenge caused 100% mortality in piglets from the mock-vaccinated group. In contrast, mortality was prevented in piglets from both vaccinated groups. CpA challenge also induced 100% mortality in piglets from the control group, while a significant reduction in mortality was observed in piglets from vaccinated sows. Clinical signs and macroscopic lesions were significantly reduced in the vaccinated groups after both challenges.

Conclusion
These results fully support the efficacy against CpA and Cdiff for the Diff/A vaccine administered alone or associated with Coli/C.

Citations
1. Songer JG, Uzal FA. Clostridial enteric infections in pigs. J Vet Diagn Invest 2005; 17:528-536.
2. "Diseases of Swine" (2019). *Veterinary Diagnostic and Production Animal Medicine Books*. 1. Pp 792-806

Poster Number: 13
Topic Area: Bacterial Disease
Title: Transfer of maternal immunity against C. difficile and C. perfringens type A from vaccinated sows to their offspring
Author(s): Merce Canal, Hipra; Gibert X., Hipra; Taberner E., Hipra; Acal L., Hipra; Sitjà M., Hipra

Introduction
Clostridioides difficile (Cdiff) and Clostridium perfringens type A (CpA) are two Gram-positive spore-forming anaerobic bacteria, responsible for neonatal diarrhoea in suckling piglets (1). Colostrum intake is one of the main factors in the multifactorial approach required for controlling complex Neonatal Diarrhoea in swine. Ensuring optimal nursing during the first hours of life is crucial to minimize the incidence of neonatal diarrhoea and thus pre-weaning mortality (2). The aim of the present study was to investigate the transfer of maternal immunity against Clostridioides difficile (Cdiff) and Clostridium perfringens type A (CpA) to piglets when the basic vaccination scheme of Suiseng® Diff/A vaccine (Diff/A) was administered to pregnant sows.

Methods
Eighteen pregnant gilts, seronegative against CpA and Cdiff, were used. Group A (n=10) was vaccinated intramuscularly (IM) with two doses of Diff/A vaccine (6 and 3 weeks before farrowing). Group B (n=8) was injected with PBS IM with the same schedule. Blood samples from sows were collected before vaccination and after farrowing. Colostrum was also collected from sows. Blood samples from piglets were collected at one day of age (n= 45 and 31 for A and B, respectively) and at weaning time (d20) (n=9 and 4 for A and B, respectively). Antibody levels against the α-toxin of CpA and TcdA and TcdB of Cdiff were determined using ELISA and seroneutralization (SN) tests. Antibody levels determined by ELISA were measured using a Relative Index Per Cent (IRPC) and by SN assay as the log2 dilution of the serum where the toxin was 50% neutralized. Group results were compared statistically using the T-test ($p<0.05$).

Results
At day 42 (farrowing), significant seroconversion was demonstrated for all vaccinated sows using both assays. The sows' colostrum contained high levels of antibodies, demonstrating the translocation of the specific immunoglobulins from serum to the mammary gland lumen before farrowing. Likewise, serum samples from all piglets of vaccinated dams contained high levels of antibodies, measured using ELISA and SN assay. The antibody levels reached were demonstrated as being protective for piglets challenged with Cdiff and CpA. All control animals (sows and piglets) remained negative throughout the study.

Conclusion
Vaccination of pregnant sows with Diff/A vaccine effectively transfers maternal immunity to their piglets at 1 day of age, which persists until weaning (d20). These antibodies have been proved to be neutralizing antibodies and protective. Therefore, this vaccine is a useful tool to transfer maternal immunity to piglets against C. difficile and C. perfringens type A.

Citations
1. Diseases of Swine 11th Edition pp 792-806.
2. Declerck, I. et al. Long-term effects of colostrum intake in piglet mortality and performance. J. Anim. Sci. 2016.94:1633–1643

Poster Number: 14
Topic Area: Bacterial Disease
Title: Improved piglet performance and reduced mortality and antimicrobial use following oral vaccination with a live non-pathogenic Escherichia coli F4/F18 vaccine against post-weaning diarrhoea
Author(s): Frédéric Vangroenweghe, Elanco Animal Health

Introduction – Post-weaning Escherichia coli (E. coli) diarrhea (PWD), also called post-weaning enteric colibacillosis, remains a major cause of economic losses for the pig industry. PWD typically causes mild to severe watery diarrhea between 5 and 10 days after weaning and is caused primarily by enterotoxigenic Escherichia coli (ETEC). The most common adhesins found on ETEC from PWD in pigs are associated with fimbriae F4 (previously called K88) and F18, while the predominant enterotoxins are heat-labile toxin (LT), heat-stable toxin a (STa), and heat-stable toxin b (STb). In addition to F4 and F18, other fimbrial adhesins, such as F5 (K99), F6 (987P), and F7 (F41), have been associated with PWD, but less frequently. Therapy to combat PWD typically consists of antibiotic treatment. However, emergence of antimicrobial resistance in E. coli strains and new EU regulations urge the need for alternative control measures, such as adapted feeding strategies or immunization. Recently, an oral live bivalent E. coli F4/F18 vaccine (Coliprotec® F4/F18; Elanco) has become available on the European market, which reduces the impact of PWD provoked by F4-ETEC and F18-ETEC.

Methods – Oral vaccination of suckling piglets using a live bivalent non-pathogenic E. coli F4/F18 vaccine was performed in 10 farrow-to-finish sow farms to prevent against PWD due to F4-enterotoxigenic E. coli (ETEC) or F18-ETEC. The vaccination strategy was compared to the standard therapeutic approach in each farm, meanwhile collecting data on Average Daily Weight Gain (ADWG), Feed Conversion Rate (FCR), mortality rate and treatment incidence with antimicrobial drugs (TI100) during the post-weaning period.

Results – Vaccine-treated groups demonstrated a significant improvement in FCR (-4.2%), mortality rate (-57.1%) and TI100 (-84.9%) as compared to the Control group. The ADWG only marginally and non-significantly improved (+2.3%) in the Vaccine-treated group.

Conclusions – In conclusion, the present study demonstrated the efficacy of an oral live non-pathogenic E. coli F4/F18 vaccine (Coliprotec® F4/F8; Elanco Animal Health) for active immunization of piglets against PWD due to F4-ETEC and F18-ETEC under field conditions. For several economically important performance parameters, such as FCR, mortality rate and TI100, E. coli vaccination performed significantly better as compared to the standard therapeutic approach. Therefore, vaccination against PWD due to F4-ETEC or F18-ETEC using an oral live non-pathogenic E. coli F4/F18 vaccinated may be considered a good alternative to consolidate post-weaning piglet performance results while meeting the new European requirements concerning prudent use of antimicrobials in intensive pig production.

Poster Number: 15
Topic Area: Bacterial Disease
Title: Convenience and economic benefit of early one-shot Mycoplasma hyopneumoniae vaccination at 3 days of age in a commercial sow farm
Author(s): Frédéric Vangroenweghe, Elanco Animal Health

Introduction – Mycoplasma hyopneumoniae (M. hyopneumoniae) is the primary pathogen of enzootic pneumonia, a chronic respiratory disease in pigs. Vaccination of piglets to protect against M. hyopneumoniae can be performed at several ages, depending on product label specifications. Early intervention in the first week of life may have advantages, since piglets can already become infected with M. hyopneumoniae during the suckling period, resulting in a significant percentage of M. hyopneumoniae-positive piglets around weaning. The current study compared convenience and economic benefits of M. hyopneumoniae vaccination in piglets of 3, 7 and 14 days of age.

Methods – The study was performed on a 2000-sow unit operating in 1-week batch management system with approx. 100 sows per week batch. Each study group (vaccination at 3, 7 and 14 days of age) included 20 sows and their respective suckling piglets. At 3 days of age, piglets were vaccinated during the regular processing activity, whereas at 7 and 14 days of age, piglets had to be vaccinated during a special vaccination moment. Total time to vaccinate the litter was recorded, including the number of piglets in the litter. Duration of vaccination per piglet at each specific vaccination moment was calculated. Based on these results, an economic calculation of vaccination cost at each age was performed taking into account pre-weaning mortality, labor cost, cost of vaccine doses and time needed to perform the vaccination.

Results – Duration of piglet vaccination at 3 days of age was significantly ($P < 0.05$) shorter (2.64 ± 0.08 seconds) as compared to 7 days of age (4.90 ± 0.18 seconds) and 14 days of age (6.04 ± 0.22 second). Economic calculation in a 1000-sow unit, using a vaccination convenience calculator, demonstrated that although the total number of piglets vaccinated is lower (- 443 and - 838 at 7 and 14 days of age, respectively) at a later vaccination age, the related increase in vaccine cost in the early vaccination group (3 days of age) was largely compensated by the decrease in cost of overall vaccination time (€ 1,115.61 and € 1,461.00 lower at 3 days of age as compared to 7 and 14 days of age, respectively).

Conclusions – In conclusion, M. hyopneumoniae vaccination at 3 days of age has several advantages over later vaccination at 7 or 14 days of age. Besides the benefits in convenience of piglet handling at that age, we could also demonstrate economic benefits of early M. hyopneumoniae vaccination.

Poster Number: 16
Topic Area: Feed/Nutrition
Title: A unique algae-clay technology mitigates mycotoxin risk in piglets
Author(s): Marie Gallissot, Olmix Group; Raquel Pereira, Consultant; Maria Angeles Rodriguez, Olmix Group; Julia Laurain, Olmix Group

Deleterious effects of mycotoxins on the health and performance of piglets have been widely described (Escriva et al., 2015). A common strategy to prevent such effects consists in using mycotoxin binders in the feed to reduce the exposure of the animals to mycotoxins. Several materials have proven their capacity to mitigate aflatoxin exposure, but very few products seem to be efficient in controlling fusariotoxins like deoxynivalenol and fumonisins (Laurain et al., 2019). Yet, these mycotoxins are the most commonly occurring in animal feeds in North America (Abbas, 2019). The aim of the present study was to measure the efficacy of a patented algo-clay complex (ACC) to mitigate the toxicity of three mycotoxins: deoxynivalenol (DON), fumonisins (FUM) and aflatoxins (AFLA) in piglets. The experiment was conducted by the Samitec Institute in Brazil and divided into three trials (one per mycotoxin). For each trial, 30 piglets with an average body weight of 10kg were distributed into 5 treatments with 6 replicates. The DON and AFLA trials lasted for 28 days and the FUM trial lasted for 42 days. The three trials tested the mycotoxins individually at 3ppm DON, 50ppm FUM and 1ppm AFLA. Treatments varied as per mycotoxin contamination (with or without) and supplementation of the algo-clay complex (0, 0.25% or 0.50%). The following performance and biological parameters were monitored for all trials: feed intake, body weight, and relative liver weight (RLW). The sphinganine to sphingosine ratio (Sa/So) in the serum was also measured in the FUM trial. The inclusion of 0.50% of ACC in the contaminated feed systematically improved feed intake and body weight of the piglets compared to the group with mycotoxin contamination alone ($P \leq 0.05$). In the FUM and AFLA trials, the RLW of the piglets supplemented with 0.50% of the ACC was significantly improved compared to the group exposed to mycotoxins with no ACC supplementation. The addition of 0.25% and 0.50% of the ACC in the FUM-contaminated diet significantly reduced the Sa/So ratio of piglets compared to the groups fed the FUM-contaminated diet with no ACC ($P \leq 0.05$). The evaluated parameters illustrate the capacity of the algo-clay complex to reduce the deleterious effects of high levels of deoxynivalenol, fumonisins or aflatoxins on biological parameters and performance of piglets. This technology appears to be a relevant strategy to manage mycotoxin risk on pig farms.

Poster Number: 17
Topic Area: Reproduction
Title: Examining the use of oxytocin on farrowing sows and stillbirth rates: A systematic review and meta-analysis
Author(s): Sarah Hill, Department of Population Medicine, Ontario Veterinary College, University of Guelph; Maria Amezcua, Department of Population Medicine, Ontario Veterinary College, University of Guelph; Eduardo Ribeiro, Department of Animal Bioscience, Ontario Agricultural College, University of Guelph; Terri O'Sullivan, Department of Population Medicine, Ontario Veterinary College, University of Guelph; Robert M. Friendship, Department of Population Medicine, Ontario Veterinary College, University of Guelph

Sows with large litter sizes often experience longer farrowing durations, which can increase the risk of dystocia, intrapartum hypoxia, and stillbirths. For decades, producers have countered prolonged farrowing, by routinely administering oxytocin to sows during parturition. The use of oxytocin has been shown to limit the number of stillbirths and fetal distress during difficult farrowings. Despite the obvious economic benefits this trend provides the pork industry, recent research has demonstrated conflicting evidence on the use of oxytocin. The objective of this systematic review and meta-analysis is to investigate the current literature on oxytocin use to create new practice guidelines for oxytocin use on sows at the time of parturition. The research questions addressed in the systematic review are: 1) Are there negative side-effects when farrowing sows receive exogenous oxytocin compared to sows that don't receive oxytocin? 2) What is the comparative effectiveness in reducing stillbirths and improving piglet viability from treating sows with oxytocin? 3) What are the appropriate dosages, treatment regimens and reason for oxytocin administration and is sow parity a factor? Several databases were used to search for relevant primary articles and a grey literature search was done through the AASV swine information library. Fifty-eight publications successfully passed both title/abstract and full-text screening. Among these, Further, data on the number of stillbirths, duration of farrowing, and litter size was extracted from each publication. A meta-analysis will be used to examine the measure of effect of oxytocin on these outcomes. To establish potential study bias, the ROB 2.0 will be used to assess risk of bias at the outcome level. Further, GRADE will be used to properly interpret and present the results. The results of this meta-analysis will hopefully create guidelines on the use of oxytocin use in sows at the time of parturition to prevent dystocia, intrapartum hypoxia, and stillbirths.

Poster Number: 18
Topic Area: Bacterial Disease
Title: Efficacy of Suiseng® Coli/C vaccine and its combined use with Suiseng® Diff/A against Enterotoxigenic Escherichia coli
Author(s): Merce Canal, Hipra; Pedernera, C., Hipra; Taberner, E., Hipra; Vidal-Mas, J., Hipra; Sitjà, M., Hipra

Introduction
Enterotoxigenic *Escherichia coli* (ETEC) is one of the most important causes of neonatal diarrhoea in piglets[1], together with *Clostridium perfringens* Type C (CpC) and Type A (CpA) and *Clostridioides difficile* (Cdiff)[2]. Piglet diarrhoea is responsible for economic losses due to mortality, morbidity and the cost of antibiotics. Suiseng® Coli/C (Coli/C) is a multivalent vaccine indicated against Neonatal Piglet Colibacillosis and Necrotic Enteritis in piglets and sudden deaths in sows. It contains subunits of *Escherichia coli (E. coli)* known as F4ab, F4ac, F5 and F6 fimbrial adhesins and LT toxoid, as well as β- and α-toxoids of *Cp*C and *Clostridium novyi*, respectively. Suiseng® Diff/A (Diff/A) is a vaccine containing *Cp*A and Cdiff toxoids. The aim of the study was to demonstrate the efficacy of Coli/C vaccine when administered alone or in association with Diff/A vaccine against ETEC.

Methods
Two ETEC strains were used to demonstrate the efficacy of the passive immunity obtained after vaccination of pregnant sows with Coli/C and with Coli/C in association with Diff/A. Challenge studies were carried out with two different strains, one strain expressed F4ac and F6 fimbrial adhesins and LT enterotoxin and the second one expressed F4ab and F5 fimbrial adhesins. Seronegative sows were vaccinated intramuscularly (IM) using the basic vaccination scheme for these two vaccines, one dose at 6 weeks before farrowing and a second dose at 3 weeks prior to farrowing. Control groups were vaccinated with a placebo (Phosphate Buffered Solution). Piglets from vaccinated and control sows were experimentally challenged via the intraoesophageal route with pathogenic strains of *E. coli* within the first 12 hours of birth. Clinical signs and mortality were monitored for 8 days after the challenge.

Results
Clinical signs were scored based on the presence of diarrhoea (slight or marked) and mortality assessed for 8 days. The offspring of sows vaccinated with Coli/C or Coli/C plus Diff/A showed a significantly lower clinical signs mean score than the piglets from the control groups. Moreover, the piglets belonging to the vaccinated sows had a lower percentage of mortality than those from the control sows.

Conclusion
Both vaccination with Coli/C alone and its associated use with Diff/A conferred a significant reduction in clinical signs and mortality caused by a challenge with ETEC strains. So, these results fully support the efficacy for the Coli/C vaccine administered alone or associated with Diff/A.

Citations
1. Melkebeek V, Goddeeris B, Cox E. (2013) ETEC vaccination in pigs. Vet Immunol Immunopathol. 152(1-2):37-42.
2. Songer JG, Uzal FA. Clostridial enteric infections in pigs. J Vet Diagn Invest 2005; 17:528-536.

Poster Number: 19
Topic Area: Bacterial Disease
Title: Clinical safety of the association of Suiseng® Coli/C and Suiseng® Diff/A in sows under field conditions.
Author(s): Merce Canal, HIPRA; Puig A., HIPRA; Scherer C.F.C, HIPRA; Gibert X., HIPRA; March R., HIPRA; Sabaté D., HIPRA

Introduction:
Neonatal diarrhoea is one of the major concerns in the swine industry. Vaccination of pregnant sows before parturition usually triggers an immune response leading to the presence of protective immunoglobulins in the colostrum. Piglets can then be passively protected by colostrum if they are nursed properly, thus preventing future cases of diarrhoea[1]. Suiseng® Coli/C (Coli/C) and Suiseng® Diff/A (Diff/A) are two vaccines covering the main causative agents of neonatal diarrhoea. Specifically, Coli/C protects against E.coli (F4ab, F4ac, F5, F6 and LT), C. perfringens type C and C.novyi type B infections while Diff/A protects against C. difficile and C. perfringens type A infections. Consequently, their use is of great interest as a strategic preventive approach on farms affected by neonatal diarrhoea. Given that both vaccines share the same vaccination schedule, simultaneous administration of these two vaccines together could be carried out by some practitioners for animal welfare and operational reasons. The objective of this field study was to assess the safety of Diff/A and Coli/C when administered together to ensure the feasibility in the event that it is carried out in practice.

Methods:
A randomized, blinded and controlled study was performed on a commercial farm in Brazil. A total of 60 pregnant sows were randomly distributed into 4 groups (n=15/group): Coli/C, Diff/A, Coli/C + Diff/A and Placebo (Phosphate Buffered Saline). Animals in all groups were administered the corresponding product intramuscularly (2 ml) twice: one dose 6 weeks prior to farrowing and a second one 21 days later. Sows in group Coli/C + Diff/A received both products together (4 ml). Local and systemic reactions, and body temperature were monitored individually from the day before each vaccination up to 2 days post-vaccination. Reproductive parameters were recorded from vaccination to farrowing.

Results:
A clinically irrelevant transient rise in rectal temperature was observed in all groups at 4h after each dose administration, returning to normal values after 24h. The maximum individual temperature was observed in group Coli/C + Diff/A at 4h after the 2nd dose (39.2ºC). No statistically significant differences were detected between groups at any timepoint. Mild local inflammatory reactions (≤3cm) were observed in some animals from all groups. The highest incidence was observed in the Coli/C + Diff/A group after the 2nd dose (20% of sows). These reactions were transient and had totally disappeared after 3 days. Finally, reproductive parameters showed no statistically significant differences between groups in the mean number of live born, stillborn and mummified piglets ($p > 0.05$).

Conclusion:
The results obtained in the present study demonstrate optimal safety for this association under field conditions in sows. Consequently, although the practice of administering together the Diff/A and Coli/C vaccines is entirely under the responsibility of the veterinarian, there does not appear to be main safety concerns that may prevent associating the products.

Citations
1. Matías J, Berzosa M, Pastor Y., Irache J., Gamazo C. (2017) Immunization of Sows during Pregnancy against ETEC Infections. Vaccines 2017, 5, 48;

Poster Number: 20
Topic Area: Bacterial Disease
Title: Comparison of avilamycin and carbadox on the incidence and severity of diarrhea and growth performance of nursery pigs naturally infected with Escherichia coli
Author(s): C. L. Puls, Elanco Animal Health, Greenfield, IN 46140; R. Evelsizer, Elanco Animal Health, Greenfield, IN 46140; S. N. Carr, Elanco Animal Health, Greenfield, IN 46140; B. Frederick, Cargill Animal Nutrition, Lewisburg, OH 45338; S. Ebarb, Cargill Animal Nutrition, Lewisburg, OH 45338; M. J. Ritter, Cargill Animal Nutrition, Lewisburg, OH 45338

Introduction:
Regulatory concerns over carbadox (Mecadox, Phibro) have prompted producers and veterinarians to investigate alternative medications for their potential application. Avilamycin (Kavault, Elanco) is an animal use only feed antibiotic for the reduction in incidence and overall severity of diarrhea in the presence of pathogenic Escherichia coli (E. coli). This study was conducted to compare effects of avilamycin and carbadox on diarrhea incidence and severity and subsequent growth performance of nursery pigs naturally infected with E. coli.

Materials and Methods:
A randomized complete block design with 3,329 pigs was used with a 2×4 factorial arrangement of treatments: 1) Stocking density (Single: 32 pigs/pen; 7.2 sq. ft/pig vs. Double: 64 pigs/pen; 3.6 sq. ft/pig) and 2) Nursery medication program (Control for 56 days vs. Kavault 73 g/ton for 21 days (Kav21) vs. Kavault 73 g/ton for 42 days (Kav42) vs. Mecadox 50 g/ton for 21 days). Pigs were weighed on days 0, 21, 42, and 56 of study. Weekly diarrhea scores were collected on each pen using a 3-point scale (0=normal feces, <5% of pigs or feces with signs of scours; 1=5% to 25% of pigs or feces with signs of scours; 2=greater than 25% of pigs or feces with signs of scours). Diarrhea incidence was determined as scores of 1 or 2 at any point whereas diarrhea severity was both the maximum weekly score and the mean weekly score. Fecal swabs confirmed presence of haemolytic E. coli.

Results:
During the first 21 days, feeding Kavault lowered ($P < 0.05$) diarrhea severity compared to controls, with Mecadox being intermediate. In the second 21 days, feeding Kav42 lowered ($P < 0.05$) diarrhea incidence and severity compared to the other treatments. In general, feeding medication resulted in lower severity and overall diarrhea scores compared to non-medicated controls. For the overall study period (day 0 to 56), there were no ($P > 0.05$) stocking density × nursery program interactions for growth performance measures. Similarly, there was no effect ($P > 0.05$) of nursery medication program on final (day 56) BW, BW Gain, ADG, ADFI, or incidence of morbidity and mortality. However, all medication treatments had numerically better final BW (+1.0 lb, +2.9 lb, and +1.7 lb for Kav21, Kav42, and Mec21, respectively) and ADG (+1.8%, +4.4%, and +2.6%, respectively) compared to controls. Overall feed conversion was improved ($P < 0.05$) for pigs fed Kavault compared to Mecadox, with controls being intermediate. Generally speaking, cost/lb gain and return over feed cost were numerically greater for medication treatments compared to non-medicated controls. Double stocking resulted in lower ($P < 0.05$) overall ADFI and improved ($P < 0.05$) feed conversion compared to single stocking, but increased ($P < 0.05$) overall morbidity and mortality and diarrhea severity, incidence, and overall scores. Single stocking had greater ($P < 0.05$) cost/lb gain than double stocking.

Conclusions:
The results of this study demonstrate similar performance and superior feed conversion for pigs fed Kavault compared to Mecadox, and suggest Kavault is a suitable replacement for Mecadox in a natural E. coli challenge.

Poster Number: 21
Topic Area: Bacterial Disease
Title: Field data of use of Porcilis Lawsonia IM and ID vaccination on a Dutch closed sow herd
Author(s): Nico Wertenbroek, MSD Animal Health The Netherlands; Jos Broere, MSD AH The Netherlands; Hans Smit, Diergeneeskundig Centrum Boven Veluwe

Introduction
Ileitis is an old disease in pigs but the Lawsonia intracellularis bacterium is still causing significant economic damage, with often no obvious clinical symptoms on the farms. Several controlling interventions are available for the veterinarian and farmer, like antibiotics, feed additives and a live oral vaccine. Recently in Europe a new killed vaccine against Lawsonia intracellularis was introduced.

The case study describes the technical performance before and after the usage of Porcilis Lawsonia under Dutch field conditions on a closed Dutch pig farm.

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

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

The monthly technical results like ADG, FCR, and mortality before – after were primary parameters used for evaluation, and antibiotic use, defined by DDD. The observed period was one year before the first Porcilis Lawsonia vaccinated pigs were slaughtered versus one year after start. Due to seasonality influences, the same corresponding months were compared before after.

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

The first Porcilis Lawsonia IM vaccinated pigs were slaughtered mid-march 2020. Comparing the corresponding months of the oral drinking water period vs IM period (April ’19 – Aug’19 vs April’20 -Aug’20) the ADG were 865 vs 920 gr/day; FCR 2,75 vs 2,68 and mortality 2,3 vs 1,9 %. Comparing the corresponding months of the oral drinking water period vs ID period the ADG were 908 vs 956 gr/day; FCR 2,79 vs 2,70 and mortality 2,1 vs 1.7 %.

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

References
1. SPC Porcilis Lawsonia EMA website.
2. Bos.2013.Consumption of Antimicrobials in pigs, veal calves and broilers in The Netherlands. Plos One https://doi.org/10.1371/journal.pone.0077525
3. Jacobs, 2019. Vaccine 37 2149-2157

Poster Number: 22
Topic Area: Production
Title: Transition of a previously modified-live vaccinated, PRRS positive stable sow herd to Prevacent® PRRS
Author(s): Matthew Ackerman, PorkVet Solutions, New Palestine, IN 46163; Dylan Lape, PorkVet Solutions, New Palestine, IN 46163; Jebediah Stevens, Ag Production Enterprises, Greensburg, IN 47240; Jessica Risser, Elanco Animal Health, Greenfield, IN 46140; Christopher L Puls, Elanco Animal Health, Greenfield, IN 46140

Introduction:
Many swine producers and veterinarians are hesitant to transition modified live (MLV) porcine reproductive and respiratory syndrome virus (PRRS) vaccines in a sow herd because of potential for shed and spread, recombination, and negative effects on production from introducing a new strain of PRRS. Prevacent® PRRS (Elanco) is a USDA licensed MLV approved for the respiratory and reproductive forms of PRRS and could be a tool to offer additional protection against negative production parameters associated with PRRS. This field evaluation set out to demonstrate the effects of transitioning a category II-vx sow herd to quarterly whole-herd vaccination with Prevacent PRRS on sow and pig performance.

Materials and Methods:
A 6,000 head category II-vx sow farm was used in this study. The sow herd had previously received quarterly whole-herd vaccinations with a commercially available MLV PRRS vaccine and weaning pigs negative for PRRS on processing fluids. A 1-3-4 RFLP PRRS strain was actively known to be circulating in nursery and finisher units. For the transition to Prevacent, two whole-herd vaccinations were given to all animals 4 weeks apart. Piglets received a full dose of MLV PRRS vaccine between 1 and 3 days prior to weaning and began receiving Prevacent one week after the initial sow herd Prevacent vaccination. Weekly processing fluids (from gilt and sow litters separately at time of processing), family oral fluids (from gilt and sow litters separately within 7 days of weaning), and nursery oral fluids (within 1 week of weaning and again 5 to 6 weeks later) were collected. Weekly sow and nursery pig group performance (i.e. closeouts) were recorded throughout the evaluation.

Results:
Sow data. Conception rate (92.9 ± 1.1%, 92.0 ± 0.8%, and 91.7 ± 1.1% for the 6 weeks prior to initial Prevacent vaccination, 4-week transition period, and 6 weeks after second vaccination, respectively), farrowing rate (89.0 ± 1.9%, 89.7 ± 1.8%, and 88.4 ± 2.2%, respectively), and stillborn and mummified pigs (6.4 ± 0.6%, 6.7 ± 0.7%, and 6.7 ± 0.5%, respectively) were similar across the evaluation period and within expected and historical farm averages. Sow processing fluids remained negative during the entire study period, with sporadic positives for gilt processing fluids. Sow family oral fluids were generally positive (32.4 to 34.9 Ct values) during the transition period but remained largely negative after the second Prevacent whole-herd vaccination. Pigs weaned during the transition period were positive on early nursery oral fluids with Ct values of 31.8 to 34.7, before returning to negative.

Nursery pig data. Comparing nursery pig performance for the 6 weeks prior to vaccination with Prevacent to the 6 weeks after, ADG increased 8.5%, FCR improved 1.3%, and nursery exit weight increased 3.1 lbs, however, nursery mortality also increased 1.2%. All metrics were within historical observed ranges for the production system.

Conclusions:
The results of this field evaluation demonstrate that Prevacent PRRS is a safe MLV PRRS vaccine in sows. Furthermore, category II-vx sow herds can successfully transition to quarterly vaccinations with Prevacent PRRS without causing disruptions in production metrics.

Poster Number: 23
Topic Area: Other
Title: Utilizing concentrated porcine IgG for conferring passive resistance to immunocompromised pigs
Author(s): Brent Pepin, Cytotheryx; Kari Allen, Cytotheryx; Brian Dacken, Cytotheryx; Kari Houg, Cytotheryx; Derek Thompson, Cytotheryx; Allie McGraw, Cytotheryx; John R Swart, Cytotheryx; Joseph B Lillegard, Cytotheryx; Robert Kaiser, Cytotheryx

Introduction
The use of immunocompromised pigs is commonplace for research purposes and for producing specific pathogen-free (SPF) animals. Bovine colostrum (BC) is the traditional source of IgG based protection from pathogens considered normal gut or environmental flora and is beneficial to maintaining piglet health long-term. The use of porcine-derived concentrated IgG antibody (cIgG) may be an alternative to BC. Animals in an internal multiplication herd provide a source for harvesting and concentrating sera-derived IgG from within the same facility, decreasing the risk of outside pathogen exposure and provide antibodies specific to the environment. Developments in plasma fractionation and heat treatment have allowed for virus inactivation and prevent pathogen transfer from cIgG administration. Replacement therapy of IgG has been used to treat human antibody deficiencies since the 1950s. The subcutaneous dosing (SQ) of IgG for human therapy is commonplace but less understood as an option for immunocompromised swine. This project entails two studies on the use of cIgG. Study 1 compares oral provision of cIgG with BC in neonatal pigs by different feeding methods. Study 2 analyzed the SQ injection of cIgG in immunocompromised pigs at different dosing frequencies monitored by specific antibody levels and by total serum porcine IgG over time.

Methods
The IgG from heparinized whole blood was highly concentrated via a series of centrifugation, filtration, and pasteurization to produce cIgG. Study 1 compares BC to the use of oral cIgG combined with ultra-pasteurized bovine whole milk by two different feeding methods (limited syringe and bowl ad libitum) in rearing cesarean derived, colostrum deprived (CDCD) piglets. Study 2 compares SQ cIgG dosing frequency in pigs (once a week dosing, twice a week dosing, and negative control) to optimize achieved titers while minimizing handling. The IgG absorption of the SQ dosing was monitored by following the measured antibody levels of pathogens for which the donor pigs were vaccinated (Mycoplasma hyopneumoniae [MHP] and Erysipelothrix rhusiopathiae [Ery]), and total porcine IgG in the serum over time.

Results
Free choice BC provided a better growth rate based on weight gained than the syringe feeding method and cIgG combined with whole milk, but ANOVA testing revealed no significant difference ($p=0.29$) in CDCD pigs. Pigs receiving 1 vs. 2 doses of SQ cIgG per week had no significant difference in total IgG or antibodies measured against specific pathogens. However, both cIgG SQ dosed groups showed a significant increase in total IgG and measured target antibodies of MHP and Ery than the negative control. Total IgG highly correlated with MHP and Ery antibody measurements ($r(115)= 0.59$, $p<0.001$ and $r(115)= 0.55$, $p<0.001$, respectively).

Conclusions
The use of cIgG orally is a viable alternative to BC. Study 2 establishes the ability to use SQ cIgG for the long-term care of immunocompromised pigs. To the authors' knowledge, this project is the first to demonstrate the use of porcine sera-derived cIgG by oral administration in piglets as an alternative to BC and demonstrate the use of SQ administration of cIgG to immunocompromised pigs for continual opportunist disease protection.

References
1. Madson DM, Arruda PHE, Magstadt DR, et al. Characterization of porcine epidemic diarrhea virus isolate US/IOWA/18984/2013 infection in 1-day-old cesarean-derived colostrum-deprived piglets. Vet Pathol. 2016; 53(1):44-52.
2. Opriessnig T, Gerber PF, Halbur PG. Refinement of a colostrum-deprived pig model for infectious disease research. MethodsX. 2018; 5:403-413.
3. Powell EJ, Charley S, Boettcher AN, et al. Creating effective biocontainment facilities and maintenance protocols for raising specific pathogen-free, severe combined immunodeficient (SCID) pigs. Lab Anim. 2018; 52(4):402-412.

4. Xinsheng L, Chun-Ming L, Annamalai T, et al. Determining the infectious titer and virulence of an original US porcine epidemic diarrhea virus PC22A strain. Vet Res. 2015; 46:109-115.
5. Huang Y, Haines DM, and Harding JCS. Snatch-farrowed, porcine-colostrum-deprived (SF-pCD) pigs as a model for swine infectious disease research. Can J Vet Res. 2013; 77(2):81-88.
6. Oliveira S, Galina L, Blanco I, et al. Naturally-farrowed, artificially-reared pigs as an alternative model for experimental infection by Haemophilus parasuis. Can J Vet Res. 2003; 67(2):146-150.
7. Boettcher AN, Loving CL, Cunnick JE, et al. Development of severe combined immunodeficient (SCID) pig models for translational cancer modeling: Future insignts on how humanized SCID pigs can improve preclinical cancer research. Front. Ocnol. 2018; 8:559.
8. Farrugia A and Quinti I. Manufacture of immunoglobulin products for patients with primary antibody deficiencies – the effect of processing conditions on product safety and efficacy. Front Immunol. 2014; 5:665.
9. Hosseini KM and Ghasemzadeh M. Implementation of plasma fractionation in biological medicines production. Iran J Biotech. 2016; 14(4):e1401.
10. Ponsford M, Carne E, Kingdon C, et al. Facilitated subcutaneous immunoglobulin (fSCIg) therapy – practical considerations. Clin Exp Immunol. 2015; 182(3):302-313.

Poster Number: 24
Topic Area: Bacterial Disease
Title: Reduction of Nursery Mortality on Canadian Farm suffering from Non-Progressive Atrophic Rhinitis
Author(s): Irene Galé, HIPRA HQ; Manon St Hilaire, Global Porc Inc; Mike De Groot, HIPRA CANADA; Marina Sole, HIPRA HQ

Introduction
Atrophic rhinitis is a disease in pigs causing upper respiratory infection leading to nasal discharge, sneezing and reduced growth rate. It is a bacterial disease caused by the coinfection of Bordetella bronchiseptica (Bb) and Pasteurella multocida type D (PmD)[1]. Atrophic rhinitis can present as a non-progressive form due to Bb producing clinical signs such as sneezing and reduced growth rate or as a progressive form, due to Bb and PmD coinfection, that additionally causes severe damage to nasal turbinates and twisted snouts.
The aim of this study was to evaluate how vaccination against Non-Progressive Atrophic Rhinitis[2] could help to control clinical signs and improve productive parameters in an antibiotic-free farm diagnosed with Bordetella bronchipseptica infection by RHINISENG® vaccine[3].

