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Journal of Swine Health & Production

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Recombinant VT2e vaccine impact on VT2e-induced edema associated mortality and morbidity

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The use of oral fluid diagnostics in swine medicine

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Lessons for managing ESFs and collecting weights on a large commercial sow farm

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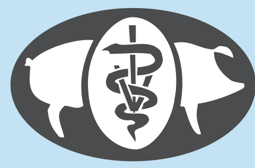

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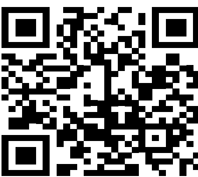

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Pigs line up at University of Missouri Swine Teaching Center

Photo courtesy of Tina Smith

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"I encourage you to think about how you are doing more with more instead of more with less."

quoted from the President's message, page 241

Rethink the norm:

Combating high pre-weaning mortality

There's a standard swine producers and veterinarians have grown accustomed to: 15 percent. It's roughly the industry average for pre-weaning mortality in piglets, an issue that has long impacted the swine industry.

The 2016 Pork Industry Analysis indicates that while the number of pigs born alive continues to rise, pre-weaning mortality is growing right alongside it. According to the report, which looks at production data from about 35 percent of the U.S. sow herds, the sow farm average for pre-weaning mortality was 17.3 percent in 2016. For operations in the top 25 percent for each production indicator, it was as high as 13.2 percent.

Average sow farm pre-wean mortality: 17.3%.

- 2016 Pork Industry Productivity Analysis

The right nutrients at the right time is key to a healthy start

A main factor of pre-weaning mortality is nutrition – getting neonatal pigs adequate nutrients so they're off to a healthy start in the first few days of life. The challenge often is getting young pigs to seek out those nutrients. Keith Aljets, DVM, Veterinary Medical Center in Williamsburg, Iowa, sees this challenge on farms.

"We're seeing the trend in more pigs born alive," says Aljets. "But many of these pigs are starving because there is not adequate nutrients in the first few days of life. Supplementation of these piglets can help them survive."

He's also seen the benefit of a unique product, Tonisity Px™, in getting pigs to drink and eat. The first isotonic protein drink for pigs, Tonisity Px has a taste profile pigs crave and provides intestinal support and hydration needed to get them off to a fast, healthy start. From Day 2, Tonisity Px can be used in farrowing and nursery settings.

"Having a product pigs will consume on Day 2 is remarkable," says Aljets. "On one of the first farm trials we did, we weaned 0.4 more pigs per litter on those given Tonisity Px. When the site manager saw that, they were all in."

Pre-weaning mortality is a challenge, but it doesn't have to impact 15 percent of piglets. Tonisity Px is one of the tools producers can reach for when combating pre-weaning mortality.

From the field:

Tonisity Px reduces pre-weaning mortality, boosts growth performance

A research study at a 7,200-sow farrow-to-finish Iowa operation investigated the effects of Tonisity Px on nursing piglets and found:

- Pre-weaning mortality fell 31.8% for piglets that received Tonisity Px for 7 days beginning at Day 2.
- Odds of survival were 1.55 times higher.
- ADG and net weight gain improved by more than 10% vs. controls from 2 days of age through 20 days post-weaning.

Pre-weaning mortality (%)	Control	Tonisity Px
Mortality	15.1%[a]	10.3%[b]
Pigs	64/425	52/506
Reduction		31.8% reduction

[ab] p=0.029

Figure 1: Pre-weaning mortality of piglets (Day 2 to approximately 19 days of age).

Data on file, Study Report TON-USA-034, Tonisity Int. Ltd.

"We weaned 0.4 more pigs per litter on those given Tonisity Px."

- Keith Aljets, DVM, Veterinary Medical Center

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Are you doing "More with Less" or "More with More"?

Sometimes we lament that the competitive nature of business forces us to do "more with less." Be honest, how often does this thought cross your mind? There is no doubt that swine production and the supporting veterinary service has become more efficient. In that way, we are doing more with less.

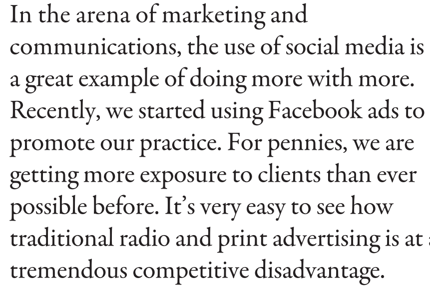
This month, I want to offer a different view. I submit we are doing "more with more." This idea stems from my own recent personal reflections on scarcity and abundance. The scarcity mentality can be defined as the belief that everything is limited, and thus it is better to be selfish than generous. It is a cynical term by nature and, as author Stephen F. Covey says, it's based around the idea that there is not enough of the pie to go around. In contrast, individuals with an abundance mentality can celebrate the success of others rather than be threatened by it. The abundance mentality is believed to arrive from having high self-worth and security, and leads to the sharing of profits, recognition, and responsibility.

What are some ways swine veterinarians do more with more? Clearly, technology is an area where we have more resources and capabilities than ever before. For example, I recently did a surgical procedure on an animal in the field without ever having done it before. I assessed the patient, then quickly referenced "Dr Google" and "Dr YouTube" on my iPhone to determine some options for treatment. I texted a veterinary colleague and called a producer I trusted on their mobile phone to get their input on the situation. After that, I consulted with the client and we agreed on the treatment and conducted the procedure. In the past, this same degree of resource investigation would have required a trip back to the office to consult with textbooks and people via a landline telephone.

"Share your story on social media using #AASVmorewithmore."

In this same example, a high level of trust between the client and myself allowed us to accomplish more with more. More trust results in less inefficiency in our interpersonal relationships. Because I was comfortable in sharing that I had never done the procedure, I was able to quickly consult with all the resources I had available in front of the client and determine the best course of action. Being transparent and vulnerable at the same time led to greater trust by the client and quicker intervention for the patient.

From an analytical perspective, the ever-increasing capability of information databases allows us to make better and faster decisions about the health and wellbeing of the pigs in our care. Sure, it's not easy all the time. Nor was it in the past. There is no doubt we have more knowledge and tools today than we have ever had before. The outcome is more accurate and effective decisions about health interventions.



In the arena of marketing and communications, the use of social media is a great example of doing more with more. Recently, we started using Facebook ads to promote our practice. For pennies, we are getting more exposure to clients than ever possible before. It's very easy to see how traditional radio and print advertising is at a tremendous competitive disadvantage.

I encourage you to think about how you are doing more with more instead of more with less. It will change your perspective and if your experience is anything like mine, it will change your attitude as well. Here are 10 ways you can foster your abundance mentality:

1. Become aware of your thoughts
2. Practice gratitude
3. Recognize the unlimited possibilities
4. Cultivate and share your passions and purpose
5. Develop mastery experiences
6. Watch what you say
7. Concentrate on personal growth
8. Maintain an attitude of openness and enthusiasm; Think like a beginner
9. Focus on what is going right
10. Make a list of positive affirmations

How are you doing more with more? Share your story on social media using #AASVmorewithmore. I'll be looking for your insights! ☺

C. Scanlon Daniels, DVM
AASV President

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A voice in organized veterinary medicine

I recently attended the 2018 annual convention of the American Veterinary Medical Association (AVMA) with the notion that it likely will be for the last time as your executive director. With that in mind, it caused me to pause and reflect on the nature of the relationship between the AASV and the rest of organized veterinary medicine.

The main reason I attend the AVMA convention is to observe the House of Delegates (HOD) meeting and support our volunteers in that body. The HOD consists of the delegates and alternate delegates from each state veterinary medical association and several allied veterinary medical associations, including the AASV. There are typically several resolutions that come before the HOD for consideration. Some of these are more pertinent for swine veterinarians than others, but all require study and scrutiny. Our HOD delegate is Dr Tara Donovan and our alternate delegate is Dr Deb Murray. They both spend a great deal of time on issues of importance to veterinary medicine within the governance of the AVMA. I extend a big thank you to them for their time and efforts!

I will be the first to admit that at times I have railed against the AVMA on various issues. As can be expected, the interests of AVMA and AASV have diverged at times. It is important, however, for AASV to maintain a viable relationship with the AVMA. Whether we agree or not, our voice needs to be heard in the broader perspective of organized veterinary medicine so that we can effectively advocate for the best interest of the pigs under our care. Albeit, sometimes we must raise our voice a bit louder and more forcefully. This has been true for the 49 years that AASV has been in existence. I have no doubt that it will continue long into the future.

The AASV is also able to send representatives to several AVMA committees. My philosophy has always been to send strong, willing, and able representatives to these committees. You never know when an issue might arise in a committee that requires immediate action. We send those who are not afraid to speak up and to hold the line on issues that might affect swine veterinary medicine. We expect our representatives to have insight into the issues at hand and to determine the appropriate course of action that best serves the needs of AASV members. It can be time consuming and even frustrating at times, but the best results arise from participation and open dialogue. Another big thank you goes to all our members who have represented AASV in the past or are currently representing AASV to the AVMA.

Organized veterinary medicine has changed over the years. The AVMA has grown in numbers but the percentage of AVMA members engaged in food animal practice has diminished. What has not diminished is the need for representation by veterinarians with experience and interest in food animal veterinary medicine. Actions taken by the AVMA have the potential to affect food animal industries with millions of animals. The health and welfare of the pig are our main concerns and deserve our full attention, not only on the farm but also in organized veterinary medicine.

"Another big thank you goes to all our members who have represented AASV in the past or are currently representing AASV to the AVMA."

Another change has occurred in veterinary medicine as alternative agendas arise in the areas of animal rights and the opposition to the use of animals for food. We need to continue to be advocates for animal protein as a food source that is safe and nutritious. It would be naïve to assume that the threat to animal agriculture does not exist within the AVMA. Let me assure you that it comes into play on a regular basis when issues such as animal welfare and use of antimicrobials come up. Beyond the AVMA, the AASV has enjoyed strong relationships with other food animal associations such as the American Association of Bovine Practitioners and the American Association of Avian Pathologists. We have often found that we can improve and strengthen our advocacy through collaboration and cooperation with our colleagues from cattle and poultry.