Methods
A 325 sows farrow-to-finish farm in Quebec, Canada, raised pigs free of antibiotics showed clinical signs of non-progressive atrophic rhinitis and increased mortality in the nursery was studies. Laboratory results confirmed infection with Bordetella bronchiseptica and lesions of non-progressive atrophic rhinitis. Rhiniseng®, a vaccine that protects against both, the progressive and non-progressive forms of atrophic rhinitis, was implemented on farm. All sows and gilts on the farm were given a primary vaccination with Rhiniseng® (2ml intramuscular injection) followed by a booster dose 3 weeks later. All sows and gilts were subsequently vaccinated with a 2ml dose of Rhiniseng® 3 weeks prior to farrowing. By passive transfer, vaccinated sows transfer immunity to their piglets. Mortality in the nursery barn was recorded each month and nursery mortality was compared for the time period before and after vaccination. A total of 2,154 piglets in the control group (before vaccination period) and 2,132 piglets in the vaccinated one were evaluated.

Results
Following implementation of Rhiniseng® vaccination on farm, overall mortality in the nursery barn decreased significantly from 10.38% in the control group to 4.63% in the vaccinated group (P-value < 0.001). In particular, the number of deaths in sick/poor-doing pigs (runts) decreased significantly as well from 8.94% in the control group to 2.98% in the vaccinated group (P-value <0.001). Other parameters such as sudden death animals or pneumonia, diarrhea or Streptococcus suis infections were also evaluated with no- statistical differences between groups

Conclusions
The results of this study show Rhiniseng® vaccine can significantly reduce mortality and the % of runts pigs in nursery farms challenged with non-progressive atrophic rhinitis. As the Canadian swine industry aims to reduce antibiotic use, some bacterial diseases such as atrophic rhinitis may become more prevalent, so prevention tools must be needed in the near future. Further studies will need to be carried out to determine the prevalence of Bordetella bronchispetica and Pasteurella multocida type D in Canada, but there are some indications these pathogens may be increasing on Canadian farms.

Citations
1. De Jong MF. 2006. Progressive and Non-Progressive Atrophic Rhinitis. Disease of swine, 10th Edition, 34:577-602.Referencia Donko.
2. Brockmeier SL et al.2012. Diseases of swine; 49:670-679.
3. Sánchez-Matamoros, A Field efficacy study of RHINISENG® in Europe in a farm with problems associated with Bordetella bronchispetica. AASV 2017.

Poster Number: 25
Topic Area: Reproduction
Title: Evaluation of the effect of a Swine Erysipelas, Porcine Parvovirus and Leptospira spp. vaccine on boar sperm output
Author(s): Irene Galé, HIPRA HQ; Daniela Bruna, HIPRA Saúde Animal Ltda; Gabriel Peixoto, HIPRA Saúde Animal Ltda; Joaquin Miguel, HIPRA HQ; Ballará Isaac, HIPRA HQ; Lorena Nodar, HIPRA HP; Poliana Silva, Coopercentral

Introduction
Reproductive vaccines against Porcine Parvovirus, Swine Erysipelas and Leptospira spp. are commonly applied in boars[1]. It is known that some vaccines can cause fever after vaccination[2]. It has been demonstrated that an increase in temperature to over 38.5ºC directly affects the sperm quality of the boar[3] and it can also be affected after vaccine administration[4]. Considering that the length of spermatogenesis is about 35 days and that maturation and transport from the testes to the epididymis takes around 15 days[5], adverse reactions at any of these times could affect sperm quality. The aim of this study was to evaluate the effect of a possible temperature increase due to vaccination or other adverse reaction, on the sperm quality.

Methods
The experiment was carried out on a 110-boar stud in the state of Minas Gerais, Brazil. 20 boars were equally distributed into two groups, considering factors such as genetics, age and semen quality. The groups were: EPL, animals vaccinated with ERYSENG® PARVO/LEPTO (inactivated vaccine against Swine Erysipelas, Porcine Parvovirus and 6 serovars of Leptospira spp., adjuvanted with HIPRAMUNE® Gd) and CON, control group (not vaccinated).The temperature of the animals was measured at the time of vaccination (0h), after 6 hours (+6h) and 24 hours after vaccination (+24h). Semen quality parameters were monitored 1 month before vaccination and 3 months later, in order to compare whether semen production was impaired by the vaccine application. Parameters evaluated were: volume, sperm concentration per ml, total sperm production (according to Topigs Norsvin methodology) and sperm morphology (using a phase contrast microscope (1000x) in an external laboratory (Ceppa-Campinas-SP). Parameters were collected from both time periods (before and after vaccination) and compared within and between groups using an ANOVA test.

Results
No statistical differences were found ($p>0.05$) at any time regarding temperature, either in group EPL comparing 0h, +6h and +24h, or comparing boars from group EPL with CON. Sperm morphology showed similar values, with no statistical differences before and after vaccination ($p>0.05$). An increase in ejaculate volume and a decrease in concentration were found in both groups ($p<0.05$) before and after vaccination but with no statistical differences between groups ($p>0.05$). Total sperm production was similar ($p>0.05$), with no differences between treatments or times of comparison.

Conclusion
This study shows that the use of ERYSENG® PARVO/LEPTO does not impair sperm production and its quality (volume, concentration per ml, total sperm production and morphology) in comparison with a group of non-vaccinated boars. Differences found in both groups in terms of sperm concentration per ml and ejaculate volume, during the periods studied before and after vaccination, could be explained by different factors such as the age of the animals or the seasonal effect[6] but it is remarkable that the total sperm output was similar.

In conclusion, the use of safe vaccines that do not cause fever after vaccination, must be taken into consideration when evaluating vaccine performance in boars, as some vaccines can cause fever and reduction of feed intake in swine[7], which could affect the sperm output.

References
1- Tizard I. R. Porcine vaccines. Vaccines for veterinarians. 2021: 225-242.e1.
2- Camprodon et al. APVS 2017. B-57
3- Lopez Rodriguez et al. Boar management and semen handling factors affect the quality of boar extended semen. Porcine Health Management (2017) p 3:15.
4- Christopher-Hennings, J et al. Effects of a modified-live virus vaccine against porcine reproductive and respiratory syndrome in boars. Am J Vet Res 1997

5- Corrêia, M. N. et al. Artificial insemination in swine. Pelotas: Printpar gráfica e editora (2001) 181p.
6- Smital J. Effects influencing boar semen. 2009, Anim Reprod Sci 110:335-346.
7- Balderrama V et al. 2019. Proceedings 50th Annual Meeting AASV. 145-52.

Poster Number: 26
Topic Area: Reproduction
Title: Comparative Reproductive Performance of ERYSENG® PARVO on Canadian Farms
Author(s): Irene Galé, HIPRA HQ; Mike De Groot, HIPRA CANADA; J. Miguel, HIPRA HQ; Isaac Ballarà, HIPRA HQ;

Introduction

Reproductive problems in sows are multifactorial and can be divided into two large groups: non-infectious problems and infectious problems[1]. When focusing on limiting the impact of disease challenges on reproductive performance, important pathogens that are widely vaccinated against are Swine Erysipelas (SE), Porcine Parvovirus (PPV), and *Leptospira spp*[2]. Although most farms will vaccinate against these pathogens, there are significant differences between the commercial vaccines available in terms of humoral immune response[3] and safety[4]. There are also differences in disease prevalence; SE and PPV are considered highly prevalent in swine production whereas Leptospirosis has a much lower prevalence[5]. Previous works has shown vaccination failures against SE and PPV on Canadian farms[6]. The objective of this study was to evaluate reproductive performance of sow farms before and after implementing ERYSENG® PARVO, an inactivated bivalent vaccine against SE and PPV, and adjuvanted with HIPRAMUNE® G[d], on seven sow farms in Canada. The farms were all previously vaccinated with two other commercial trivalent vaccines (adjuvanted with an oil based adjuvant Amphigen® and an aqueous adjuvant respectively) widely used against SE, PPV, and *Leptospira spp.*

Methods

This study examined seven commercial swine farms enrolled in the Canadian Quality Assurance (CQA) program in Ontario, Canada that switched from a commercial trivalent vaccine to ERYSENG® PARVO in 2019 and 2020. Farm size on these seven farms varied from 350 sows to 2,500 sows. All gilts entering the farms received ERYSENG® PARVO vaccination plan on arrival and given a booster dose 3 weeks later. Timing of vaccination on sows varied on the seven farms, but all sows were given a booster dose each gestation period (3-4 weeks before farrowing or day 7-10 of lactation). Farm reproductive data was collected for a period of 4-6 months before ERYSENG® PARVO vaccination and 6-14 months post vaccination. Data were analyzed comparing reproductive performance 4-6 months before ERYSENG® PARVO vaccination and 3-11 months after implementing ERYSENG® PARVO in the farm's vaccination plan and all the animals had been received at least 1 shot.

Results

Average total piglets born per sow significantly increased *(P<0.05)* during the period the sows were vaccinated with ERYSENG® PARVO (15.20 ± 0.59 vs 14.66 ± 0.59) and consequently the average live born piglets (13.52 ± 0.43 vs 13.15 ± 0.66). Other parameter improved *(P<0.05)* related to the change on the vaccine, was the percentage of weaned sows bred by 7 days (91.23 ± 3.42 vs 88.97 ± 4.18). There were no significant changes in other reproductive parameters monitored.

Conclusions

Following implementation of ERYSENG® PARVO, different reproductive parameters improved significantly, which entails greater productive and economic profitability for the farms. A reason for this improvement could be starting to use a vaccine with greater efficacy[7] against SE and PPV and safety[8]. Moreover, removing the antigens of *Leptospira spp.* from the vaccination plan did not cause any reproductive problems in the farms evaluated, demonstrating that vaccination with reproductive bivalent vaccines (SE and PPV) could play a great role in the improvement of reproductive parameters in the country.

References

1. Muirhead R *et al.* Managing Pig Health. P201-255.
2. Adler B and De la Peña Moctezuma A, 2010, Vet Microbiol., 140:292-4
3. Camprodon *et al.* APVS 2017. B-53
4. Camprodon *et al.* APVS 2017. B-57
5. Prescott J. F. and Mcewen S. A.. Canadian Journal of Veterinary Research, 1989 53(3):290-4
6. DeGroot M et al. Banff Pork Seminar 2018. 2018-0197
7. Jordà R et al. Banff Pork Seminar 2018. 2018-137S
8. Jordà R et al. Banff Pork Seminar 2018. 2018-138S

Poster Number: 27
Topic Area: Other
Title: Comparison of time taken by intradermal and intramuscular vaccination at different production stages.
Author(s): Isaac Ballarà, Laboratorios HIPRA S.A; Almudena Sánchez-Matamoros, Laboratorios HIPRA S.A; Silvia Ramirez, Laboratorios HIPRA S.A; Alba Puigredon, Laboratorios HIPRA S.A; Jorge Molina, Laboratorios HIPRA S.A; Montserrat Ortiz, Laboratorios HIPRA S.A

Introduction:
One of the main concerns of farmers is time optimisation, which is directly associated with different worker tasks, such as vaccination. Therefore, herd management should be improved by analysing the duration of the vaccination procedure using the available devices. The objective of this study was to compare the time taken by intradermal (ID) and intramuscular (IM) vaccination procedures on conventional farms: farrowing sows, suckling piglets and the nursery stage.

Method:
The study was conducted as a parallel group study (ID using Hipradermic® vs IM using Henke or Hauptner) on 3 different farm sites (farrowing, suckling piglets and nursery) representing different vaccination scenarios (Table 1). All the vaccinations were performed by herd personnel with experience in the use of all the devices and were recorded for further analysis. The time parameters recorded were inoculation time per animal, device preparation, vaccine vial and needle changes.

Table 1: Farm, scenarios and vaccination procedure.

Results:
If we consider the individual parameters included in the vaccination procedure, the time taken for ID sow inoculation in the different scenarios was significantly less than for IM (Table 2). In contrast to other published articles, the time taken to inoculate piglets in farrowing or nursery was significantly longer in the ID vaccinated group (Table 2). The time spent on device preparation was longer with ID versus IM (30.5 ± 4.6 vs 22.2 ± 8.3); however, vial change was less in the ID group (15.0 ± 4.3 vs 23.3 ± 12.3). Likewise, no needle change was necessary (21.5 ± 8.6). Finally, if we consider all the factors affecting the vaccination procedure, the duration was shorter in the ID vaccinated group compared to the IM group on all farm and in all scenarios.

Table 2: Number of vaccinated animals and inoculation time per animal (seconds).

Conclusion:
This field study demonstrated that ID vaccination using the Hipradermic® device could be performed in less time than IM vaccination as no vaccine vial and needle changes are needed. Intradermal vaccination with Hipradermic® allows workers' time to be optimised in herd management on the farm.

Poster Number: 28
Topic Area: Production
Title: Reduction in sow mortality and ROI after vaccination against Clostridium novyi on two commercial farms in Brazil.
Author(s): Irene Galé, HIPRA HQ; Tatiana de Souza, HIPRA Saúde Animal Ltd; Ferradin Daniela Bruna, HIPRA Saúde Animal Ltd; Gabriela Ibanez, HIPRA HQ; Lorena Nodar, HIPRA HQ; Oriol Boix, HIPRA HQ

Introduction
Clostridium novyi (C.novyi) is an anaerobic, motile, non-capsulated, spore-forming Gram-positive bacterium producing a lethal alpha toxin that can cause sudden death in swine[1]. The course of the disease is usually acute, with no previous clinical signs and occurs mainly in females during the peripartum period2. Immediately after dying, the animals present rapid post-mortem decomposition and macroscopic evaluation shows distension of the carcass, purple skin colour, subcutaneous infiltration with blisters and bloody fluid with an unpleasant odour, generalized oedema and a dark and enlarged liver[2]. The aim of this study was to calculate the return on investment (ROI) after the introduction of a C. novyi alpha toxin vaccine on a sow farm suffering high levels of sudden death in sows.

Material and Methods
The study was carried out on two farms over a period of 3 years in the State of Paraná in Southern Brazil (Farm A with 1,817 sows and Farm B with 2,500 sows). On both farms, the sows were immunized in the first 18 months with a vaccine against neonatal diarrhoea without C. novyi alpha toxin (P1 August/2017 - December/2018). In the subsequent 18 months (P2, January/2018 - April/2020), both farms were immunized with SUISENG®, including C.novyi in its composition. To distinguish sow sudden death from general sow mortality, immediately after death sows were necropsied and macroscopic lesions suggestive of C. novyi infection were monitored. The economic cost of sow mortality was calculated according to farm-based parameters, including sow and genetics amortization, the gestation value, and costs associated with feeding, housing and management.

Results
Farm A showed a mortality rate of 3.74% (68/1817) during P1, representing 41,410 $. During P2, the mortality rate decreased to 1.98% (36/1817), representing 21,923 $. This was a 47% reduction in the sow mortality rate due to sudden death (p-value < 0.05), generating savings of 17,051 $ and a ROI of 7.32 after the vaccination plan was changed.
Farm B showed a mortality rate of 2.3% (64/2500) during P1, representing 38,974 $. During P2, the mortality rate decreased to 1% (24/2500), representing 14,615 $. This was a 62% reduction in sow mortality due to sudden death (p-value < 0.05), generating savings of 23,004 $ and a ROI of 5.42 after the vaccination plan was changed.

Conclusion
The decrease in mortality after vaccination was statistically significant on both farms. Therefore, the connection between the decrease in mortality and the use of vaccines indicates that immunization against Clostridium novyi could be responsible for a significant reduction in the mortality rate and its associated costs.

Citations
1. Skarin H, Segerman B. Plasmidome interchange between Clostridium botulinum, Clostridium novyi and Clostridium haemolyticum converts strains of independent lineages into distinctly different pathogens. PLoS One. 2014;9(9):e107777.
2. Akiyama N, Shibahara T, Ushiyama K, Shimizu H, Koizumi I, Osaki M. Fatal Clostridium novyi type B infection in a sow. Jarq-Jpn Agr Res Q. 2017;51(1):85–9.

Poster Number: 29
Topic Area: Production
Title: Evaluation of UVB light on pre-wean piglet Vitamin D3 synthesis and productivity
Author(s): Benjamin C. Smith, Iowa State Univeristy; Brett C. Ramirez, Iowa State Univeristy; Laura L. Greiner, Iowa State Univeristy; Justin T. Brown, Iowa State Univeristy

Introduction
Piglets reared in indoor production systems are commonly deficient in Vitamin D and are typically administered low efficacy and expensive oral supplements (Flohr et al., 2016; Matte & Audet, 2020). Vitamin D, specifically vitamin D3, is used by pigs to improve digestion and immune system function. Pigs can synthesize vitamin D3 in epithelial cells when exposed to ultraviolet light (280 to 320 nm, UVB; Stricker Jakobsen et al., 2020).objective of this study was to evaluate low intensity UVB-295 nm LEDs as a supplement Vitamin D3 source for neonatal piglets.

Methods
A two-room laboratory was utilized with each room housing four farrowing stalls. Two stalls in each room were randomly assigned either UVB treatment or no treatment. Two UVB-295nm LEDs (2 mW optical output) were attached to the supplemental heat sources. A novel supplemental heat source was utilized in room 1 housing the LEDs 36 cm above the floor while heat lamps were used in room 2 housing the LEDs 4 cm above the floor. Eight parity 6 and 7 sows were procured from a commercial producer with sow parity selected by the producer. UVB treatment was started at seven days of age through weaning. This was performed to minimize the lactation capacity and colostrum impact on serum levels of vitamin D3. Blood samples were collected from one piglet per litter at seven and fourteen days of age and at weaning, this assumed that the probability of each pig entering the UVB treatment area under the heat source was the same and similar research with nursery pigs (Flohr et al., 2016). Individual piglet weights were collected at seven days of age, fourteen days of age, and at weaning. A Standard Least Squares model was utilized to analyze the serum levels of 25 (OH) D3 with fixed effects of treatment, day of experiment and the interaction of the two, and a Standard Least Squares model was used to evaluate the weight and growth with fixed effects of age, starting weight and treatment.

Results and Conclusions
No erythema or mortalities were attributed to the UVB treatment in this study. There was a significant difference between the seven and fourteen day of age serum levels for the UVB-295 nm group ($P < 0.05$), and a trend for significant difference between control and UVB treatment at fourteen days of age. No other differences were noted for serum levels or growth ($P > 0.05$). This study suggests that the use of low intensity UVB LEDs are promising for supplementation of piglets in the creep area, but requires further investigation.

References
Flohr, J. R., Woodworth, J. C., Bergstrom, J. R., Tokach, M. D., Dritz, S. S., Goodband, R. D., & DeRouchey, J. M. (2016). Evaluating the impact of maternal vitamin D supplementation on sow performance: II. Subsequent growth performance and carcass characteristics of growing pigs1,2. Journal of Animal Science, 94(11), 4643–4653. https://doi.org/10.2527/jas.2016-0410

Matte, J. J., & Audet, I. (2020). Maternal perinatal transfer of vitamins and trace elements to piglets. Animal, 14(1), 31–38. https://doi.org/10.1017/S175173111900140X

Stricker Jakobsen, S., Nielsen, J. P., & Jakobsen, J. (2020). Effect of UVB light on vitamin D status in piglets and sows. The Journal of Steroid Biochemistry and Molecular Biology, 200, 105637. https://doi.org/10.1016/j.jsbmb.2020.105637

Poster Number: 30
Topic Area: Viral Disease
Title: Antiviral Effects of Glycerol Monolaurate on Wild-Type African Swine Fever Virus in Primary Swine Cells
Author(s): Charles Elrod, Natural Biologics, Inc./Cornell University; Joshua A. Jackman, School of Chemical Engineering, Sungkyunkwan University; Erik Arabyan, Group of Antiviral Defense Mechanisms, Institute of Molecular Biology of NAS; Zaven Karalyan, Group of Antiviral Defense Mechanisms, Institute of Molecular Biology of NAS; Hovakim Zakaryan, Group of Antiviral Defense Mechanisms, Institute of Molecular Biology of NAS

The development and commercial translation of chemical mitigants to inhibit African swine fever virus (ASFV) is encumbered by two challenges: (1) mitigant testing is mainly limited to a laboratory-adapted ASFV strain that cannot infect pigs; and (2) wild-type ASFV strains only infect and multiply in primary swine cells and are incompatible with common model cell lines used in laboratories. To address these shortcomings and to validate glycerol monolaurate (GML) as a promising anti-ASFV mitigant against a wild-type ASFV strain, the objective of this study was to determine the effect of GML on inhibiting the infectivity of the highly virulent ASFV Armenia/07 strain in freshly isolated porcine alveolar macrophage (PAM) cells.

To isolate PAM cells, healthy, ~30 kg pigs were euthanized according to ethical guidelines and the pig lungs were removed and processed to recover free alveolar cells. The PAM cells were resuspended in culture media with appropriate supplements for antiviral assays. Pig blood cells were also collected and processed to have enriched blood erythrocytes.

Briefly, suspensions of ASFV Armenia/07, at a multiplicity of infection of 0.5 50% hemadsorption doses ($HADU_{50}$) per well, were first treated with varying GML concentrations, 250, 125, 63, or 31 µM in a two-fold dilution series, for one hour at room temperature along with negative control (virus-only without GML). The treated virus samples were then added to PAM cells seeded at 2×10^5 cells per well in a 96-well plate and cultured for 48 or 72 hours. After 24 hours, aliquots of the erythrocyte preparation were added to each well. ASFV presence was quantified by the formation of erythrocyte rosettes around infected macrophages and expressed in $HADU_{50}$/mL units. Each experiment was repeated three times and the results were averaged for each concentration and time point. Statistical analysis was performed by one-way analysis of variance (ANOVA).

The results indicate that GML exhibited highly potent antiviral activity against ASFV Armenia/07 in the 63 µM to 250 µM concentration range, with corresponding 2.5 to 2.75 log reductions in ASFV infectivity after 48 hours and 2.50 to 3.42 log reductions in ASFV infectivity after 72 hours (Table 1). By contrast, 31 µM GML treatment was inactive and did not reduce ASFV infectivity after either 48 or 72 hours, which also agrees with the molecular characteristics of GML.

Table 1. Dose response of GML antiviral activity against ASFV Armenia/07 (log $HADU_{50}$/mL)

Time Post-Inoculation	Control (virus only)	31 µM	63 µM	125 µM	250 µM
48 hours	5.08	5.00	2.58**	2.42**	2.33**
72 hours	5.42	4.92	2.92**	2.17**	2.00**

** $P<0.01$

Notably, these results demonstrate that GML exhibits equal or superior antiviral activity against the highly virulent wild-type ASFV Armenia/07 strain, as compared to activity against laboratory-adapted strains as reported in past works. Taken together, the findings in this study support that GML has high antiviral potency against wild-type ASFV, even at low concentrations, and is therefore a promising mitigant to be further investigated for feed and drinking water applications.

Poster Number: 31
Topic Area: Viral Disease
Title: Flavonoid Library Screening Reveals Highly Antiviral Mitigants of African Swine Fever Virus
Author(s): Charles Elrod, Natural Biologics, Inc./Cornell University; Joshua A. Jackman, School of Chemical Engineering, Sungkyunkwan University; Erik Arabyan, Group of Antiviral Defense Mechanisms, Institute of Molecular Biology of NAS; Hovakim Zakaryan, Group of Antiviral Defense Mechanisms, Institute of Molecular Biology of NAS

Naturally occurring plant flavonoids are a promising class of antiviral agents that may be used to inhibit African swine fever virus (ASFV). Published studies of specific flavonoids have reported a wide range of antiviral mechanisms against ASFV and other viruses, which motivates a broader search for flavonoids with greater anti-ASFV potency and unique mechanisms of action. The objective of this study was to screen a library of 90 flavonoid compounds in a Vero cell-based antiviral assay against the ASFV Ba17V strain.

First, the flavonoids were tested for cytotoxic effects in Vero cells and a test concentration of 20 µg/mL was judged as nontoxic for all flavonoids except diosmin, khellin, and 7,8-bezoflavone, which were used at 10 µg/mL. For screening, ASFV suspensions of 2 X 10^5 50% tissue culture infectious doses ($TCID_{50}$) were incubated with the test compounds for 1 hour and then the virus-compound mixtures were diluted 20-fold before being added to infect Vero cells. The cell culture media was collected 24 hours post-infection and titrated by cytopathic effect assay. Virus titers were calculated by the Spearman-Karber endpoint method and expressed as $TCID_{50}$/mL. Each experiment was repeated three times and the results were averaged for each compound. Statistical analysis was performed by one-way analysis of variance (ANOVA).

From the screen, flavonoids that exhibited >40% inhibition of ASFV and showed no sign of Vero cell monolayer damage were selected as positive hits and included 7,8-benzoflavone, calycosin, diosmin, isosenensetin, kaempferol, khellin, maackiain, sakuranetin, and sinensetin. More detailed assays focused on evaluating antiviral activity and virucidal activity were then performed on these compounds (Table 1).

For the antiviral assay, Vero cells were infected with ASFV and treated simultaneously with a flavonoid. After 24 hours, by which time the first cycle of viral replication had occurred, the cell culture media was collected for virus titration. Six of the nine flavonoids demonstrated statistically significant antiviral activity: 7,8-benzoflavone, diosmin, kaempferol, maackiain, sakuranetin and sinensetin. Kaempferol had the greatest antiviral activity, reducing the virus titer from 5.14 ± 0.19 log $TCID_{50}$/ml to 3.98 ± 0.19 log $TCID_{50}$/ml ($P < 0.01$), which corresponds to a 92% reduction in viral yield. For the virucidal assay, the virus suspension was incubated with the flavonoids for one hour, then diluted 20-fold and added to the Vero cells. The media was harvested 24 hours post-infection and titrated by cytopathic effect assay. Only 7,8-benzoflavone demonstrated significant virucidal activity, reducing the virus titer from 5.24 ± 0.21 log $TCID_{50}$/ml to 4.64 ± 0.09 log $TCID_{50}$/ml ($P < 0.05$), indicating that this compound possesses virucidal activity.

Table 1. Antiviral and virucidal activities of flavonoids against ASFV *in vitro* (log $TCID_{50}$/mL)

	Control (virus only)	7,8-benzo.	Diosmin	Kaempferol	Maackiain	Sakuranetin	Sinensetin
Antiviral	5.14	4.47*	4.48*	3.98*	4.2*	4.3*	4.75*
Virucidal	5.24	4.64*	5.02	5.5	5.2	5.14	5.7

* $P<0.05$

In conclusion, several flavonoids were identified as potential mitigants against ASFV. One flavonoid was found to have direct-acting virucidal activity, while other flavonoids were found to disrupt another step in the viral infection or replication stages of ASFV.

Poster Number: 32
Topic Area: Viral Disease
Title: Antiviral Effects of Kaempferol on African Swine Fever Virus Infection
Author(s): Charles Elrod, Natural Biologics, Inc./Cornell University; Joshua A. Jackman, School of Chemical Engineering, Sungkyunkwan University; Erik Arabyan, Group of Antiviral Defense Mechanisms, Institute of Molecular Biology of NAS; Tamara Hakobyan, Group of Antiviral Defense Mechanisms, Institute of Molecular Biology of NAS; Astghik Hakobyan, Group of Antiviral Defense Mechanisms, Institute of Molecular Biology of NAS; Rafaella Grigoryan, Group of Antiviral Defense Mechanisms, Institute of Molecular Biology of NAS; Roza Izmailyan, Group of Antiviral Defense Mechanisms, Institute of Molecular Biology of NAS; Aida Avetisyan, Group of Antiviral Defense Mechanisms, Institute of Molecular Biology of NAS; Zaven Karalyan, Group of Antiviral Defense Mechanisms, Institute of Molecular Biology of NAS; Hovakim Zakaryan, Group of Antiviral Defense Mechanisms, Institute of Molecular Biology of NAS

Naturally occurring plant flavonoids are a promising class of antiviral agents to inhibit African swine fever virus (ASFV), which causes highly fatal disease in pigs and is a major threat to the swine industry. We recently performed a library screen of anti-ASFV flavonoid candidates and identified that kaempferol was the most potent one. The objective of this study was to characterize the antiviral mechanisms of kaempferol in order to understand how it inhibits ASFV in vitro and to assess its feasibility as an ASFV mitigant.

Using a laboratory-adapted ASFV strain, we first treated ASFV-infected Vero cells with kaempferol at different concentrations. The strongest antiviral effect, a 1.26 log decrease, was recorded at 20 μg/ml concentration. This inhibitory effect occurred in a dose-dependent manner down to around 2.5 μg/ml.

Next, we determined the optimal treatment duration required for the strongest antiviral effect on ASFV replication. 20 μg/ml kaempferol was added at 0 h post-infection and was then removed through medium replacement after 2, 4, 8, 12, or 16 h. After 24 h post-infection, the virus was collected and quantified. Gradual declines in viral titers were observed as the interval between infection and compound removal increased.

To determine the specific replication stages in the ASFV life cycle that can be affected by kaempferol, we conducted time-of-addition experiments. The compound at 20 μg/ml concentration was added at seven time points prior to and after infection followed by virus titration at 24 h post-infection. A sharp drop in virus titer was observed at early stages of infection. Notably, the most potent antiviral effects were observed when kaempferol was added at 0 and 2 h post-infection, which led to viral titer reductions of 1.25 log ($P < 0.05$) and 1.1 log ($P < 0.05$), respectively. In marked contrast, there was no significant inhibitory effect on ASFV infection when kaempferol was added at later stages of infection.

In addition, we conducted a liquid plaque assay to define whether 20 μg/ml kaempferol can inhibit virus spread in vitro. Kaempferol was added to Vero cells together with ASFV (0 h) or 1 h post-infection. The results showed a significant reduction in plaque numbers in a dose-dependent manner, indicating that the viral spread was diminished in the presence of kaempferol.

Since macrophages are primary target cells for ASFV replication in pigs, we also studied the antiviral activity of kaempferol against a highly virulent ASFV isolate (Arm/07) in porcine alveolar macrophages. For this purpose, ASFV-infected macrophages were treated with kaempferol at increasing concentrations up to 20 μg/ml. Anti-ASFV activity was observed at 10 and 20 μg/ml concentrations in a dose-dependent manner. Kaempferol at 20 μg/ml concentration reduced the viral titer from 5.3 ± 0.11 log HADU50/ml to 3.4 ± 0.14 log HADU50/ml ($P < 0.01$), thereby demonstrating more potent antiviral activity than in Vero cells.

Together, these findings support that kaempferol is a promising anti-ASFV agent that inhibits early stages of virus infection and can be further explored to treat ASFV infection in pigs as part of preventative health strategies.

Poster Number: 33
Topic Area: Bacterial Disease
Title: Fighting enteropathogenic E. coli with algal polysaccharides
Author(s): Marie Gallissot, Olmix Group; Maria Garcia Suarez, Olmix Group; Mustapha Berri, INRAE; Pi Nyvall-Collen, Olmix Group; Danièle Marzin, Olmix Group

Young piglets raised in commercial conditions are highly sensitive to enteropathogenic Escherichia coli infections (colibacillosis), which can colonize the piglets' intestinal epithelium and trigger diarrhea with important economic losses. Thus, supporting the integrity of the intestinal mucosa and the immune system is essential to ensure gut health in piglets (Turner, 2009). Recent research has shown, in vitro, that a red algal extract from Solieria chordalis upregulates the gene expression of tight junction and mucin proteins (Gallissot et al., 2017) which are essential for the proper functioning of the intestinal epithelial barrier. Other research has shown that a green algal extract from Ulva sp, has the capacity to modulate the immune response as demonstrated in vitro (Berri et al., 2016 and 2017). The first objective of the present work was to evaluate, in vitro, the capacity of the red algal extract to reinforce the gut barrier in the presence of an enteropathogenic E. coli strain. Also tested, in vivo, was the ability of this red algal extract, together with the green algal extract from Ulva sp, to improve the health status and performance of nursery piglets. In the in vitro assays, IPEC-1 cells were isolated from the jejunum and the ileum of a newborn piglet and incubated with the cell-culture medium only (negative control) or with the red algal extract prior to incubation with E. coli K88 1305 and E. coli K88 968. Trans-epithelial electrical barrier (TEER) and bacterial adhesion to cell culture were measured. Results showed that the red algal extract maintained a higher TEER than the negative control in the first ten hours of infection with E. coli K88 1305. Moreover, the red algal extract reduced the percentage of adhesion of E. coli K88 968 to IPEC-1 cells at the three concentrations tested compared to the negative control. In vivo, two hundred and eleven piglets from gilts weaned at 21 days were homogenously distributed to 8 post-weaning pens of 27±1 piglets and divided in 2 experimental groups: a control group (not supplemented), and a test group receiving the algae-based supplement for 43 days (until transfer to grow-finish units). Results showed an improved health status of the tested piglets when compared to the control animals as shown by a decreased inflammatory status (-16% haptoglobin level at 26 days of age), better fecal consistency during the first two weeks post-weaning and a lower number of animals needing a medical treatment (-57%, $P<0.01$). The nursery piglets were better prepared for this stressful period and showed improved performance (+300g at 64 days, -1pt FCR), proving good potential of this algae-based supplement to promote health and performance of pigs in the nursery phase.

Poster Number: 34
Topic Area: Feed/Nutrition
Title: A unique algae-clay technology mitigates mycotoxin risk in piglets
Author(s): Marie Gallissot, Olmix Group; Raquel Pereira, Independant consultant; Maria Angeles Rodriguez, Olmix Group; Julia Laurain, Olmix Group

Deleterious effects of mycotoxins on the health and performance of piglets have been widely described (Escriva et al., 2015). A common strategy to prevent such effects consists in using mycotoxin binders in the feed to reduce the exposure of the animals to mycotoxins. Several materials have proven their capacity to mitigate aflatoxin exposure, but very few products seem to be efficient in controlling fusariotoxins like deoxynivalenol and fumonisins (Laurain et al., 2019). Yet, these mycotoxins are the most commonly occurring in animal feeds in North America (Abbas, 2019). The aim of the present study was to measure the efficacy of a patented algo-clay complex (ACC) to mitigate the toxicity of three mycotoxins: deoxynivalenol (DON), fumonisins (FUM) and aflatoxins (AFLA) in piglets. The experiment was conducted by the Samitec Institute in Brazil and divided into three trials (one per mycotoxin). For each trial, 30 piglets with an average body weight of 10kg were distributed into 5 treatments with 6 replicates. The DON and AFLA trials lasted for 28 days and the FUM trial lasted for 42 days. The three trials tested the mycotoxins individually at 3ppm DON, 50ppm FUM and 1ppm AFLA. Treatments varied as per mycotoxin contamination (with or without) and supplementation of the algo-clay complex (0, 0.25% or 0.50%). The following performance and biological parameters were monitored for all trials: feed intake, body weight, and relative liver weight (RLW). The sphinganine to sphingosine ratio (Sa/So) in the serum was also measured in the FUM trial. The inclusion of 0.50% of ACC in the contaminated feed systematically improved feed intake and body weight of the piglets compared to the group with mycotoxin contamination alone ($P \leq 0.05$). In the FUM and AFLA trials, the RLW of the piglets supplemented with 0.50% of the ACC was significantly improved compared to the group exposed to mycotoxins with no ACC supplementation. The addition of 0.25% and 0.50% of the ACC in the FUM-contaminated diet significantly reduced the Sa/So ratio of piglets compared to the groups fed the FUM-contaminated diet with no ACC ($P \leq 0.05$). The evaluated parameters illustrate the capacity of the algo-clay complex to reduce the deleterious effects of high levels of deoxynivalenol, fumonisins or aflatoxins on biological parameters and performance of piglets. This technology appears to be a relevant strategy to manage mycotoxin risk on pig farms.

Poster Number: 35
Topic Area: Bacterial Disease
Title: Slaughterhouse visual and palpation method for monitoring economic losses of porcine proliferative enteropathy (PE)
Author(s): Szabó István, ENVIROSCIENCE Kft., Hatvan, Hungary; István Makkai, Intervet Hungaria Kft., Budapest, Hungary; Péter Máté, Intervet Hungaria Kft., Budapest, Hungary; Tamás Molnár, ENVIROSCIENCE Kft., Hatvan, Hungary; Jessica Laurin Seate, Merck Animal Health, Madison, New Jersey; Rika Jolie, Merck Animal Health, Madison, New Jersey; Hanny Swan, MSD CDS, Boxmeer, Netherlands; Derald Holtkamp, Iowa State University, Ames, IA; Róbert Glávits, Autopsy Path Kft, Budapest, Hungary; István Szabó junior, ENVIROSCIENCE Kft., Hatvan, Hungary

Introduction:
Lawsonia intracellularis causes porcine proliferative enteropathy (PPE), a disease characterized by thickening in the small intestine due to the proliferation of enterocytes, typically in the ileum, but also in the colon's mucosa. Economic losses due to ileitis have been estimated at $4.65 per fattening pig, with US pig farmers losing $56.1 million annually.

Currently there is no monitoring tool at the slaughterhouse for PPE evaluation and it has become necessary to develop an ileitis monitoring tool that is inexpensive, simple, fast, sensitive and provides immediate results.

Materials and methods:
Our studies were performed on pig herds and slaughterhouses in five Central European countries.
Ileitis slaughterhouse monitoring method (EnteriPig)
The ilea of slaughtered pigs were evaluated based on visual assessment and palpation. During the assessment, the following evaluation system was developed and used (table 1.):

Table 1.
0 = no perceptible lesion, no hard to the touch tact formula in the intestine, no thickening at the ileocecal intestinal cross section, no color change visible through serosa
1 = one or two palpable hard tact 0.5-1 cm formula in the intestine, slightly noticeable folds in the intestine, slight increase in intestinal wall thickness
2 = multiple, hard tact oval formula in the intestine, pronounced folds in the intestine, thickening at the ileocecal intestinal cross section, discoloration
2 + = blood content plus as described in the 2 evaluations already visible from the serosa

Hypothetic losses due to the disease were evaluated by Holtkamp's ileitis economic losses study 2019. In the EnteriPig tool, animals with an ileum scored for "0" corresponded to Holtkamp PPE-free, those with a value of "1" in lower, and those with a value of "2" in upper bound category. The percentage incidence of individuals with acute PPE lesions "2+" was used to represent acute.

Results:
More than 1.500 fattening pigs from 27 pig farms, Lawsonia seropositive, were checked at slaughterhouses. Ileum examinations performed with this new tactile method scored "0" in 46%, "1" in 26.9%, "2" in 26.7% of the animals. "2+" rated ileums were scored in 0.5%.

Cohen's kappa coefficient (κ) analyses were applied to the mucosal results on visual and palpation methods. There was significant agreement observed in these two methods.

Evaluation of economic losses of the disease by visual and palpation monitoring tool at the slaughterhouse show that only 60-70% of the achievable profit have been obtained. In a dedicated farm, animals' acute ileitis EnteriPig score (2+) projected significant losses for producing fatteners due to Lawsonia intracellularis infection. In 2 cases, 20-42 % of the achievable profit; in 9 cases, 62-76% and in 1 case (Ileitis vaccination started 5 months prior) 82% of the achievable profit were obtained.

Discussion:

The method developed is similar to lung lesion scoring tools and can be performed at slaughterhouses. The results of the procedure are correlated with the results of other laboratory diagnostic tests for ileitis (histology, immunohistochemistry, serology, fecal PCR). The results can provide immediate interpretation of the status of ileitis.

Poster Number: 36
Topic Area: Viral Disease
Title: Rethinking the uncertainty of African swine fever virus contamination in feed ingredients and risk of introduction into the United States
Author(s): Rachel Schambow, University of Minnesota College of Veterinary Medicine; Fernando Sampedro, University of Minnesota; Pedro Urriola, University of Minnesota; Jennifer van de Ligt, University of Minnesota; Gerald Shurson, University of Minnesota; Andres Perez, University of Minnesota

Transoceanic models of feed ingredient transport have demonstrated the survival of experimentally inoculated economically relevant pathogens such as African swine fever virus (ASFV). However, these models did not characterize the likelihood of virus survival under various time and temperature processes that feed ingredients undergo before they are added to swine diets. Here, we developed a quantitative risk assessment model to estimate the probability that one or more corn or soybean meal containers (25,000 tonnes) contaminated with ASFV would be imported into the United States annually. This final probability estimate was conditionally based on five likelihoods: the probability of initial ASFV contamination (p0), ASFV inactivation during processing (p1) and transport (p2), recontamination (pR), and ASFV inactivation while awaiting customs clearance at United States entry (p3). The probability of ASFV inactivation was modeled using corn and soybean (extruded or solvent extracted) processing conditions (times and temperatures), D-values (time to reduce 90% or 1-log) estimated from studies of ASFV thermal inactivation in pork serum (p1), and survival in feed ingredients during transoceanic transport (p2, p3). “What-if” scenarios using deterministic values for p0 and pR (1, 10, 25, 50, 75, and 100%) were used to explore their impact on risk. The model estimated complete inactivation of ASFV after extrusion or solvent extraction processes regardless of the initial ASFV contamination probability assumed. The value of recontamination (ranging from 1-75%) was highly influential on the risk of one ASFV-contaminated soybean meal container entering the United States. Median risk estimates ranged from 0.064% (0.006-0.60%; 95% Probability Interval [PI]), assuming a pR of 1.0%, up to 4.67% (0.45-36.50% 95% PI) assuming a pR of 75.0%. This means that at least one container with ASFV-contaminated soybean meal would be imported once every 1,563 to 21 years, respectively. When all raw corn was assumed to be contaminated (p0=100%), and no recontamination was assumed to occur (pR=0%), the median probability of one container with ASFV-contaminated corn entering the United States was 2.02% (0.28-9.43% 95% PI) or once every 50 years. Values of recontamination between 1-75% did not substantially change the risk of corn. Days of transport, virus survival during transport (D-value), and number of containers shipped were the parameters most influential for increased likelihood of a container with ASFV-contaminated soybean meal or corn entering the United States The model helped to identify knowledge gaps that are most influential on output values and serves as a framework that could be updated and parameterized as new scientific information becomes available. We propose that the quantitative risk assessment model developed in this study be used as a framework for estimating the risk of ASFV entry into the United States and other ASFV-free countries through other types of imported feed ingredients that may potentially become contaminated. Ultimately, this model can be used to develop risk mitigation strategies and critical control points for inactivating ASFV during feed ingredient processing, storage, and transport, and contribute to the design and implementation of biosecurity measures to prevent the introduction of ASFV into the United States and other ASFV-free countries.

Poster Number: 37
Topic Area: Other
Title: Feed Mill and Swine Production Site Investigations After Several Outbreaks of Porcine Deltacoronavirus
Author(s): C. Grace Elijah, Kansas State University; Olivia L. Harrison, Kansas State University; Allison K. Blomme, Kansas State University; Jason C. Woodworth, Kansas State University; Cassandra K. Jones, Kansas State University; Chad B. Paulk, Kansas State University; Jordan T. Gebhardt, Kansas State University

Three swine breed-to-wean herds were diagnosed with porcine deltacoronavirus (PDCoV) within one week in fall 2020. Site A and B were operated by the same production system, whereas Site C was independent. However, Site C receives replacement gilts from Sites A and B. Two feed mills were also included in this feed safety investigation after clients of feed mill 2 were diagnosed with PDCoV. There was suspicion that feed mill 2 was the origin or helped spread the disease to Sites A and B. Therefore, the objectives of this investigation were to understand if feed mills were202021 involved in these disease events as an epidemiological link and determine if trucks or people served as vectors and helped spread this virus. Samples from Sites focused on feed contact and non-feed contact surfaces outside of the barn. Feed mill locations focused on high-risk areas, like those with high foot or vehicle traffic, feed trucks going from farm to feed mill, and bulk feed bins. Environmental sampling was performed using one of two methods to sample locations; either utilizing a pre-moistened 10 cm square cotton gauze surgical sponge or pre-moistened paint roller covers with a paint roller extension set. Site B took and submitted a feed sample during the outbreak of clinical signs. All samples were processed at Iowa State's veterinary diagnostic laboratory (ISU VDL) for triplex qPCR of porcine epidemic diarrhea virus (PEDV), PDCoV, and transmissible gastroenteritis virus (TGEV). Extractions from all samples were analysed twice; the first time samples were analyzed using 36 PCR cycles and the second time samples were analyzed up to 45 cycles. For the Ct cut off of 36, 17 samples had detectable PEDV or PDCoV RNA. Site A had four environmental swabs with detectable PDCoV RNA, Site B had six environmental swabs with detectable PDCoV; Site C had five environmental swabs with detectable PDCoV RNA. Feed mill 2 had two environmental swabs with detectable PEDV RNA - the feed truck pedals and floor and feed truck steering wheel and dashboard. Feed mill 1 had no samples with detectable PEDV, PDCoV, and TGEV RNA. For the Ct cut off of 45, 13 additional samples had detectable PEDV or PDCoV RNA. Site A had no additional locations with detectable PDCoV RNA; Site B had nine additional locations with detectable PDCoV RNA; Site C had two additional locations with detectable PDCoV RNA. Feed mill 1 had two more sites with detectable PDCoV RNA – the feed truck steps and inside the feed truck cab. Feed mill 2 had no additional sites with detectable PEDV RNA. The Site B feed sample submitted was non-detectable for PEDV, TGE, and PDCoV RNA at both cut off values. The primary locations with detectable PDCoV or PEDV RNA were areas of high traffic and transportation vehicles suggesting that these moving locations can serve as fomites for pathogen transmission. When utilizing environmental swabs to determine sources of pathogen for disease outbreaks, environmental swabs should be analyzed using PCR threshold limit of 45 to maximize diagnostic sensitivity.

Poster Number: 38
Topic Area: Bacterial Disease
Title: Performance of Mycoplasma hyopneumoniae serum antibody ELISAs
Author(s): Ana Paula Serafini Poeta Silva, Iowa State University; Ronaldo L. Magtoto, Iowa State University; Henrique M. Souza Almeida, Iowa State University; Precy D. Magtoto, Iowa State University; Rachel J. Derscheid, Iowa State University; David H. Baum, Iowa State University; Maria J. Clavijo, Iowa State University; Bailey L. Arruda, Iowa State University; Jeffrey J. Zimmerman, Iowa State University; Luis G. Giménez-Lirola, Iowa State University

In the field, Mycoplasma hyopneumoniae (MHP) surveillance based on serum antibody detection is an economical and practical approach. The performance of six ELISAs (diagnostic specificity and cross-reactivity) was evaluated using serum samples from 50 8-week-old cesarean-derived, colostrum-deprived pigs allocated to 5 inoculation groups of 10 pigs each, 1) sham-inoculated, 2) M. flocculare-inoculated, 3) M. hyorhinis-inoculated, 4) M. hyosynoviae-inoculated, and 5) MHP-inoculated. Pigs were housed in pairs and sera (weekly) and oral fluids (daily) were collected through 56 days post inoculation. Pig infection status was established by PCR testing of the oral fluid samples.

The six MHP ELISAs were evaluated on the basis of false positive rates using the manufacturers' recommended cutoff and alternative cutoffs (calculated using known MHP-negative samples), cross-reactivity across mycoplasma species, and misclassification error score (Poisson regression). Three ELISAs in which no false positives and cross-reactivity were observed using either the manufacturers' or alternative cutoffs achieved the lowest misclassification error scores ($P < 0.05$) and were selected for further investigation under field conditions.

Thereafter, the diagnostic sensitivity of the three best-performing ELISAs was assessed using serum samples from 6-week-old pigs exposed to MHP infection under field conditions. MHP inoculum was administered in 10 pigs housed in one pen at the center of a room with ~1,250 six-week-old pigs housed in 46 pens. Matched tracheal and serum samples (n = 318) from MHP-exposed pigs were collected at 7- to 14-day intervals through 98 days post inoculation. ROC analyses of test results from the three ELISAs estimated similar diagnostic sensitivity (ELISA 1: 47%, 95 CI% 40%, 55%; ELISA 2: 56%, 95% CI 49%, 62%; and ELISA 3: 62%, 95% CI 54%, 68%) and diagnostic sensitivity (ELISA 1: 99%, 95 CI% 96%, 100%; ELISA 2: 99%, 95% CI 96%, 100%; and ELISA 3: 99%, 95% CI 96%, 100%), with no statistical difference ($P > 0.05$) in the area under the curve (ELISA 1: 0.80, 95 CI% 0.77, 0.84; ELISA 2: 0.83, 95% CI 0.79, 0.86; and ELISA 3: 0.85, 95% CI 0.81, 0.97). Therefore, either of these ELISAs (or a combination thereof) could support MHP surveillance.

Poster Number: 39
Topic Area: Bacterial Disease
Title: Probability of detection for Mycoplasma hyopneumoniae DNA in oral fluid samples with different protocols
Author(s): Ana Paula Serafini Poeta Silva, Iowa State University; Gabriel Y Storino, Iowa State University; Franco S Matias Ferreyra, Iowa State University; Min Zhang, Iowa State University; Chong Wang, Iowa State University; Jessica M Miller, Iowa State University; Karen M Harmon, Iowa State University; Philip C Gauger, Iowa State University; Wendy Witbeck, IDEXX Laboratories Inc.; Kent Doolittle, IDEXX Laboratories Inc.; Silva Zimmerman, IDEXX Laboratories Inc; Rachel J Derscheid, Iowa State University; Maria J Clavijo, Iowa State University; Bailey L Arruda, Iowa State University; Jeffrey J Zimmerman, Iowa State University

Control of Mycoplasma hyopneumoniae (MHP) is dependent upon detection of infected pigs using effective monitoring protocols. Oral fluid sample (OF) is routinely used in disease monitoring programs in swine production systems because sampling does not require animal restraint and offers an assessment of population status. However, the optimal detection parameters of MHP DNA in OF have not been clearly defined. Therefore, the objective of this study was to compare the probability of detecting MHP DNA in OF as a function of within pen MHP prevalence and test protocol.

OFs (n = 322) were collected daily from 5 groups of PRRSV- and MHP-free 7-week-old pigs from day post inoculation (DPI) -4 through DPI 59. The negative control group consisted of 3 pigs; the 4 treatment groups contained 9 pigs each. Treatment groups differed in the proportion of pigs inoculated with MHP (lung homogenate, 1 × 105 CCU/mL MHP 232) at time zero: 1/9, 3/9, 6/9, and 9/9 pigs. Tracheal swabs were collected twice weekly to establish individual pig MHP infection status.

MHP DNA detection in OF was evaluated using 4 test protocols based on a combination of two extraction methods and three PCRs. Extraction methods were (E1) MagMAXTM-96 Pathogen RNA/DNA kit, Applied BiosystemsTM, Carlsbad, CA; and (E2) IDEXX RealPCR* DNA/RNA Magnetic Bead Kit, IDEXX Laboratories Inc., Westbrook, ME. PCRs were (PCR1A) TaqMan® Fast Virus 1-Step Master Mix (Life Technologies, Carlsbad, CA) and primer/probe described for Mhp183; (PCR1B) TaqMan® Fast Virus 1-Step Master Mix with the addition of AmpliTaq® 360DNA Polymerase (5U/uL) (ThermoFisher Scientific), and primer/probe described for Mhp183; and (PCR2) RealPCR* Master Mix and RealPCR* M hyo DNA Mix, IDEXX Laboratories Inc. Results obtained with PCR1A and PCR1B were considered positive when Ct <37, while with PCR2 when Ct <40. Mixed-effect logistic regression coupled with a piecewise exponential Cox proportional hazard model was used to compare the probability of detecting MHP DNA in OF among test protocols. MHP DNA detection in OF was considered the dependent variable, protocols and estimated within-pen MHP prevalence as fixed effects, and group as random effect.

On DPI 3, tracheal swabs from 17 of 19 inoculated pigs were MHP DNA positive (E1+PCR1B). The remaining 2 pigs were MHP DNA positive on DPI 7. For the 322 OF tested, MHP DNA was detected in 173 OFs using E1+PCR2, 148 OFs using E1+PCR1B, 134 OFs using E2+PCR2 and 109 OFs using E1+PCR1A. The probability of detecting MHP DNA in OF increased as the pen-level MHP prevalence (based on PCR on individual pig tracheal swabs) increased (coefficient = 4.7, 95 CI% 4.0, 5.5), but significantly differed among test protocols. With 10% within-pen prevalence, the probability of detecting MHP DNA in OF was highest using E1+PCR2 (13%, 95% CI 6%, 25%), followed by E2+PCR2 (5%, 95% CI 2%, 11%), E1+PCR1B (4%, 95% CI 2%, 9%), and E1+PCR1A (3%, 95% CI 1%, 6%).

The significant differences detected among protocols (extraction and PCR) on the probability of detecting MHP DNA in OF suggest that further improvements in laboratory methods may be possible.

Poster Number: 40
Topic Area: Bacterial Disease
Title: Alura® PCV2 and M. hyopneumoniae vaccines field study in Colombia
Author(s): Luis-Miguel Gomez-Osorio, Alura Animal Health & Nutrition; Ricardo Henao, Alura Animal Health & Nutrition; Fabian Chamba, Pharmgate Inc

Introduction: Alura® vaccines (Pharmgate Circo/MycoGard® vaccines in the USA) are PCV2b/Mhp stand alone or combination vaccines with high safety, antigen purity and cell-mediated immunity due to the triple adjuvant system (StimGard® Plus). We compared Alura® vaccines against Fostera® PCV MH and FlexCombo® in a commercial and observational prospective study in Colombia.

Methods: A total of 7 sow farms (flows) with continuous flow nursery and finishing barns were enrolled starting May 2020. Five flows switched from Fostera® PCV MH one dose at weaning to Alura® vaccines (4 flows switched to PCV2/Mhp alone or in combination one dose at weaning, and 1 flow switched to 2 Mhp doses and one PCV2 dose at weaning). The other 2 flows switched from FlexCombo® to Alura® PCV2/Mhp combination vaccine. A total of 146 closeouts (9458 pigs) recorded until May 2021 were divided in 3 comparisons (nursery Alura® vs Fostera®, nursery Alura® vs FlexCombo® and Alura® vs Fostera® growing-finishing). Nursery and finishing mortality, ADG, FCR, live weight/ton feed (LWTF) and gross margin/ton feed (GMTF) were the outcomes in linear models adjusted by age, start weight, end weight, month, and farm/flow effects. Price per ton of finishing feed was 346 USD and income per kg live weight sold was 1.94 USD.

Results: For the nursery Alura® and Fostera® comparison, there were 5 farms/flows and a total of 28 and 48 closeouts for each vaccine. We did not find any significant differences in performance indicators summarized as adjusted mean±95%CI, p-value (Mortality, %: 3.1±2.0 vs 4.6±1.4, p=0.27; ADG, g/d: 415±18 vs 414±12, p=0.92; FCR: 1.67±0.10 vs 1.65±0.06, p=0.31; and LWTF, kg: 778±38 vs 784±26, p=0.80), and adjusted to 21.6 days of age, 5.5 kg start weight and 26.8 kg end weight. In the nursery Alura® and FlexCombo® comparison, there were 2 farms/flows and a total of 22 and 21 closeouts for each vaccine. We did not find any significant differences in performance indicators (Mortality, %: 2.2±2.4 vs 2.7±1.4, p=0.77; ADG, g/d: 420±12 vs 436±14, p=0.17; FCR: 1.52±0.10 vs 1.46±0.10, p=0.44; and LWTF, kg: 881±64 vs 905±68, p=0.69) adjusted to 23.9 days of age, 6.0 kg start weight and 25.3 kg end weight. Finally, in the finishing Alura® and Fostera® comparison, there were 2 farms/flows and a total of 8 and 19 closeouts for each vaccine. We did not detect any significant differences in performance and economic indicators (Mortality, %: 2.2±2.4 vs 2.7±1.4, p=0.77; ADG, g/d: 762±58 vs 832±30, p=0.06; FCR: 2.33±0.12 vs 2.30±0.06, p=0.69; LWTF, kg: 606±28 vs 615±14, p=0.62; and GMTF, USD: 831±28 vs 849±14,p=0.62) adjusted to 26.0 kg start weight and 92.3 kg end weight.

Conclusion: Alura® and Fostera® PCV2/Mhp vaccines performed similarly in commercial continuous flow nursery and finishing barns from 5 Colombian farms/flows. Additionally, Alura® and FlexCombo® PCV2/Mhp vaccines performed similarly in commercial continuous flow nursery barns from 2 Colombian farms/flows. Overall, Alura® PCV2/Mhp vaccines were competitive in a commercial/observational comparative study in Colombia.

Poster Number: 41
Topic Area: Viral Disease
Title: Shedding patterns and virus diversification of primary and secondary influenza infections in pigs infected with two distinct subtypes
Author(s): Chong Li, University of Minnesota; M. Culhane, University of Minnesota; M. Cheeran, University of Minnesota; D. Schroeder, University of Minnesota; L. Galina Pantoja, Zoetis; M. Jansen, Zoetis; M. Torremorell, University of Minnesota

Introduction
Influenza is one of the top diseases in the US swine industry and influenza A virus (IAV) is hard to control due to its rapid spread and diversification in swine populations [1]. Pigs can replicate IAVs from various hosts, and the co-circulation of different subtypes and strains of IAVs is common in swine herds, which facilitates virus evolution and makes vaccination difficult [2]. However, how a co-infection with a heterosubtypic IAV impacts virus shedding and overall diversity at the individual pig level is poorly understood. In this study, we assessed the shedding patterns of IAV infected pigs upon a secondary infection of IAV of a distinct subtype and we evaluated its impact on virus diversity.

Materials and methods
Fourteen naive pigs were inoculated with either an H1N1 or an H3N2 IAV, and distributed in 7 rooms with one pig of each subtype housed together. Nasal swabs were taken daily, and bronchoalveolar lavage fluid (BALF) samples were collected during necropsy at seven days post-contact. Samples were tested by matrix and HA subtyping real-time PCR. Any nasal swabs or BALF samples with Ct values under 35 were quantified for viable virus (TCID50) and whole genome sequenced by Illumina Nextseq platform. Forty plaques were isolated from three BALF samples to identify virus reassortment in pigs with a confirmed heterosubtypic IAV secondary infection.

Results and Conclusions
During the 7-day observation, we found 43% of pigs (6/14) with a heterosubtypic IAV secondary infection in the lungs or nasal cavities. Among these pigs, five pigs apparently cleared their primary IAV infection and at necropsy (7 dpc) they had mainly the subtype of the other pig. Of importance is that the simultaneous shedding of both challenge viruses was detected through the upper respiratory tract in one pig at 2 to 5 dpc, a second pig at 3 to 5 dpc, and a third pig at 2 to 3 dpc. We observed similar genetic variation patterns between the primary and secondary IAV infections even though we found a positive selection on H1 and M1 IAV proteins in pigs that had a primary H1N1 infection. Purified selection and antigenic drift were the primary selection forces shaping IAV within-host diversity regardless of infection statuses. About 10% (4/40) of plaques isolated from 3 pigs (BALF samples) were identified as reassortants which resulted in 2 distinct genotypes. Overall, there was limited IAV reassortment in pigs infected with the secondary IAV infection. Our study demonstrated that pigs that become infected consecutively by multiple subtypes of IAVs can have extended IAV shedding patterns over time which may affect virus diversity in the pigs. More research is needed to validate these results under field conditions.

Reference
1. Diaz A, Marthaler D, Culhane M, Sreevatsan S, Alkhamis M, Torremorell M. Complete genome sequencing of influenza A viruses within swine farrow-to-wean farms reveals the emergence, persistence, and subsidence of diverse viral genotypes. J Virol. 2017;91: e00745-17.
2. Nirmala J, Perez A, Culhane MR, Allerson MW, Sreevatsan S, Torremorell M. Genetic variability of influenza A virus in pigs at weaning in Midwestern United States swine farms. Transbound Emerg Dis. 2020.

Poster Number: 43
Topic Area: Feed/Nutrition
Title: A summary of evidence for differences in serum 25(OH)D3 status across different life stages, fortification levels, and dietary vitamin D sources
Author(s): Sara Hough, DSM Nutritional Products; Jon Bergstrom, DSM Nutritional Products; Jeremiah Nemechek, DSM Nutritional Products

Introduction
Vitamin D is essential for health and function of the skeletal system by maintaining calcium and phosphorus homeostasis.[1] Vitamin D must be ingested or acquired endogenously through exposure to sunlight. A deficiency can result in rickets, lameness and unthriftiness in young pigs, while bone fractures can occur in older animals.[2] Sustained hypovitaminosis D, evidenced by low serum levels of 25-hydroxyvitamin D, is common in cases of metabolic bone disease.[3] Swine are susceptible to skeletal disorders because of their rapid growth rate and limited exposure to sunlight in modern confinement facilities.[4] Growing evidence supports the importance of vitamin D in other metabolic processes, including immune functionality.[5] Serum 25(OH)D3 is the best biomarker of vitamin D status.[6] Hy-D® is a source of 25(OH)D3 for feed or water supplementation and has greater bioavailability compared to vitamin D3. Fortifying pig diets using Hy-D® should provide a more acceptable vitamin D status.

Method
The objective of this compilation was to compare serum levels of 25(OH)D3 in relation to the type and level of dietary vitamin D fortification, either as vitamin D3 or Hy-D®. Thirty-one studies of animals receiving their assigned dietary vitamin D level for at least two weeks, using either vitamin D3, Hy-D®, or a combination were identified. Production phase, animal number, study duration, and associated serum levels were also captured. The feeding levels ranged from 0-9600 IU/kg for D3 and 0-2000 IU/kg for 25(OH)D3 from Hy-D®.

Results
Of the 31 trials, 4 were in gilt development, 8 in sows, 15 in nursery and 9 in grow-finish. When comparing the most common level fed of total vitamin D (2000 IU/kg), Hy-D® increased serum status roughly 200% (15.2 vs. 49.2 ng/ml) in 8 nursery trials and 100% (31.1 vs 72 ng/ml) in 3 gilt development trials when compared to vitamin D3. Sows fed 2000 IU/kg of Hy-D® had higher levels of serum 25OHD3 during the gestation and lactation phases, 36.5 vs 61.7 ng/ml, respectively. When considering dietary levels greater than 2000 IU/kg, one nursery trial showed a benefit of using 2000 IU/kg of Hy-D® with 1500 IU/kg of D3. This resulted in nursery pigs with an average serum level of 40 ng/ml, whereas, serum levels from pigs fed 3500 IU/kg of D3 alone were 11 ng/ml.[7]

Conclusions
Differences in serum status occurred when comparing pigs supplemented with vitamin D3 versus Hy-D®. At equivalent dietary levels of vitamin D, an improved serum status from feeding Hy-D® demonstrated its greater bioavailability. With increased vitamin D status using Hy-D®, its use has been associated with improved structural soundness in gilts.[8] A higher number of liveborn and weaned pigs have been reported when feeding Hy-D® to gilts and sows, compared to control.[9,10] In growing animals, Hy-D® has reduced the severity of osteochondrosis lesions.[11] More research is needed to fully understand how vitamin D can improve various performance parameters. Current evidence shows that, regardless of production stage and geography, pigs benefit from a more bioavailable form of dietary vitamin D and an improved serum status.

References
1. DeLuca, H. (2004) Overview of general physiologic features and functions of vitamin D. American Journal of Clinical Nutrition. 80(Suppl), 1689-1696.
2. Meisinger, D. (Ed). The National Swine Nutrition Guide. US Pork Center of Excellence. 2010. Page 41.
3. Arnold, J., D. Madson, S. Ensley, J. Goff, C. Sparks, G. Stevenson, T. Crenshaw, C. Wang and R. Horst. Survey of serum vitamin D status across stages of swine production and evaluation of supplemental bulk vitamin D premixes used in swine diets. Journal of Swine Health and Production.2015; 23 (1), 28-34.
4. Dittmer K.E. and K.G. Thompson. Vitamin D metabolism and rickets in domestic animals: a review. Vet Pathol. 2011; 48, 389–407.

5. Baeke, F., T. Takiishi, H. Korf, C. Gysemans, and C. Mathieu. Vitamin D: modulator of the immune system. Current Opinion in Pharmacology.2010; 10(4), 482–496.
6. Jones, G. Metabolism and biomarkers of vitamin D. Sc and J Clin Lab Invest. 2012; 72, 7–13.
7. Puls, R. Vitamin Levels in Animal Health: Diagnostic Data and Bibliographies. Sherpa International. 1994.
8. Brana, D., J. Gabriel-Landon, J. Cervantes-Lopez and J.A. Cuaron. Nonruminant nutrition: Vitamins and Minerals. Journal of Animal Science. 2012; 98(Supp 1), 114.
9. Planchenault, D., B. Renouf, A. Meuter and J.M. Thoby. Effect of dietary 25-hydroxycholecalciferol and cholecalciferol on the performance of gestation and lactating sows and their litter. Journees Recherche Porcine. 2018; 50, 143-144.
10. Coffey, J.D., E.A. Hines, J.D. Starkey, C.W. Starkey and T.K. Chung. Feeding 25-hydroxycholecalciferol improves gilt reproductive performance and fetal vitamin D status. Journal of Animal Science. 2012; 90, 3783-3788.
11. Sugiyama, T., T.K Chung, H. Yonekura, E. Azem and T. Hayakawa. Effects of 25-hydroxycholecalciferol on the development of osteochondrosis in swine. Journal of Animal Science. 2013; 84, 341-349.

Poster Number: 44
Topic Area: Bacterial Disease
Title: Prevalence of Lawsonia intracellularis in Peru through diagnosis of lesions in slaughterhouses
Author(s): Marcelo Figueiredo, MSD Animal Health Peru; Kiara Solange Garcia Garro, MSD Animal Health Peru; Orlando Javier Hernandez Salcedo, MSD Animal Health Peru

Introduction:
Ileitis is caused by the bacterium Lawsonia intracellularis and causes huge economic losses to pig production in the last phase of pig production for fattening.

Serological studies have shown that the prevalence of herds positive for proliferative enteropathy varies between 60 and 90% in different countries (Lawson and Gebhart, 2000; Dunser and others 2000; Prieto et al., 2002), at the same time that in most cases clinical manifestations become increasingly rare due to the excessive use of antibiotics.

The need to develop a diagnosis of the prevalence of Ileitis in its subclinical form led to the present work, to perform the analysis of lesions in the ileal of 10 different farms in Peru, totaling 1231 intestines, in the refrigerators of Lima-Peru, during the first half of 2021.

Methods:
Through a study carried out by T. K. Jensen, K. Moller, G. Christensen, T. D. Leser, S. E. Jorsal in 1999, the degree and type of lesion was determined, based on a scoring system, both by palpation and by the visualization of the walls and the intestinal mucosa.

This study has 3 degrees of scoring (0, 1, 2, 3), the method of evaluation is visual and palpatory; grade 0 are normal proportions; grade 1 a slight increase in the thickness and stiffness of the intestinal wall is observed; grade 2 is observed with an increase of up to 4 mm in thickness of the intestinal wall, hyperplasia of the lymphoid tissue and slight edema of the tissues; grade 3 increase of more than 4 mm in the thickness of the intestinal wall is observed with partial stenosis (Jensen et al, 1999).

This methodology was implemented by reviewing intestines, first locating the ileocecal junction to cut approximately 15 to 20 cm from the end of the ileum, where you can observe majority of the lesions compatible with Lawsonia intracellularis. We placed the corresponding degrees according to the lesions observed macroscopically. We compared what was observed by sending samples to the histopathology laboratory of which 5 samples of each degree were selected hoping that it coincided to the touch or palpation with the histopathological result and thus have a validated methodology between the macroscopic and microscopic evaluation.

Results:
Of the 1231 samples the evaluation showed 16% in grade 0, 48% in grade 1, 24% in grade 2 and 12% in grade 3.

Conclusions:
It was possible to establish a diagnosis at the field level and then supported by the laboratory analysis. The intention being of creating a tool to also be used during the necropsies on the farm and that the producer can understand and visualize the importance of Lawsonia intracellularis being present in their farm, also in a subclinical way reducing the use of nutrients. The evaluations have found a 36% of lesions of score 2 and 3 where a substantial and silent economic loss is occurring.

Poster Number: 45
Topic Area: Production
Title: Evaluation of Metaphylactic Gentamicin Use at Processing on the Pig Respiratory and Fecal Microbiome at Weaning
Author(s): Jana Morgan, Boehringer Ingelheim; Lauren Glowzenski, TriOak Foods; Fernando Leite, Boehringer Ingelheim

Introduction
The respiratory and gut microbial community, or microbiome, has become increasingly recognized for its influence on overall health and production performance. [1,2] Yet there is a paucity of studies to evaluate how common on farm management practices may influence these microbial communities. The days following birth until weaning are a period of major immune and organ development of the pig, of which the microbiome plays a major role.[1,3] Metaphylactic use of antimicrobials at processing is common in the swine industry and should be carefully evaluated considering farm specific pathogen challenges. In this study, we evaluated the effect of metaphylactic gentamicin treatment in a herd that was experiencing post weaning Escherichia coli scours and parainfluenza infection and questioning the impact and need for gentamicin treatment at processing. The objective of this study was to evaluate the effect that this antimicrobial treatment was having on the respiratory and fecal microbiome of pigs.

Materials and Methods
This investigation was done in a PRRSV negative nucleus herd. Two treatment groups were evaluated in this study, one group received the labeled dose of gentamicin IM at processing and another group did not. Each group comprised of three randomly chosen piglets from 5 randomly chosen litters, for total of 15 pigs per treatment. Just before weaning, fecal and nasal swabs were taken from all pigs. Samples were analyzed for microbiome composition by 16S rRNA gene sequencing.