The archives at our office reveal that AASV (then AASP) and the AVMA have had a relationship dating back to 1969. With almost 50 years invested in organized veterinary medicine, it would be unwise to abandon relationship building with representatives from diverse areas of veterinary medicine. We can continue to advocate for our profession while serving the best interest of swine veterinary medicine. We can't be shy if we need to confront other veterinarians or veterinary medical associations on issues affecting pig health and welfare. To do otherwise would not be sustaining the strong voice established almost 50 years ago by our founders.

Tom Burkgren, DVM
Executive Director

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Dosage and Administration: Must be diluted before administration to animals. Include in the drinking water to provide a concentration of 200 mg tilmicosin per liter (200 ppm). One 960 ml bottle is sufficient to medicate 1200 liters (320 gallons) of drinking water for pigs. The medicated water should be administered for (5) five consecutive days.

Use within 24 hours of mixing with water. Do not use rusty containers for medicated water as they may affect product integrity.

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See product label for complete dosing and administration information.

RESIDUE WARNING: Swine intended for human consumption must not be slaughtered within 7 days of the last treatment with this product.

Precautions:

Do not allow horses or other equines access to water containing tilmicosin. The safety of tilmicosin has not been established in male swine intended for breeding purposes.

Always treat the fewest number of animals necessary to control a respiratory disease outbreak. Prescriptions shall not be refilled. Concurrent use of Pulmotil AC and another macrolide by any route is not advised. Use of another macrolide immediately following this use of Pulmotil AC is not advised.

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AH0470 YL089279AMX W1a

Veterinary communication

Changes implemented over the past decade in veterinary curriculum in universities across North America, have reflected the need for entry-level veterinarians to have well developed communication skills as a core competency. Not surprisingly, many Doctor of Veterinary Medicine (DVM) programs incorporate some form of training within the curriculum.

I recently attended a course focused on veterinary communications that was delivered by the Institute for Healthcare Communication (IHC; **healthcarecomm.org/veterinary-communication/**) in New Haven, Connecticut. The educational materials for this workshop were originally developed through the generous financial support of Bayer Animal Health from 2001 to 2016 and perhaps many of you have been trained using some of these modules. Thankfully the program has continued, and I had the opportunity to attend a session intended for faculty engaged in veterinary curriculum training us how to deliver evidence-based communication modules that were specifically developed by the IHC for veterinary medicine. It was a train-the-trainer type of course.

The module examples and scenarios were strongly focused on companion-animal practice, so to be fully engaged I had to dust off some of my companion-animal communication skills and knowledge to participate. There were some food-animal production scenario examples as well and the IHC is working to develop more.

I wanted to write about this experience because I felt that the concepts behind the training, while companion-animal focused, were directly translatable to food-animal practice and food-animal veterinarians. The training covers basic communication skills such as maintaining eye contact with the person you are speaking with, using open-ended questions more frequently to encourage open dialogue, maintaining appropriate facial expressions, and body language and posture (no slouching as my Mom would say). The premise behind these basic skills is that by building a relationship through optimum communication, then we as veterinarians can improve outcomes such as the optimization of animal health. I think all swine veterinarians would agree that a major goal of our work is to improve outcomes, whether it is improving pig average daily gain or improving overall health by maximizing client compliance.

The course really challenged me to stretch outside my comfort zone. Similar to the veterinary curriculum here at my home university, we participated in client simulations with actors portraying clients. The actors were very talented and versatile and kept the scenarios real. The experience has provided me with the opportunity to develop my train-the-trainer skills and provided me with a fresh outlook on the subject. It has also motivated me to further develop this area in my swine DVM teaching. This topic seems appropriately timed in the September-October issue of the journal as veterinary students are returning to school from their summer experiences.

"The premise behind these basic skills is that by building a relationship through optimum communication, then we as veterinarians can improve outcomes such as the optimization of animal health."

I hope my message encourages other educators and mentors to consider their own communication skills and how we can continue to improve how we train new swine veterinarians. I also believe that as swine veterinarians, further development and practice in communication skills can help us educate and engage our clients and their farm staff, truck drivers .... and many more in improving and maintaining swine health.

If you are unfamiliar with the IHC program or have not had the opportunity to engage in any type of communication workshop I encourage you to look it up. My experience at the IHC workshop not only reinforced my current skills, but it helped me to develop new skills, and helped me to recognize how other people, clients or students for example, can struggle with communication themselves.

Terri O'Sullivan, DVM, PhD
Executive Editor

Comparison of postmortem airway swabs and lung tissue for detection of common porcine respiratory pathogens by bacterial culture and polymerase chain reaction assays

Eric R. Burrough, DVM, PhD; Andrea P. Schwartz; Philip C. Gauger, DVM, PhD; Karen M. Harmon, PhD; Adam C. Krull, DVM, PhD; Kent J. Schwartz, DVM, MS

Summary

Objective: To compare pathogen detection from tracheobronchial swabs with lung tissue in diagnostic submissions from pigs with reported respiratory disease.

Materials and methods: Individual lung samples (n = 153) from 133 laboratory submissions were included in this study. Inclusion criteria were a lung sample where the tracheal bifurcation or major bronchus was readily identifiable and a clinical report of respiratory disease symptoms. Sterile, nylon-flocked swabs were used to sample the largest available airway before the lung tissue was routinely processed for diagnostic testing. Swabs were placed in Amies transport medium and tested in blinded parallel with the lung tissue by bacterial culture and polymerase chain reaction (PCR) for common swine respiratory pathogens.

Results: There was excellent agreement between PCR detection from lung and bronchial swab samples for porcine reproductive and respiratory syndrome virus, influenza A virus, *Mycoplasma hyopneumoniae*, and porcine circovirus 2 (kappa > 0.8, all assays). Agreement between bacterial culture from lung and swabs was substantial for *Pasteurella multocida* and *Salmonella* spp. and fair for *Streptococcus suis*. Lung tissue was culture positive more often than swabs for *Haemophilus parasuis* and *Actinobacillus* spp.; however, in these cases, PCR for the respective pathogen was 100% positive on swab samples regardless of culture status of the swab.

Implications: Tracheobronchial swabs are a single, uniform sample that can be easily collected at postmortem and transported to the laboratory for detection of swine respiratory pathogens by culture and PCR. Such swabs may serve as a rapid screening tool for unexpected mortalities in a population.

Keywords: swine, respiratory disease, diagnostic sensitivity, airway swabs

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Resumen – Comparación de tejido pulmonar y muestras de vía respiratoria post mórtem para la detección de patógenos respiratorios porcinos comunes mediante cultivo bacteriano y pruebas de reacción en cadena de polimerasa

Objetivo: Comparar la detección de patógenos de muestras traqueobronquiales con tejido pulmonar en muestras diagnósticas de cerdos con reporte de enfermedad respiratoria.

Materiales y métodos: En este estudio se incluyeron (n = 153) muestras de pulmón individuales de 133 entregas de laboratorio. El criterio de inclusión fue una muestra de pulmón en la que la bifurcación traqueal o bronquio principal fuera fácilmente identificable y con reporte clínico de síntomas de enfermedad respiratoria. Se utilizaron hisopos estériles de nylon agrupado para tomar muestras de las vías respiratorias más grandes disponibles antes de que el tejido pulmonar fuera procesado de forma rutinaria para pruebas de diagnóstico. Los hisopos se colocaron en un medio de transporte Amies y se probaron a ciegas y en paralelo con el tejido pulmonar mediante cultivo bacteriano y reacción en cadena de la polimerasa (PCR por sus siglas en inglés) para detectar patógenos respiratorios porcinos comunes.

Resultados: Hubo una concordancia excelente entre la detección del PCR del pulmón y los hisopos de muestra bronquial para el virus del síndrome reproductivo y respiratorio porcino, virus de la influenza A, *Mycoplasma hyopneumoniae*, y circovirus porcino 2 (kappa > 0.8, todas las pruebas). La concordancia entre el cultivo bacteriano del pulmón y los hisopos fue sustancial en la detección de *Pasteurella multocida* y *Salmonella* spp. y media para *Streptococcus suis*. El tejido de pulmón resultó positivo en cultivo más frecuentemente que en hisopos en la detección de *Haemophilus parasuis* y *Actinobacillus* spp.; sin embargo, en estos casos, el PCR para los patógenos respectivos fue 100% positivo en muestras de hisopos independientemente del estado del cultivo o del hisopos.

Implicaciones: Los hisopos traquobronquiales son una muestra única y uniforme que puede recolectarse fácilmente post mórtem y transportarse al laboratorio para detección de patógenos respiratorios porcinos mediante

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This article is available online at http://www.aasv.org/shap.html.

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cultivo y PCR. Esos cotonetes pueden servir como una herramienta rápida de revisión para mortalidades inesperadas en la población.

Résumé – Comparaison d'un écouvillonnage post-mortem des voies respiratoires et de tissu pulmonaire pour la détection d'agents pathogènes communs du système respiratoire porcin par culture bactérienne et réaction d'amplification en chaîne par la polymérase

Objectif: Comparer la détection d'agents pathogènes à partir d'écouvillons trachéo-bronchiaux à du tissu pulmonaire provenant de porcs avec des problèmes respiratoires et soumis pour diagnostic.

Matériels et méthodes: Des échantillons individuels de poumon (n = 153) provenant de 133 soumissions au laboratoire étaient inclus dans l'étude. Les critères d'inclusion étaient un échantillon de poumon où la bifurcation avec la trachée ou une bronche majeure était facilement identifiable et un rapport clinique de symptômes de maladie respiratoire. Des écouvillons stériles en nylon étaient utilisés pour échantillonner la voie respiratoire la plus grosse qui était disponible avant que le tissu pulmonaire ne soit utilisé pour les tests diagnostiques de routine. Les écouvillons étaient placés dans le milieu de transport Amies et testés à l'aveugle en parallèle avec le tissu pulmonaire par culture bactérienne et réaction d'amplification en chaîne par la polymérase (PCR) pour les agents pathogènes respiratoires fréquents chez le porc.