Results
Gentamicin treatment led to a significant reduction in the diversity of fecal microbiota (Chao1 index, $p<0.05$). No significant impact on the diversity of respiratory microbiota was found. Gentamicin treatment also led to a significant change in the community structure of the fecal microbiota (Bray-Curtis index, $p<0.05$), without a significant change to community structure of the respiratory microbiota.

Discussion and Conclusions
In this study, metaphylactic gentamicin treatment of pigs at processing led to a change in the diversity and community structure of fecal microbiota at weaning. This raises questions as to the implications of this for the health of the pig, as fecal microbial diversity has been found to be correlated to the ability to respond to PCV2/PRRSV among other pathogens.[4,5] Further research is needed to better understand potential unintended consequences of gentamicin and metaphylactic antimicrobial treatment when a clear intended targeted pathogen is not present.

References:
1. Isaacson R, et al. The intestinal microbiome of the pig. Anim Health Res Rev. 2012.
2. Niederwerder MC. Role of the microbiome in swine respiratory disease. Vet Microbiol. 2017 Sep;209:97-106.
3. Moeser AJ, et al. Weaning stress and gastrointestinal barrier development: Implications for lifelong gut health in pigs. Anim Nutr. 2017 Dec;3(4):313-321.
4. Ober RA et al, Increased microbiome diversity at the time of infection is associated with improved growth rates of pigs after co-infection with porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2). Vet Microbiol. 2017 Sep;208:203-211.
5. Argüello H. et al, Influence of the Intestinal Microbiota on Colonization Resistance to Salmonella and the Shedding Pattern of Naturally Exposed Pigs. mSystems. 2019 Apr 23;4(2):e00021-19.

Poster Number: 46
Topic Area: Viral Disease
Title: Assessment of the relationship between breeding herd PRRSv occurrence and manure pumping events in the U.S.
Author(s): Carles Vilalta, Upnorth analytics; Juan Sanhueza, Departartamento de Ciencias Veterinarias y Salud Pública, Facultad de Recursos Naturales, Universidad Católica de Temuco; Mariana Kikuti, Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota; MSHMP Participants ; Catalina Picasso, Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota; Cesar A Corzo, Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota

Introduction
During the last decade, a repetitive and seasonal epidemic pattern has been reported for the Porcine Reproductive and Respiratory Syndrome (PRRS) in the United States breeding herd. Data obtained from producers and practitioners and analyzed through the Morrison Swine Health Monitoring Project (MSHMP) demonstrated a consistent onset of the epidemic of this disease occurring between mid-October and November which peaks during the winter months[1]. Unfortunately, there are no solid explanations for the root cause of this consistent onset. Neither air nor fomite related transmission during cold months fully explain the seasonal onset of the disease since the virus continues to disseminate year-round at a lower rate. Therefore, there may be other pig farming events during fall besides temperature that could play an important role creating the yearly pattern. A noticeable event that occurs at the same time as the onset of the national epidemic involves manure agitating, pumping, and spread. The aim of this study was to describe the relationship between manure management and reporting a breeding herd PRRS outbreak to MSHMP, with the goal of understanding the drivers of the seasonal dynamics observed.

Methods
Outbreak data and manure pumping activities were directly obtained from MSHMP participants between July 1st, 2019 and June 30th, 2020. The dataset was composed of on-site manure pumping dates, outbreak onset, manure storage (e.g. lagoon or deep pit) together with all the data already collected through MSHMP (e.g. status, location, air filtration, and herd size). Two hypothetical incubation periods of 15 and 30 days after manure agitation and pumping were considered. The ratio between the incidence rate occurring within the hypothesized incubation period (15 or 30 days) and the incidence rate outside the period was calculated for the overall population and each variable of interest.

Results
A total of 216 breaks were reported during the study period from which, 150 provided information regarding manure pumping dates and storage. The proportion of outbreaks occurring during the first 15 and 30 days after agitation and pumping was 25% (38/150) and 41% (61/150), respectively. The incidence risk ratio (IRR) was 9.55 (95%CI: 6.59-13.84) for the 15-day and 9.08 (95%CI: 6.81 - 13.49) for the 30-day incubation period, respectively. The three months in which farms had higher number of pumping events were October (40), April (29), and November (24). The risk of having an outbreak during the first 15 days after pumping was similar during the fall (IRR: 16.42 [95%CI: 9.03-29.21]) and the spring (IRR:17.18 [95%CI: 7.33-39.06]) compared with outside of the period.

Conclusions
Two main periods in which manure pumping in breeding herds occurred were during the fall and spring. The risk of having an outbreak within the following 15 days of manure pumping remained the same in farms that either pumped during the fall or the spring. These findings suggest that the occurrence of PRRS within the 15 -30 days of on-site manure pumping was temporally associated with reporting a PRRS outbreak between 15 and 30 days after. More studies are needed to understand the relationship between the two events.

1. Tousignant, S.J., et al., Temporal and spatial dynamics of porcine reproductive and respiratory syndrome virus infection in the United States. Am J Vet Res, 2015. 76(1): p. 70-6.

Poster Number: 47
Topic Area: Viral Disease
Title: Comparative efficacy of two modified-live PRRS vaccines against a heterologous PRRSV 1-7-4 challenge
Author(s): Reid Philips, Boehringer Ingelheim Animal Health USA Inc; Greg Haiwick, Boehringer Ingelheim Animal Health USA Inc; David Whiteman, Boehringer Ingelheim Animal Health USA Inc

Introduction
The objective of this study was to evaluate the efficacy of two commercially available PRRSV vaccines in a three-week-old pig respiratory challenge model, using a heterologous PRRSV lineage-1 RFLP 1-7-4 field strain that was isolated in 2016.

Materials and Methods
At approximately three weeks of age (Day 0 of study), 104 PRRSV naïve piglets pigs were randomized into groups, blocked by weight and intramuscularly vaccinated with 2 ml of either a phosphate buffered saline-placebo (challenge controls, N=34), INGELVAC PRRS MLV (N=34) or 1 ml intramuscularly of a competitor PRRS vaccine* (N=36). Pigs were housed in rooms by group during the vaccination period. At day 27 of the study (D27), all pigs were comingled and challenged with 2.0 mL intramuscularly and 2.0 mL intranasally (1 mL per nostril) with $10^{4.83}$ $TCID_{50}$/dose of PRRSV RFLP 1-7-4. Serum samples and weights were collected periodically from D0 through termination of the study on D42. On D42 (14 days post-challenge), all pigs were necropsied, and lungs were scored for the presence of macroscopic lesions. Serum samples were tested by RT-PCR for the presence of viremia and by ELISA for the presence of anti-PRRSV antibody. Pairwise comparisons between groups were conducted using a level of confidence of 0.05 to indicate statistical significance.

Results
Both vaccinated treatment groups demonstrated significant reduction in gross lung lesions (mean percent) compared to the placebo group.

The percent lung lesions were 1.83 (95% CI 0.76, 3.33) for INGELVAC PRRS, 3.87 (95% CI 2.28, 5.87) for the competitor vaccine and 8.31 (95% CI 5.87, 11.14) for the placebo group. There was no difference in average daily weight gain (ADWG) between vaccinated and placebo groups during the pre-challenge phase of the study (Day 0 – Day 27). The pre-challenge ADWG (lbs) was 1.05 (95% CI 0.93, 1.17) for INGELVAC PRRS, 1.12 (95% CI 1.00, 1.24) for the competitor vaccine and 0.94 (95% CI 0.82, 1.06) for the placebo group. In the challenge phase of the study (Day 27 – Day 42), the INGELVAC PRRS vaccinated group had significantly higher ADWG than the competitor vaccine and placebo groups. The challenge phase ADWG (lbs) was 0.73 (95% CI 0.63, 0.82) for INGELVAC PRRS, 0.59 (95% CI 0.50, 0.68) for the competitor vaccine and 0.53 (95% CI 0.44, 0.63) for the placebo group.

Conclusion
An objective of this study was to evaluate the efficacy of INGELVAC PRRS and a competitor vaccine against a current heterologous PRRSV 1-7-4 isolate in a respiratory challenge model. There was no difference in ADWG between vaccinated treatment groups during the pre-challenge period of the study. Both vaccines demonstrated heterologous protection by significantly reducing lung lesions compared to the placebo group. Pigs vaccinated with INGELVAC PRRS had significantly higher post-challenge ADWG compared to the competitor vaccinated group and the placebo group. This study further confirms the ability of INGELVAC PRRS MLV to protect against a relevant and contemporary PRRSV lineage-1 challenge.

*PRRSGard®, Pharmgate Animal Health, Wilmington, NC.

Poster Number: 48
Topic Area: Viral Disease
Title: Field Safety Evaluation of FLEX ParvoPRRS®
Author(s): Rex Smiley, Boehringer Ingelheim Animal Health USA Inc; Reid Philips, Boehringer Ingelheim Animal Health USA Inc

Introduction
Porcine Reproductive and Respiratory Syndrome virus (PRRSV) is a pathogen in swine causing reproductive failure in dams and respiratory disease in pigs. Infection of pregnant sows in late-term gestation with PRRSV commonly causes severe reproductive failure including spontaneous abortions, the birth of stillborn pigs, mummified fetuses, and non-viable piglets.[1] The objective of this study was to evaluate the safety of a new combination vaccine FLEX PARVOPRRS containing Ingelvac PRRS® MLV and an adjuvanted Porcine Parvovirus (PPV, killed baculovirus vector), under typical field conditions, when administered to adult breeding age swine.

Materials and Methods
This was a non-blinded, uncontrolled observational field safety study conducted in two swine farm sites. A total of 420 adult sows and gilts from pre-breeding (~5-7 wks pre-breeding, n=173), early gestation (~35 days gestation, n=124), and mid-late gestation (~70 days of gestation, n=123) stages of production across both sites received FLEX PARVOPRRS (2mL, I.M.) on D0 of the study. On D21, all dams received a booster dose of the PPV monovalent vaccine (2 mL, I.M.). Individual health observations were recorded daily for all enrolled dams until removal from the study. The pregnant vaccinated dams were observed until farrowing, loss of pregnancy, removal for humane reasons or mortality occurred. At farrowing, the number of healthy, weak born, stillborn, and mummified piglets per litter was recorded. All data was imported into SAS version 9.4 (SAS, Cary, USA/North Carolina, SAS Institute Inc.) for management and summarization.

Results
Across both sites, 396 (94.3%) of vaccinated sow/gilts were observed as healthy for all days enrolled in the study. Of the dams that remained in the study through farrowing (384), 368 (95.8%) were observed as healthy on all days. The most observed adverse events included anorexia in six dams (1.4%) and lameness in five dams (1.2%). None of the recorded AEs were determined to be vaccine-related by the attending veterinarians. The reproductive performance and litter characteristics for the 384 dams that remained in the study through farrowing had an average of 13.33 healthy piglets/litter (90.73%), 0.21 mummies/litter (1.3%), 0.84 stillborn/litter (5.53%) and 0.36 weak born/litter (2.44%).

Conclusion
Overall, 94.3% of vaccinated sows/gilts were observed as healthy for all days enrolled in the study. Of the dams that remained in the study through farrowing (384), 368 (95.8%) were observed as healthy on all days. None of the adverse events (AE's) recorded were considered to be vaccine-related. At both sites, the mean number of stillborn and mummified fetuses per litter was low and overall average number of healthy piglets per litter (13.33) was above industry standards, indicating vaccination had no negative effect on reproductive performance.[2] The results of this study demonstrate that FLEX PARVOPRRS is safe for use in pregnant animals and "gilts" vaccination prior to breeding.

References
1. Benfield, D., et al. 1999. Diseases of Swine (8th edition).
2. NAHMS, USDA, Baseline Reference of Swine Health and Management, 2012.
3. Data On File. BIAH USA Inc. study #2019003, approved by CVB Jan. 2021

Poster Number: 49
Topic Area: Viral Disease
Title: Efficacy of FLEX ParvoPRRS® against a virulent PRRSV challenge in gestating gilts
Author(s): Rex Smiley, Boehringer Ingelheim Animal Health USA Inc; Reid Philips, Boehringer Ingelheim Animal Health USA Inc

Introduction
Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is an important swine pathogen causing reproductive failure in dams and respiratory disease in pigs. Infection of pregnant sows in late-term gestation with PRRSV commonly causes severe reproductive failure including spontaneous abortions, the birth of stillborn pigs, mummified fetuses, and weak, non-viable piglets. The objective of this study was to evaluate the efficacy of a new combination vaccine FLEX PARVOPRRS containing Ingelvac PRRS® MLV and adjuvanted Porcine Parvovirus (PPV, killed baculovirus vector) in a reproductive heterologous PRRSV challenge model.

Materials and Methods
At approximately 6 months of age on Day 0 of the study (D0), 40 PRRSV naïve pre-breeding gilts were randomized into two groups and intramuscularly (I.M.) vaccinated with 2 ml of either FLEX PARVOPRRS (Group VC, N=20), or a monovalent adjuvanted PPV vaccine as controls (Group NVC, N=20). Both groups received a 2 mL I.M. booster dose of the monovalent PPV vaccine on D21. The two groups were housed in separate rooms during the vaccination/pre-challenge phase of the study. Gilts' estrous cycles were synchronized and artificially inseminated between D26 and D32. On D115, pregnant gilts were re-randomized to rooms and farrowing crates for equal distribution of treatments across rooms for the challenge phase of the study. On D120 (~92 days of gestation) all gilts were challenged with 2.0 mL (I.M.) and 2.0 mL intranasally (1 mL per nostril) with 104.24 TCID 50/dose of virulent PRRSV Strain SDSU-73 (RFLP type 1-4-4). Clinical observations, and serum collections were conducted on gilts through the challenge phase to weaning. Piglets farrowed from the gilts were evaluated for health status as live-healthy or non-viable, and weights were taken at day of farrowing and weaning to assess average daily weight gain (ADWG). Serum samples were tested by RT-PCR for the presence of PRRSV viremia (in genomic copies or GC/ml). Data were imported into SAS® version 9.4 for management and analysis.

Results
Table 1 summarizes the percent viremia at post-challenge and reproductive performance of each group. Figure 1 summarizes the average viral load of the study dams by group and day of the study.

Table 1. Post-challenge Viremia and Reproductive Performance

Group**	- Viremic Dams*	- Healthy pigs born	- Viremic pigs born	- Pig survival to wean	- Pigs' ADWG (lbs)
VC (N=20)	15% [a]	54.7% [a]	38.1% [a]	42.0% [a]	0.42 [a]
NVC (N=15)	100% [b]	16.9% [b]	88.6% [b]	1.3% [b]	0.02 [b]

a,b Different superscripts indicate significant (P≤0.05) differences.
*9 Days post-challenge.
** Vaccinated Challenged (VC) and Non-Vaccinated Challenged (NVC)/Control

Figure 1. Average Viral Load of Study Dams (GC/mL)

Conclusion
This vaccination-challenge study demonstrated that a single 2 mL dose of FLEX PARVOPRRS is efficacious in mitigating reproductive failure caused by virulent PRRSV when administered to gilts prior to breeding. The efficacy is primarily supported by the significant differences in healthy, live pigs at farrowing between vaccinated and non-vaccinated/control gilts. Efficacy is also supported by the detected differences in incidence, magnitude and duration of viremia and viral load in gilts post-challenge. Significantly more pigs from vaccinated gilts survived to wean, and these pigs also had significantly higher ADWG than pigs farrowed from non-vaccinated/control gilts. FLEX PARVOPRRS is a new combination vaccine that offers protection against both PRRSV and PPV in reproductive vaccination protocols.

References

1. Data on File. BIAH USA Inc. study #2018009 approved by CVB May 2020.

Poster Number: 50
Topic Area: Viral Disease
Title: A genetic analysis of the ORF5 gene from recent Porcine Reproductive and Respiratory Syndrome virus isolates from Vietnam
Author(s): Hongyao Lin, MSD Animal Health Innovation; Nguyễn Ngọc Hải, Department of Infectious Diseases and Veterinary Public Health Faculty of Animal Science and Veterinary Medicine, Nonglam University HCMC, Ho Chi Minh City, Vietnam; Trần Hoàng Anh Thư, Han-Viet Veterinary Diagnosis Laboratory, Faculty of Animal Science and Veterinary Medicine, Nong Lam University, Ho Chi Minh City, Vietnam; Nguyễn Trung Quân, Han-Viet Veterinary Diagnosis Laboratory, Faculty of Animal Science and Veterinary Medicine, Nong Lam University, Ho Chi Minh City, Vietnam; Nguyễn Thị Phương Bình, Han-Viet Veterinary Diagnosis Laboratory, Faculty of Animal Science and Veterinary Medicine, Nong Lam University, Ho Chi Minh City, Vietnam; Lê Thị Thu Hà, MSD Animal Health Vietnam, Ho Chi Minh City, Vietnam; Doãn Đức Chung, MSD Animal Health Vietnam, Ho Chi Minh City, Vietnam; Hồ Viết Hiền, MSD Animal Health Vietnam, Ho Chi Minh City, Vietnam; Phan Văn Hiệu, MSD Animal Health Vietnam, Ho Chi Minh City, Vietnam

Introduction
Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is an acute infectious disease in pigs that first appeared in Vietnam in 1997. PRRSV is divided into PRRSV-type 1 (European genotype - EU) and PRRSV-type 2 (North American genotype – NA-PRRSV). Furthermore, PRRSV-type 2 (NA-PRRSV) can be divided into major subtypes: classical North American PRRSV (C-PRRSV), Highly-Pathogenic PRRSV (HP-PRRSV) and the NADC30-like PRRSV subtype (NADC30-PRRSV). To date, these three NA-PRRSV subtypes are circulating in China (Li et al., 2017). Reports to date suggest that PRRSV strains in Vietnam from 2007 to 2015 mainly belonged to HP-PRRSV (136/144 strains), with the rest classified as US-PRRSV (6/144 strains), and no reports of PRRSV NADC30-like subtype PRRSV strains.

Methods
28 samples were collected from the field in 2020 – 2021 across provinces in Vietnam (Dongnai, Daknong, Binh Phuoc, Binh Thuan, Haiphong, Laichau, and Vinhphuc). Tissue samples (lymph nodes, kidneys, spleen) were homogenized in distilled water and the mixture was centrifuged and collected for RNA extraction with GeneJET Viral DNA/RNA Purification Kit (Thermo, USA), then cDNA synthesized by reverse transcriptase via RevertAid First Strand cDNA Synthesis Kit (Thermo, USA). To detect the presence of PRRS virus in field samples, RT-PCR was performed with one pair of primers complementary to the target gene segment on ORF5 with an amplicon size of 508 bp. The product was then electrophoresed on a 1.5% agarose gel and observed under UV light. After positive confirmation for PRRSV through RT-PCR was continued to be used to obtain the 764 bp PRRSV ORF5 gene fragment. The ORF5 gene cloned RT-PCR product of 28 samples was sent for bidirectional sequencing. The results were processed and assembled using Sequencher 5.4.6 software. Nucleotide and amino acid sequences of the samples were analyzed and compared with reference sequences published in the gene bank using Bioedit 7.2.6 software. Genetic tree was constructed using MEGA 7.0.26 software, by Neighbor Joining method with bootstrap 1000 repetitions.

Results
In this study, 28 samples from lymph nodes, spleen, kidney and serum of infected pigs collected from Dong Nai, Dak Nong, Lai Chau, Vinh Phuc provinces showed positive results for PRRSV virus.

Analysis of ORF5 sequences showed that 17/28 strains belonged to the highly virulent PRRS subtype (HP-PRRSV), 10 strains belonged to the classical North American PRRS subtype (US-PRRSV), and 1 recombinant combination of HP-PRRSV and US-PRRSV.

The US-PRRSV line included 6 field PRRSV strains (4 Dong Nai strains: VN/HVDN1/2021, VN/HVDN2/2021, VN/HVDN3/2021, VN/HVDN4/2021 and 2 Binh Phuoc strains: VN/HVBP3/2021 , VN/HVBP4/2021) belonging to the same clade as NADC30-Like strains. The percentage of homology of these newly found Vietnamese NADC30-like strains and NADC30-like reference strains in the literature was low, in the range of 83.0 - 86.2%.

Conclusions

This to our knowledge is the first isolation of NADC-30 like isolates outside of mainland China. Through genetic analysis, we showed that the PRRSV strains obtained in the study have clear genetic diversity. However, more in-depth studies are needed to monitor the variation of PRRSV and to match field strains to vaccine strains in Vietnam.

Poster Number: 51
Topic Area: Viral Disease
Title: Evaluation of air filters in swine farms as a surveillance method to assess the spread of porcine reproductive and respiratory syndrome and influenza A viruses
Author(s): Lan Wang, University of Minnesota; Jayaveeramuthu Nirmala, University of Minnesota; My Yang, University of Minnesota; Montserrat Torremorell, University of Minnesota

Introduction
Among all infectious agents affecting pigs, airborne pathogens are the most costly and difficult to control[1]. Porcine reproductive and respiratory syndrome virus (PRRSV) and influenza A virus (IAV) are considered two important airborne viruses in pigs that result in significant economic losses to the pig industry. Air filtration of incoming air is being used to effectively reduce the incidence of airborne PRRSV infections in breeding herds in the Midwestern US[2]. Therefore, testing of used air filters from filtered farms offers a unique largely unexplored opportunity to monitor the regional spread of PRRSV and IAV. In this study, we aimed to evaluate the use of air filters as a surveillance method to monitor the regional spread of these two viruses, and to enhance our understanding of the epidemiology and control of airborne diseases.

Methods
We selected 7 breeding herds from high pig density areas which were either PRRSV negative or stable at the beginning of the study. Twenty brand new air filters were installed in each farm, and five filters were removed each time at approximately 6, 8, 11 and 14 months post installation. Five samples of twelve square inches were cut from each filter, and these samples were processed by grinding. Viral RNA was extracted from each sample and quantified by real time RT-PCR for PRRSV and IAV[3]. A filter was considered positive if at least one sample tested positive. Samples positive for PRRSV or IAV were further analyzed by whole genome sequencing.

Results
A total of 136 air filters were analyzed. Filters were installed in July 2019 at the earliest, and removed successively until October 2020. These filters were cut into 680 samples for testing. Out of the 136 filters, ten (1.5%) samples corresponding to seven (5%) filters from three farms tested positive for PRRSV. During the study, PRRS outbreaks were reported in four farms, however, only one positive filter originated from farms that had PRRSV positive filters. In contrast, sixty five (47.8%) filters from all seven farms tested positive for IAV, with a total of 131 samples positive (19.3%). Six IAV positive samples were sequenced and one sample was successfully subtyped as an H3N2 human-like influenza virus. In addition, multiple lineages were identified for the influenza internal genes from different samples.

Conclusions
Testing of used air filters in swine farms did not result in an enhanced surveillance method for airborne PRRSV. However, used filters for influenza surveillance should be further evaluated. Overall, detection of PRRSV and IAV in the air filters showed some potential evidence of airborne transmission for these viruses, but additional investigations are needed to better understand the factors that contribute to airborne transmission of these viruses.

References
1. Hyslop, N S. “Factors influencing the epidemiology and epizootiology of airborne diseases.” Journal of the American Veterinary Medical Association vol. 159,11 (1971): 1500-7.
2. Dee, Scott et al. “Evaluation of the long-term effect of air filtration on the occurrence of new PRRSV infections in large breeding herds in swine-dense regions.” Viruses vol. 4,5 (2012): 654-62.
3. Nirmala, Jayaveeramuthu, et al. "Evaluation of viral RNA extraction methods to detect porcine reproductive and respiratory syndrome and influenza A viruses from used commercial HVAC air filters from swine farms." Journal of Aerosol Science 151 (2021): 105624.

Poster Number: 52
Topic Area: Viral Disease
Title: Evaluation of parity, personnel and cross-fostering in influenza infections during the pre-weaning period
Author(s): Gustavo Lopez-Moreno, University of Minnesota; Peter Davies, University of Minnesota; Marie Culhane, University of Minnesota; Emily McDowell, Pipestone Veterinary Services; Eduardo Fano, Boehringer Ingelheim Animal Health; Christa Goodell, Boehringer Ingelheim Animal Health; Montserrat Torremorell, University of Minnesota; Jorge Garrido-Mantilla, Pipestone Veterinary Services

Introduction
Influenza A virus (IAV) is an important respiratory pathogen affecting pig's health. IAV has an impact on growth efficiency and can lead to increased morbidity and mortality. Piglets are born IAV negative but in endemically infected farms they often become infected prior to weaning. Therefore understanding the sources of IAV exposure to piglets is key to controlling IAV.

In this study, we evaluated an internal biosecurity protocol that included changes in management practices during the lactation period that consisted of limiting movement of pigs and/or nurse sows between litters (cross-fostering) and implementing internal biosecurity measures by the workers while handling piglets. We also evaluated the effect of sow parity in IAV prevalence and assessed the levels of IAV contamination in farm worker's hands, which could represent a risk for mechanically transmitting IAV to naïve piglets.

Material and methods
Three IAV positive farms were selected for the study. At enrollment, litters were allocated to either a "control" or "treatment" group. Litters within the control rooms were processed (tail-docking and castration) and handled with no changing of gloves, and cross-fostering was allowed between farrowing rooms when it was warranted. In contrast, cross-fostering was not allowed in the treatment litters after processing, and piglet handling was done by farm personnel after changing gloves, and wearing boot covers if personnel was to enter the crates. To study the effect of parity, litters were stratified into young (≤P3) and older parities (>P3) and selected randomly within each strata. A total of 360 litters (120 per farm) were enrolled into the study. Sampling of litters took place at 1, 8, 13 and 18 days of age of the piglets using udder skin wipes[1]. Samples were also collected from materials used in the farrowing rooms and from worker's hands after piglet handling. Samples were tested using an IAV rRT-PCR[2]. Statistical differences between groups and parities were assessed with a multivariate statistical model using R statistical software [3].

Results
Udder wipes collected from litters from the treatment group had a lower IAV prevalence of 29% (209 out of 720) compared to the 43% prevalence (318 out of 720) in the control group. However, this difference was only significant at day 8 and 13 of lactation. At day 18, IAV prevalence was not different between groups. Furthermore, no differences in IAV prevalence were seen between young parity sows 254/676 (37.5%) and older parity sows 273/764 (35.7%) at any sampling point. Samples collected from farm worker's hands had 58% (65/111) positivity rate to IAV.

Discussion
Our results indicate that specific management practices directed at minimizing spread of IAV in pigs prior to weaning can slow down IAV transmission within farrowing rooms. However, these practices by themselves were not sufficient to fully halt transmission during the pre-weaning period. We found no evidence that sow parity was associated with IAV litter prevalence during the lactation period. Lastly, we found high prevalence of IAV in the samples collected from farm worker's hands and tools, which is most likely facilitating the indirect transmission of IAV within farrowing rooms.

Acknowledgements
This study was funded by Boehringer Ingelheim Animal Health USA Inc. Duluth, GA 30096.

References
1. Garrido-Mantilla J, Alvarez J, Culhane M, Nirmala J, Cano JP, Torremorell M. Comparison of individual, group and environmental sampling strategies to conduct influenza surveillance in pigs. BMC Vet Res. 2019;15:61:1-10. doi:10.1186/s12917-019-1805-0

2. Slomka MJ, Densham ALE, Coward VJ, et al. Real time reverse transcription (RRT)-polymerase chain reaction (PCR) methods for detection of pandemic (H1N1) 2009 influenza virus and European swine influenza A virus infections in pigs. Influenza Other Respi Viruses. 2010;4(5):277-293. doi:10.1111/j.1750-2659.2010.00149.x
3. R Core Team. R: A Language and Environment for Statistical Computing. 2017. https://www.r-project.org/.

Poster Number: 53
Topic Area: Viral Disease
Title: Evaluation of internal biosecurity practices combined with sow vaccination to wean influenza negative piglets
Author(s): Gustavo Lopez-Moreno, University of Minnesota; Marie Culhane, University of Minnesota; Cameron Schmitt, Pipestone Veterinary Services; Montserrat Torremorell, University of Minnesota

Introduction
Influenza A virus (IAV) is widespread in swine populations worldwide[1]. Piglets of weaning age are one of the sub-populations most likely to test IAV positive in breeding herds[2]. Commonly at weaning pigs are transported to distant pig locations where pigs may become commingled with pigs from other herds creating an opportunity for co-circulation of distinct influenza viruses. As a result, new strains of IAV may emerge.

Vaccination of pregnant sows has been the main tool for controlling IAV infections in breeding herds[3]. This strategy has been effective at reducing IAV transmission and prevalence at weaning, but is not sufficient to consistently wean IAV negative pigs[4]. A recent field study that evaluated enhanced biosecurity measures resulted in a significant delay in IAV infections during lactation, but it was not enough to reduce IAV prevalence at weaning [5]. The objective of the present study was to evaluate the impact of combining both, internal biosecurity practices directed at minimizing IAV infection in piglets and sow vaccination, in IAV prevalence at weaning.

Material and methods
Six IAV positive breeding herds were selected for the study. Five herds were assigned to the treatment group, which consisted in implementing an enhanced internal biosecurity protocol combined with mass sow vaccination. The internal biosecurity protocol consisted of implementing practices of no cross fostering after the first 3 days of age, no usage of nurse sows, changing of disposable gloves between litters when handling piglets and daily disinfection of tools used in farrowing rooms. The mass sow vaccination included vaccinating all females with an autogenous herd-specific vaccine with a booster 3 weeks later. One farm served as control, in which there was no change in management or vaccination practices. Prior to the intervention, all farms were screened for IAV for 3 consecutive weeks, collecting 90 udder skin wipes from litters of weaning age[6]. Six weeks after the booster vaccination, litters at weaning were sampled for 3 more consecutive weeks to assess IAV prevalence after intervention. All collected samples were then tested individually using an IAV rRT-PCR test.

Results
Three of the five farms (60%) that were assigned to the treatment group tested IAV negative in all 3 sampling points post-intervention. One of the treatment farms had a significant decrease in IAV prevalence post intervention but IAV could still be detected in low levels in the last sampling of the study. In contrast, IAV prevalence in one of the treatment farms was not altered indicating that the treatment failed and had no effect on decreasing prevalence at weaning. As expected, the control farm tested IAV positive throughout the study.

Conclusions
The study presented here provides a protocol that combines sow vaccination and enhanced internal biosecurity practices to help wean negative pigs to IAV or pigs with low risk of influenza dissemination. This protocol can serve as a guide to pork producers that have the goal of controlling IAV infections in their breeding herds.

References
1. Baudon E, Peyre M, Peiris M, Cowling BJ. Epidemiological features of influenza circulation in swine populations: A systematic review and meta-analysis. *PLoS One*. 2017;12(6):1-25. doi:10.1371/journal.pone.0179044

2. Diaz A, Perez A, Sreevatsan S, Davies P, Culhane M, Torremorell M. Association between influenza A virus infection and pigs subpopulations in endemically infected breeding herds. *PLoS One*. 2015;10(6):1-11. doi:10.1371/journal.pone.0129213

3. Van Reeth K, Ma W. Swine influenza virus vaccines: to change or not to change-that's the question. *Springer Int Publ*. 2017;410(September 2012):173-200. doi:10.1007/82

4. Chamba FO, Matthew P, Marie WA, Morrison RB, Davies PR, Perez A. Effect of influenza A virus sow vaccination on infection in pigs at weaning : A prospective longitudinal study. 2020;(June):1-11. doi:10.1111/tbed.13688

5. Lopez-Moreno G, Davies P, Culhane M, et al. Evaluation of parity, personnel, and cross-fostering in influenza virus infection during the pre-weaning period. *AASV*. 2021. doi:10.1186/s12917-019-1805-0

6. Garrido-Mantilla J, Alvarez J, Culhane M, Nirmala J, Cano JP, Torremorell M. Comparison of individual, group and environmental sampling strategies to conduct influenza surveillance in pigs. *BMC Vet Res*. 2019;15:61:1-10. doi:10.1186/s12917-019-1805-0

Poster Number: 54
Topic Area: Reproduction
Title: Variation of estrus rates in gilts after consumption of different Altrenogest containing products in Thailand
Author(s): Nutthee Am-in, Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand; Junpen Suwimonteerabutr, Swine Reproduction Research Unit, Chulalongkorn University, Bangkok, Thailand

In Thailand, altrenogest is commonly used for adjusting the number of gilts for batch and weekly farrowing system. Variation in the start of oestrus post withdrawal of altrenogest is often observed between different commercial products. The variation of altrenogest withdrawal to estrus interval (WHEI) is speculated to be due to different rates of excretion from the serum of altrenogest, the sole active compound found in altrenogest containing products. Known metabolites of altrenogest are not known to have biological activity. The aims of this present study are to determine gilt plasma concentrations the withdrawal to estrous interval and pre-ovualted follicles of each altrenogest product, which can be used to guide clinical reproductive plan for gilt replacement program in Thailand. Three altrenogest containing products (R, A and V) were assigned to feed for 6 gilts in each group. 20 mg oral doses altrenogest/gilt/day were given to the healthy gilts at an interval of 24 hr for 18 days. Plasma samples were collected, and altrenogest was determined by ultra-high-performance liquid chromatography with mass spectrometry to calculate the pharmacokinetic parameters through noncompartmental model analysis. The stop hormone to estrus interval and the number of pre-ovulated follicle were compared among group. After the first administration (D 1), the elimination half-life ($T1/2\lambda z$) of R production was significantly shorter than A and V product ($P<0.05$) on D1 and D18 and also found that the plasma clearance of R product trend to be shorter than A and V product ($P= 0.09$) on D1 and D18. The stop hormone to estrus interval of R product are significantly shorter than A and V product (($P<0.05$). These results showed that after 18 consecutive days of oral administration of altrenogest, there is the plasma concentrations of altrenogest which had a certain degree of fluctuation and variation of the stop hormone to estrus interval. This result may use as the guideline for gilt replacement planning for the pig farm.

Poster Number: 55
Topic Area: Bacterial Disease
Title: Immunological evaluation of an autogenous vaccine used in sows to protect piglets against Streptococcus suis infections
Author(s): Alison Jeffery, University of Montreal; Melina Gilbert, University of Montreal, Quebec; Lorelei Corsaut, University of Montreal, Quebec; Simon Cloutier, S.V.A triple-V Inc, Acton-vale, Quebec; Marie-Christine Frenette, S.V.A triple-V Inc, Acton-vale, Quebec; Charles Surprenant, F. Menard, Ange-Gardien, Quebec; Marcelo Gottschalk, University of Montreal, Quebec; Mariela Segura, University of Montreal, Quebec

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

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

Results
Results targeting the response against serotypes 2, 5 and 7, showed that vaccinated sows present higher levels of antibodies against these serotypes than the placebo control group. Maternal antibody transfer to their litters was higher in piglets born from vaccinated sows compared to controls, which lasted until 3 to 5 weeks of age. A mixed IgG1/IgG2 response was observed. Antibody functionality (as measured by OPA) was high in one-week-old piglets but showed a decline at 5 weeks of age.

Conclusion
This study provides evidence for the first time that a gilt vaccination program with an autogenous vaccine increases antibody levels in piglets after one week of age and up to 3 and/or 5 weeks of age depending on the S. suis serotype. Nevertheless, more studies are required to fully characterize the clinical protective effect of this vaccine during the complete nursery period.

References:
1. Segura, M. Streptococcus suis vaccines: candidate antigens and progress. Expert Review of Vaccines, 14: 1587-1608, 2015.
2. Corsaut, L., M. Misener, P. Canning, G. Beauchamp, M. Gottschalk, and M. Segura. Field study on the immunological response and protective effect of a licensed autogenous vaccine to control Streptococcus suis infections in post-weaned piglets. Vaccines, 8: e384, 2020.
3. Corsaut, L., L. Martelet, G. Goyette-Desjardins, G. Beauchamp, M. Denicourt, M. Gottschalk, and M. Segura. Immunogenicity study of a Streptococcus suis autogenous vaccine in preparturient sows and evaluation of passive maternal immunity in piglets. BMC Veterinary Research, 17: e72, 2021.

Poster Number: 56
Topic Area: Bacterial Disease
Title: Aivlosin® WSG and Pulmotil® AC comparison after a Mycoplasma hyopneumoniae challenge in finishing pigs
Author(s): Chad Smith, Pharmgate Animal Health; F. O. Chamba Pardo, South West Ontario Veterinary Services; G. Greaves, South West Ontario Veterinary Services; C. Lichty, South West Ontario Veterinary Services; J. Mora, Eco Animal Health

Introduction: We compared the efficacy of Aivlosin® WSG (AIV) and Pulmotil® AC (PUL) following a Mhp aerosol and intra-tracheal seeder challenge model in a 74-day study.

Methods: A total of 402 feeder pigs were sourced from a herd negative for PRRSV, PEDV and Mhp. After pigs were raised in an off-site nursery, each pig was randomly allocated to a finishing pen dividing pigs into 3 weight categories (large, medium, and small) and same proportion from each sex. Following placement, the entire barn was challenged with a clinically virulent Mhp strain on day 1. Lung homogenate Mhp+ (PCR Ct=27) and negative for PRRSV was aerosolized using 2 foggers. Additionally, 25% of pigs (101 pigs total) were intra-tracheally (IT) inoculated on day 29 with 10 mL/pig of lung homogenate Mhp+ (PCR Ct=34) and negative for PRRSV to worsen Mhp related clinical signs. Mhp challenge was confirmed by daily barn cough index, laryngeal swabs (LS) (8 pools of 3) tested by Mhp PCR (day 21), and lung lesions in 7 euthanized pigs on day 48. After confirming Mhp associated lung lesions, pigs were weighed, and pens randomly assigned to one of 3 treatments on day 54: Control (CON) or no water medication (3 pens, 180 pigs), AIV, Tylvalosin tartrate at 50ppm for 5 days (5 pens, 180 pigs), and PUL, Tilmicosin phosphate at 200ppm for 5 days (5 pens, 180 pigs). Pen-level cough index was estimated daily during water treatment. Lung lesions were scored 5 days after water treatment completion in IT pigs (9 CON, 15 AIV and 15 PUL) using a modified Goodwin et al. method.

Results: Challenge was confirmed by PCR Mhp+ LS pools (Ct ranged from 35 to 40). Barn daily cough index increased about 2 weeks post aerosol challenge and stayed steady around 0.6 throughout the study. Lung lesions to confirm challenge ranged from 0.3 to 31 with a median of 11 out 55 max score. We did not find any significant differences among treatment groups in pen-level cough index. Lung lesions after water treatment were numerically higher in AIV pigs, followed by CON and PUL pigs. Pigs in this study faced tail biting problems and higher lameness removals in a few pens that were assigned as controls to avoid non-Mhp related variability in the water treatments comparison. Dead or removed were 15% (AIV), 13% (PUL), and 23% (CON) with lameness representing 22% of the total dead/removed and 47% of the dead/removed in CON group. Pen level median±mad ADG was 952±58 (AIV), 945±21 (PUL), 832±6 (CON) grams per day. Feed conversion ratio (FCR) was 2.70±0.08 (AIV), 2.69±0.05 (PUL), and 3.15±0.34 (CON). Gross margin per ton of feed (GMTF) using 1.78 CAD/kg live weight and 364 CAD/ton feed was 593±8 (AIV), 601±11 (PUL) and 514±6 (CON).

Conclusions: Control pens had lower performance and economic benefits given higher removals due to lameness. The results of the current study indicated that both Aivlosin® WSG and Pulmotil® AC water treatments performed similarly and highlighted the difficulty of Mhp aerosol models in finishing pigs.

Poster Number: 57
Topic Area: Production
Title: Relationship of tissue dimensions and three captive bolt application sites on cadaver heads from mature swine (Sus scrofa domesticus) > 200 kg body weight.
Author(s): Karly Anderson, University of Minnesota AND University of Wisconsin - River Falls; Kaysie J. Allen, University of Wisconsin - Madison; Angela Baysinger, Merck Animal Health; Madonna Benjamin, Michigan State University; Jennifer Berger, Abbyland Pork Pack; James R. Claus, University of Wisconsin - Madison; Brian J. Greco, University of Wisconsin - River Falls; Brett O'Brien, Merck Animal Health; Emily A. Pintens, University of Wisconsin - River Falls; A Ramirez, Iowa State University; A.K. Rendahl, University of Minnesota; A.A. Reyes, University of Wisconsin - River Falls; Pearl E. Zhitnitskiy, University of Minnesota; K.D. Vogel, University of Wisconsin - River Falls

Three penetrating captive bolt (PCB) placements were tested on cadaver heads from swine with estimated body weight (BW) >200 kg (sows = 232.9 ± 4.1 kg; boars = 229.3 ± 2.6 kg). The objectives were to determine tissue depth, cross-sectional brain area, visible brain damage (BD), regions of BD, and bolt-brain contact for each placement; and to determine the relationship between external head dimensions and tissue depth at each placement. A Jarvis PAS – Type P 0.25R PCB with a Long Stunning Rod Nosepiece Assembly and 3.5 gr power loads was used at the following placements on heads from 111 sows and 46 boars after storage at 2-4° C for approximately 62 h before treatment: FRONTAL – 3.5 cm superior to a line drawn across the top of the eyes at midline, TEMPORAL – at the depression posterior to the lateral canthus of the eye within the plane between the lateral canthus and the base of the ear, or BEHIND EAR – directly caudal to the pinna of the ear on the same plane as the eyes and targeting the middle of the opposite eye. For sows the bolt path was in the plane of the brain for 42/42 (100%, 95% CI: 91.6-100.0%) FRONTAL heads, 39/40 (97.5%, 95% CI: 86.8-99.9%) TEMPORAL heads, and 34/39 (87.5%, 95% CI: 72.6-95.7%) BEHIND EAR heads; for the heads that could reliably be assessed for BD, damage was detected in 25/26 (96.2%, 95% CI: 80.4-99.9%) FRONTAL heads, 24/35 (68.6%, 95% CI: 50.7-83.2%) TEMPORAL heads, and 5/40 (12.5%, 95% CI: 4.2-26.8%) BEHIND EAR heads. For boars, the bolt path was in the plane of the brain for 17/17 (100.0%, 95% CI: 80.5-100.0%) FRONTAL heads, 18/18 (100.0%, 95% CI: 81.5-100.0%) TEMPORAL heads, and 14/14 (100.0%, 95% CI: 76.8-100.0%) BEHIND EAR heads; damage was detected in 11/12 (91.7%, 95% CI: 61.5-99.8%) FRONTAL heads, 2/15 (13.3%, 95% CI: 1.7-40.5%) TEMPORAL heads, and 7/14 (50.0%, 95% CI: 23.0-77.0%) BEHIND EAR heads. Tissue depth was reported as mean ± standard error followed by 95% one-sided upper reference limit (URL). For sows, total tissue thickness was different ($P < 0.05$) between placements (FRONTAL: 52.7 ± 1.0 mm, URL: 64.1 mm; TEMPORAL: 69.8 ± 1.4 mm, URL: 83.9 mm; BEHIND EAR: 89.3 ± 1.5 mm, URL: 103.4 mm). For boars, total tissue thickness was different ($P < 0.05$) between placements (FRONTAL: 41.2 ± 2.1 mm, URL: 56.3 mm; TEMPORAL: 73.2 ± 1.5 mm, URL: 83.4 mm; BEHIND EAR: 90.9 ± 3.5 mm, URL: 113.5 mm). The data indicated that the FRONTAL placement may have the greatest likelihood for successful euthanasia due to the least total tissue thickness and greatest brain area and may present less risk of failure than the alternative TEMPORAL or BEHIND EAR placements.

Poster Number: 58
Topic Area: Production
Title: Evaluation of biosecurity measures on commercial swine operations using Glo Germ powder as a visible learning aid
Author(s): Olivia Harrison, Kansas State University; Payton L. Dahmer, Kansas State University; Jordan T. Gebhardt, Kansas State University; Chad B. Paulk, Kansas State University; Jason C. Woodworth, Kansas State University; Cassandra K. Jones, Kansas State University

Introduction:
Farm biosecurity is an integral part in maintaining the health status of swine. Proper use of entry benches, clean/dirty lines, and showers can reduce the risk of pathogen introduction into swine farms. Unfortunately, the positive effects of farm biosecurity are difficult to visualize and the fortitude of employees to maintain the biosecurity standards may wane, especially during periods of high health. Teaching aids can be used to demonstrate pathogen entry and flow into a farm without risking the health of the herd. Glo Germ is a fluorescent powder which can be used as a visual aid to track the movements of individuals throughout a system. The objective of this study was to use Glo Germ in different areas of a university research swine farm to evaluate the efficacy of biosecurity measures to stop the spread of Glo Germ.

Methods:
Four different locations at a farm were photographed weekly for 7 weeks to provide an assessment of the efficacy of the biosecurity measures to prevent movement of the fluorescent powder. These locations were 1) the clean side of the entry bench, 2) the flooring within the shower, 3) the clean side of the locker room after completing the required shower, and 4) within the barn (control – no biosecurity measure). These clean areas were cleared of any remaining Glo Germ from the prior week in the evening prior to the farm's heaviest trafficked day and pictures were taken of these areas to serve as "Before" pictures. Following personnel movement, "After" pictures were taken of the same areas. These before and after pictures were blindly evaluated by 47 untrained panelists to determine the amount of Glo Germ coverage visible within each picture. The difference between the before and after pictures were averaged across all the panelists. These averaged differences would represent the increased amount of Glo Germ visible between the before and after pictures. Data were analyzed using the GLIMMIX procedure of SAS, v 9.4 (Cary, NC).

Results:
There was a significant difference between the control (no biosecurity measure) and the three other locations ($P < 0.0001$). On average, the three locations with biosecurity measures did not have an increased Glo Germ coverage above 1% following movement of personnel through the four locations. The average difference in Glo Germ coverage of the control, however, was 19.5% across the 7 weeks.

Conclusions:
The use of entry benches and showers were able to stop the spread of Glo Germ throughout the office of the farm, while employing no biosecurity measures allowed for Glo Germ to be tracked throughout the barns. Due to Glo Germ's effectiveness as a teaching aid, operations can use this to help visualize biosecurity gaps. Different colors of Glo Germ are available to differentiate areas of the barns and breaches of biosecurity are easily identifiable with and without a blacklight. In conclusion, the use of Glo Germ is an effective way to demonstrate the efficacy of practical biosecurity measures; furthermore, entry benches and showers appear to be effective biosecurity steps in the farm's biosecurity plan.

Poster Number: 59
Topic Area: Bacterial Disease
Title: Laboratory diagnosis in polyserositis: traditional culture vs. qPCR.
Author(s): Isaac Ballará Rodriguez, HIPRA; Gonzalo García, HIPRA; Sofía Lázaro, EXOPOL; Silvia del Caso, EXOPOL; Emili Barba, HIPRA; Daniel Angelats, HIPRA

INTRODUCTION
Polyserositis complex is a very common disease in the post-weaning phase, with well-known non-specific signs. There are several pathogens involved in this complex, with Glaesserella parasuis and Streptococcus suis being the two main pathogens involved. Laboratory diagnosis is essential to establish correct preventive methods or treat this disease. Traditionally, diagnosis of the pathogens has been confirmed by molecular isolation, as this method permits supplemental characterisation of the strain, such as antimicrobial susceptibility. However, it has been reported that the culture of G. parasuis is not always successful, as it is a fastidious bacterium to grow in the lab1. Alternatively, molecular detection by PCR is increasingly being used to speed up diagnosis and easily obtain more information such as the serovar.

Against this background, the objective of this study was to analyse the differences that exist between traditional culture and molecular techniques (PCR) in the diagnosis of two of the main pathogens involved in polyserositis: S.suis and G. parasuis.

METHODS
A total of 163 reports were obtained from 109 farms in a Spanish reference laboratory (Exopol, Zaragoza). Molecular diagnosis (quantitative PCR, qPCR) and traditional culture were performed on the same samples to assess the presence of the 2 main pathogens involved in the polyserositis complex.

Regarding bacterial culture, a portion of the tissue was spread on a plate of blood agar medium (OXOID PB5039A) with a streak of S. aureus, as G. parasuis grows as a satellite of S. aureus, which provides the NAD factor. After incubation of the plates for 18-24 hours at 37 °C in 5% CO2, individual compatible colonies were transferred to blood agar plates (S. suis) or to BHI plates supplemented with NAD factor (G. parasuis) and incubated at 37 °C. Identification of each pure culture was performed by Maldi-Tof technology.

For the qPCR, total DNA was extracted from samples using the commercial kit MagMax Core RNA/DNA Kit and the automated system KingFisher™ Flex (Thermofisher Scientific). qPCR was performed using commercial qPCR kits EXOone Streptococcus suis and EXOone Glaesserella parasuis (Exopol), following the manufacturer's instructions.

In addition, the detection of other pathogens including environmental bacteria and other secondary pathogens were detected by culture and qPCR and included in the group "others".

RESULTS
From all the analyses performed, the following percentages of the samples were found to be positive in each group.

Table 1.- Positive samples for PCR and Traditional Culture.

	G.parasuis	S.suis	Others
Culture	23%	39%	82%
qPCR	56%	50%	53%

As can be seen in Table 1, there was a higher detection of both pathogens with PCR. Regarding S. suis, qPCR was found to be 1.2 times more sensitive than culture, while with G. parasuis the detection was 2.4 times as high with qPCR than with culture.

CONCLUSIONS
In view of these results, although culture in the laboratory is still indicated in order to assess microbial susceptibility, it is highly recommended that molecular diagnosis is performed by qPCR, which is the most sensitive method.

Poster Number: 60
Topic Area: Bacterial Disease
Title: Prevalence study of polyserositis on Spanish farms
Author(s): Isaac Ballará Rodriguez, HIPRA; Gonzalo García, HIPRA; Sofía Lázaro, EXOPOL; Silvia del Caso, EXOPOL; Emili Barba, HIPRA; Daniel Angelats, HIPRA

INTRODUCTION
Polyserositis complex is a very common disease in the post-weaning phase, with well-known non-specific signs such as high fever, anorexia, dyspnoea, and finally death. There are several pathogens involved in this complex, although Streptococcus suis is commonly believed to be predominant in these situations. The objective of this study was to analyse the prevalence of the different pathogens: Glaesserella parasuis, Streptococcus suis and Mycoplasma hyorhinis, in polyserositis cases on Spanish pig farms.

METHODS
A total of 184 diagnostic reports were obtained from 140 farms. The prevalence of G. parasuis, S. Suis and M. hyorhinis was analysed by real time PCR. From these results, 131 reports were obtained from farms sampled by field vets and sent for analysis to the local reference laboratory Exopol (Zaragoza) during the years 2016-2020. The other 53 reports were from the HIPRA Diagnos laboratory (Amer) and were obtained by active screening by HIPRA Technical Services on farms during the latter part of 2020 and the first quarter of 2021.

RESULTS
Of the 131 reports from farms analysed by Exopol, 86.25% (113 farms) out of the total were positive for at least one of the 3 pathogens. A similar prevalence was obtained with the 53 reports obtained at HIPRA Diagnos, 83.9% (47 farms). Table 1 indicates the prevalence of each pathogen or combination of pathogens on the different farms.

Table 1. Prevalence of each pathogen or combination of pathogens on the different farms.

Another interesting aspect observed was the positive correlation between several of the pathogens in both labs. When analysed together, the correlation was highly significant between G. parasuis and M. hyorhinis (Spearman coef. 0.3; $P<0.001$), significant between S. suis and M. hyorhinis (Spearman coef. 0.18; $P>0.02$) and with a tendency between G. parasuis and S. suis (Spearman coef. 0.15; $P>0.06$).

CONCLUSIONS
In view of the results, the prevalence of the 3 agents studied in the polyserositis complex had a similar distribution in both labs, co-infection with the 3 agents being the most prevalent. Moreover, these results confirm the importance of other agents besides S. suis involved in the polyserositis complex, with a highly significant positive correlation between Mycoplasma hyorhinis and Glaesserella parasuis.

Poster Number: 61
Topic Area: Viral Disease
Title: Monoglyceride reduces PEDV viability in feed
Author(s): Farrah Phillips, Kemin Industries, Inc.; Dillon Mellick, Kemin Industries, Inc.

Outbreaks of African Swine Fever virus (ASFv) and Porcine Epidemic Diarrhea virus (PEDv) have revealed the susceptibility of livestock to disease transmitted through feed. Several viruses, including PEDv, survive in feed and may introduce disease that causes significant morbidity and mortality.[1–4] In 2013, PEDv – which causes severe diarrhea and vomiting – escaped its endemic locations in Europe and Asia and spread rapidly across the United States, killing approximately seven million pigs over a 12-month period.[5] The global exchange of ingredients has created demand for products that prevent disease transmission from feed. Formaldehyde-based products are highly effective against enveloped viruses when applied at ≥ 6.5 lb/ton.[6,7] Alternative products to formaldehyde, including carboxylic acids, essential oils and medium chain fatty acids (MCFAs), have exhibited mixed efficacy against PEDv and require application rates ≥ 6 lb/ton.[6,7] Amphiphilic molecules like MCFAs disrupt the bilayer-lipid membranes that protect viral nucleic acids through the formation of micelles.[8,9] Monoglycerides form micelles at lower concentrations than MCFAs, which suggests they may be more potent against enveloped viruses.[10] The potential efficacy of monoglycerides against enveloped viruses in feed led to the identification and examination of an experimental monoglyceride.

Three different mitigants were added to 1 g of feed and mixed thoroughly. A formaldehyde-based product Sal CURB® (Kemin Industries, Des Moines, IA) at 6.25 lb/ton and a proprietary blend of MCFAs (20 lb/ton) were included as positive controls. Feed was treated with the experimental monoglyceride at 3, 5 and 7 lb/ton. The treated feed samples were inoculated with ~1×105.1 TCID50 of PEDv (Colorado-13) and held at room temperature for 0, 12, 24, 48 and 120 hours post inoculation. Each treatment and time point were replicated three times in two independent experiments (n = 6). Statistical analyses were conducted in JMP® (SAS Institute Inc., Cary, NC) using one-way ANOVA followed by Tukey-Kramer. Viable virus was eluted from the feed and titrated onto monolayers of Vero-81 cells and viability was calculated by TCID50.[11] Both positive control products reduced virus viability by 2 logs within 24 hours of contact. Over the entire 120-hour period, no additional reduction in virus viability was detected ($p > 0.05$). The experimental monoglyceride blend was applied at 3, 5 or 7 lb/ton and after 24 hours of contact, the 5 and 7 lb/ton treatments were just as effective as the positive controls ($p > 0.05$) and reduced virus viability by more than 2 logs. The 3 lb/ton monoglyceride treatment also significantly ($p < 0.0001$) reduced virus viability compared to the untreated control by 1.83 logs. This experimental monoglyceride at 3 lb/ton has the potential to mitigate transmission of enveloped viruses through feed. Higher inclusion levels of 5 or 7 lb/ton exhibited similar efficacy as MCFAs and formaldehyde against PEDv in feed.

1. Jung, K. & Saif, L. J. Porcine epidemic diarrhea virus infection: Etiology, epidemiology, pathogenesis and immunoprophylaxis. Vet. J. 204, 134–143 (2015).
2. Wang, Q., Vlasova, A. N., Kenney, S. P. & Saif, L. J. Emerging and re-emerging coronaviruses in pigs. Current Opinion in Virology vol. 34 39–49 (2019).
3. Wang, Q., Vlasova, A., Kenney, S. P. & Saif, L. J. Porcine epidemic diarrhea virus (PEDV): An update on etiology, transmission, pathogenesis, and prevention and control. Curr. Opin. Virol. 34, 39–49 (2019).
4. Dee, S. et al. An evaluation of contaminated complete feed as a vehicle for porcine epidemic diarrhea virus infection of nai¨ve pigs following consumption via natural feeding behavior: Proof of concept. BMC Vet. Res. 10, 176 (2014).
5. Niederwerder, M. C. & Hesse, R. A. Swine enteric coronavirus disease: A review of 4 years with porcine epidemic diarrhoea virus and porcine deltacoronavirus in the United States and Canada. Transboundary and Emerging Diseases vol. 65 660–675 (2018).
6. Cochrane, R. A. et al. Assessing the effects of medium-chain fatty acids and fat sources on PEDV infectivity. Transl. Anim. Sci. 4, 1051–1059 (2019).
7. Dee, S. A. et al. An evaluation of additives for mitigating the risk of virus-contaminated feed using an ice-block challenge model. Transbound. Emerg. Dis. 1–13 (2020) doi:10.1111/tbed.13749.
8. Yoon, B. K., Jackman, J. A., Valle-González, E. R. & Cho, N. J. Antibacterial free fatty acids and monoglycerides: Biological activities, experimental testing, and therapeutic applications. International Journal of Molecular Sciences vol. 19 (2018).
9. Yoon, B. K., Jackman, J. A., Kim, M. C., Sut, T. N. & Cho, N. J. Correlating Membrane Morphological Responses with Micellar Aggregation Behavior of Capric Acid and Monocaprin. Langmuir 33, 2750–2759 (2017).

10. Jackman, J. A., Boyd, R. D. & Elrod, C. C. Medium-chain fatty acids and monoglycerides as feed additives for pig production: Towards gut health improvement and feed pathogen mitigation. J. Anim. Sci. Biotechnol. 11, (2020).
11. Karber, G. 50% end-point calculation. Arch Exp Pathol Pharmak 162, 3 (1931).

Poster Number: 62
Topic Area: Feed/Nutrition
Title: BioPlus® 2B – Repeating Results in the face of a Lateral PRRS Infection
Author(s): Keith Kinsley, Chr Hansen Inc; Steve Kitt, First Choice Livestock; Mark Bertram, First Choice Livestock; Steve Lerner, Chr Hansen Inc

Introduction: Probiotics have been available and used for decades. Unfortunately, a history of poorly repeatable results has left some key-decision-makers skeptical about their efficacy. BioPlus® 2B (a combination of unique Bacillus licheniformis and Bacillus subtilis strains) is a probiotic product for the swine industry. The goals of this study were to validate the ability of BioPlus 2B to support normal growth in the face of a lateral PRRS challenge and to determine the interaction of a probiotic and a commercially available modified-live vaccine for PRRS.

Methods: Two groups of 200 piglets (~21-day old) were acquired from a PRRS-naive sow farm that applied BioPlus 2B or nothing (Control) as a top dress to birthing dams for a minimum of 31-days pre-farrowing through lactation. Upon weaning, piglets were moved to an offsite nursery and consumed rations matching the BioPlus 2B (B2B) or Control (C) feeding programs of their dams. Within each group, B2B or C animals, half (n=100 pigs) were vaccinated with Ingelvac PRRS® MLV vaccine (+V) on day 0 thereby establishing four treatment groups: B2B, B2B+V, C, and C+V. On day 21, all pigs were challenged with a wild-type PRRS virus by intramuscular injection. Animals were monitored for common performance variables (ADG, ADFI, F:G) throughout the study, and on day 42, 25 pre-identified pigs per treatment were euthanized and examined for gross lung lesions and differences in immunological variables.

Results: Pigs weaned from B2B-fed sows were heavier ($p<0.05$) upon entering the nursery. From entry until the day of PRRS challenge, no differences in performance were identified across treatments. In the post-challenge period (day 21+), significant improvements ($p<0.05$) in performance were noted between identified groups compared to C: ADG and ADFI for B2B+V and C+V and F:G for B2B, B2B+V, and C+V. Regardless of treatment group, body weights of pigs at day 41 were significantly better ($p<0.05$) than those in the C group. Severity of gross lung lesions was lower ($p<0.01$) in groups fed B2B versus that of the C group. Daily feeding of B2B, with or without vaccination, resulted in a demonstrable change in immune response, as measured by PRRS-specific ELISPOT for interferon gamma (INF-γ). In addition, more antibodies were present earlier in the post-challenge period (days 28 and 35), as measured by the percentage of ELISA positive animals. Feeding BioPlus 2B resulted in a tendency for more PRRS-negative animals on day 42, based on PCR, and fewer genomic copies of the virus per mL of serum.

Conclusions: This is the second study designed to ascertain the impact of daily feeding of an effective probiotic to sows and post-weaning piglets combined with a severe PRRS challenge. In both studies, daily feeding of BioPlus 2B supported the capacity of challenged piglets to survive, defend themselves against the challenge, and grow and convert feed in a normal manner. Additional work is needed to elucidate the exact modes of action by which the interactions of BioPlus 2B with feed, other microorganisms, and the immune system of their host results in these beneficial effects.

Poster Number: 63
Topic Area: Viral Disease
Title: Targeted Development of Rotavirus Vaccines against Current Circulating Strains using Medgene's Prescription Platform (RxP)
Author(s): Ashley Petersen, M.S., Medgene Labs; Alan Young, Ph.D., Medgene Labs

Introduction:
Swine rotaviruses are a prevalent and economically devastating disease that plague the swine industry (VanderWaal and Deen, 2018). Rotaviruses exist in multiple genotypes due to reassortment of viral genes. Current vaccines rely on outdated strains that may not cover current circulating strains. More flexible and modern vaccine technology is required to match the needs of producers. We have shown that a baculovirus-produced, rotavirus specific bivalent vaccine elicited high titers of neutralizing antibodies against target Rotavirus A. We have established critical epitopes within the target proteins as evidenced by effective cross-neutralization in vitro, combined with in silico analysis of predicted protein structure. Data indicated cross-protection against significantly divergent Rotavirus A isolates can be generated. This work opened the door to the availability of targeted vaccines against circulating strains of Rotavirus A, and the potential for development of vaccines against Rotavirus B and C. Various field evaluation studies - utilizing RVA, RVB, and RVC vaccines (monovalent and multivalent) - have been conducted with swine integrators across the Midwest. Overall, clinical impressions from attending veterinarians at these swine facilities have been positive.

Materials and methods:
Sequences of target Rotavirus isolates were obtained from routine diagnostic samples. Recombinant baculoviruses were produced to generate proteins corresponding to the obtained sequences. Experimental prescription platform vaccines were produced against each isolate. Mice and pigs were vaccinated twice at 3-week intervals and serum harvested about two weeks post second vaccination. Sera was then tested in standard virus neutralization assays using either homologous or heterologous Rotavirus A isolates (Feng et al., 2019; Ward et al., 1996). In addition, viral sequences corresponding to the native virus, or the adapted sequence used in recombinant baculovirus, were analyzed for predicted secondary structure and immune epitopes.

Results:
Mice immunized with a bivalent vaccine developed neutralizing titers whereas control mice did not. It was evident that one of the two selected viral proteins appeared to provide the majority of the neutralizing response and that the sequence homology between the target viral protein and the vaccine construct was not highly predictable. However, further analysis of individual epitopes conserved between the virus and vaccine construct was predictive, and several neutralizing epitopes were identified based on this work.

Veterinarians utilizing our Rotavirus vaccines observed positive outcomes during field evaluation. In one such study, our Rotavirus A killed baculovirus vaccine was compared to a traditional Rotavirus A autogenous vaccine. Serological response to vaccination, determined by standard virus neutralization assay, was stronger with Medgene’s vaccine than with a traditional autogenous vaccine.

Conclusions:
Based on our work, we identified two proteins that together appear to provide high levels of neutralizing antibody. Further observation of the sequence differences between these virus proteins and the target construct identified several target regions that may correspond to target neutralizing epitopes. Together with positive clinical impressions from veterinarians, these data indicate that the RxP approach can generate highly successful vaccines, and greater understanding of the nature of target epitopes within the viral proteins may extend the success of these vaccines against heterologous challenge.

References:
Erdman, M.M., N.E. Clough, and P.J. Hauer. 2020. Review of updated regulations and product license categories for veterinary vaccines in the United States. *Journal of the American Veterinary Medical Association* 257:1142-1147.

Feng, N., L. Hu, S. Ding, M. Sanyal, B. Zhao, B. Sankaran, S. Ramani, M. McNeal, L.L. Yasukawa, Y. Song, B.V.V. Prasad, and H.B. Greenberg. 2019. Human VP8* mAbs neutralize rotavirus selectively in human intestinal epithelial cells. *J Clin Invest* 129:3839-3851.
Jiang, B., J.R. Gentsch, and R.I. Glass. 2002. The role of seerum antibodies in the protection against Rotavirus Disease: An Overview. *Clinical Infectious Diseases* 34:1351-1361.
Jiang, B., Y. Wang, and R.I. Glass. 2013. Does a monovalent inactivated human rotavirus vaccine induce heterotypic immunity? Evidence from animal studies. *Hum Vaccin Immunother.* 9:1634-1637. doi: 1610.4161/hv.24958. Epub 22013 Jun 24956.
VanderWaal, K., and J. Deen. 2018. Global trends in infectious diseases of swine. *Proc Natl Acad Sci U S A* 115:11495-11500.
Wang, Y., M. Azevedo, L.J. Saif, J.R. Gentsch, R.I. Glass, and B. Jiang. 2010. Inactivated rotavirus vaccine induces protective immunity in gnotobiotic piglets. *Vaccine.* 28:5432-5436. doi: 5410.1016/j.vaccine.2010.5406.5006. Epub 2010 Jun 5415.
Ward, R.L., A.Z. Kapikian, K.M. Goldberg, D.R. Knowlton, M.W. Watson, and R. Rappaport. 1996. Serum rotavirus neutralizing-antibody titers compared by plaque reduction and enzyme-linked immunosorbent assay-based neutralization assays. *J Clin Microbiol* 34:983-985.
Wilhelm, B., L. Waddell, J. Greig, A. Rajic, A. Houde, and S.A. McEwen. 2015. A scoping review of the evidence for public health risks of three emerging potentially zoonotic viruses: hepatitis E virus, norovirus, and rotavirus. *Prev Vet Med* 119:61-79.

Poster Number: 65
Topic Area: Viral Disease
Title: Evaluation of the serological response to SEQUIVITY® Rotavirus vaccine administered pre-farrowing to gilts
Author(s): Sabra McCallister, North Carolina State University; Brett O'Brien, Merck Animal Health; Justin Cagle, Merck Animal Health; Jeremy Pittman, Smithfield Hog Production; Hunter Everett, North Carolina State University; Michelle Allen, Merck Animal Health; Joanna Piasecka-Srader, Merck Animal Health

Introduction
Rotavirus is a major cause of diarrhea in neonatal pigs and causes significant production losses in U.S. swine herds.[1,2] The SEQUIVITY® Rotavirus RNA Particle (RNA-P) vaccine is administered to help reduce these losses.[3] To date, rotavirus vaccine evaluations have not included strain specific serological testing. This study's objective was to assess the post-vaccination serological response in gilts, their colostrum, and offspring after a single dose of SEQUIVITY® Rotavirus RNA-P vaccine administered two weeks prior to farrowing.

Methods
The study was conducted on a 1750 head commercial sow farm that is endemic with rotavirus. One hundred gilts were enrolled in the study two months pre-breeding when housed in the gilt development unit. The gilts were orally inoculated twice, one day post-enrollment and one week later, with 10 mL of rotavirus gut homogenate (Natural Planned Exposure (NPE) method); which contained farm-specific rotavirus types A and C (CT= 18 and 16, respectively). Serum was collected from all gilts at enrollment and four weeks post-NPE; and from a subset of 30 gilts at six- and eight weeks post-NPE. Gilts in the same breeding group were blocked by their antibody titers four weeks post-NPE and randomly allocated to T01 (control, 1mL sterile water IM, n=20) or T02 (SEQUIVITY® Rotavirus vaccine, 1mL IM , n=21). Treatments were administered two weeks pre-farrowing. Within 24-hours of farrowing, serum from sows and five milliliters of colostrum were collected. Four randomly selected piglets from all litters were bled and tagged at 3-5 days of age. All serum and colostrum were tested by G5 Rotavirus A Virus Neutralization (VN) assay developed at Merck Animal Health.
Rotavirus A VN values were log transformed and the means per treatment group were analyzed by T test and Chi squared for each time point. Pearson correlation coefficient was generated to compare mean Rotavirus A VN of sow serum pre-farrowing, colostrum, and piglet serum.

Results
There was no difference (P=0.9) at allocation in geometric mean Rota A VN log titers between treatment groups (Table 1). There was a trend (P=0.08) of higher geometric mean Rota A VN log titers in controls at the time of vaccination (T01=904 and T02=564). There was a significant difference ($p<0.01$) in the change in log titers following-vaccination (T01= -0.15 and T02=1.05). Likewise, there was a significant Rota A VN titer increase in the mean, max, and mode in piglets from vaccinated dams compared to controls ($P<0.01$). Across sample types, there was moderately strong agreement between sow titers post-vaccination and piglet sera at processing (Pearson coefficient=0.6) and colostrum and the piglet sera (Pearson coefficient=0.59) yet low agreement between sow titers post-vaccination and colostrum (Pearson coefficient=0.37).

Conclusions
This study demonstrated a significant increase in the post-vaccination serological response of NPE exposed gilts vaccinated with the SEQUIVITY® Rotavirus RNA-P vaccine administered pre-farrowing compared to controls. Furthermore, the study demonstrated that vaccinated gilts conferred this immunity to their offspring.

References
1. Chepngeno, J. et al. Rotavirus C: prevalence in suckling piglets and development of virus-like particles to assess the influence of maternal immunity on the disease development. Vet Res 50, 84 (2019). https://doi.org/10.1186/s13567-019-0705-4
2. Zimmerman J. et al. Diseases of Swine, Tenth Edition. Edited by Jeffrey. 2012 John Wiley & Sons, Inc. Published 2012 by John Wiley & Sons, Inc.
3. Boyd, W. et al. Comparison of prefarrow rotavirus vaccine and natural planned exposure on suckling pig performance. AASV. 2020. 228-229.