Résultats: Il y avait un excellent accord entre la détection par PCR à partir du tissu pulmonaire et les écouvillons bronchiaux pour le virus du syndrome reproducteur et respiratoire porcins, le virus de l'influenza A, *Mycoplasma hyopneumoniae* et le circovirus porcin de type 2 (kappa > 0.8 pour tous les tests). L'accord entre la culture bactérienne du tissu pulmonaire et les écouvillons était substantiel pour *Pasteurella multocida* et *Salmonella* spp. et raisonnable pour *Streptococcus suis*. Le tissu pulmonaire était positif pour la culture plus souvent que les écouvillons pour *Haemophilus parasuis* et *Actinobacillus* spp.; toutefois, pour ces cas, l'analyse par PCR pour les agents respectifs était 100% positive sur les écouvillons indépendamment du résultat de la culture à partir de l'écouvillon.

Implications: Les écouvillons trachéo-bronchiaux sont un échantillon unique uniforme qui peut être facilement prélevé en post-mortem et transporté au laboratoire pour la détection d'agents pathogènes du système respiratoire porcin par culture et PCR. De tels écouvillons peuvent servir d'outils rapides de tamisage lors de mortalités inattendues dans une population.

There is considerable variation in sample collection, handling, preservation, and shipping of specimens from swine populations to diagnostic laboratories. Each of these factors can affect the results of diagnostic tests for the multitude of infectious agents of disease. For detection of swine respiratory pathogens, lung tissue has been the sample type of choice for bacterial culture and molecular testing by polymerase chain reaction (PCR) assays. However, lung tissue has potential disadvantages for detection of pathogens because of variation in sample size, variation in location from which the sample is selected within the organ (sampling bias), uneven pathogen distribution within the lung, packaging selected to contain the sample, and speed of tissue cooling post-collection based on size and packaging. Moreover, swine diagnostic samples are commonly collected in the field by lay personnel (animal owners, farm operators, and farm managers) not formally trained in nuances of agent pathogenesis, pathology, and intricacies of diagnostic testing.

When respiratory disease is present, offending pathogens are usually in high concentrations but may not be uniformly distributed throughout the lung, nor are all contributors to the porcine respiratory disease complex all found at a single location or in a single sample of lung. Detection of offending pathogens in high numbers, along with compatible gross and microscopic lesions, is a core concept for disease diagnosis. Since the mucociliary system continuously moves material up from the deeper lung, airways theoretically contain any pathogens that may be contributing to respiratory disease distal to where collection occurs. Collection of conducting-airway exudates from primary bronchi at the tracheal bifurcation should thereby reflect the entire associated lung lobe and may serve as an alternative to individual lung lobe samples for diagnostic testing. Such sampling could serve to better standardize the collection process and reduce sampling bias inherent in individual lung lobe samples without substantially influencing diagnostic sensitivity.

The objective of this study was to determine the correlation between results of parallel testing of lung tissue and bronchial swabs for the detection of common porcine respiratory pathogens by bacterial culture and PCR. Pathogens included in this analysis were porcine reproductive and respiratory syndrome virus (PRRSV), influenza A virus (IAV), porcine circovirus 2 (PCV2), *Actinobacillus* spp. (ACT), *Bordetella bronchiseptica* (BB), *Haemophilus parasuis* (HPS), *Mycoplasma hyopneumoniae* (MHP), *Pasteurella multocida* (PM), *Salmonella* spp. (SAL), and *Streptococcus suis* (SS).

Materials and methods

No animal use approvals were required for this study as all samples used in this investigation were derived from routine diagnostic submissions to the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) between May 10, 2017 and June 30, 2017. The samples used were limited to those cases in which respiratory disease was reported and at least a majority of one lung lobe was submitted such that the main conducting airway could be easily visualized. One hundred fifty-three individual lung samples from 133 unique diagnostic submissions were utilized for this investigation. At the time of initial case processing, a sterile nylon-flocked swab (ESwab, Copan Diagnostics, Inc, Murrieta, California) was introduced into the largest available conducting airway (tracheal bifurcation, primary bronchus, or secondary bronchus) and swabbed 3 to 5 times before removal and placement into the polypropylene screw-cap tube containing 1 mL of Amies transport medium that is provided with each swab. The swab samples were individually labeled, processed, and tested in parallel, yet independently, thus blinded from the corresponding lung tissue from which they were obtained. The lung tissue was then processed routinely by ISU VDL staff for bacterial culture and PCR. Each swab sample was subjected to the same PCR testing and bacterial culture as was requested on the corresponding lung tissue and this testing varied in the context of the specific diagnostic question for each submission. At the completion of the study, PCR for ACT, HPS, or both was performed on the swab samples from all cases where ACT or HPS was recovered by culture of either lung or swab samples.

Lung and swab samples were processed routinely for the detection of PRRSV, IAV, PCV2, MHP, ACT, and HPS nucleic acid

Table 1: Primers and probes used for rPCR reactions for *Mycoplasma hyopneumoniae*, porcine circovirus type 2, *Haemophilus parasuis*, *Actinobacillus pleuropneumoniae*, and *Actinobacillus suis* at the ISU VDL

	Primer, nM	Probe, nM	Internal control	Forward primer	Reverse primer	Probe
MHP	*	*	Xeno	†	†	†
PCV2	400	200	XIPC	TGGCCCGCAGTATTCTGATT	CAGCTGGGACAG-CAGTTGAG	CCAGCAAT-CAGACCCCGTTG-GAATG
HPS	400	200	XIPC	TTACGAGTAGGGCTACAC	CTTCATGGAGTC-GAGTTG	CGCGATTGCATA-CAGAGGGYGAC-GAAGCATCGCG
APP	200	200	Xeno	GGGGACGTAACTCGGTGATT	GCTCACCAACGTTT-GCTCAT	CGGTGCGGA-CACCTATATCT
A suis	400	350	none	GAGCTGGGAAGCTCGACTAT	CCCCCATCTTCAAA-CAGGAT	AGCTAACGACAAG-TAGGGCG

* For each reaction, 0.08 mL VetMAX Primer-probe mix was added.
† Sequence data not supplied.
rPCR = real-time polymerase chain reaction; ISU VDL = Iowa State University Veterinary Diagnostic Laboratory; MHP = *Mycoplasma hyopneumoniae*; PCV2 = porcine circovirus type 2; XIPC = exogenous internal positive control; HPS = *Haemophilus parasuis*; APP = *Actinobacillus pleuropneumoniae*; *A suis* = *Actinobacillus suis*.

by real-time reverse transcriptase polymerase chain reaction (rRT-PCR) for RNA viruses or real-time polymerase chain reaction (rPCR) for DNA virus and bacteria. To extract RNA, the MagMAX Viral RNA Isolation Kit (Thermo Fisher Scientific, Waltham, Massachusetts) and a Kingfisher 96 or Flex instrument (Thermo Fisher Scientific, Waltham, Massachusetts) were used according to manufacturer's instructions. Lung tissue homogenate and processed bronchial swabs were extracted using the standard lysis (SL) procedure with 50 µL of sample added to 130 µL of lysis-binding solution and carrier RNA mixture prepared according to the kit insert, 20 µL magnetic bead mix, and 90 µL of elution buffer. The SL protocol used 150 µL of wash solution I and II provided with the kit. The SL extractions were conducted using the Kingfisher program *AM1836 DW 50 v3* (supplied by Thermo Fisher) with a 5 min pause added at the end of the program to allow the eluate to reach room temperature.

Real-time reverse transcriptase PCR or rPCR was performed on nucleic acid extracts using commercially available reagents for PRRSV (Applied Biosystems TaqMan NA and EU PRRSV Real-Time PCR assay, Thermo Fisher Scientific, Waltham, Massachusetts), IAV (VetMAX-Gold SIV Detection Kit, Thermo Fisher Scientific, Waltham, Massachusetts), and MHP (VetMAX *M hyopneumoniae*, Thermo Fisher Scientific, Waltham, Massachusetts) with proprietary primer and probe information. For both PRRSV and IAV, rRT-PCR setup and thermal cycling conditions were performed according to manufacturer's recommendations. For MHP rPCR, the primer-probe mix was used with the TaqMan Fast 1-Step Master Mix (Thermo Fisher Scientific, Waltham, Massachusetts). Real-time PCR was performed using previously published primers and probes for PCV2[1], *Actinobacillus suis*[2], and *Actinobacillus pleuropneumoniae* (APP).[3] For HPS, an in-house rPCR assay was utilized. See Table 1 for primer and probe sequences.

An exogenous internal positive control (XIPC, based on Schroeder et al[4]) or Xeno RNA (Thermo Fisher Scientific, Waltham, Massachusetts) was included in the extraction (50,000 or 20,000 copies per sample for XIPC or Xeno, respectively) and appropriate primers and probe included in each master mix to monitor PCR amplification and inhibition. Two positive extraction controls, one negative extraction control, and a negative amplification control are also included with each extraction and PCR run.

Each rRT-PCR or rPCR reaction for PCV2, MHP, and HPS was set up using TaqMan Fast 1-Step Master Mix (Thermo Fisher Scientific, Waltham, Massachusetts). The APP PCR utilized the Quanta ToughMix and *A suis* PCR was set up with VetMAX-Plus qPCR Mix (Thermo Fisher Scientific, Waltham, Massachusetts). All these reactions were set up according to manufacturer's recommendations, using 5 µL extracted nucleic acid per reaction. See Table 1 for agent-specific details.

Real-time reverse transcriptase PCR or rPCR for PRRSV, PCV2, MHP, and HPS was performed using an AB 7500 fast thermocycler (Thermo Fisher Scientific, Waltham, Massachusetts) in fast mode with the following cycling conditions: 1 cycle of 50°C for 5 min, 1 cycle of 95°C for 20 sec, and 40 cycles of 95°C for 3 sec and 60°C for 30 sec. Amplification curves were analyzed with commercial thermal cycler system software. Cycling conditions for IAV include 1 cycle of 48°C for 10 min, 1 cycle of 95°C for 10 min, and 40 cycles of 95°C for 15 sec and 60°C for 45 sec. The APP PCR was conducted on a Qiagen RGQ (Qiagen, Germantown, Maryland) with the following cycling conditions: 1 cycle of 95°C for 2 min followed by 40 cycles of 95°C for 10 sec and 56°C for 1 min. For *A suis*, the following thermal cycling profile was utilized: 1 cycle of 95°C for 15 min followed by 45 cycles of 94°C for 15 sec and 60°C for 1 min.