Poster Number: 66
Topic Area: Other
Title: Evaluation of the Residual Binding Time of ArmatrexTM on Common Swine and Poultry Surfaces
Author(s): Brett O'Brien, Merck Animal Health; Abigail Redalen, Merck Animal Health; Todd Williams, Pipestone Research; Scott Dee, Pipestone Research; Roy Edler, Pipestone Research; Ivan Alvarado Ortiz, Merck Animal Health; Derald Holtkamp, Iowa State University; Chong Wang, Iowa State University

Introduction
The disease risk to naïve populations that enter contaminated facilities can result in significant disease and production losses. To help reduce the disease prevalence in swine facilities, the application of an antimicrobial finish can help reduce bioburden levels in the environment as well as increase surface longevity. ArmatrexTM is an antimicrobial finish consisting of a silane quaternary ammonium salt, offering residual protection on treated surfaces and is approved for treating livestock facilities. The purpose of this study was to evaluate the longevity of ArmatrexTM on different surface types in order to determine the appropriate application frequency.

Methods
Fifty-four coupons made of aluminum, tribar, plastic farrowing pen panel, plastic nursery flooring, plastic tote, plastic chick boxes, painted metal, rubber boots, two plastic chick trays, stainless steel, plastic sort board, two incubator curtains, FRP wall panel, concrete, and galvanized metal were randomly allocated to treatment group (T01: Control; T02: Armatrex) in replicates of three. All surfaces were stored at room temperature except aluminum which was also stored at 0°F and 30°F. Armatrex was applied using the Max Charge Electrostatic Sprayer (model BP1) and surfaces were positioned either vertically or horizontally depending on how they would normally be positioned in the barn. The presence of Armatrex was determined using 0.5% Bromophenol Blue (BPB) solution on study day 0 (4 hours post-Armatrex application) and every two weeks thereafter until study day 98. Five droplets of BPB were applied within a 2x2 cm2 grid using a polyester swab and allowed to sit on the surface for 5 minutes. The surface was then rinsed with distilled water and allowed to dry for 5 minutes. Each plate was then given a BPB score (0=no score, 1=light blue, 2=dark blue) by a trained scorer blinded to treatment group and photographed. The digital image was divided into quadrants and scored on Hue, Saturation, and Value using the ColorMeter RGB colorimeter (White Marten GmbH).

Descriptive statistics were used for summarizing frequency of BPB scores at each time point, average hue, saturation, and value differences between controls and treated. For the comparison of BPB score vs. hue differences, the data were further dichotomized such that BPB score 0 = 0, BPB score ≥ 1 = 1, Hue difference≤0 = 0, and Hue difference ≥0 = 1 and then analyzed (adjusted kappa).

Results
Armatrex was present on all treated surfaces through study day 98 except plastic nursery flooring which was negative after day 0 and stainless steel which only had 1/3 treated plates BPB positive for almost all days after day 0. Concrete, plastic chick trays (horizontal and vertical), and galvanized metal had BPB staining on the controls which prevented comparison with Armatrex treated coupons. Saturation and value means were not different numerically between the treated and control coupons, and therefore was not used to detect BPB staining. Average hue differences were strongly correlated with BPB scores (Kappa=0.61).

Conclusions
Armatrex demonstrated residual binding out to day 98 on a wide variety of metals, plastic, and rubber materials found in swine and poultry operations.

Poster Number: 68

Topic Area: Bacterial Disease
Title: Comparison of post-vaccination serological profiles of different vaccination strategies with Porcilis® Ileitis in a controlled challenge study
Author(s): Brett O'Brien, Merck Animal Health; Nathan Winkelman, Swine Services Unlimited Inc.; Adam Mueller, Swine Services Unlimited Inc.; Brad Thacker, Merck Animal Health; Kimberly Crawford, Merck Animal Health

Introduction

Porcilis® Ileitis from Merck Animal Health is an inactivated, ready-to-use vaccine for the control of ileitis caused by *Lawsonia intracellularis*.[1] The product offers flexible dosing options: 1) a single injection given to pigs at 3 weeks of age (WOA) or older or 2) two injections starting as early as 3 days of age with a booster 3 weeks later. The objective of this study was to compare the post-vaccination serological responses for both vaccine options in a controlled *Lawsonia* challenge study.[2]

Methods

Crossbred pigs were blocked according to their litter, weight, and gender, and then randomly assigned to treatment group prior to weaning. Treatment group 1 (T01) pigs (n=32) were vaccinated at 3 WOA with 2 mL IM, treatment group 2 (T02) pigs (n=31) were vaccinated at 3 and 6 WOA with 1 mL IM, and treatment group 3 (T03) pigs (n=32) served as controls and were injected at 3 and 6 WOA with 1 mL of saline IM. Pigs were housed such that each pen contained pigs from the same litter with one pig from each treatment group. At 9 WOA, all pigs were individually challenged by oral gavage with 40 mL of a gut mucosal tissue homogenate containing approximately 2.04 X 10^9 *L. intracellularis*. All pigs were necropsied 23 days post-challenge. Serum was collected from all pigs immediately prior to each vaccination, at challenge, and at necropsy. A subset of 10 sera from each treatment group (except all from T01 at challenge) were tested for serum antibody testing, the bioScreen Ileitis Antibody ELISA (Svanova, Uppsala Sweden) by Merck Animal Health and the Immunoperoxidase Monolayer Assay (IPMA) at the University of Minnesota's Veterinary Diagnostic Laboratory. ELISA percent inhibition (PI) and log transformed IPMA titers were analyzed by Kruskal-Wallis One-Way AOV and linear regression (Statistix™ 10, Analytic Software, Tallahassee, FL).

Results

All pigs were confirmed Lawsonia IPMA and ELISA negative prior to initial vaccination. Controls remained negative on both serological assays until challenge. All pigs bled three weeks post- vaccination had a geometric mean IPMA titer of 137.8 and mean PI's of 37.7 (T02) and 41.6 (T01). At the time of challenge, pigs that had received two doses of vaccine had significantly higher serological response than those receiving either one dose of vaccine or controls (geomean IPMA T01= 65 and T02=4728 and mean PI T01=62 and T02=81; $P<0.01$). Interestingly, at 23 days post-challenge both controls and one dose vaccine group titers continued to rise while the two dose vaccine group lowered (geomean IPMA T01=591, T02=1267, and T03= 182 and PI's T01=90, T02=89, and T03=85). Post-challenge serology results suggest an increased sensitivity of the IPMA test. There was also a significant correlation between Lawsonia IPMA and ELISA results with a linear regression coefficient of 10.99 ($P<0.01$).

Discussion

It is important for veterinarians using Porcilis Ileitis to understand the expected post-vaccination serological profile depending on the vaccine strategy employed. In this study, the two dose vaccination strategy yielded a more pronounced antibody response in comparison to the one dose strategy and this protective immunity yielded no rise in antibodies following challenge.

References

1. Thacker B et al. Field experiences with Porcilis™ Ileitis: The newest tool for controlling *Lawsonia intracellularis*. AASV. 2017; 191.
2. Thacker B et al. Efficacy of a two dose Porcilis™ Ileitis regimen against experimental *Lawsonia intracellularis* challenge. AASV. 2021; 138-139.

Poster Number: 69
Topic Area: Other
Title: Using analytics models for description, prediction and prescription of contagious risk of African Swine Fever in Colombia
Author(s): Mario Pena, Porkcolombia; Fernando Rojas, Porkcolombia; Francisco Gomez, Universidad Nacional de Colombia; Fausto Moreno, Universidad Nacional de Colombia; David Gonzalez, Universidad Nacional de Colombia; Maria Morales, Universidad Nacional de Colombia; Gabriel Lozano, Universidad Nacional de Colombia; Lina Prado, Universidad Nacional de Colombia; Diego Hernandez, Universidad Nacional de Colombia; Juan Canas, Universidad Nacional de Colombia

Introduction
African Swine Fever (ASF) is a highly contagious and deadly hemorrhagic disease affecting pigs, with devastating consequences for economies of infected countries (1,2,3). Several countries are free of ASF, including Colombia. Nevertheless, because of its transboundary nature and the lack of vaccines, threat and vulnerability of contagion in most free countries are considerably high (4). Threat and vulnerability assessment of disease contagion aims to establish levels of risk of contagious of this disease quantitatively (5). This surveillance strategy is fundamental for devising cost/effective epidemiological vigilance strategies (1,2,3). Nevertheless, the levels of threat and vulnerability to ASF depend on local, highly complex multifactorial environmental, ecological, and social factors, which can be challenging to identify and quantify even for domain experts.

In this work, we introduce a novel data analytics-based model aimed to quantify the ASF levels of threat of contagious and propagation, for scenarios when the disease is not present yet.

Materials and Methods
An indicator-based model provided the threat quantification (5). For this, a systematic review based on the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) method guided by expert criteria provided a set of risk factors for Colombia (6). Broad categories of threat grouped these factors. Quantification of each factor was performed by using multiple data sources describing different aspects of ASF, including biosecurity surveys, animal mobilization, and productive process descriptions, and among others. Specific mathematical models were devised to compute a normalized 0-1 index of vulnerability per municipality - per factor. These indices were linearly mixed per municipality by using a convex combination. Weights for these combinations were defined by 24 Colombian domain experts using linguistic quantifies and ordered weighted average operators (7). A similar strategy was used to combine the resulting categories. Finally, a visualization tool and thematic cartography were developed to explore the risk factors.

Another systematic review based on the PRISMA was conducted in order to identified ASF spread models. The infected rate, the simulated days, the infected town and the mobilization network, were parameters for ASF spread model through direct contacts.

Results
Table 1 reports the 22 risk factors and the 7 risk categories of threat, together with the corresponding relevance weights obtained from the experts.

Figure 1 shows the user interface devised to explore different risk factors. As observed, different risk categories and factors were quantified in different threat levels and probabilities for different regions of Colombia

Figure 2 shows the final integrated map of threat levels of 7 risk categories and 22 risk factors for swine areas in Colombia.

A total of 1000 different spread simulations were run. Over each scenario the probability was calculated like to show in the Figure 3.

In general, ASF simulations produced small and short propagations highly dependent on the time and number of animals mobilized.

Conclusions and discussion

Implementation of successful epidemiological surveillance strategies requires objective evidence for cost/effective resource allocation. In the case of ASF free areas, where probably there is no available information on previous contagious, models based on data may provide these quantifications. Our results show that a combination of evidence coming from existing literature, mathematical modeling and expert knowledge may provide quantifications of threat for ASF.

Simulation studies conducted here were useful for estimating the potential risk of propagation of ASF over specific initial infected town throught assessing the main routes of spread transmission.

Acknowledgments

This work was funded by PorkColombia-Fondo Nacional de Porcicultura.

References

1. Boklund A, Cay B, Depner K, Földi Z, Guberti V, Masiulis M, et al. Epidemiological analyses of African swine fever in the European Union (November 2017 until November 2018). EFSA J. 2018;16(11).
2. Gulenkin VM, Korennoy FI, Karaulov AK, Dudnikov SA. Cartographical analysis of African swine fever outbreaks in the territory of the Russian Federation and computer modeling of the basic reproduction ratio. Prev Vet Med 2011;102(3):167–74.
3. De la Torre A, Bosch J, Iglesias I, Munoz MJ, Mur L, Martinez-Lopez B, et al. Assessing the Risk of African Swine Fever Introduction into the European Union by Wild Boar. Transbound Emerg Dis. 2015 Jun;62(3):272–9.
4. Sanchez-Vizcaino JM, Mur L, Martinez-Lopez B. African swine fever: an epidemiological update. Transbound Emerg Dis. 2012 Mar;59 Suppl 1:27–35.
5. Caribbean Handbook on Risk Management (CHARIM). Introduction to Exposure, Vulnerability and risk assessment. C.J. van Westen (http://www.charim.net/methodology/51), accessed February 29 of 2019..
6. Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gøtzsche, P. C., Ioannidis, J. P., ... & Moher, D. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. Annals of internal medicine, 151(4), W-65..
7. Lamata, M. T., & Pérez, E. C. (2012). Obtaining OWA operators starting from a linear order and preference quantifiers. International Journal of Intelligent Systems, 27(3), 242-258.

Poster Number: 70
Topic Area: Other
Title: Piglets coccidiosis in US farms- evaluation of farm positivity by newly developed qPCR
Author(s): Daniel Sperling, Ceva Animal Health; Daniel Goza, Ceva Animal Health; Corey White, Ceva Animal Health; Joseph Thomas, Iowa State University; Todd Williams, Pipestone; Erin Heffley, Iowa State University; Zalan Homonnay, Ceva Animal Health; Istvan Kiss, Ceva Animal Health; John El-Attrache, Ceva Animal Health

Introduction
Cystoisosporosis caused by *Cystoisospora suis* is one of the most important causes of preweaning diarrhea in intensive pig production worldwide. The annual financial loss due to porcine cystoisosporosis was calculated to be up to € 100 per sow.[1] Diagnostis is usually based on clinical signs, gross lesions, and detection of oocysts in the feces by flotation with confirmation by histopathology. Interpretation of fecal floatation is frequently hampered by the short individual excretion period of oocysts and the high fat content of fecal samples. Recently, a new real-time PCR assay for rapid detection and quantification of *C. suis* has been developed.[2] With this study we aimed to investigate the occurrence of *C. suis* on selected conventional pig breeding farms across the US with real-time PCR.

Materials and Methods:
A total of 540 fecal samples were gathered from 321 litters from the Midwestern United States, and stored at 4°C until DNA extraction. From each farm, pooled litter samples were obtained in the 2nd and 3rd week of life of piglets. To increase sensitivity, each litter was sampled twice in one week intervals due to the short periods of oocyst excretions. A litter was considered coccidia-positive when it was positive at least in one out of the two samplings.

For each sample, 0.1 grams of feces was used in the Qiagen PowerFecal Extraction kit. All qPCR reactions were run using the Life Technologies QuantStudio 7 Flex, and Qiagen's QuantiNova Probe Real Time PCR kit, with a primer/probe set targeting the ITS1 region in the *C. suis* genome.

Results
Overall, we examined 540 samples and 321 litters from 22 farms (mean 14.6 litters/farm) in the 2nd and 3rd week of life. In total 14/22 (63.6%) farms were positive for *C. suis,* in at least one sampling and 11/22 (50%) were positive by PCR in both samplings. In total 102/321 (31.8%) litters were at least once positive for *C. suis* by PCR. The number of litters that were positive on the farms varied greatly from 0.0 to 100%. The highest percentage of litters, that were positive by state was observed in Missouri (50% in 1st sampling and 45% in 2nd round). On the opposite, lowest % was confirmed in Minnesota (5% and 35% in 1st and 2nd sampling round respectively).

Discussion
C. suis was detected on 63.6% of sampled farms with up to 100% positive litters per farm. These rates are in line with a study from EU countries, that showed herd prevalence of 71% of farms where autofluorescence technique (AF) was used for detection (Hinney et al., 2020). qPCR provides a sensitive assay for detection of *C.suis*, which may improve detection of this important pathogen of piglets.

References:
1. Kreiner T, Worliczek HL, Tichy A, Joachim A (2011) Influence of toltrazuril treatment on parasitological parameters and health performance of piglets in the field – an Austrian experience. Vet Parasitol 183:14–20
2. Homonnay et al., 2021, in Proceeding of 12th ESPHM conference, Bern, Switzerland
3. Hinney et al., 2020, *Cystoisospora suis* control in Europe is not always effective Front. Vet. Sci., 7 (2020), p. 113, 10.3389/fvets.2020.00113

Poster Number: 71
Topic Area: Reproduction
Title: Different combinations of cryopreservation extender and thawing solution for improving boar sperm viability
Author(s): Andre Andrade, University of Illinois at Urbana-Champaign; Rudolf Großfeld, Minitübe GmbH, Germany; Robert Knox, University of Illinois at Urbana-Champaign

Introduction
Cryodamage is severe in boar sperm cells due to their high cold shock sensitivity, resulting in low survivability during the freezing-thawing process (Passarelli et al., 2020). There is evidence that thawing extender can improve the morphofunctional characteristics of boar sperm (Knox et al., 2015). This study evaluated whether there were differences in the viability of cryopreserved sperm using combinations of two different freezing (Minitube Cryoguard – F1 or Androstar® CryoPlus – F2, Minitub GMBH, Germany) and thawing extenders (Minitube Cryoguard Thawing solution – T1 or Androstar® Plus – T2).

Methods
Eleven ejaculates collected from high fertility boars used in a weekly collection rotation, were diluted (1:1) and cooled before overnight shipping at 17 °C to the freezing lab. At the freezing lab, samples were evaluated and processed if motility was 75% or greater. Each ejaculate was split into the treatment cryopreservation extender F1 or F2. Samples were processed as previously reported, loaded into 0.5 mL straws, and frozen using a controlled rate freezer (IceCube 14s). Four straws from each treatment were thawed at 50 °C for 20 s and diluted in the treatment thawing extenders, T1 or T2. The combinations gave rise to four treatments (F1-T1, F1-T2, F2-T1, and F2-T2). Subsequently, 30 min after thawing, diluted samples were evaluated for motility kinetics using a computerized analysis system (CASA, HTM-IVOS, Version 12.3). The integrity of the plasma and acrosomal membranes were individually assessed by counting 200 sperm for each using a Zeiss Axio- CamHRc, at 600X following staining with Hoechst 33342, Propidium Iodide, and Pisum sativum agglutinin conjugated to fluorescein. The data were submitted for analysis of variance using PROC MIXED (SAS® software) for the 2 × 2 experimental design.

Results
Sperm parameters (mean ± SE) are presented in Table 1. There was no interaction between F × T (P>0.05) and therefore only main effects are presented. The Total (TMOT) and Progressive (PMOT) motility were greater (P≤0.05) in F1 compared with F2. Samples thawed in T1 exhibited greater (P≤0.05) TMOT and PMOT than T2. Sperm kinetics VAP, VCL, BCF, STR, LIN, and RAPID (data not shown) differed by the thawing extender used, but with no effects of F (P>0.05). Plasma membrane integrity (MI) was greater (P≤0.05) in F1 than F2; while AI is not affected (P>0.05). Thawing extender T2 improved (P≤0.05) MI and AI compared to T1. However, when considering both plasma and acrosomal membranes together, integrity was improved (P≤0.05) in F1 compared to F2. Thawing extender also affected AIMI, with T2 improving integrity compared to T1.

Table 1.

	Treatments					Probabilities		
	F1 - T1	**F1 - T2**	**F2 - T1**	**F2 - T2**	**Stderr**	**F**	**T**	**F × T**
TMOT	40.18^{aA}	30.36^{aB}	33.91^{aA}	24.64^{aB}	1.84	0.172	**0.008**	0.57
PMOT	26.82^{aA}	19.27^{aB}	21.18^{bA}	15.09^{bB}	1.36	0.015	**0.033**	0.18
AIMI	39.59^{aA}	47.14^{aB}	32.83^{bA}	41.92^{bB}	2.08	**0.009**	**0.008**	0.28
AI	72.93^{aA}	78.73^{aB}	76.46^{aA}	79.98^{aB}	1.32	0.088	**0.001**	0.79
MI	39.68^{aA}	47.32^{aB}	32.91^{bA}	42.13^{bB}	2.09	**0.009**	**0.009**	0.28

Lower case letters (a and b) different in the same line identify the difference between cryopreservation extenders (F). Capital letters (A and B) different in the same line identify the thawing extenders' difference (T). TMOT - Total spermatozoa with motility (%); PMOT - Spermatozoa with progressive motility (%), AIMI - Spermatozoa with simultaneously acrosome and plasma membrane integrity (%), AI - Spermatozoa with acrosome membrane integrity (%), and MI - Spermatozoa with plasma membrane integrity (%).

Conclusion

We conclude that different combinations of the cryopreservation extender with the thawing solution could improve sperm viability during the cryopreservation/thawing process.

References

Knox, R. V., Ringwelski, J.M., McNamara, K.A., Aardsma, M., Bojko, M., 2015. The effect of extender, method of thawing, and duration of storage on in vitro fertility measures of frozen-thawed boar sperm. Theriogenology 84, 407–412. https://doi.org/10.1016/j.theriogenology.2015.03.029

Passarelli, M. da S., Pinoti Pavaneli, A.P., Mouro Ravagnani, G., Pasini Martins, M., Pedrosa, A.C., Maria Massami Kitamura Martins, S., Rocha, N.R. de A., Bittar Rigo, V.H., Yasui, G., Yeste, M., de Andrade, A.F.C., 2020. Effects of different equilibration times at 5 °C on boar sperm cryotolerance. Anim. Reprod. Sci. 219. https://doi.org/10.1016/j.anireprosci.2020.106547

Poster Number: 72
Topic Area: Viral Disease
Title: PCV2d and contemporary PRRSV 1-7-4 coinfection model: pathology, virology, and PCVAD diagnosis
Author(s): Molly Kroeger, Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, IA, USA; Eduardo Fano, Boehringer-Ingelheim Animal Health Inc, USA; Kent J Schwartz, Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, IA, USA; Pablo E Piñeyro, Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, IA, USA

Porcine Circovirus Type 2 (PCV2) and Porcine Respiratory and Reproductive Syndrome Virus (PRRSV) are globally and economically important swine pathogens. The pathological impact of experimental PCV2/PRRSV coinfection of commonly circulating subtypes has not been reported under controlled, field conditions. Therefore, a PCV2d and a contemporary PRRSV isolate coinfection model under field conditions was developed to evaluate mortality, severity of histological lesions, IHC, tissue viral load (PCR), and viremia. Porcine Circovirus-Associated Disease (PCVAD) was defined by the presence of multisystemic clinical disease, and by gross and microscopic lesions including PCV2 antigen in lesions, lymphoid depletion and histiocytic inflammation.

Twenty weanling pigs were sourced from a PRRSV/Mhp negative, PCV2 low prevalence, commercial sow farm. Animals were vaccinated with Ingelvac PRRSV MLV at 21 days of age. On D0 (49 days-of-age), pigs received 1 mL IM and 1 mL IN of PCV2d (5 log10/2ml dose), and 2 mL IM of 1-7-4 PRRSV (4.0 TCID50/mL). Blood samples were collected weekly for four weeks and tested for PCV2 and PRRSV by PCR. Oral fluids were collected for IAV-S PCR on D0, 14, and 28. At necropsy on D28, gross lesions were scored and fresh and formalin-fixed tonsil, lung, and lymph node samples were collected. Tissues were assessed by histopathology, IHC, and PCV2 PCR. For pigs that died before D28, etiological and histological evaluation was performed.

Mortality was 60% before D28, with 12 deaths occurring D15-26. Tonsil and lymph node histopathology in deceased pigs (DP) had an average score maximum of 3 (severe inflammation and lymphoid depletion), while survived pigs (SP) averaged a score of 1 and 2 (moderate histiocytic inflammation and lymphoid depletion). In DP, lungs had severe and diffuse interstitial lesions, moderate and patchy suppurative bronchopneumonia, and peribronchiolar nonsuppurative cuffs with mild fibrosis; SP had moderate-to-extensive interstitial pneumonia. Lung and tonsil IHC of DP had an average score of 3 (>50% positive cells); SP had IHC lung and tonsil scores averaged 0 and 1 respectively (normal and <10% positive cells). PCV2 PCR performed on lung, lymph node, and tonsil of DP had average Ct values of 8.0, 9.4, and 8.2, respectively; for SP the PCV2 PCR Ct values were 14.0, 14.1, and 12.1, respectively. Average PCV2 Ct values in the serum of the DP were 24.9 on D7, 17.1 on D14, and 17.2 on D21. SP had averages of 30.8 on D7, 21.7 on D14, 26.3 on D21, and 26.2 on D28. Average Ct values for PRRSV in the lung were 16.8 for DP and 22.7 for SP. All oral fluids samples tested IAV-S positive on D0, 14, and 28.

Experimental coinfection by inoculation with contemporary PCV2d/PRRSV isolates resulted in high mortality and severe PCVAD. The roles of PRRSV, endemic IAV, and endemic potentially pathogenic bacteria greatly influence the outcome of PCV2d infection. Death was correlated to high PCV2d detection by IHC and PCR. SP had a higher serum Ct by day 7. However, survivors are likely to be stunted and more susceptible to ongoing insults, demonstrating the long-lasting consequences of PVC2d/PRRSV coinfection.

Poster Number: 73
Topic Area: Bacterial Disease
Title: Suilysin (SLY) is an indicator of Streptococcus suis derived capsule 1 pathogenesis only
Author(s): Paul Lawrence, Bimeda Biologicals

Introduction:
Among the systemic Streptococcus suis infections in pigs, serotypes 1, 2 and 1/2 are the most common types in the USA. These 3 serotypes account for at least 50% of the infections resulting in septicemia, arthritis, meningitis mostly in pigs 2-6 weeks. There are hundreds of virulence genes described in the literature. The most studied ones are suilysin (SLY), muramidase-released protein (MRP), and extracellular factor (EF). Capsular polysaccharides (cps) genes are critical for immune evasion; however, antibodies directed against CPS offer protective immunity. The correlation between other virulence genes and pathogenesis is sketchy at the best in the literature.

This study was done with the objective of understanding the degree of association of SLY, MRP and EF proteins among pathogenic and commensal serotypes 1, 2 and 1/2 isolated from clinically diseased pigs or obtained from nasal or lung swabs during necropsy. High throughput sequencing (HTS) was used to generate the whole genome nucleotide sequence data and amino acid sequence derived to obtain structural genes. Derived capsule (DC) was assigned based on curated genomic sequence data using specialized software.

Materials and Methods:
S. suis isolates were selected from the swine belt within the USA. The samples were carefully collected for systemic (brain, joints, meninges, heart surface) infection or commensal (nasal/lung swabs). The isolates were sequenced via iSeq 100 (illumina Inc) HTS system to generate at least 20x coverage for 150 bp pair reads. The sequence generated was compared with curated cps genes and structural protein for SLY, MRP and EF, (https://www.ncbi.nlm.nih.gov/).

Results:
Among the systemic DC1 isolates, 99% had functional SLY and 80-90% expressed MRP and EF. However, within the commensal DC1 types only 42% expressed SLY followed by MRP and EF. In systemic DC2 types, 60-70% expressed all 3 proteins. Only 18-50% commensal DC2 expressed all 3 genes. Among the DC 1/2 types, commensal express (60%) SLY more than systemic isolates (12%).

Conclusions:
Among the 3 derived capsule (DC) types SLY was the tightly associated with systemic DC1 followed by MRP and EF. There was no strong association of any of these genes among pathogenic or commensal DC 2 or 1/2 types.

Poster Number: 74
Topic Area: Bacterial Disease
Title: In-vivo tracheal fluids collection for Mycoplasma hyopneumoniae gilt exposure
Author(s): Clint Lichty, South West Ontario Veterinary Services; Cathy Templeton, South West Ontario Veterinary Services; Gillian Greaves, South West Ontario Veterinary Services; Courtney Werth, South West Ontario Veterinary Services; Guy Moser, South West Ontario Veterinary Services; Fabian Chamba, South West Ontario Veterinary Services

A farrow to wean producer in Ontario, Canada expanded from 600 to 1200 sows in early 2020. During that process, the herd became unstable for Mycoplasma hyopneumoniae (Mhp). Despite control efforts, respiratory problems were reported in nursery pigs. Euthanizing gilts to harvest lungs to prepare exposure material was not possible in this herd. We field piloted an in-vivo tracheal fluid collection method and evaluated whether it was effective for Mhp gilt exposure.

Methods
The sow herd, negative for PRRSV and IAV introduces replacement gilts four times per year. A group of approximately 160 gilts of 3 ages (2, 3, and 4-months) are purchased quarterly from a Mhp negative source. Gilts were vaccinated with RespiSure-One® two weeks prior to delivery and entered an offsite gilt development barn (GDU), where they showed no clinical signs of Mhp. When gilts were moved into the sow herd, they showed mild, but on-going, clinical signs of Mhp, despite treatment efforts. Five clinically infected untreated gilts that had entered the sow farm within 14 days were selected for in-vivo tracheal fluids collection. Each gilt was snared, and a flexible catheter was inserted into the trachea through a speculum where 5mL of phosphate buffer saline (PBS) was delivered. Immediately, as much fluid as possible was withdrawn from the gilt's trachea through the same catheter and a syringe[1]. The catheter was then flushed with 5mL of PBS to recover residual tracheal fluids. A tracheal fluid aliquot from each gilt was Mhp PCR tested. The total tracheal fluid collected from those 5 gilts (~35mL) was then diluted in 500mL of PBS to create the gilt exposure material. An aliquot of the exposure material was Mhp PCR tested. On the same day, exposure material was used to intra-tracheally (IT) inoculate ~half of the gilts in 10 pens (70/160 gilts) in the GDU. In each pen, ~half of gilts were randomly selected and inoculated with 5mL of the tracheal fluid exposure material. Finally, 3 weeks after IT inoculation, 21 gilts (2 gilts/pen) were randomly selected for laryngeal swabbing (LS) and Mhp PCR testing.

Results
Time to collect in-vivo tracheal fluids from 5 gilts was ~1 hour. The amount of collected fluid from each gilt was ~0.5 from 4 gilts and ~10mL for 1 gilt, without including the 5mL used to flush the catheter. Gilt tracheal fluid Mhp PCR Ct values ranged from 28 to 38 (median=33, n=5). The total volume collected from 5 gilts including the flushed material was ~35mL with a Mhp PCR Ct value of 33. Diluted exposure material (~35mL in ~450mL PBS) was not Mhp PCR tested. Exposed gilts in the GDU began to show mild Mhp clinical signs ~23 days post IT inoculation. Of the 21 LS samples, 16 (76%) tested Mhp PCR positive with Ct values ranging from 30 to 40 (median=36).

Conclusion
In-vivo tracheal fluid collection from gilts was feasible in a commercial sow barn where euthanizing gilts to harvest lung is not possible. Diluted tracheal fluid as Mhp gilt exposure material was effective to expose gilts in a commercial GDU.

References:
1 Pieters, M., Daniels, J. & Rovira, A. Comparison of sample types and diagnostic methods for in vivo detection of Mycoplasma hyopneumoniae during early stages of infection. Veterinary microbiology 203, 103-109, doi:10.1016/j.vetmic.2017.02.014 (2017).

Poster Number: 75
Topic Area: Bacterial Disease
Title: Actinobacillus suis: an emerging pathogen in finishing pigs in Brazil
Author(s): Lucas Dos Santos, Microvet Laboratory; Amanda GS Daniel, Microvet Laboratory; Geysa P. Paes, Microvet Laboratory; Manuella M. Granja, Microvet laboratory; Flaviane S. Coutinho, Microvet Laboratory; Carlos Eduardo R. Pereira, Universidade Federal de Vicosa; Daniel L. Santos, Microvet Laboratory; Walter Guimaraes, Microvet Laboratory; Jose Lucio Santos, Microvet Laboratory

Introduction
Actinobacillus suis is a Gram negative, facultative anaerobe and commensal bacteria of the upper respiratory tract of pigs. It is an important cause of pneumonia, septicemia and death in suckling and, recently, weaned piglets. Sudden death and disease resembling pleuropneumonia can also be attribute to A. suis. The gross lesions observed are pleuritis, pericarditis, multifocal abscesses like nodules can be observed mostly in the diaphragmatic lobes in the lung. Only a few studies and reports describe A. suis infection in growing pigs. In general, syndromes involving growing or finishing pigs have been recognized in high-health status herds. In the last years an increase in A. suis outbreaks in grow/finishing pigs was observe in our diagnostic laboratory in Brazil. The aim of this study was to quantify and demonstrate the increase of A. suis isolation in grow/finishing pigs in Brazil from January of 2017 to May of 2021.

Methods
A total of 630 isolates were included in this study. The isolates were obtained from diseased pigs from cases submitted to the Veterinary Diagnostic Laboratory (VDL) of the Microvet laboratory, Vicosa, Brazil during January 2017- May 2021.

Results
A total of 21 and 11 isolates of A. suis were identified in grow/finishing pigs in 2017 and 2018, respectively. A substantial increase in A. suis isolation in grow/finishing pigs was observed in the following years, with a total of 166 isolates in 2019, 277 isolates in 2020 and 161 during the first 5 months of 2021. Those findings demonstrate a substantial increase in isolations from A. suis from 2019 onwards. 63%, 62% and 83% of the confirmed cases were observed between 71 and >180 days of age in 2019, 2020 and 2021, respectively. Those percentages were much higher than the values obtained in 2017 (29%) and 2018 (20%).

Conclusion
The results from the past years demonstrate the increase of A. suis isolations in grow/finishing pigs in Brazil. Outbreaks occur predominantly in populations without previous exposure and timely production of antibodies. Some cases can occur in farms with high health status, low environmental contamination rate and consequently low titers of antibodies. Therefore, the perception of A. suis as a swine pathogen in grow/finishing pigs in Brazil must be changed. Vaccination of nursery pigs can be performed to maintain antibody titers of A. suis in the final stages and prevent the disease.

Poster Number: 76
Topic Area: Viral Disease
Title: Partitioning: A cost-effective approach for the pork industry to manage ASF risks
Author(s): Solenne Costard, EpiX Analytics; Francisco Zagmutt, EpiX Analytics; Andres Perez, University of Minnesota; Huybert Groenendaal, EpiX Analytics

As ASF continues to expand geographically, new strategies are needed to reduce disease spread and economic losses in affected areas. Depopulation is central to current ASF control programs, and its efficacy depends on effective surveillance and prompt disease reporting by producers. But depopulation is typically done at the farm level, resulting in large losses regardless of the producer's effort to detect and contain the disease early. An alternative approach is to perform partial depopulation (e.g., depopulate some, but not all units on the farm), which requires producer investment in biosecurity and on-farm surveillance for early detection and reporting. Such measures need to be cost-effective to benefit individual producers implementing them.

We argue that commercial swine farms can be partitioned in separate epidemiological units, to which biosecurity, surveillance and control can be applied. With such partitioning approach, individual producers can reduce the risk of ASF introduction and spread on a farm, in turn reducing ASF outbreak losses (culling and time to recovery) via partial depopulation. The partitioning framework relies on three main components:
1. Biosecurity guidelines to help producers prioritize external and internal biosecurity practices, to prevent ASF introduction into the farm, and to maintain separate epidemiological units within the farm
2. Guidelines and tools to help optimize on-farm ASF surveillance to enhance early detection while considering management and costs for the individual producer
3. Guidelines and tools for ASF response plans, including monitoring of units to demonstrate freedom of disease

Partitioning should be led and funded by industry, as the ASF risk reduction benefits are accrued mainly by individual producers, and its design should be tailored to fit individual farms. For its successful implementation, it requires regulators to recognize the notion of within-farm epidemiological unit and provide a regulatory framework for partial depopulation. This provides producers with regulatory clarity and certainty regarding their investment in the various components of partitioning.

By providing a regulatory framework for individual farmers to invest in ASF prevention and surveillance at the farm level, partitioning can be a useful public-private partnership (PPP) approach for ASF risk reduction. Partitioning can be implemented as a stand-alone program, or in complement to other efforts and programs, such as the US Swine Health Improvement Plan (SHIP) or the US Secure Pork Supply (SPS) programs. In addition, while partitioning is currently most applicable in ASF-affected regions, ASF-free areas could also benefit as it would reduce losses in case of a potential ASF introduction. As such, partitioning will result in the improved resilience and sustainability of the global pork industry and will benefit consumers and society through improved food security.

Poster Number: 77
Topic Area: Bacterial Disease
Title: Utilization of a fiber technology to agglutinate pathogenic bacteria associated with post weaning diarrhea in nursery pigs.
Author(s): Dr. Stacie Crowder, PMI; Kobe Lannoo, Agri-Improve; Dr. Dari Brown, PMI

Introduction
The economic losses associated with post weaning diarrhea are two-fold, with treatment costs and loss of animal performance1. This combined with the incidence of drug resistant bacteria increasing over the past few years has led researchers to seek out alternative approaches to support gut health. At weaning the gut is under stress from dietary changes as well as enteric challenges2. This stress leads to an increase in Gr-bacteria, which in turn increases the presence of endotoxins that can lead to inflammation of the intestines. Dietary fiber has been used to increase stool consistency and lower inflammation pressure in nursery pigs, however, this increased fiber takes up valuable space in the diet. Therefore, the objective of this experiment was to evaluate the effectiveness of a fiber technology to agglutinate pathogenic bacteria commonly associate with post weaning diarrhea in nursery pigs.

Materials and Methods
Agglutination was estimated by observing the fiber technology mixed with pathogenic bacteria under a microscope. While this assay did not provide quantification of the agglutination it provided visual representation of the clumping action indicative of agglutination. The next step in the agglutination assay was to quantify the results seen under the microscope with agglutination by filtration. Two identical samples each of E.Coli (K88), E.coli (F18), and Salmonella typhimurium were put into a 100ml solution and the fiber technology was added to one of the 100ml samples. Samples were mixed for 15 minutes and filtered through a Whatmann filter with a pore size of 4-7µm. Both filtrates were then plated and E.Coli colonies counted to determine amount recovered. E.Coli amounts were determined on the start culture, on filtered E.coli , and on the residue and filtrate of E.Coli + fiber technology, and calculated to the total CFU amounts. HEK-Blue™-4 cells were used to evaluate the anti-inflammatory properties of the fiber technology. HEK-Blue™-4 cells were maintained in a Dulbecco modified Eagle medium (DMEM) containing Quanti-Blue 1:10. NF-κB activation leads to the expression of a protein soluble embryonic alkaline phosphatase which colors a Quanti-Blue solution from pink to blue signaling a proinflammatory response.

Results and Conclusions
Results from this experiment demonstrate that a fiber technology can agglutinate 99% of the E.coli K88 and inhibit 78% of the agglutinated E.coli from growing when plated. Agglutination results with E.coli F18 and Salmonella typhimurium demonstrated 96.3% and 83.3%, respectively. LPS challenged HEK-Blue™-4 cells exhibited a 13.2-fold increase in the activation of NF-κB. Treating HEK-Blue™-4 cells with a fiber technology lowered the activation of NF-κB by 12 fold signaling the anti-inflammatory properties of the fiber technology. Further research is needed to determine the effectiveness of agglutination in an in-vivo challenge model as well as to determine the effects on animal performance.

Poster Number: 78
Topic Area: Production
Title: Optimal time interval between Improvest doses in gilts
Author(s): Marnie Mellencamp, Zoetis; Manuel Alexander Vasquez-Hidalgo, North Dakota State University; Larry Rueff, Progressive Sires; Steve Pollmann, DSP Consulting LLC; Deb Amodie, Zoetis

Introduction
Improvest® is approved in the U.S. for temporary estrus suppression in gilts. The benefits of estrus suppression include reduction in negative gilt behavior like mounting and inconsistent feed consumption. As a secondary effect of estrus suppression, Improvest has shown performance advantages in gilts, including increased live (+4.0 kg) and carcass weight (+3.2 kg)1. Improvest-treated gilt carcasses resemble carcasses from physically castrated barrows, with more uniform weight and thicker, heavier bellies1. The objective of this study was to determine the optimal time interval between the first and second Improvest doses to deliver the highest weight gain and best carcass quality. Our hypothesis was that a shorter time interval would be advantageous for weight gain.

Materials and Methods
Gilts (n=240) were allocated to 10 pens (24 gilts/pen) and 3 treatment groups (n=80): Improvest short interval (4 wk; administered at 16 and 20 wk of age), Improvest long interval (8 wk; administered at 12 and 20 wk of age) and control (no Improvest). All treatments were in each pen. Individual pig weights were obtained at the beginning and end of the project. Gilts were harvested 3- and 4-weeks after the second Improvest dose. At harvest, ovaries were scored (0-3) for reproductive maturity. Hot carcass weight (HCW), standard and primal carcass data, and meat quality measurements were collected. The PROC MIXED procedure of SAS 9.4 was used to analyze the results; treatment, harvest date and their interaction were the fixed effects and the residual error was the random effect.

Results
Examination of ovarian structures showed that both Improvest treatments were effective in hindering follicular development. Performance results showed that the short interval had greater ($P<0.0001$) final body weight compared to control and a tendency ($P=0.06$) to be higher than the long interval (135.77 vs 129.54 and 133.23 ± 0.93 kg, respectively). Short and long intervals had greater ($P<0.001$) ADG compared to control (0.90 and 0.88 vs 0.85 ± 0.005 kg/day, respectively). HCW showed an interaction ($P=0.0285$) between treatment and harvest date, with the short interval having the heaviest HCW on harvest day #2 (103.2 ± 1.32 kg), and control showing the lightest HCW on harvest day #1 (95.6 ± 1.32 kg). Short and long interval gilt carcasses had greater ($P=0.0011$) backfat compared with control (1.78 and 1.80 vs 1.65 cm, respectively). The long interval had the greatest ($P=0.003$) visual marbling, with control being intermediate and short showing the lowest marbling (2.59, 2.39 and 2.21 ± 0.07, respectively). Belly weights from short and long Improvest gilts were numerically ($P=0.27$) heavier than controls (7.02 vs. 7.01 vs. 6.8 ± 0.13 kg, respectively).

Conclusions
Estrus suppression by both Improvest treatments was associated with increased final body weight, ADG and HCW compared to control gilts. The short interval resulted in heavier HCW compared to the long interval (103.22 vs 97.84 kg at harvest #2). These results show strategic management of estrus by using Improvest offers the opportunity to increase return on investment while delivering heavier, more uniform gilts to the packer.

Poster Number: 79
Topic Area: Feed/Nutrition
Title: Mycotoxin Contamination in US Corn and Corn By-product
Author(s): Lan Zheng, BIOMIN America, Inc.; Maryn Ptaschinski, BIOMIN America, Inc.; Shelby Ramirez, BIOMIN America, Inc.; Ursula Hofstetter -Schähs, BIOMIN Holding GmbH, Getzersdorf, Austria; G. R. Murugesan, BIOMIN America, Inc.; BIOMIN Holding GmbH, Getzersdorf, Austria

Mycotoxins are harmful secondary fungal metabolites that may possess detrimental impacts on animal health. The classic signs of mycotoxicosis include reduced feed intake, vomiting, impaired growth, and oral and intestinal lesions; however, impacts of mycotoxins on immune dysfunction, inflammation, and modulation of the gastrointestinal environment are often underestimated. Mycotoxins such as deoxynivalenol (DON) and fumonisins predispose animals to infectious diseases, while zearalenone contributes to poor reproductive performance and sow pelvic organ prolapse. Additionally, the immunosuppressive effects of mycotoxins can compromise vaccine efficacy, potentially contributing to vaccine failure. The objective of this study was to determine the effect of harvest year on five major mycotoxin groups: aflatoxins (Afla), type A trichothecenes (A-Trich), type B trichothecenes (B-Trich; including DON), FUM, and ZEN in corn and corn by-product samples. For each mycotoxin group within ingredient, the 2019 harvest (329 corn and 54 corn by-product) were compared with the 2020 harvest (280 corn and 92 corn by-product). Samples were analyzed utilizing Liquid chromatography tandem mass spectrometry (LC-MS/MS). Average B-Trich contamination level in corn is significantly ($P<0.05$) affected by harvest year; however, the level remained consistent over the last 3 years. Contamination levels in corn for Afla, A-Trich, FUM, and ZEN have remained consistent over the last five years. In corn by-product, B-Trich and ZEN contamination have been significantly ($P<0.05$) affected by harvest year. The B-Trich and ZEN contamination levels were decreased ($P<0.05$) from 2019 to 2020, whereas FUM contamination level remained similar. The 2020 crop risk profile is likely to change as the sample pool expands. A combination of hot weather, storm events, and drought during the 2020 growing season resulted in crop stress and damage, ultimately leading to grain quality and mycotoxin contamination concerns. Due to the continued risk of mycotoxin co-occurrence, expanding mitigation strategies beyond adsorption by mycotoxin deactivation with biotransformation process and support of immune and liver function is essential.

Poster Number: 80
Topic Area: Viral Disease
Title: In-use stability of PRRS MLVs when mixed with PCV2 vaccines
Author(s): Oliver Gomez-Duran, Boehringer-Ingelheim Animal Health; W.D. Strachan, Boehringer-Ingelheim Animal Health

Introduction
To reduce labour requirements on farms and the number of injections administered to pigs, combinations of vaccines are increasingly attractive. Combinations of PCV2, Mhp and PRRS, can address critical diseases to successful pig production. To ensure these vaccine combinations are efficacious, in-use stability of the PRRS MLV component and lack of interference between the vaccines must be demonstrated. PRRS Modified Live virus (MLV) vaccines are lyophilised and require reconstitution prior to injection. The objective of these studies was to determine the in-use stability over 4 hours of PRRS MLVs reconstituted with different vaccines.

Materials and Methods
Combinations (all Boehringer-Ingelheim Vetmedica unless indicated) tested were: A) Ingelvac CircoFLEX® and Ingelvac PRRSFLEX® EU, B) Ingelvac CircoFLEX® and Ingelvac PRRS MLV, C) Ingelvac PRRS MLV and Reprocyc ParvoFLEX D) FLEXcombo and Ingelvac PRRSFLEX® EU, E) Porcilis PCVMhyo and Porcilis PRRS (MSD Animal Health) F) Suvaxyn CircoMH and Suvaxyn PRRS MLV (Zoetis). After reconstituting the PRRS MLV vaccines with diluent or combination vaccine, the mixtures were stored at room temperature (15-25°C) for a maximum of 4 hours. Tests for potency (virus titration) were performed in triplicate at 0, 2, and 4 hours after reconstitution using 4 -fold dilutions according to SOP at an accredited laboratory. For all products, commercial release batches were used and the diluents for reconstitution of each PRRS MLV vaccine were used according to manufacturers' instructions and used as controls. Results were reported as the mean log10 TCID50/ml +/- SD. The change over time and differences between diluent and combination were determined.

Results
For combinations A to D, the mean titre of the mixed samples over 4 hours was not, or very slightly, reduced (< 0.4 log10 TCID50/ml) and remained at all times above the minimum release titre for the PRRS MLV vaccine. The viral titres of the combination and the diluent (PBS) were very similar during the evaluated time points.
Combination E exhibited a 1 log reduction at time 0 and no viable PRRS vaccine virus was titrated at 2h and 4h, while combination F showed approximately 1.13 log titre reduction after mixing with combination vs PBS diluent and 0.7 log10 by the end of the experiment.

Conclusions
Under in vitro conditions, in-use stability determination of PRRS MLV (both PRRS FLEX EU and Ingelvac PRRS MLV) mixed with Ingelvac CircoFLEX, FLEXcombo or Reprocyc ParvoFLEX have shown no negative effect on virus titre during a 4-hour period. These results conform with guidelines for stability and in-use testing for vaccines [1,2]. The combinations A, B and C have completed lack of interference studies (data not shown) and are licensed combinations available to veterinarians and producers. Other off-label mixes would result in reduced potency of the PRRS MLV even if used within the 4-hour period.

References
1. VICH Guideline GL17 Stability testing of new Biotechnological/Biological Veterinary Medicinal Products
2. EMEA/CVMP/IWP/250147/2008. Guideline on data requirements to support in-use claims for veterinary vaccines.

Poster Number: 81
Topic Area: Viral Disease
Title: THE EFFECT OF BETA-1, 3 GLUCANS (ALETA™) ON SWINE INFLUENZA VACCINE SEROLOGY IN BREEDING GILTS
Author(s): Clayton Johnson, Carthage Veterinary Service, LTD; Gustavo Silva, Iowa State University; Tom Marsteller, Stellar Swine Health; Marc White, Kemin Industries, Inc.; Vanessa Iseri, Kemin Industries, Inc.

Introduction and Objective: Aleta™ is a bioactive source of greater than 50% beta-1,3 glucans, which can be utilized to help animals minimize the impact of disease challenges by priming the immune system. The beta-1,3 glucans in Aleta are provided as bioactive granules and shown to be readily taken up by macrophages in vitro and shown to enhance innate immunity in vivo (Kemin internal data). Aleta activated T cells, reduced gut inflammation, improved gut barrier function and boosted immune response in a swine E. coli challenge model.[1] In addition, Aleta has been shown to positively impact growth, Feed:Gain and morbidity in PRRSv-positive weaned piglets administered in-feed antibiotics.[2] The objective of this study was to evaluate the efficacy of Aleta in gilt development diets to improve the serologic response to a swine influenza killed vaccine (SIV) with four influenza isolates (Isolate 1, 2, 3 and 4) for a 30-day (d) period.

Materials and Methods: Sixty 6-month-old breeding gilts in eight rooms which were housed along with other gilts were randomly assigned to 1 of 2 treatments (8 rooms/treatment; 800 pigs/room): 1. Negative Control feed, SIV vaccinated; 2. Aleta feed - 250 ppm Aleta in feed, SIV vaccinated. On d0 (pre-vaccination), 60 gilts were bled per treatment (15 gilts per room; 4 rooms/treatment). On d30 (post-vaccination), the same animals were bled to establish a before and after serological profile. Since only 46 animals remained in the 8 rooms on d30 due to gilt selection rates at the breeding farm, d0 and d30 serum antibody titers were performed only on these 46 gilt samples and the change in titers between the two samples were evaluated. Serum antibody titers were evaluated by the University of Minnesota Veterinary Diagnostic Laboratory by using the HI antibody test. Statistical analyses were performed using JMP® (SAS Institute, Cary, NC) software and significance was determined at $P \leq 0.05$.

Results: Administration of vaccine lead to an increase in serum antibody titers for all four isolates compared to pre-vaccination. Adding Aleta significantly improved Isolate 1 serum antibody titers at d30 compared to the negative control at d30 ($P<0.01$; 326 vs. 158). There was a trend for Aleta improving Isolate 2 antibody titers compared to negative control at d30 ($P=0.07$; 80 vs. 52). There was no effect of Aleta on Isolate 3 and 4 titers ($P>0.05$). Further, there was an association of Aleta on improving the percentage of animals of different titer category (high, medium, low) post-vaccination for Isolate 2 and 4 (Chi-square test $P=0.02$ and $P=0.007$).

Conclusions and Discussion: Feeding Aleta during SIV vaccination demonstrated an ability to increase the antibody titer response for different isolates, which warrants further research. Overall, these results provide evidence that Aleta was able to improve serum antibody titers in breeding gilts vaccinated with four SIV isolates.

Keywords: Aleta, nursery pigs, health, growth, 1,3-beta glucans, immune stimulation

References
1. Kim K. et al, 2019 Algae-derived β-glucan enhanced gut health and immune responses of weaned pigs experimentally infected with a pathogenic E. coli.
2. Marsteller, Tom. et al, 2019. Comparison of Aleta™ with and without in-feed antibiotics for growth performance, morbidity, and Full Value Pigs™ in PRRSV-positive nursery pigs. Abstract presented at Allen D. Leman Swine Conference.

Poster Number: 82
Topic Area: Feed/Nutrition
Title: The Use of Activated Medium Chain Fatty Acids to Address Disease Risks Associated with Swine Feed
Author(s): Dr. Stacie Crowder, PMI; Dr. Scott Dee, Pipestone Applied Research; Dr. Dari Brown, PMI; Dan Hanson, Pipestone Applied Research; Jenna Schuld, Pipestone Applied Research

Introduction

Operational Biosecurity has been the focus of the swine industry for many years to address disease challenges. The operational biosecurity protocols are aimed at the movement of people, supplies, equipment, and the newest area of concern is feed. The potential for virus transmission in feed has prompted the search for natural mitigation products. The objective of this study was to evaluate the efficacy of two MCFA products from PMI: Vitacy FL-1 and Vitacy FL-2 for reducing the risk of infection following consumption of feed contaminated with PRRSV 174, PEDV and SVA using a novel, highly robust challenge model, employing controlled field conditions and multiple metrics.

Materials and Methods

In a challenge study, 146 pigs (44 lb. body weight; 46 pigs/treatment) were used to evaluate the efficacy of Vitacy FL-1 and Vitacy FL-2 as a feed biosecurity tool. Complete feed was mixed to either contain no additive as Control, or to contain 0.25% Vitacy FL-1 and 0.125% Vitacy FL-2. Viral challenge was delivered via a 1.0 lb. ice block, consisting of 100 mL SVA (5 logs TCID50/mL, Ct = 20.72), 100 mL PRRSV 174 (5 logs TCID50/mL, Ct = 21.38), 100 mL PEDV (5 logs TCID50/mL, Ct = 24.25) and balanced with 154 mL MEM. Blocks were frozen at -80C and placed into each feed bin on days 0 and 6 of the study on top of the feed. The blocks proceeded to melt, with liquid permeating the feed, which was then augured into room for pigs to consume via natural feeding behavior. Inoculated feed was delivered by auger to pens for 15 days. The percentage of infected pigs was determined by viral recovery using tonsil tissue for SVA, serum samples for PRRSV, and rectal swabs for PEDV (n = 30 pigs/treatment), and disease was determined by observation of clinical signs (n = 6 pens/treatment).

Results

Challenge study results demonstrated 100% of control-fed pigs tested were positive for PRRSV, 70% were positive for PEDV, and 20% were positive for Seneca Valley A compared to 0% for PRRSV, 0% for PEDV, and 0% for Seneca Valley A in both PMI Vitacy FL-1 and Vitacy FL-2-fed pigs. Additionally, providing activated MCFA in the feed reduced the incidence of clinical signs for each virus when compared to control-fed pigs. Finally, the overall average daily gain was higher in challenged pigs that were provided diets containing MCFA compared to control-fed pigs.

Conclusions

Feed in the bins was successfully contaminated using the ice block method, resulting in delivery of all 3 viruses to all rooms. Transmission of PRRSV, PEDV and SVA via the feed was documented in the control group. These data support the risk of contaminated feed as a means of viral entry to farms. Pigs on both PMI products remained free of infection with PRRSV, PEDV, and SVA, and performed significantly better than controls, with no mortality observed in either group. Activated medium chain fatty acids (MCFA) are a tool that can be used for feed biosecurity.

Poster Number: 83
Topic Area: Bacterial Disease
Title: Mycoplasma hyopneumoniae aerosol challenge evaluation in finishing pigs
Author(s): Gillian Greaves, South West Ontario Veterinary Services; Fabian O. Chamba Pardo, South West Ontario Veterinary Services; Clint Lichty, South West Ontario Veterinary Services; Guy Moser, South West Ontario Veterinary Services; Courtney Werth, South West Ontario Veterinary Services

Introduction:
Aerosol Mycoplasma hyopneumoniae (Mhp) exposure methods have been described for gilt acclimation[1], but scarce studies are available in finishing pigs. The feasibility of Mhp aerosol challenge was evaluated in finishing pigs for Mhp vaccines or antibiotic comparisons in commercial settings.

Methods:
In 4 studies, pigs were confirmed naïve/negative for Mhp by ELISA (serum) and PCR (laryngeal swabs, LS) testing at entry. The aerosol Mhp challenge method consisted of fogging Mhp positive lung homogenate (Ct range 24 to 28) for 30-mins using two hurricane foggers simultaneously and 1-2-hours of reduced ventilation, in a 400-head (13 pen) commercial finishing site. Pigs were challenged ~13 weeks (3-weeks after entry, study 1) and ~10 weeks of age (immediately after entry, studies 2, 3, 4). Natural (study 1) or inoculated (study 4) PRRSV co-infection was also evaluated. Studies 2 and 3 had only Mhp challenge. In studies 3 and 4, pigs were also Mhp challenged intra-tracheally (25% pigs in study 3, 50% pigs in study 4, Ct range 28-34). Challenge was confirmed by Mhp PCR detection in LS 2-3 weeks post-challenge, lung lesions, and daily barn-level cough indices. Lung lesions were confirmed (Goodwin method)[2] at 7-weeks post-aerosol challenge/entry (3-weeks post-intra-tracheal challenge) in study 3, and 3-weeks post-challenge/entry in study 4. Lesions were not assessed in studies 1 and 2. In all studies, a daily barn-level cough index (CI) was estimated by counting: number of coughing bouts (CC), number of pigs assessed (n) and duration of assessment (t) (CI=(CC/n*t)*100). Studies 2, 3 and 4 included control pigs. Production and economic indicators were evaluated in 2 Mhp antibiotic comparisons (studies 1 and 3) and 1 Mhp vaccine comparison (study 2).

Results:
Mhp was PCR detected in LS at 2-3 weeks post-challenge in study 1 (n=24, Ct range 25 to 39), study 2 (n=59, Ct range 29 to 40) and study 3 (n=8 pools of 3, Ct range 35 to 40). LS were not collected in study 4. Lung lesions in study 3 (n=7) ranged from 0.3 to 31 (median 11/55 max. score), and in study 4 (n=5) ranged from 12 to 43 (median 29/55). In study 4, lung tissues and bronchial swabs (5 pigs) were PRRSV and Mhp PCR positive 3 weeks after challenge/entry. Study and time adjusted mean±CI95 daily barn-level cough index was higher (0.4±0.08 vs 1.3±0.1, p<.01) and increased a week earlier (2 vs 3-weeks after challenge) with PRRSV co-infection. Mortality was higher in PRRSV co-infected pigs: 3.7% (28/763) vs 8.3% (29/350), p<.01. Start weight (30kg), end weight (108kg), pen, block, treatment, and study adjusted mean±CI95 feed conversion ratio (2.38±0.17 vs 3.04±0.47, p=0.04), live weight (kg)/ton feed (586±22 vs 537±58, p=0.21) and gross margin (CAD)/ton feed (679±38 vs 592±104, p=0.21) were lower with PRRSV co-infection. Controls performed lower than vaccinates (study 2) and slightly lower than treated pigs in study 3.

Conclusion:
An aerosol Mhp challenge in finishing pigs provides sufficient infection and mimics field conditions. However, it seems to lack clinical severity as recently described [3,4]. PRRSV co-challenge increased clinical and production severity.

References:
1 Robbins, R. C., Betlach, A. M., Mondragon-Evans, M. R. & Pieters, M. Development of a herd-specific lung homogenate for exposure to Mycoplasma hyopneumoniae under field conditions. J Swine Health Prod 27, 221-227 (2019).
2 Garcia-Morante, B. et al. Assessment of Mycoplasma hyopneumoniae-induced pneumonia using different lung lesion scoring systems: a comparative review. Journal of comparative pathology 154, 125-134 (2016).
3 Garcia-Morante, B. et al. Induction of mycoplasmal pneumonia in experimentally infected pigs by means of different inoculation routes. Veterinary research 47, 54, doi:10.1186/s13567-016-0340-2 (2016).
4 Silva, A. P. S. P. et al. Comparison of Mycoplasma hyopneumoniae response to infection by route of exposure. Veterinary microbiology, 109118 (2021).

Poster Number: 84
Topic Area: Viral Disease
Title: Influenza neuraminidase virus-like particle vaccine is highly immunogenic and protects pigs against heterologous challenge
Author(s): Vasilis Pliasas, Auburn University; Zach Menne, Emory University; Virginia Aida, Auburn University; Ji-Hang Yin, Auburn University; Maria C. Naskou, Auburn University; Peter J. Neasham, Auburn University; J. Fletcher North, Auburn University; Katharine Horzmann, Auburn University; Ioanna Skountzou, Emory University; Constantinos S. Kyriakis, Auburn University

Introduction: Swine influenza (swIAV) is one of the most important diseases of the pork industry, with outbreaks of swIAV infections resulting to significant economic losses. Influenza vaccines in pigs mainly target the hemagglutinin (protein) and often fail to induce protection against antigenically drifted strains. The neuraminidase (NA) glycoprotein is the second most immunogenic influenza virus protein and has been shown to elicit broad immune responses against heterologous strains. The purpose of this study was the evaluation of the immunogenicity and protective efficacy elicited by a recently developed N2 VLP vaccine, comprised of the NA protein from the A/Perth/16/2009 (H3N2) strain in pigs, as a candidate for a broadly protective vaccine.

Methods: A total of 18 influenza-seronegative piglets were used in the study, divided into 3 groups of six and were prime-boost vaccinated with a 3 week interval with (a) the N2 VLP experimental vaccine combined with a water-in-oil-in-water adjuvant, (b) a commercial quadrivalent whole virus inactivated swIAV vaccine or (c) adjuvant alone. Four week after boost vaccination, pigs were intranasally challenged with A/sw/NC/KH1552516/2016, an H3N2 swIAV field isolate. Amino acid homology between the vaccine and challenge NA was 90.9%. Multiple serological assays were performed to assess HA or/and NA specific immunological protection, including NA ELISA, NA-star Neuraminidase Inhibition, Hemagglutination Inhibition and Virus Neutralization assays. After challenge, nasal swabs were collected daily and bronchoalveolar lavage fluid (BALF) and tissue samples from the entire respiratory tract (nasal turbinates, trachea and pulmonary tissues) were harvested at euthanasia (day 5). Vaccine-induced protection was assessed based on five parameters, (i) cellular immune responses, (ii) cytokine profile at euthanasia (day 5), (iii) virus titers in nasal swabs and tissue homogenate samples, (iv) Bronchoalveolar lavage fluid (BALF) cytology and (v) respiratory tract histopathology.

Results: The NA VLP vaccine, was highly immunogenic in pigs and significantly reduced pulmonary virus titers, BALF neutrophilic infiltration and lung tissue histopathology compared to unvaccinated controls.

Conclusions: Overall, our results suggest that while neither the commercial nor the NA VLP vaccine elicited sterilizing protection, the NA VLP construct performed comparably to the commercial swIAV vaccine, inducing a robust heterologous protection.

Poster Number: 85
Topic Area: Viral Disease
Title: A double intervention to stabilize Porcine Reproductive and Respiratory Syndrome - PRRS in a one site farm with continuous flow
Author(s): Mishelle Moral, Trouw Nutrition Latam; Juan Peralvo Vidal, Trouw Nutrition Latam; Eduardo Rosa Medina, University of Minnesota; Jorge Campos Loyola, Universidad Cientifica del Sur; Marco Coral Almeida, Universidad de las Americas; Jorge Garrido Mantilla, University of Minnesota

Introduction
Porcine Reproductive and Respiratory Syndrome (PRRS) is the most relevant infectious disease in the swine industry. PRRS virus (PRRSv) live inoculation has been used to stabilize and even eliminate PRRS in positively confirmed farms. Batch farrowing is a production system used to maximize productivity and could be used it to interrupt disease transmission in susceptible populations. This report captures the stabilization of a one site farm with a continuous production flow by virus inoculation and implementation of batch farrowing management.

Methods
In 2018, a commercial farm (200 sows per year) experienced reproductive and respiratory problems in both sows and growing finisher pigs (PRRS positive by ELISA). Serum samples were randomly collected from 35 days old piglets with acute clinical signs to confirm PRRS infection by ELISA. PRRS positive serum was used to amplify the PRRSv in 21 days old piglets, these pigs were euthanized and bled to harvest serum to be used as PRRSv inoculum. 1 ml of serum titrated to 103 PRRSv /ml was inoculated by muscular injection the whole herd. Simultaneously, a batch farrowing system was implemented in groups of three. The pig production flow was moved into another barn within the same farm emptying the nursery area. All-in all-out, washing and disinfection processes were implemented along with McREBEL1 procedures in all farrowing rooms to avoid transmission. Herd closure for 40 weeks was stablished. PRRSv surveillance was implemented throughout and after the herd closure following the AASV recommendations. Differences in production parameters between periods were assessed using paired samples t-test, repeated measures ANOVA and post-hoc Tukey test, significance level was established at $p<0.01$.

Results and Conclusions
PRRSv circulation was present before intervention. After PRRSv inoculation compatible clinical signs with infection were observed, including 12.2% abortion. Three batch farrowing groups were successfully implemented with 15 sows / group. Summary of the production results is shown in table 1 and summary statistics is presented in table 2 and 3. Farrowing rate decreased from 85.63% to 74.43% ($p<0.01$) during the PRRS outbreak, after the intervention the farrowing rate increased to 87.6% ($p<0.01$). After intervention, the abortion rate decreased from 25.57% to 3.08% ($p<0.01$), this recovery reached similar levels than before the outbreak. In addition, total number of piglets born alive increased from 8.89 to 10.7 ($p<0.01$), this increment was higher than before the outbreak; wean to finish mortality rate decreased from 55% to 5.11% ($p<0.01$); ADG increased from 726 g to 897 g ($p<0.01$). Finally, PRRSv PCR tests were consistently negative in four consecutive sampling processes after PRRSv inoculum.

A significant production improvement was obtained after the implementation of live PRRSv inoculation and the implementation of the batch farrowing system. Moreover, after 40 weeks of herd closure, the PRRSv circulation was eliminated. Immunity development by inoculation and interruption of PRRSv transmission by modification of epidemiological subpopulation might be the main reasons to obtain these results. Altogether, this report shows that virulent PRRSv infection can be controlled in a one site farm with a continuous production flow.

Poster Number: 86
Topic Area: Other
Title: Evaluating the uses of geofencing to characterize networks of swine facilities within production systems under field conditions
Author(s): Nicholas Black, The Ohio State University; Ting-Yu Cheng, The Ohio State University; Andreia Arruda, The Ohio State University

Introduction
The swine industry has evolved over the past decades, and is now dominated by vertically integrated multisite production systems designed for large-scale production[1]. However, this has also facilitated the spread of swine diseases within and between production sites. Thus, biosecurity has been an integral part of preventing the spread of diseases in swine production systems. Technological applications, e.g., geofencing, could be of assistance by allowing producers and veterinarians to monitor biosecurity breaches related to employee movements between swine facilities. The objectives of this study were: (1) to validate a geofencing platform under field conditions in a multi-site production system; (2) to describe the social network of movements between sites and investigate site connectivity patterns according to employees' roles; and (3) to identify and quantify movements between sites that are potential breaches to standard "nights down" biosecurity protocol.

Methods
Two large multisite production systems containing over 200 swine sites were recruited. System 1 and 2 were located in Ohio and Iowa, respectively. A mobile application-based geofencing technology platform[2] using GPS coordinates to establish virtual barriers around individual sites was implemented in both systems. Animal health-related personnel (veterinarians, caretakers, etc.) and industry service providers (e.g., truck drivers, maintenance, etc.) were asked to install the application on either their personal or a supplied mobile device. Site entries were recorded when an employee crossed the virtual geofence barriers with an application-installed device. Data validation was carried out by comparing physical entry logs maintained by a subset of employees from System 1 for a duration ranging from 1-6 weeks and digitally recorded logs from the geofencing platform captured during the same period. This provided a proportion of the observations in which the geofencing technology was correct as compared to the written logs, which was used as a "gold standard" (currently used by most swine production systems). Within System 2, site entries were recorded prospectively for one month. Consecutive site entries from an individual within a single day were used to create direct movements between sites. A multiplex network of indirect site connections was constructed and comprised of multilayers corresponding to categories of employee role. Additionally, movements within System 2 were investigated for potential beaches to the company's standard operating nights down protocol.

Results
Out of a total of 398 manually recorded site entries from System 1, 379 (95.23%) were also digitally captured by the geofencing platform. Results from the social network analysis were implicative of the importance of employees within administrative and support services roles with respect to increasing the indirect connections between different production sites. Furthermore, 1861 total site connections were established by connecting consecutive site entries of an individual employee for the duration of the study. Of these, 12 (0.6%) were identified as potential breaches using the system's downtime guidelines. Employees of the department of communication and information contributed 9 of the 12 (75%) identified breaches, implying their roles in connecting different production sites pose risks of disease spread.

Conclusion
This study demonstrated the potential benefits using geofencing technology and its application to vertically integrated production systems.

References:
[1] McBride, W.D., Key, N., 2013. U.S. Hog Production From 1992 to 2009: Technology, Restructuring, and Productivity Growth (Economic Research Report No. 158). Economic Research Service, United States Department of Agriculture.
[2] Barn360: Production Management Platform, 2020.. BarnTools. URL https://barntools.com/barn360 (accessed 7.7.21).

Poster Number: 87
Topic Area: Production
Title: Turnover events of animal caretakers and its impact on productivity in swine farms
Author(s): Nicholas Black, The Ohio State University; Andreia Arruda, The Ohio State University

Introduction
Attracting and retaining quality animal caretaking personnel is one of most pressing issues the US swine industry currently faces. Turnover rates among animal caretakers have been reported to be between 20 and 35%, depending on farm size and geographical region[1]. On-farm employee turnover can be costly and may have an impact on productivity due to general work disturbances and subsequent hiring and training needs. The primary objectives of this study were to describe the amount of animal caretaker turnover events that occurred in a single year in eleven swine farms, and to investigate associations between employee turnover events and two subsequent production parameters of interest: number of pigs weaned per sow (PWS) and pre-weaning mortality (PWM).

Methods
A retrospective cohort study was conducted with eleven commercial farrow-to-wean swine farms belonging to two vertically integrated multi-site production systems within two swine production companies in the state of Ohio. Human resources and production data for the year of 2019 were obtained monthly from each farm. The primary predictor of interest was the occurrence of an employee turnover event, defined as 'voluntary' (employee decided to leave or quit) or 'involuntary' (employee was terminated by company decision). The primary outcomes of interest included the monthly average PWS and PWM. These associations were assessed with 1-, 2-, 3-, and 6- months between the turnover events and the outcome. Linear mixed effects models were fit in STATA 15, with system and farm included as random effects. To account for temporal and seasonal trends of production, season and the monthly production were included in the models.

Results
There were a total of 152 turnover events, with 4 and 148 turnover events in systems 1 and 2, respectively. The average turnover, calculated as a percent of total turnover among full time employee positions, was 92 % (SD = 62 %; Range = 8–217 %). Improvements in both productivity measures (increase of 3 pigs weaned per every 4 sows ($p = 0.01$) and decrease of 1.15% in monthly PWM ($p = 0.02$)) were significantly associated with the occurrence of an involuntary turnover event 2-months prior, after controlling for season, previous month production, farm, and system. For the PWS outcome, there was a significant interaction between an involuntary turnover event two months prior and monthly county-level unemployment rate ($p = 0.02$), indicative of the improved performance being most profound at the lowest levels of unemployment rate and diminishing at the highest levels. This association could reflect the effect that the local labor market has on the farm's ability to find a quality replacement following the termination of former employees.