Assays conducted on the AB 7500 Fast instrument used the auto baseline to determine fluorescence baselines and cycle thresholds (Ct) set at 0.1 for all agents except type 1

PRRSV, which was set at 0.05, and IAV, which was set according to the manufacturer's kit insert. For APP samples run on the RGQ instrument, the threshold was set at 0.02. Internal control Xeno or XIPC RNA Ct values were set at 10% of maximum. Paired lung and swab samples were tested separately as previously described under different accession numbers to keep the molecular diagnostics staff blinded to the pairing and to prevent any potential reporting bias. The number of pairs tested for each assay was as follows: PRRSV (111 pairs), IAV (118 pairs), MHP (49 pairs), and PCV2 (24 pairs).

For bacterial culture, all lung and swab samples were plated onto 5 different agar plates and atmospheric conditions for isolation of pathogens associated with respiratory disease including the 6 bacteria of interest in the study (ACT, BB, HPS, PM, SAL, and SS). The 5 plates included (1) blood agar (2% agar) with a *Staphylococcus* nurse, incubated with 5% CO_2, (2) blood agar (4% agar) with a *Staphylococcus* nurse, incubated with 5% CO_2, (3) blood agar incubated anaerobically, (4) Hektoen enteric agar incubated at normal atmosphere, and (5) Tergitol-7 agar incubated at normal atmosphere (all media, Thermo Fisher Scientific, Lenexa, Kansas). All plates were incubated at 35°C for a minimum of 48 hours. Identification of pathogens was done via matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry following standard laboratory protocol. A minimum MALDI-TOF confidence score of 2.10 was required for a confirmatory identification. The 153 paired lung and swab samples were cultured separately under different accession numbers to keep the bacteriology staff blinded to the pairing and to prevent any potential interpretation bias.

Statistical analyses were performed using a commercial statistical software package (JMP Pro 11, SAS Institute, Cary, North Carolina). A kappa coefficient was calculated to determine the degree of agreement for detection of each pathogen between sample types and coefficients are reported and interpreted as follows: values > 0.8 indicate excellent agreement, values ≤ 0.8 and > 0.6 indicate substantial agreement, values ≤ 0.6 and > 0.4 indicate moderate agreement, and values ≤ 0.4 indicate no better than fair agreement.[5] A McNemar test was used to determine if sample type specifically contributed to disagreement. A Wilcoxon signed rank test was used to compare differences in PCR Ct values between sample types. For all tests, $P < .05$ was considered significant.

Results

Contingency tables summarizing the results of PCR testing for PRRSV, IAV, MHP, and PCV2 are presented in Table 2. For all four assays, there was excellent agreement between the results of detection from lung tissue and swab samples (kappa > 0.8, all assays). While neither sample type was statistically associated with any observed disagreement, the results of disagreement analysis approached significance ($P = .08$) for PRRSV and MHP with swab samples being occasionally positive when tissue samples were negative while the reverse did not occur.

Mean PCR Ct values for PRRSV, IAV, MHP, and PCV2 and associated differences between results from lung tissue and swab samples are summarized in Table 3. For PRRSV, Ct values were lower in lung samples compared to swabs ($P = .02$) while MHP Ct values were lower in swabs than in lung samples ($P = .002$). Differences in Ct values were not detected for IAV or PCV2 PCR ($P > .05$).

Contingency tables reflecting the results of bacterial culture are presented in Table 4. For the six species of bacteria included in this analysis, there was substantial agreement between detection from lung tissue and swab samples for three (ACT, PM, and SAL), fair agreement for one (BB), and poor

Table 2: Contingency tables for results of PCR assays applied to conducting airway swabs and lung tissue homogenates from the same tissue sample submission

	Swab, positive	Swab, negative	Kappa statistic (SE; 95% CI)*	McNemar test†
PRRSV				
Lung, positive	40	0	0.94 (0.03; 0.87-1)	0.08
Lung, negative	3	68		
IAV				
Lung, positive	27	3	0.85 (0.06; 0.73-0.96)	0.71
Lung, negative	4	84		
PCV2				
Lung, positive	7	1	0.90 (0.09; 0.71; 0.71-1)	0.32
Lung, negative	0	16		
MHP				
Lung, positive	18	0	0.87 (0.07; 0.73-1)	0.08
Lung, negative	3	28		

* Values > .8 indicate excellent agreement.
† Values < .05 were considered significant.
PCR = polymerase chain reaction; PRRSV = porcine reproductive and respiratory syndrome virus; IAV = influenza A virus; PCV2 = porcine circovirus type 2; MHP = *Mycoplasma hyopneumoniae*.

Table 3: Comparison of differences in PCR Ct values for assays applied to conducting airway swabs and lung tissue homogenates from the same tissue sample submission

	Mean Ct from lung	Mean Ct from swab	Mean difference (SE)	Correlation	*P* value*
PRRSV	32.11	32.64	-0.53 (0.22)	0.95	.02
IAV	34.8	34.56	0.24 (0.26)	0.90	.36
PCV2	32.54	33.66	-1.12 (0.52)	0.95	.11
MHP	33.78	32.59	1.19 (0.39)	0.88	.002

* Wilcoxon signed rank test for matched pairs; $P < .05$ are considered significant.
PCR = polymerase chain reaction; Ct = cycle threshold; PRRSV = porcine reproductive and respiratory syndrome virus; IAV = influenza A virus; PCV2 = porcine circovirus type 2; MHP = *Mycoplasma hyopneumoniae*.

agreement for the remaining two (HPS and SS). Additionally, only fair agreement was observed between sample types for the reporting of 'no significant growth' by the laboratory. Disagreement analysis revealed a statistically significant association between sample type and detection for ACT and HPS ($P = .046$ and .03, respectively) with lung tissue being culture positive while swabs were culture negative more often than the reverse. A similar trend was observed for PM with results approaching significance ($P = .08$). For BB, there was also an association between sample type and detection ($P = .046$) but with swabs being culture positive while lung tissue was negative more often than the reverse.

For all cases where ACT or HPS was recovered by culture of either lung or swab samples, PCR was positive for the cultured organism (10 ACT and 17 HPS) even when the swab had been culture negative. For HPS there was no difference in mean PCR Ct by culture status with culture positive swabs having a mean Ct of 16.88 ± 3.99 and culture negative swabs a mean Ct of 16.58 ± 2.29. For ACT, mean PCR Ct values also did not differ ($P > .05$) with culture positive swabs having a mean Ct of 21.70 ± 6.42 and negative swabs a mean Ct of 23.88 ± 7.25.

Discussion

Overall there was excellent agreement between lung tissue and swab samples for the PCR assays tested indicating that swabs can be a reliable alternative sample for routine PCR detection of these agents in swine. This sample type should also be effective for use in multiplex PCR assays targeting PRRSV, IAV, MHP, and PCV2 and there is a critical need for the development and implementation of such assays in routine veterinary diagnostics to reduce turnaround time and costs. In sufficiently large-sized animals, collection of postmortem swab samples from the tracheal bifurcation or large conducting airways from affected lobes can easily be standardized and incorporated into field personnel training such that a consistent sample is provided to the laboratory and can reduce shipping costs associated with large volumes of tissue. Submission of formalin-fixed lung sections from any abnormal lung tissue in tandem with bronchial swabs would differentiate mere agent detection from agent-associated disease, that is, associate what may otherwise be an endemic agent with a lesion to have greater confidence that the agents detected are truly causing disease.

Interestingly, for both PRRSV and MHP PCR, there were a few cases where swab samples were positive while tissue homogenates were negative. Moreover, there were no instances where swabs were negative while tissues were positive suggesting swabs from conducting airways may be a more sensitive sample for detection of these pathogens. This is consistent with previous reports for MHP, where tracheobronchial samples were preferred for detection[6,7]; however, additional testing of a larger sample set is warranted to further explore this observation for PRRSV. It is worth emphasizing that the focus of this study was for pathogen detection in diseased tissue and not merely agent detection. The PCR Ct values were also significantly lower in swab samples for MHP which further supports that swabs collected from the primary bronchi are of higher diagnostic sensitivity than lung tissue samples for the diagnosis of enzootic pneumonia. This aligns with historic recommendations to include large conducting airways in fresh tissue sections submitted for MHP testing.[8]

For bacterial isolation, there was lower agreement between lung tissue culture and culture of swabs collected from primary bronchi. This is not entirely unexpected given that microbial culture requires organism viability and there are likely differences between maintenance in a transport medium such as Amies medium and lung parenchyma. This was particularly an issue for the more fastidious organisms ACT and HPS, which were recovered more frequently from lung tissue. This is consistent with a recent study where culture for HPS was a more sensitive assay for detection than direct PCR and lung was a preferred sample type.[9] In the present study, PCR of bronchial swabs for ACT and HPS was 100% sensitive for detecting cases where either organism was recovered by culture of lung tissue or swab samples suggesting PCR testing for these agents should be performed in parallel with culture when lesions suggest these agents are involved and only swab samples are tested. Common colonizing bacteria (BB and SS) were readily recovered from both sample types and with fair to poor agreement indicating that culture results from either sample type should be interpreted in the context of any observed gross and microscopic lesions. As with any endemic pathobiont, while the organism may not be active in the individual animal sampled, its presence remains a risk factor for the population and increases the likelihood that other animals may have clinical infections with these agents.

Limitations of this study include the use of routine diagnostic samples, which can vary in their preservation and handling prior to analysis and potential biases impacting the kappa statistic when determining agreement. The use of diagnostic samples replicates field conditions and thereby reflects the applicability of results to practitioners; however,

Table 4: Contingency tables for results of bacterial culture applied to conducting airway swabs and lung tissue homogenates from the same tissue sample submission

	Swab, positive	Swab, negative	Kappa statistic (SE; 95% CI)*	McNemar test†
Actinobacillus spp.				
Lung, positive	6	4	0.74 (0.13; 0.49 to 0.98)	0.046
Lung, negative	0	143		
Bordetella bronchiseptica				
Lung, positive	9	4	0.47 (0.11; 0.26 to 0.69)	0.046
Lung, negative	12	128		
Haemophilus parasuis				
Lung, positive	0	13	-0.04 (0.02; -0.07 to -0.01)	0.03
Lung, negative	4	137		
Pasteurella multocida				
Lung, positive	19	9	0.71 (0.08; 0.56 to 0.87)	0.08
Lung, negative	3	122		
Salmonella spp.				
Lung, positive	5	1	0.76 (0.13; 0.5 to 1)	0.56
Lung, negative	2	145		
Streptococcus suis				
Lung, positive	26	19	0.33 (0.08; 0.17 to 0.49)	0.37
Lung, negative	25	83		
No significant growth				
Lung, positive	44	23	0.46 (0.07; 0.32 to 0.61)	0.34
Lung, negative	17	69		

* Kappa values > 0.8 indicate excellent agreement, values ≤ 0.8 and > 0.6 indicate substantial agreement, values ≤ 0.6 and > 0.4 indicate moderate agreement, and values ≤ 0.4 indicate no better than fair agreement.

† Values < .05 are considered significant.

this also increases potential for variation in PCR Ct values from testing on different PCR plates and on different days. Variation between plates is continuously monitored at the ISU VDL through quality management software and by maintaining statistical process control charts that require Ct values of positive controls to remain within specified limits. For the kappa statistic, results are not interpretable when there is significant disagreement detected by the McNemar test as with ACT, BB, and HPS in this report. Additionally, the kappa statistic becomes unstable when prevalence is below 20% or above 80%.[5] Low prevalence may have impacted the agreement analysis of several bacteria in this study (ACT, BB, HPS, and SAL).

Taken together the results of this study show that swabs with Amies transport medium provide a single uniform sample that can be easily collected at postmortem and transported to the laboratory for detection of common swine respiratory pathogens. This dual-use sample has enough fluid for multiple PCR assays and the swab itself is used for culture of bacterial agents. Swabs are also easily adapted to automated bacterial culturing systems.[10] For detection of fastidious bacteria such as HPS and ACT, either PCR from tracheobronchial swabs or culture from lung tissue are appropriate and similar in sensitivity; however, lung tissue remains a preferred sample for cultural confirmation of those agents. Submission of formalin-fixed tissue in parallel with tracheobronchial swabs remains an important practice to differentiate the presence of an agent from an actual causal role in disease.

Implications

- Tracheobronchial swabs are an effective sample for detecting PRRSV, IAV, PCV2, MHP, ACT and HPS by PCR.
- For MHP, tracheobronchial swab samples are more sensitive than lung tissue.
- For common colonizing bacteria such as BB and SS, tracheobronchial swabs are often positive when lung tissue is negative.
- Fastidious bacteria, such as ACT and HPS, are more reliably cultured from lung tissue than from tracheobronchial swabs, therefore parallel application of both culture and PCR is recommended to detect these pathogens from swab samples.

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Conflict of interest

None reported.

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Reduced mortality and morbidity associated with verotoxin 2e-induced edema disease in pigs using a recombinant verotoxin 2e vaccine

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Summary

Objective: The efficacy of Vepured, a recombinant verotoxin 2e (VT2e) vaccine, against clinical signs and mortality of VT2e-induced toxemia was evaluated in a controlled experimental challenge.

Materials and methods: Piglets free of VT2e neutralizing antibodies (NAb) were selected and blocked by weight and litter and randomly allocated between three groups: vaccinated (n = 32); non-vaccinated (n = 32); and non-vaccinated, non-challenged (n = 10). Piglets were vaccinated intramuscularly with 1 mL of Vepured (vaccinated) or phosphate-buffered saline (non-vaccinated) at two days of age. The onset and duration of protection were investigated via intravenous VT2e challenge, using mortality and clinical signs related to VT2e-induced toxemia.

Results: Mortality in the non-vaccinated piglets was 92.3% and 68.8% at the onset of immunity and through the experiment duration, respectively, whereas all vaccinated piglets survived the challenge. The total clinical score and percentage of animals with clinical signs were greater ($P < .05$) in the non-vaccinated group. Also, vaccinated pigs had better growth performance than non-vaccinated pigs.

Neutralizing antibodies against VT2e were detected in most (78.6%) vaccinated piglets at 21 days and in all vaccinated piglets at 28 days and mean NAb titers (log2) were 3.9 and 4.3, respectively. Moreover, NAb persisted for at least 112 days in most (94.1%) vaccinated animals (mean NAb titer was 3.8).

Implications: In this study, active immunization with Vepured conferred effective protection against VT2e-induced toxemia, reducing the presence and severity of clinical signs and preventing mortality related to VT2e-induced toxemia from 21 to 112 days after vaccination.

Keywords: swine, edema disease, vaccine, verotoxin, Vepured

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Resumen – Mortalidad y morbilidad reducida asociadas con la enfermedad del edema en cerdos inducida por verotoxina 2e utilizando una vacuna de verotoxina 2e recombinante

Objetivo: En un desafío experimental controlado, se evaluó la eficiencia del Vepured, una vacuna de verotoxina 2e recombinante (VT2e por sus siglas en inglés), contra los signos clínicos y la mortalidad de la toxemia provocada por la VT2e.

Materiales y métodos: Se seleccionaron lechones libres de anticuerpos neutralizantes (NAb por sus siglas en inglés) contra VT2e y se organizaron en bloques por peso y camada, y se distribuyeron al azar en tres grupos: vacunados (n = 32); no vacunados (n = 32); y no vacunados, no desafiados (n = 10). A los dos días de edad, los lechones se vacunaron intramuscularmente con 1 mL de Vepured (vacunado) o con solución salina tamponada con fosfato (no vacunado). Se investigaron el inicio y la duración de la protección por medio de un desafío intravenoso de VT2e, midiendo signos clínicos y mortalidad relacionados con la toxemia inducida por VT2e.

Resultados: La mortalidad en los lechones no vacunados fue, respectivamente, de 92.3% y 68.8% durante el inicio de la inmunidad y en el experimento de duración de la inmunidad, respectivamente, mientras que todos los lechones vacunados sobrevivieron a la prueba. La puntuación clínica total y el porcentaje de animales con signos clínicos fue mayor ($P < .05$) en el grupo no vacunado. Así mismo, los cerdos vacunados tuvieron un mejor desempeño de crecimiento comparado con los cerdos no vacunados.

Se detectaron anticuerpos neutralizantes contra la VT2e en la mayoría (78.6%) de los cerdos vacunados a los 21 días y en todos los lechones vacunados a los 28 días, la media de los títulos NAb (log2) fue de 3.9 y 4.3, respectivamente. Además, los NAb persistieron por lo menos 112 días en la mayoría (94.1%) de los animales vacunados (la media de los NAb fue de 3.8).

Implicaciones: En este estudio, la inmunización activa con Vepured confirió protección efectiva contra la toxemia inducida por VT2e, reduciendo la presencia y severidad de los signos clínicos y previniendo la mortalidad relacionada con la toxemia inducida por VT2e entre 21 y 112 días después de la vacunación.

Résumé – Réduction de la mortalité et de la morbidité associées avec la vérotoxine 2e induisant la maladie de l'œdème chez des porcs en utilisant un vaccin recombinant de la vérotoxine 2e

Objectif: Évaluer l'efficacité de Vepured, un vaccin recombinant de la vérotoxine 2e (VT2e), à réduire les signes cliniques et la mortalité associés à une toxémie induite par VT2e dans un challenge expérimental contrôlé.

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Mallorquí J, Simon-Grifé M, Ferrer-Soler L, et al. Reduced mortality and morbidity associated with verotoxin 2e-induced edema disease in pigs using a recombinant verotoxin 2e vaccine. *J Swine Health Prod.* 2018;26(5):253-261.

Matériels et Méthodes: Des porcelets exempts d'anticorps neutralisants (AcN) contre VT2e ont été sélectionnés et bloqués en fonction du poids et de la portée et distribués de manière aléatoire en trois groupes: vaccinés (n = 32); non-vaccinés (n = 32); non-vaccinés, non-challengés (n = 10). Les porcelets étaient vaccinés par voie intramusculaire avec 1 mL de Vepured (vacciné) ou de la saline tamponnée (non-vacciné) à 2 jours d'âge. Le début et la durée de la protection étaient étudiés par challenge intraveineux avec VT2e, se servant de la mortalité et des signes cliniques associés à une toxémie induite par VT2e.

Résultats: La mortalité chez les porcelets non-vaccinés était de 92.3% et 68.8% au début de l'immunité et au long de la durée de l'expérience, respectivement, alors que les porcelets vaccinés ont survécu au challenge. Le pointage clinique total et le pourcentage des animaux avec des signes cliniques étaient supérieurs ($P < .05$) dans le groupe des animaux non-vaccinés. De plus, les porcs vaccinés avaient des meilleures performances de croissance que les porcs non-vaccinés.

Des AcN contre VT2e ont été détectés dans la majorité (78.6%) des porcelets vaccinés à 21 j d'âge et chez tous les porcelets vaccinés à 28 j d'âge et les titres d'AcN moyens (log2) étaient 3.9 et 4.3, respectivement. De plus, les AcN ont persisté pour au moins 112 j dans la plupart (94.1%) des animaux vaccinés (titre AcN moyen était de 3.8).

Implications: Dans la présente étude, l'immunisation active avec Vepured a conféré une protection efficace contre une toxémie induite par VT2e, réduisant la présence et la sévérité des signes cliniques et prévenant la mortalité reliée à la toxémie causée par VT2e entre les jours 21 et 112 après la vaccination.

Edema disease (ED) is an enterotoxemia caused by certain *Escherichia coli* colonizing the small intestine and producing verotoxin (VT2e). The toxin, also known as Stx2e, is absorbed from the intestine into the bloodstream and damages endothelial cells.[1] The endothelial cell damage induces an increase in vascular endothelium permeability of the blood vessels resulting in edema in target tissues such as the brain, intestine, eyelids, lungs, kidneys, and spleen.[1] Edema disease is mainly observed in recently weaned piglets, although it can also be observed during the growing and finishing phases.[1] Clinical signs associated with ED are mainly neurological: ataxia, convulsions, paralysis, and rigidity as a consequence of edema in the nervous system.[2] Experimentally, intravenous injection of pigs with VT2e reproduced clinical signs of ED.[3,4] Swelling of the eyelids and throat and dyspnea may also appear in pigs affected by ED.[3,5,6] Average morbidity of ED is 10% to 30% and the associated mortality ranges from 50% to over 90%.[1]

Traditional control of ED is mainly the use of antimicrobial therapy however, its efficacy is poor because VT2e has already been absorbed into circulation when clinical signs become apparent.[1] Furthermore, with ongoing international pressure to decrease antibiotic use in agriculture due to its perceived link with increasing antibiotic resistance,[7,8] an efficacious vaccine is required to induce a protective immune response against this disease.