Conclusion
Turnover of animal caretaking personnel in farrow-to-wean farms was confirmed to be highly variable and high for the majority of farms in this study. Furthermore, animal caretaker turnover was associated with subsequent trends of production efficiency, warranting closer consideration of prioritizing managerial efforts in worker recruitment, training and retention.

References:
[1] Boessen, C., Artz, G., Schulz, L., 2018. A baseline study of labor issues and trends in U.S. pork production. National Pork Producers Council, Urbandale, IA, USA.

Poster Number: 88
Topic Area: Viral Disease
Title: Enhanced Passive Surveillance for African swine fever and Classical swine fever
Author(s): Daniella Schettino, University of Minnesota; Gustavo Lopez, University of Minnesota; Oriana Beemer, CEAH/USDA/APHIS; Marta Remmenga, CEAH/USDA/APHIS; Karyn Havas, Cornell University and Pipestone Veterinary Services; Jonathan Arzt, FADRU/ARS/USDA/ Plum Island; Andres Perez, University of Minnesota

African swine fever (ASF) and Classical swine fever (CSF) are viral diseases of pigs (*Suidae* family) with an important impact on trade. Because the far-reaching losses that ASF and CSF epidemics cause, disease-free areas are interested in designing and implementing systems for early detection of and reaction to hypothetical disease incursions, allowing for the reestablishment of the free status. Enhanced passive surveillance (EPS) protocols may serve as an awareness tool aiding pig producers and practitioners to quantify and balance procedures and signs at the farm regarding risk of introduction of these hemorrhagic fevers of swine. We propose here an EPS protocol for ASF, and CSF based on three basic components, namely, (1) pig farm-level risk factors (biosecurity background), (2) syndromic surveillance data, and (3) necropsy findings. Whereas the first two components of the surveillance system are conducted at the swine system and farm level, the third one is primarily led by veterinary diagnostic laboratories (VDLs). Each component at the farm will generate a score, associated with risk for an ASF or CSF outbreak, ranging from 0 (lowest) to 10 (highest). We calibrated our protocol with scores according to ASF/CSF experts. Combinations of components are added leading to a final score that the risk for an ASF/CSF outbreak in the farm. The use and application of the system were tested comparing the scores that would be estimated for alternative situations. For example, (1) a sow farm located in an area with feral pigs would be considered at 18 times higher risk of being an ASF/CSF case compared to a sow farm in an area without feral pigs; (2) for two commercial pig farms with increased mortality rate, and showing signs of splenomegaly, if one of them did not implement biosecurity and surveillance protocols, the risk for an ASF/CSF would almost double the risk of the farm with biosecurity in place and performing daily syndromic surveillance. The proposed protocol may be implemented as part of a dynamic process with frequent updates performed by pig producers and private veterinarians with an active collection of information that will feed a passive surveillance model, and these adjustments will work as an indicative of improvement or maintenance of actions at the farm-level. We expected that this system will help pig producers and practitioners perform an active collection of information that will feed our model of surveillance, and the outputs of this model will bring a categorization of risk of introduction of ASF, or CSF based on these three components. The system will also help the official veterinary services to target surveillance activities at high-risk farms detected by the model. Additionally, this scoring system may also help inform the definition of cases in areas with outbreaks or the vulnerability of swine farms in preparation for planning response to epidemics.

Poster Number: 89
Topic Area: Bacterial Disease
Title: Development of antigen based diagnostic ELISA for Mycoplasma hyopneumoniae
Author(s): Rachel Bradley, University of Minnesota; Venkatramana D. Krishna, University of Minnesota; Maria Pieters, University of Minnesota; Jian-Ping Wang, University of Minnesota; Maxim C-J. Cheeran, University of Minnesota

Introduction
Mycoplasma hyopneumoniae is one of the most prevalent respiratory bacterial pathogens that causes enzootic pneumonia, a chronic infection that leaves pigs vulnerable to other respiratory pathogens. Timely detection of disease is critical for treatment, control and preventing spread. Current diagnostic testing such as culture and PCR are time consuming, require specialized laboratory facilities, and skilled personnel. Using monoclonal antibodies specific to M. hyopneumoniae, an ELISA was optimized and evaluated for antigen detection using various clinical samples. The ELISA developed in this study will be used in a magnetic particle spectroscopy (MPS) device, which detects alterations in the harmonics of antibody-functionalized magnetic nanoparticles (MNP) when bound to mycoplasma antigen. MPS technology will give producers access to quick, reliable, point-of-care diagnostics that can be applied in the field.

Methods
Mice monoclonal and rabbit polyclonal antibodies specific to M. hyopneumoniae were purified using Protein A IgG purification kit. The antibodies were confirmed to be specific to M. hyopneumoniae with Western blot testing. The concentration of purified antibodies was determined using nanodrop. Initial testing showed that the monoclonal antibody used as the capture antibody and the polyclonal antibody used as the detection antibody resulted in the best signal and lowest background noise. Using this sandwich design, the antigen-based ELISA was optimized. The optimized ELISA was then tested with serial dilutions of M. hyopneumoniae to determine dynamic range and limit of detection. Next the concentration curve for the ELISA was established. Analytical specificity of the optimized ELISA was determined using strains of M. flocculare, M. hyorhinis, M. hyosynoviae , Influenza A virus (IAV) strain H1N1, IAV strain H3N2, Porcine Respiratory Reproductive virus strain VR2332, Pasteurella multicoda, and Streptococcus suis. Finally, the ELISA will be evaluated using spiked clinical samples and M. hyopneumoniae experimentally infected pig clinical samples. For detection of M. hyopneumoniae in various clinical matrices (laryngeal swabs, nasal swabs, tracheal wash, and bronchial lavage fluid), samples will be first confirmed negative for M. hyopneumoniae by PCR. These samples will then be spiked with known concentrations of a reference strain of M. hyopneumoniae and the results compared to a standard concentration curve for the agent. Clinical samples from experimentally infected M. hyopneumoniae pigs will also be tested and ELISA results compared to PCR.

Results and Conclusions
The optimum concentration of monoclonal capture antibody was 1ug/mL. The optimum concentration of polyclonal detection antibody is 1 ug/mL. The optimum blocking buffer was 1% BSA. These concentrations resulted in the best signal with the least background absorbance readings. The dynamic range for the optimized assay was 226.57 ng/mL - 7,250 ng/mL- of M. hyopneumoniae lysate, and the limit of detection is 226.57 ng/mL. The ELISA was confirmed to not cross-react with any of the other respiratory viral or bacterial pathogens or commensal M. flocculare. Testing of the spiked and experimentally infected samples is underway. After optimizing the ELISA for clinical sample testing, the monoclonal antibodies used will be attached to MNPs. These nanoparticles will be used to detect antigens using MPS devices, which will be evaluated for diagnostic ability to detect M. hyopneumoniae.

Poster Number: 90
Topic Area: Other
Title: Evaluating the effect of mixing and half dosing Prevacent PRRS when administered with various other vaccines
Author(s): Brandi Burton, Suidae Health & Production; Robert Evelsizer, Elanco Animal Health; Patrick Hoffmann, Elanco Animal Health

Introduction
It is common to administer multiple injections to piglets around the time of weaning as handling frequency increases. Multiple injections cause extra stress on the piglets and can make it more difficult for those administering the products. Being able to mix vaccines without sacrificing the pig's ability to elicit an immune response to vaccines would make the process more efficient.

The goal of this study was to evaluate PRRS antibody response in pigs when administering Prevacent PRRS separately or mixed with FosteraGold PCVMH, Circumvent G2-PCVM, Circo/MycoGard, Circo/MycoFLEX, and ParvoShield L5E. Additionally, the difference between a full dose and a half dose of Prevacent PRRS was assessed in the same manner.

Materials and Methods
The study was conducted in a wean-to-finish barn located in north-central Iowa. Five vaccines were selected to be administered with Prevacent PRRS: Fostera Gold PCVMH, Circumvent G2-PCVM, Circo/MycoGard, Circo/MycoFLEX, and ParvoShield L5E. Within each vaccine group, there were 5 treatment groups: saline, full and half dose Prevacent administered separately, and full and half dose Prevacent mixed with treatment vaccine. A full dose of the selected vaccines was administered per label.

There was a total of 25 treatment groups and 450 total pigs enrolled in the study. On day 0, serum was collected from each pig, the pig was tagged, and vaccinated according to its treatment group. Serum was collected from each pig 9 days and 2, 3, and 5 weeks post-vaccination. Serum samples were analyzed for PRRS antibody response by PRRSV X3 ELISA. Oral fluid samples were collected at each time point and tested for PRRS PCR and, if positive, ORF5 sequencing was requested. All samples were sent to ISU-VDL.

Results
There were no consistent differences between mixing and half dosing Prevacent in vaccine groups except Circo/MycoGard group. Pigs administered Prevacent mixed with Circo/MycoGard had 0% PRRS ELISA positives at 2 weeks post-vaccination, whereas the other 4 vaccines and their respective Prevacent treatment subgroups were all above 80% positive. At 3 weeks post-vaccination, Circo/MycoGard groups 3 and 5 (mixed groups) were 35-40% PRRS ELISA positive; however, this is consistent with group 1 (saline) so those results are likely attributed to shedding of Prevacent PRRS within the pen. All other vaccines and their treatment subgroups were above 95% (except in saline groups). At 5 weeks post-vaccination, oral fluids were positive and ORF5 sequence revealed a lateral wild-type PRRSV, so results were not analyzed.

Conclusions
This study shows that there was an appropriate antibody response elicited when mixing Prevacent PRRS with various other vaccines that are commonly given at the same time; however, results indicate Prevacent PRRS should not be mixed with Myco/CircoGard. Even though antibody response does not necessarily equate to protection against wild-type PRRS, this study shows the body is able to respond to vaccine in these conditions.

Poster Number: 91
Topic Area: Bacterial Disease
Title: GILT ACCLIMATION PROGRAM WITH LUNG HOMOGENATE FOR THE CONTROL OF MYCOPLASMA HYOPNEUMONIAE UNDER FIELD CONDITIONS
Author(s): Paula Cardona, Grupo Porcícola Mexicano - Kekén; Alberto Alfonso, Grupo Porcícola Mexicano - Kekén; Felipe Bedoya, Grupo Porcícola Mexicano - Kekén; María José Clavijo, Iowa State University and Pig Improvement Company, PIC®; Alejandrina Da Silva, Grupo Porcícola Mexicano - Kekén; Esteban Ramírez, Grupo Porcícola Mexicano – Kekén

Keywords:
Mycoplasma hyopneumoniae, acclimatization, lung homogenate.

Introduction
Mycoplasma hyopneumoniae (MHP) is the causative agent of chronic respiratory infection in pigs known as enzootic pneumonia. MHP can increase the production cost between $ 1 and $ 10 USD per pig either as a primary pathogen or by coinfection with other viral pathogens.[1] The piglet is colonized through sow-to-pig transmission and the prevalence at weaning has been associated with development of disease in grower-finisher pigs.[2] The gilt acclimation programs have been developed for early exposure at ~50 days of age, which allows for the introduction of immune non-shedding gilts.[3] During the last five years, our system (~87,000 sows) has confirmed a high incidence of MHP (75%) in grow-finish clinical cases. The objective of this work was to develop and implement a system wide early gilt acclimation program to reduce the incidence of MHP in the growing-finishing phase.

Methods
The acclimation program started on week 46 of 2019 and was implemented in a continuous flow gilt development unit (32,000 gilts) that sourced 15 breeding herds (~67,000 sows), located in the state of Yucatán, México. Lung homogenate (LH) was obtained from gilts between 100-110 days of age with clinical MHP infection confirmed by PCR from tracheal samples.[4-5] The areas with lung consolidation were weighed and liquefied (Ninja Blender) for six minutes with liquid Friss Medium (Teknova) while preserving the 60:40 ratio (Lung tissue: Friss Medium). The product was filtered, placed in 50 ml tubes, and stored in an ultra-freezer at -80 ° C. In addition, a sample from the LH was quality tested in the laboratory. When each room (1,500 gilts) reached ~60 days of age, the lung homogenate was thawed in a water bath at 24 ° C and mixed with reconstituted liquid Friis Medium, in a ratio of 1:80 (LH: Medium Friis). This mixture was fogged (Hurricane fogger) for 45 minutes in the room with curtains closed (four liters of LH for 180 gilts). Thereafter, serum samples of four percent of gilts within each inoculated group was tested six weeks post-exposure by ELISA (IDEXX M. hyo). The proportion of clinical cases confirmed MHP by PCR and histopathology in grower-finisher pigs was compared before and after the acclimation program.

Results and Conclusions
A total of 61 groups (~63,000 gilts) have been exposed and introduced to the breeding herds since November 2019. On average, 74% of gilts tested ELISA positive at six weeks post-exposure and increased to 87% in the last 23 groups. MHP was confirmed as causative agent of respiratory disease in 32% of all reported grow-finish clinical cases. Thus, the proportion of MHP cases was reduced by 43%, evidencing the effectivity of early gilt exposure programs to reduce MHP transmission from dam to piglet.

Aerosolization of MHP lung homogenate has been an effective method for repeated and safe exposure of large groups of gilts at ~60 days of age, under field conditions. The reduction in the incidence of MHP cases in the system is encouraging. Additional studies are needed to estimate the ROI of this program in our system.

References
1. Haden DC, et al. 2012, AASV; 43:75-76.
2. Fano E, et al. 2007, Can J Vet Res; 71: 195 – 200.
3. Pieters M y Fano E. 2016, Veterinary Records; 178: 122 – 123.
4. Silva, A. et al. 2021. Veterinary Microbiology, 258, 109118.
5. Clavijo MJ, et al. 2021, J. Clin. Microbiol; Apr 20.

Poster Number: 92
Topic Area: Bacterial Disease
Title: Evaluation of nursery mortality and production performance using controlled and natural Glaesserella parasuis exposure
Author(s): Katie Parker, Iowa State University; T. Lee Girard, Iowa Select Farms; Cesar Amorim Moura, Iowa Select Farms

Introduction: Glaesserella parasuis is known to be a main pathogen causing polyserositis, fibrinous exudate, and systemic infection in nursery pigs. Glaesserella parasuis (GPS) often increases nursery mortality as a secondary disease to swine influenza and porcine reproductive and respiratory syndrome virus (PRRSV).[1] There are multiple ways to assist in controlling GPS within a herd, including commercial and autogenous vaccines, as well as using a controlled GPS exposure. In previous studies, a controlled GPS exposure program has been more effective than vaccination programs.[2] Early introduction of GPS can reduce the risk of systemic infection and improve performance. In the current study a live GPS inoculum was given with the intent of reducing nursery mortality and increasing production performance compared to those that did not receive the inoculum.

Objective: To compare levels of GPS challenge by evaluating nursey mortality, mortality with fibrin, and treatment rate between controlled GPS exposure and natural exposure.

Materials and Methods: The study is a systematically randomized field trial on a wean-to-finish site including pigs from a gilt only, PRRSV endemic sow farm with a history of GPS disease in offspring post-weaning. Pigs were inoculated by litter via oral exposure at three days old with a live GPS inoculum that matched a pre-existing serotype present within the flow. The control pigs did not receive the inoculum and were identified by ear tags. Experimental and control pigs remained separate through weaning, transportation, and placement at the finisher. Pigs were placed in a single-stocked wean to finish site. The study groups were held within the same air space but did not have nose-to-nose contact. Necropsies were conducted on all mortalities to grossly detect fibrin. All treatments and mortality were recorded by pen. Four sampling events took place where lung, lymph node, and tonsil samples were taken from acutely dead pigs and submitted for histopathology and serotyping of GPS. The pens in the finisher were the experimental units, while the individual pigs were the observational units. The proportion of mortality and mortality with fibrin between groups was evaluated by binomial regression while the treatment rate between the groups was evaluated by Poisson regression.

Results: Mortality in the experimental group was 10% post-weaning. Mortality in the control group was 11.34% post-weaning. Total post-weaning mortality was 10.68%. On post-mortem examination 38.67% of the control group mortalities had fibrin present while 33.07% of the experimental group mortalities had fibrin. Of the 42 pigs that samples were submitted from, 6 returned positive for GPS, all were positive for PRRSV, and one grouped sample was positive for influenza A. In addition to GPS, multiple other respiratory bacterial challenges were detected in samples. Overall, mortality in the experimental group was less than the control group. The experimental group had significantly less treatments administered than the control group ($p=0.0002$).

Discussion: Results show improvement in mortality and treatment rate within the experimental group (GPS inoculated) indicating this procedure may provide value to offspring from disease challenged farms. Limitations of this study include minimal replication and therefore a small power.

References:
1. “Haemophilus parasuis (Glasser’s Disease).” *Iowa State University Veterinary Diagnostic and Production Animal Medicine,* https://vetmed.iastate.edu/vdpam/ FSVD/swine/index-diseases/glasser-disease. Accessed 7 July 2021.
2. Oliveira S, Pijoan C, Morrison R. Evaluation of *Haemophilus parasuis* control in the nursery using vaccination and controlled exposure. *J Swine Health Prod.* 2004;12(3):123-128.

Poster Number: 93
Topic Area: Viral Disease
Title: Detection of PRRSv in manure pits under field and experimental conditions
Author(s): Julian Montoya Lopez, University of Minnesota; Matt Allerson, Holden Farms, Northfield, Minnesota; Andy Kryzer, Fairmont Veterinary Clinic, Fairmont, Minnesota; Cesar A. Corzo, University of Minnesota

Introduction
Breeding herd PRRS epidemics have followed the same trend during the last decade with the onset of the epidemic occurring during the fall (e.g., October-November). During the same time, farmers agitate, pump and spread manure from pig barns (Arruda et al., 2018; Tousignant et al., 2015). These two events led us to conduct an exploratory study to determine whether PRRSv could be detected in manure pits and detected it by RT-qPCR in 13.87% (95% CI 9.5%, 19.81%) of the barns (Montoya et al., 2021 published). Results from this study lead us to continue asking more questions including the understanding of PRRSv shedding patterns and viral viability in manure pits as a way to assess potential risk from this activity. Therefore, we conducted a longitudinal follow-up study in both field and experimental conditions to understand frequency of detection, shedding patterns and viability of the virus in manure pits.

Materials and methods
The field study was conducted at two wean-to-finish barns (Farm A and B) by following groups of recently weaned pigs born to viremic sows from 2 different sow farms by collecting four oral fluid samples and manure pit samples. Manure pit content was collected directly from the pit using a clean polyvinyl chloride (PVC) tube and then transferred to a Falcon® tube. Samples were collected every four weeks starting at weaning.

The experimental infection study was conducted at the University of Minnesota (UMN) veterinary facility. A total of six three-week-old PRRSv naïve pigs were housed in pairs in 42 x 24 x 38 inches air-filtered isolators with two pairs (Group 1 and 2) being infected with a lineage 5 virus (RFLP 2-5-2) and the remaining pair serving as the negative control (Group 3). On day 1 of the study, groups 1 and 2 were challenged intramuscularly and intranasally with a 1x103, 1x105, respectively. A rectal swab was collected from each pig on days 4, 7, 14, 21, 28, 35, 42, 49. Each isolators' manure collection canister was sampled on days 6, 13, 20, 27, 34, 42. All samples were submitted to the UMN Veterinary Diagnostic Laboratory for RT-qPCR testing for PRRSv. Virus isolation was attempted in manure pit samples.

Results
Field study: Farm A yielded a total of three positive samples out of 24 manure samples. The positive manure samples were detected in week 12, 16, and 20. No virus was isolated from positive samples. Farm B yielded a total of one positive sample out of 20 manure samples. The positive manure sample was detected in week 16.
Experimental infection: At four days post-infection (dpi), the first positive results from the samples collected were obtained from all pigs, with the Ct values from the rectal swabs ranging between 28 and 38 for the group 1. The negative control group remained negative throughout the whole experiment. Manure pit samples were positive starting on dpi four with the last positive sample detected four weeks post-infection (pi) with Ct values gradually increasing from 32 to 35 at week seven for group 1. For group 2, positive samples were detected only for the first two weeks pi. Rectal swabs were positive during the first, fourth, fifth, and sixth week dpi. Virus isolation attempts were made in all manure samples, but none were positive.

Discussion
Results from this study raise awareness from a risk perspective as manure pit samples were positive for RT-qPCR test in both experiments. However, the frequency of detection varied between groups in which under field conditions first detection was approximately 10 to 14 after pig arrival. On the experimental infection study, the waste canister used as a proxy for the manure pit was emptied daily; regardless of this process, we were able to detect the virus. Failing to detect viable virus in manure pits generates further questions related to the risk of manure. More studies are needed to further understand the diagnostic test used and the sample preparation to increase the chances of detecting viable virus.

Poster Number: 94
Topic Area: Viral Disease
Title: IMPROVED GROWTH WITH THE USE OF FURST PROTECT™ FOR NURSERY PIGS DURING A NATURAL PORCINE EPIDEMIC DIARRHEA VIRUS (PEDV) INFECTION
Author(s): Fredrik Sandberg, Furst-McNess Company; Greg Hartsook, Furst-McNess Company; Kevin Soltwedel, Furst-McNess Company; Megan Bible, Furst-McNess Company

Introduction: Furst Protect™ is a combination of fatty acids, monoglycerides, antioxidants, and phytonutrients. Fatty acids are important and are vital to aid the immune system (1). Field studies have demonstrated that Furst Protect™ reduces stillborns, improves wean to 1st interval, increases pigs weaned and reduces mortality when fed to sows. Research shows that Furst Protect™ reduces mortality and improves growth performance during a multi-viral challenged infection via the feed (2). Thus, the response of Furst Protect™ was evaluated during a natural Porcine Epidemic Diarrhea virus infection (PEDv).

Materials & Methods: A total of 640 (DNA x DNA 600 sired) weaned, commercial pigs (21 days of age, average 14.4 lb (P=0.26); 10 reps; 32 pigs/pen) were tagged individually using the LeeO RFID system. Pigs were blocked by sow litter and sow parity. There were 2 treatments: 1) standard nursery feed without antibiotics, control (CON); 2) CON + 8 lb per ton of Furst Protect™ for first 21 days (FP). Experiment utilized a 3-phase feeding program. A FANCOM feeding system was used to deliver feed. Pens of pigs were weighed and feed disappearances were recorded on d 0, 21, and 42. Data were used to calculate ADG, ADFI, and F:G. At day 21, pigs went through a natural exposure of PEDv. To simulate commercial practice, pigs were treated with injectable antibiotic if required, and those that did not respond were removed and placed in sick pens. At the end of 42 days, the number of dead (% mortality) and viable pigs (% morbidity) were calculated, as well as the total cost of injections were calculated. Data were analyzed as a randomized complete block design using the GLM procedure in Minitab with Fisher's t-test to determine differences between dietary treatments.

Results and Discussion: For d 0-21, ADG (P=0.10; 0.51 lb vs. 0.54 lb) and ADFI (P=0.02; 0.75 lb vs. 0.79 lb) were increased by 5.8% (P≥0.10) and body weight was improved (P=0.02; 25.0 lb vs. 26.0 lb) by 1 lb. No differences (P=0.15) were observed for F:G. There were no differences (P≥0.84) observed in mortality per pen, morbidity per pen, mortality plus morbidity per pen, or total injections cost for d 0-21 or 0-42. Overall (d 0-42), ADG was improved (P=0.01) by 6.6% (0.75 lb vs. 0.80 lb) and ADFI increased (P=0.04; 1.17 lb vs. 1.25 lb) by 6.8%. No differences (P=94) in F:G Pigs fed FP were 2.5 lb heavier (P=0.02; 46.4 lb vs. 48.9 lb) compared to pigs fed CON. Pigs fed FP continued to grow and consume more feed, even during a PEDv infection. This suggests that Furst Protect™ may be acting as an immune nutritional supplement for nursery pigs during a viral infection.

Conclusions: Furst Protect™ is a nutritional supplement that aids nursery pigs during a viral infection and further testing to support lifetime growth is warranted.

References:
1. Radzikowska et al. (2019) Nutrients 11:2290.
2. Dee et al. (2003) Transboundary and Emerging Diseases: 61:833-845.

Poster Number: 95
Topic Area: Viral Disease
Title: FURST PROTECT DIRECT™ NOURISHES THE IMMUNE SYSTEM BEFORE A NATURAL PORCINE EPIDEMIC DIARRHEA VIRUS (PEDV) INFECTION IN NURSERY PIGS
Author(s): Fredrik Sandberg, Furst-McNess Company; Greg Hartsook, Furst-McNess Company; Kevin Soltwedel, Furst-McNess Company; Megan Bible, Furst-McNess Company

Introduction: Furst Protect Direct™ is a unique combination of fatty acids, monoglycerides, antioxidants, and phytonutrients that are delivered via drinking water. Research has shown that fatty acids are important and are vital to the immune system (1). Research shows that Furst Protect™ in the feed reduces mortality and improves growth performance during a multi-viral challenged infection via the feed (2). Thus, the response of Furst Protect Direct™ was evaluated during a natural Porcine Epidemic Diarrhea virus infection (PEDv).

Materials & Methods: A total of 641 (DNA x DNA 600 sired) weaned, commercial pigs (21 days of age, average 14.4 lb (P=0.13); 10 reps; 32 pigs/pen) were tagged individually using the LeeO RFID system. Pigs were blocked by sow litter and sow parity. There were 2 treatments: 1) no water treatment, control (CON); 2) 16 oz of Furst Protect Direct™ in 5 gallons of stock solution (metered at 1:128) for the first 5 days continuously (FPD). Experiment utilized a standard, 3-phase feeding program without antibiotics. A FANCOM feeding system was used to deliver feed. Pens of pigs were weighed and feed disappearances were recorded on d 0, 21, and 42. Data were used to calculate ADG and ADFI. At day 21, pigs went through a natural exposure of PEDv. To simulate commercial practice, pigs were treated with injectable antibiotic if required, and those that did not respond were removed and placed in sick pens. At the end of 42 days, the number of dead (% mortality) and viable pigs (% morbidity) were calculated, as well as the total cost of injections were calculated. Data were analyzed as a randomized complete block design using the GLM procedure in Minitab with Fisher's t-test to determine differences between dietary treatments.

Results and Discussion: For d 0-21, there were no differences (P≥1.00) observed for any measured parameters. However, during the PEDv infection (d 21-42), ADG was increased (P=0.03; 0.99 lb vs. 1.05 lb) by 6.1%. Numerically, there was a 42.9% reduction (P=0.19; 4.38% vs. 2.50%) in % morbidity plus mortality and a $0.63 reduction (P=0.18; $1.67 vs. $1.04) in cost of injectable treatments during d 21-42. No other differences (P≥0.56) were observed for d 21-42. Overall (d 0-42), ADG was improved (P=0.07; 0.75 lb vs. 0.78 lb) by 4.0% and pigs were 1.4 lb heavier (P=0.06; 46.4 lb vs. 47.8 lb). No differences (P≥0.60) were observed in any other parameter measured. This suggests that Furst Protect Direct™ may be setting up the immune system during the early nursery so the pigs are better able to handle a viral infection during the late nursery.

Conclusions: Furst Protect Direct™ is a nutritional water supplement that nourishes the immune system during the first 5 days of the nursery phase for a natural PEDv infection occurring during the late nursery. Further testing is needed to evaluate lifetime performance.

References:
1. Radzikowska et al. (2019) Nutrients 11:2290.
2. Dee et al. (2003) Transboundary and Emerging Diseases: 61:833-845.

Poster Number: 98
Topic Area: Other
Title: Prescription Platform Vaccines: Next-Generation Technology Combats Emerging Diseases
Author(s): Mike Roof, Iowa State University

Introduction:
Vaccines are a critical tool for the Animal Health Industry to prevent disease, reduce antibiotic usage, ensure animal welfare, and support optimal growth and production. The current USDA regulatory process ensures a pure, safe, potent and efficacious product, but does take several years to develop and license. The industry needs to utilize new technology and have a more agile vaccine process. The USDA Center for Veterinary Biologics (CVB) recently created new subcategories for platform and prescription platform vaccines, based on established vector-expression systems (VES), standardized manufacturing, and other specific requirements. These new regulatory pathways can now by used by veterinarians and animal health companies to use advanced recombinant technology to rapidly respond with products that address pathogens with high diversity as well as new and emerging diseases

Methods
The new USDA guidance is based on a platform product is comprised of the VES, expressed gene material of interest (GOI), a manufacturing process, and safety and efficacy data associated with fully licensing an initial product. VS Memo 800.213 describes an expedited licensing pathway for inactivated or non-replicating vaccines derived from licensed platforms. Prescription platform (RxP) vaccines are an extension of platform technologies and CVB guidance, which is outlined in VS Memo 800.214. Manufacturers of RxP vaccines must have a licensed platform on which subsequent vaccines are based; thus, the GOI is the only aspect that differs from the original licensed platform. Each RxP vaccine is prepared on a custom basis after a written prescription order is issued by a licensed veterinarian with a valid veterinarian-client-patient relationship (VCPR). The prescribing veterinarian may provide a microbial isolate from which a GOI will be derived or may work with the manufacturer to select the most appropriate insert from a bank of known gene sequences. Safety, purity, customization, and shorter turnaround time to market are the focus of RxP vaccine manufacturers and the CVB.

Prescription platform vaccines may be used in animals that the attending veterinarian determines could benefit from their use. RxP vaccines can also fulfill a need in cases where commercially available (off the shelf) vaccines are not a viable option. A distinct advantage of RxP products is that they can be used for emerging diseases where there is no other way to possibly create a vaccine. Many agents causing emerging diseases cannot be grown in vitro, so a traditional autogenous vaccine cannot be used, and a commercial vaccine cannot be developed in a timely manner.

Conclusions
In conclusion these new USDA policies give veterinarians a new tool to address genetic variability and emerging pathogens. The process uses advanced technology and a streamline process to allow a faster response. It is important todays veterinarians understand the new process and utilize when most advantageous to their clients.

Poster Number: 100
Topic Area: Other
Title: Applications of next generation sequencing for detection and genomic characterization of novel and emerging pig pathogens
Author(s): Sunil Kumar Mor, Minnesota Veterinary Diagnostic Laboratory and Department of Veterinary Population Medicine, UMN; Vikash Kumar Singh, Minnesota Veterinary Diagnostic Laboratory and Department of Veterinary Population Medicine, UMN; Albert Rovira, Minnesota Veterinary Diagnostic Laboratory and Department of Veterinary Population Medicine, UMN; Matthew Sturos, Minnesota Veterinary Diagnostic Laboratory and Department of Veterinary Population Medicine, UMN; Fabio Vannucci, Minnesota Veterinary Diagnostic Laboratory and Department of Veterinary Population Medicine, UMN; Stephanie Rossow, Minnesota Veterinary Diagnostic Laboratory and Department of Veterinary Population Medicine, UMN

The hypothesis-free metagenomics strategy enables NGS to simultaneously detect mixed infections with different microorganisms and identify novel and/or uncharacterized pathogens directly from clinical samples without any prior knowledge of the pathogen(s). The Minnesota Veterinary Diagnostic Laboratory (MVDL) has invested in this latest technology and is offering this as a non-validated test from 2017 for disease diagnosis and whole genome sequencing. We have applied targeted and non-targeted methods for whole genome sequencing of viruses. We found NGS to be helpful in detection and whole genome sequencing of novel and divergent viruses such as a divergent and reassortant porcine rotaviruses, astrovirus, sapelovirus, sapovirus, porcine reproductive and respiratory syndrome virus, swine influenza virus, porcine circovirus and other pathogens in different disease conditions of pigs. The whole genome sequencing is helpful in better characterization of emerging viruses, comparison with vaccine strains as well as to see trend in change in virus sequences over time in a particular farm, state and country. NGS is helpful to see the viral load of different viruses associated with disease syndrome cases such as in enteric disease syndrome astrovirus, calicivirus, picornaviruses, picobirnavirus, and rotaviruses have been detected in different combinations. In addition, we identified viruses associated with uncharacterized CPE in different cell lines. NGS was used for whole genome sequencing and characterization of different bacteria such as Streptococcus suis, Glaesserella parasuis, E. coli, and Salmonella. The bacterial whole genome sequencing is helpful to gain information of predicted serotype, sequence type, antimicrobial resistance and virulence genes. The validation of NGS as a diagnostic test is a challenge especially for clinical samples. The results reporting is also not easy. For example, how to share results, what information is useful to share, and what is the meaning of mixed infections and the presence of novel and divergent pathogens. The advantages and challenges related to NGS will be discussed in detail.

Poster Number: 101
Topic Area: Viral Disease
Title: Inactivation of two swine vruses on shoes by BioSec, a shoe sanitizing station
Author(s): Angie Quiñonez Muñoz, Veterinary Diagnostic Laboratory and Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota; Trisha Sharma, Veterinary Diagnostic Laboratory and Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota; Madeeha Gohar, Veterinary Diagnostic Laboratory and Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota; Faisal Ahmad, Veterinary Diagnostic Laboratory and Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota; Cesar Corzo, Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota; Sagar M. Goyal, Veterinary Diagnostic Laboratory and Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota

Enhancement of biosecurity measures is critical to prevent and control pathogen transmission in swine farms. Soles of shoe are known as a significant source of pathogenic microorganisms. Indirect transmission of high economic impact diseases such as those caused by Porcine Reproductive and Respiratory Syndrome virus (PRRSv) and Porcine Epidemic Diarrhea virus (PEDv) by shoes and other fomites, has been described. BioSec is a shoe sanitizing station that combines Ozone + UVC (UVZone) technology and has been found effective against bacterial pathogens. The objective of this study was to assess the inactivation rate of PRRSv and PEDv on rubber soles and polyblend boot material by BioSec.

The PRRSv and PEDv were propagated and titrated in Marc-145 and Vero-81 cells, respectively. Approximately 1cm 2 coupons of rubber sole and polyblend boot material were cut. The coupons were placed in a sterile 24-well tissue culture plate and were contaminated with 40 µl of PEDv or PRRSv per coupon. The virus inoculum was air-dried for about 1 hour in a biosafety cabinet (BSC). Subsequently, four of the virus-spiked coupons were placed on the left side of the BioSec machine. An operator stepped on the right side of the machine to activate it for a standard time of eight seconds as specified by the manufacturer. After treatment, coupons were removed aseptically, and the surviving virus was eluted from them using an eluent solution (3% beef extract-0.05M glycine). Four virus-spiked non-BioSec treated coupons were used as negative control. Serial 10-fold dilutions of eluates from treated and non-treated coupons were prepared inMEM. All dilutions were inoculated in monolayers of appropriate cells contained in 96-well microtiter plates using 3 wells per dilution. Plates were examined daily for the appearance of virus-induced cytopathic effects (CPE). After 7 days of incubation, virus titers were calculated (Karber, 1931) and expressed as log 10 TCID 50 /mL.

On an average, ≥99% of the PRRSv was inactivated on both rubber sole and polyblend boot material. In addition, an average of 98.55 % and ≥99% of PEDv was inactivated on rubber sole and polyblend material, respectively. These findings demonstrate the efficacy of BioSec inactivating swine pathogens in shoe materials and its potential use to enhance biosecurity practices in swine farms.