Therefore, immunoprophylaxis seems to be a promising approach. Active immunity against an intravenous challenge with VT2e toxoid, prepared with glutaraldehyde or formaldehyde, was shown to be efficacious in previous studies.[9,10] However, these vaccine candidates were not safe because of residual toxicity.[9,10] Conversely, recombinant VT2e vaccines have proved to induce protection against ED and also proved safe due to no residual toxicity both in challenge experiments and in field trials.[4,11-13] In the present study, we evaluated the protection induced in piglets against VT2e-induced toxemia by a recombinant VT2e vaccine. The onset of immunity (OOI) and the duration of immunity (DOI) against VT2e-induced toxemia were studied.

Materials and methods

All procedures involving animals were conducted in accordance with the European Union Guidelines for Animal Welfare (Directive 2010/63/UE) and approved by the Ethical Committee of HIPRA Scientific SLU and The Department of Agriculture, Livestock, Fisheries and Food of the Catalonia Government (file: 8294).

VT2e toxin production

The VT2e toxin used in the seroneutralization assays and in animal challenges was prepared from *E coli* strain 107/86 (O139:K12:H1).[14,15] *Escherichia coli* 107/86 was cultured in Bacto Tryptic Soy Broth (Becton Dickinson, Le Pont-de-Claix, France) with Bacto Yeast Extract (Becton Dickinson, Erembodegem, Belgium) medium at 37°C for 3 hours. Bacterial cells were then collected by centrifugation (8000*g*, 20 minutes) and resuspended in phosphate-buffered saline (PBS; Sigma-Aldrich Company Ltd, Dorset, England) with Polymyxin B (1×10^4 IU/mL; Xellia, Copenhagen, Denmark). After incubation at 37°C for 1 hour, the supernatant was recovered by centrifugation (11,500*g*, 30 minutes), filtered, and stored at -20°C.

The 50% cytotoxic dose (CD_{50}) of VT2e was 3.1×10^5 CD_{50}/mL, evaluated in vitro using Vero cells according to the procedure published by Gentry and Dalrymple.[16]

Test product

One milliliter of Vepured vaccine (Laboratorios HIPRA S.A., Amer, Spain) contains 600 ELISA Units of Antigenic Mass of purified recombinant verotoxin 2e adjuvanted with 2117 mg of aluminum hydroxide and 10 mg of diethylaminoethyl-dextran hydrochloride.[17]

Experimental design

The piglets included in this study were obtained from a commercial farm located in Catalonia, Spain. The farm was considered free of ED because it did not have a history of ED outbreaks, the animals did not present ED clinical signs, and verotoxigenic *E coli* was not found in the feces as analyzed by bacteriological diagnosis. One week before the trial, blood samples were collected from 15 sows to confirm that they did not have VT2e neutralizing antibodies (NAb). Seventy-four piglets from VT2e NAb-free sows were selected and blocked by weight and litter and assigned into 3 groups: vaccinated (n = 32); non-vaccinated (n = 32); and non-vaccinated, non-challenged (sentinel, n = 10). Between each block group, the average weight was equivalent and piglets from each litter were represented in each group. Blood samples were obtained from these piglets before vaccination to confirm that they did not have VT2e NAb on day 0 of the study.

Piglets were weaned at approximately 21 days of age and raised on the commercial farm until the challenge period when they were moved to the experimental isolation unit of the HIPRA Scientific SLU facilities.

Piglet immunization and challenge

Vaccinated piglets were injected intramuscularly at two days of age with 1 mL of Vepured (batch number: P.86YG). Non-vaccinated piglets received 1 mL of PBS intramuscularly. To evaluate the safety of Vepured vaccine, the piglets were monitored daily during the post-vaccination period for local clinical signs such as inflammation and nodules and general clinical signs including edema and neurological signs related to ED.

The OOI experiment was conducted 21 days after treatment administration, when 14 piglets from the vaccinated group (one piglet died before the challenge) and 13 piglets from the non-vaccinated group (two piglets died before the challenge) were given VT2e toxin (4.7×10^4 CD_{50}/kg) intravenously. The DOI experiment was conducted 112 days after treatment administration, when an additional 17 piglets from the vaccinated group and 16 piglets from the non-vaccinated group (one piglet died before the challenge) were given VT2e toxin (6×10^3 CD_{50}/kg) intravenously. In the DOI experiment, piglets were anaesthetized intramuscularly with 0.2 mL/kg of a mixture of Xilagesic (Calier, Barcelona, Spain) and Zoletil 100 (Virbac, Barcelona, Spain) before the challenge to ensure the correct administration of VT2e toxin intravenously. Piglets from the sentinel group were distributed equally to each experiment (Table 1). All piglets were observed three times the day of the VT2e toxin challenge and twice a day for 7 days thereafter.

The experiment was carried out under blinded conditions, as staff involved in the animal experimental phase, specifically those who performed clinical evaluations, were not aware of the treatment received by each individual animal. During the post-challenge period, clinical signs were scored depending on their severity. Palpebral and throat edema, tremors, ataxia, or mild dyspnea were scored as 1 (mild clinical signs). The presence of paralysis, opisthotonos, extensor rigidity, or severe dyspnea were scored as 2 (severe clinical signs). The total clinical signs score was calculated for each animal as the ratio between the summation of the daily clinical signs score and the number of days for which the animal lived. During the post-challenge period, piglets were euthanized for ethical reasons with an intravenous overdose of sodium pentobarbital *ante finem* after showing paralysis or opisthotonos at two consecutive observations. Before euthanasia, pigs were anaesthetized intramuscularly with 0.2 mL/kg of a mixture of Xilagesic and Zoletil 100.

Blood samples were collected before vaccination (day 0), before the challenge (OOI = day 21; DOI = day 112) and at the end of the study (7 days after challenge) to evaluate the presence of VT2e NAb. In the DOI experiment, blood samples were collected periodically between vaccination and challenge (days 28, 56, 84, 98, and 105 post vaccination) to follow the antibody response evolution. Additionally, pigs from the DOI experiment were weighed immediately before the challenge and at the end of the study.

Bacteriological isolation

To identify verotoxin-producing *E coli*, rectal swabs were inoculated into 5 mL peptone water and incubated overnight at 37°C. Samples were then cultured on blood agar plates (Becton Dickinson, San Agustin de Guadalix, Spain) and on MacConkey agar plates (Becton Dickinson, Heidelberg, Germany) and incubated overnight at 37°C. The blood agar plates were used to evaluate the presence of hemolytic *E coli* in the samples. The MacConkey agar plates were used to confirm the presence of virulence factors related to VT2e producing *E coli* (adhesion F18 and toxin VT2e genes) by polymerase chain reaction.[18]

Seroneutralization assay

Fresh Vero cells were counted, suspended to 4×10^4 cells/mL in Glasgow Minimum Essential Medium (GMEM; Gibco, Paisley, United Kingdom), and 0.1 mL were pipetted into 96-well microtiter plates. Monolayers were established by 24 h of incubation at 37°C in 5% CO_2.

Serial two-fold dilutions of each serum sample, starting with a 1:2 dilution, were prepared using medium with 2% antibiotics (Penicillin 1×10^4 IU/mL and Streptomycin 10mg/mL; Gibco, Paisley, United Kingdom).

Table 1: Experimental design used to assess the onset and duration of immunity conferred by Vepured against VT2e-induced toxemia

Experiment	Treatment*	Age at vaccination, d	No. of pigs at vaccination	Toxin challenge dose, CD_{50}/kg	Age at challenge, d	No. of pigs on challenge day
OOI	Vaccinated	2	15	4.7×10^4	23	14†
	Non-vaccinated		15	4.7×10^4	23	13†
	Sentinel		5	None	NA	5
DOI	Vaccinated	2	17	6×10^3	114	17
	Non-vaccinated		17	6×10^3	114	16‡
	Sentinel		5	None	NA	5

* Vaccinated piglets were injected intramuscularly with 1 mL of Vepured, non-vaccinated piglets were injected intramuscularly with 1 mL of phosphate-buffered saline; sentinel pigs were non-injected and non-challenged.

† Three pigs (one from the vaccinated group and two from the non-vaccinated group) from the OOI experiment died post vaccination and prior to the challenge. Necropsy was performed on these animals to establish the cause of death. The deaths were not related to vaccination. The vaccinated piglet died due to severe anorexia and growth retardation. Intestinal lesions such as congestive small intestine with gas-hemorrhagic content were observed in one piglet from the non-vaccinated group. The other piglet from the non-vaccinated group died due to septicemia and paralysis resulting from tail docking.

‡ On day 112, one animal from the non-vaccinated group died before the challenge as a consequence of anesthesia. This animal had a severe cough on the morning of day 112 and the necropsy revealed macroscopic pneumonia lesions.

VT2e = verotoxin 2e; OOI = onset of immunity; DOI = duration of immunity; NA = not applicable.

Sixty microliters of diluted serum samples were added to a 96-well microtiter plate with 60 μL of VT2e toxin solution (adjusted to 20 CD_{50}/mL in GMEM) or with 60 μL of GMEM. Negative (60 μL of GMEM and 60 μL of GMEM with 2% antibiotics) and positive (60 μL of VT2e toxin solution and 60 μL of GMEM with 2% antibiotics) controls were included on each plate. Diluted serum samples and controls were incubated at 37°C in 5% CO_2 for 1 hour.

After incubation, 100 μL of diluted serum samples and controls were added to Vero cell monolayers and incubated at 37°C for 24 hours in 5% CO_2. All serum samples and controls were plated in duplicate.

Supernatant was removed and the wells were washed twice with water (0.2 mL/well). The remaining cells were fixed by adding 37% formaldehyde (0.2 mL/well; Merck, Darmstadt, Germany) for 3 min. Formaldehyde was removed and the plates were stained with crystal violet (0.2 mL/well; Merck, Darmstadt, Germany) for 20 min. Excess stain was removed by rinsing with water and the stain was eluted with ethanol (0.1 mL/well; Panreact, Darmstatd, Germany). Absorbance was determined at 595 nm in a spectrophotometer.

Using optical density (OD), the percentage of live cells was calculated for the positive control (average of positive control OD/average negative control OD × 100). The percentage of live cells was calculated for each diluted serum sample (average OD of toxin-diluted serum sample/average OD of GMEM-diluted serum sample ×100).

The NAb titer was defined as the reciprocal of the highest serum dilution that neutralized 50% of the cytopathic effect of the VT2e toxin used in the assay. A serum dilution neutralized at least 50% of the cytopathic effect of the VT2e when the percentage of live cells was greater than the percentage of live cells in the positive control + ([100 – percentage of live cells in the positive control]/2). Sera with a NAb titer equal or greater than 2 were considered positive for VT2e NAb, while sera with a NAb antibody titer less than 2 were considered negative for VT2e NAb.

Statistical analysis

Data analyses were conducted using the IBM SPSS Statistics 22 software (IBM Corp, Armonk, New York). The total clinical signs score observed after the challenge in the vaccinated groups were compared with the control group using a Kruskall-Wallis test. The percentage of animals with clinical signs after the challenge and mortality observed in the vaccinated groups were compared with the control group using the Chi-square test. Analysis of variance with a post-hoc Scheffé test was used to compare body weight between groups. A significance level of $P < .05$ was used for all variables evaluated.

The NAb titers were transformed to base 2 logarithms to calculate the means and confidence intervals.

Results

Safety of Vepured

No evidence of clinical signs or local reactions caused by the intramuscular administration of Vepured to two-day old piglets negative for VT2e NAb were observed.

Protection against VT2e-induced toxemia after OOI

Antibody response. On day 21 of the study, the majority (11 of 14) of piglets from the vaccinated group had NAb against VT2e, with a 3.9 mean NAb titer. Seven days after the challenge, all vaccinated piglets were positive for VT2e NAb and the mean NAb titer increased to 8.4. The animals from the non-vaccinated group were negative for VT2e NAb at day 21 and the sole surviving animal was negative for VT2e NAb at the end of the study. Animals from the sentinel group remained negative for VT2e NAb throughout the study (Table 2).

Mortality. In the non-vaccinated group, rapid disease progression was observed, with 92.3% (12 of 13) mortality within three days post challenge. Four of the piglets were euthanized *ante finem* and the others died after showing mild or severe clinical signs of VT2e-induced toxemia. In contrast, there was 0% mortality observed in vaccinated piglets after the challenge (Figure 1A).

Clinical signs. The onset of clinical signs of VT2e-induced toxemia, such as palpebral and throat edema were observed at 5 hours after challenge in both vaccinated and non-vaccinated groups. During the 7 days post challenge, all piglets from the non-vaccinated group showed clinical signs related to VT2e-induced toxemia and most of them (8 of 13) showed severe clinical signs. In fact, there was 100% mortality of piglets that demonstrated severe clinical signs. In contrast, none of the vaccinated piglets showed severe clinical signs associated with VT2e-induced toxemia. Only 8 of 14 vaccinated piglets showed mild clinical signs related to VT2e-induced toxemia and only 2 of 14 showed clinical signs 7 days post challenge. Clinical signs associated with VT2e-induced toxemia were not observed in piglets from the sentinel group (Figure 2A and B). The total clinical score was greater ($P < .05$) in the non-vaccinated group than in the vaccinated group (Figure 2C). Additionally, the percentage of animals with clinical signs related to VT2e-induced toxemia was greater ($P < .05$) in the non-vaccinated group (100%) compared to the vaccinated group (57.1%; Figure 2D).

Duration of immunity against VT2e-induced toxemia

Antibody response. At 112 days post vaccination, most piglets (16 of 17) remained positive for VT2e NAb (Table 2). The mean NAb titers remained persistent from day 28 to 112 post vaccination, fluctuating from 3.8 to 4.3. However, after the DOI challenge (119 days after vaccination), the mean NAb titers increased from 3.8 to 6. The animals from the non-vaccinated group and the sentinel group remained negative for VT2e NAb throughout the study (Table 2).

Mortality. Rapid disease progression was observed in the non-vaccinated group, with 68.8% (11 of 16) mortality within 4 days post challenge. Nine pigs from the non-vaccinated group were euthanized *ante finem* after showing severe clinical signs related to VT2e-induced toxemia during two consecutive observations. In contrast, there was 0% mortality observed in the vaccinated group post challenge (Figure 1B).

Clinical signs. The onset of clinical signs of VT2e-induced toxemia in the non-vaccinated group was observed 24 hours post challenge. Thirteen of 16 pigs from this group showed clinical signs related to VT2e-induced toxemia during the post-challenge period and in most of them (10 of 13), the clinical signs were severe. In contrast, none of the 17 vaccinated piglets showed severe clinical signs during the 7 days post challenge. Only one of the vaccinated piglets developed mild clinical signs associated with VT2e-induced toxemia from days 3 to 5 post challenge. Clinical signs associated with VT2e-induced toxemia were not observed

Table 2: Presence of VT2e neutralizing antibodies after vaccination with Vepured*

Experiment	Day of study	Vaccinated		Non-vaccinated
		Pigs positive for NAb, No. (%)	NAb titer, mean (95% CI)†	Pigs positive for NAb, No. (%)
OOI	0	0 (0)	0	0 (0)
	21	11 (78.6)	3.9 (2.5-5.3)	0 (0)
	28	14 (100)	8.4 (7.6-9.3)	0 (0)
DOI	0	0 (0)	0	0 (0)
	28	17 (100)	4.3 (3.2-5.4)	0 (0)
	56	17 (100)	4.8 (3.8-5.9)	0 (0)
	84	17 (100)	4.8 (4.0-5.6)	0 (0)
	98	17 (100)	4.2 (3.5-5.0)	0 (0)
	105	16 (94.1)	3.8 (3.1-4.5)	0 (0)
	112	16 (94.1)	3.8 (3.1-4.5)	0 (0)
	119	17 (100)	6.0 (4.7-7.3)	0 (0)

* All pigs (5 animals in each experiment) in the sentinel non-vaccinated, non-challenged group remained negative for neutralizing antibodies against VT2e throughout the study.

† Antibody titers are expressed as log2 value of the reciprocal of the highest dilution causing neutralization.

VT2e = verotoxin 2e; NAb = neutralizing antibody; OOI = onset of immunity; DOI = duration of immunity.

in piglets from the sentinel group (Figure 3A and B). The total clinical score was greater ($P < .05$) in the non-vaccinated group than in the vaccinated group (Figure 3C). Additionally, the percentage of animals with clinical signs related to VT2e-induced toxemia was greater ($P < .05$) in the non-vaccinated group (81.3%) compared to the vaccinated group (5.8%; Figure 3D).

Weight gain. At the end of the study, the body weight of vaccinated pigs was greater than that of non-vaccinated pigs ($P < .05$). Seven days after challenge with VT2e toxin, vaccinated pigs had gained an average of 4.7 kg whereas non-vaccinated pigs that survived the challenge had lost an average of 8.3 kg. No statistical differences were observed between the vaccinated and the sentinel groups (Table 3).

Discussion

In the present study, the efficacy of Vepured was evaluated in laboratory conditions by means of an intravenous VT2e toxin challenge with the intent to demonstrate the reduction of morbidity and mortality attributed to VT2e-induced toxemia. The challenge model used produced clinical signs typically associated with ED. It is expected that this vaccine will protect against outbreaks of ED. However, further large-scale clinical trials on commercial pig farms are needed to confirm the efficacy of Vepured against ED under natural field conditions.

The clinical signs of ED are most commonly observed in the first weeks after weaning. For this reason, early vaccination of piglets and a rapid onset of immunity after weaning are required. In the OOI experiment, two-day old piglets were immunized and were challenged with wild-type VT2e 21 days later. The results support that Vepured vaccination induced an immune response within 21 days that reduced morbidity and mortality associated with VT2e-induced toxemia. In contrast, all but one pig in the non-vaccinated group died after the challenge. Clinical signs of ED can also appear in the fattening herd, resulting in morbidity, mortality, and affecting pig performance.[1] The DOI study suggested that one vaccine dose was able to protect piglets in the fattening period, for at least 112 days after vaccination, reducing morbidity and mortality associated with VT2e-induced toxemia. Previous studies have demonstrated that vaccination protected pigs from weight loss associated with VT2e toxemia.[10,11,19] In the present DOI experiment, body weight before the challenge was equivalent in both the vaccinated and non-vaccinated groups. However, after the challenge, pigs from the vaccinated group performed better than those from the non-vaccinated group.

The administration protocol of Vepured to two-day old piglets could allow a farmer to incorporate this vaccination with other routine management practices, such as iron administration. Additionally, the small volume of vaccine (1 mL) is desirable for two-day old piglets. In a previous study, Vepured vaccine efficacy was demonstrated even in the presence of maternally derived antibodies (MDA), although MDA to VT2e seems to be atypical in the field.[20]

In previous studies, VT2e NAb in pigs from farms with and without clinical signs of ED were not detected, suggesting that VT2e does not normally induce detectable levels of antibodies after a natural infection.[21] In the present study, VT2e NAb were detected in most of the vaccinated piglets at 21 days after vaccination and in all piglets at 28 days after vaccination (mean neutralization antibody titers of 3.9 and 4.3, respectively). Moreover, NAb persisted for at least 112 days in all the vaccinated animals (mean neutralization antibody titers of 3.8) except for one animal, which was found to be negative for VT2e NAb from day 105. Interestingly, this was the only animal from the vaccinated group affected by mild clinical signs related to VT2e-induced toxemia after the experimental challenge but was still protected from mortality. This specific animal presented VT2e NAb seven days after infection (day 119 after vaccination) indicating that vaccination generated an im-

mune memory, which allowed the immune system to respond more rapidly and effectively to the toxin. This circumstance was also observed with three vaccinated animals that were negative for VT2e NAb at day 21 post vaccination but before the challenge. Neutralizing antibodies were not detected in any of the non-vaccinated animals at any time during the study. The results of this study suggest that the presence of NAb may predict protection against VT2e-induced toxemia. However, this was not evaluated in this study and further research is warranted in this area.

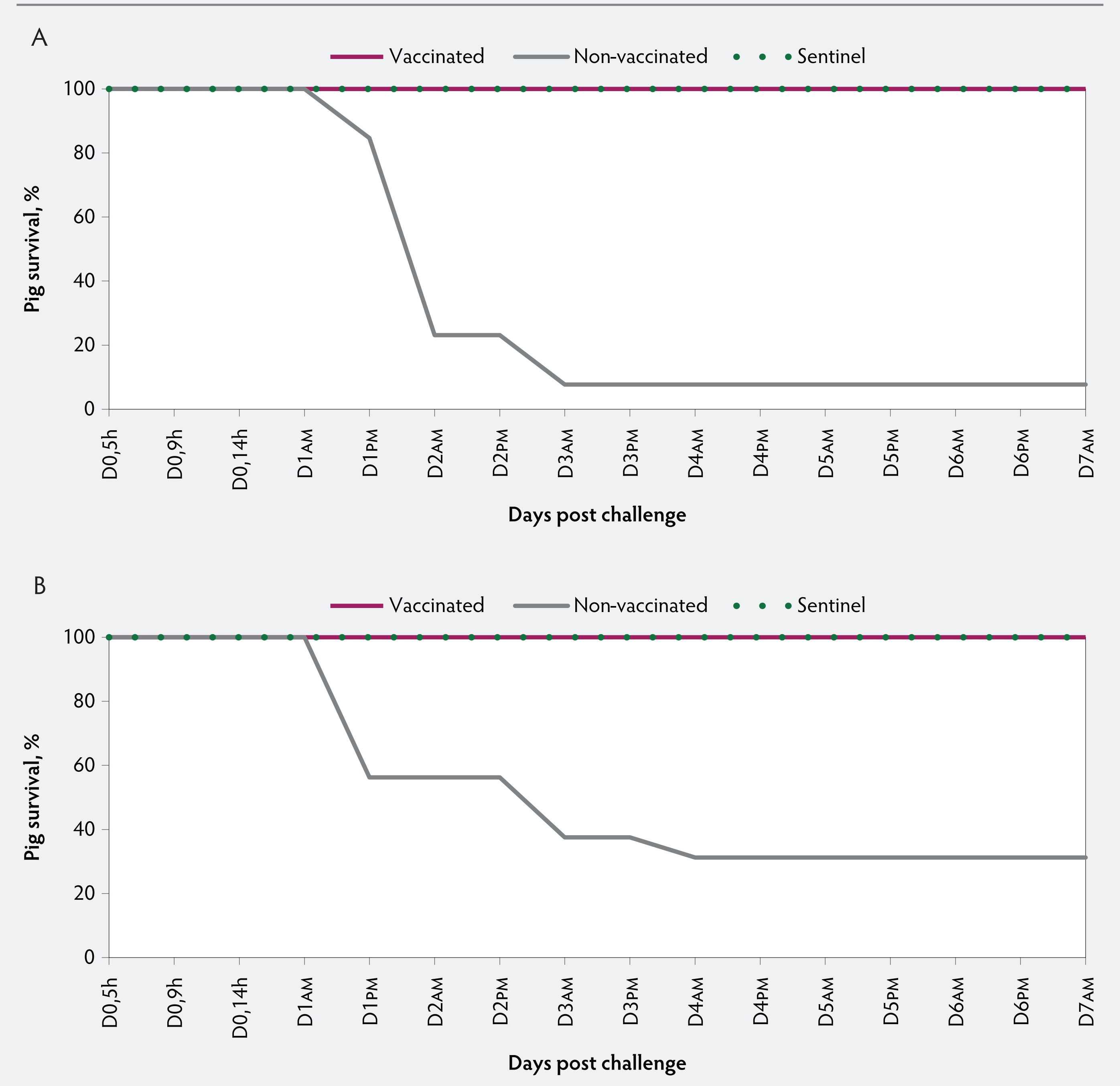
In agreement with previous reports where experimental vaccines were tested,[4,11] the results of this study demonstrated a reduction in the presence and severity of clinical signs of VT2e-induced toxemia after experimental challenge in the vaccinated group.

Implications

Under the conditions of this study:

- Mortality associated with VT2e-induced toxemia was reduced in piglets immunized with Vepured vaccine.
- Morbidity and severity of clinical signs associated with VT2e-induced toxemia was reduced in piglets immunized with Vepured vaccine.

Figure 1: Survival curve after VT2e challenge. Observation of clinical signs occurred at 5, 9, and 14 hours post challenge and twice a day for 7 days thereafter. A, Percentage of piglets alive after a VT2e challenge given 21 days post vaccination (OOI experiment). B, Percentage of piglets alive after a VT2e challenge given 112 days post vaccination (DOI experiment). VT2e = verotoxin 2e; OOI = onset of immunity; DOI = duration of immunity.



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Conflict of interest

The authors are employees of HIPRA. This study was funded by HIPRA, which provided the animals, the *E coli* strain, and the Vepured vaccine and carried out the study. The study design and all procedures, data collection, registries, manipulation, and analysis of samples and data were conducted at HIPRA by HIPRA personnel.

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Figure 2: Clinical signs associated with VT2e-induced toxemia observed in piglets after a VT2e challenge given 21 days post vaccination (OOI experiment). Observation of clinical signs occurred at 5, 9, and 14 hours post challenge and twice a day for 7 days thereafter. A, Percentage of animals with mild clinical signs scored as 1 after challenge. B, Percentage of animals with severe clinical signs scored as 2 after challenge. C, Total clinical signs score from day 0 to day 7 post challenge. D, Percentage of animals with clinical signs from day 0 to day 7 post challenge. Significant differences are represented with different superscript letters (Figure C: Kruskall-Wallis; $P < .05$ and Figure D: Chi-square statistic; $P < .05$). VT2e = verotoxin 2e; OOI = onset of immunity.

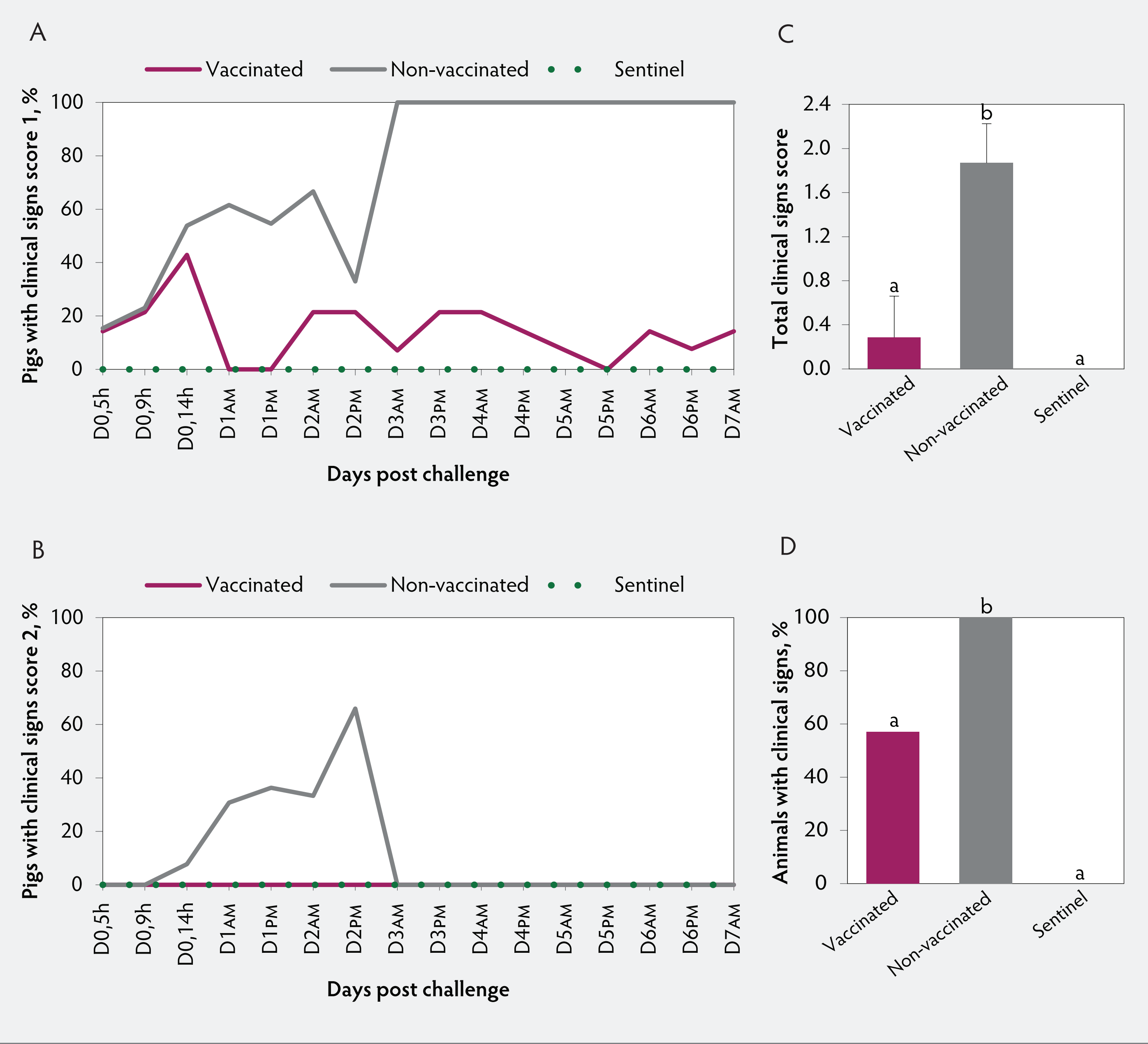

Figure 3: Clinical signs associated with VT2e induced toxemia observed in piglets after a VT2e challenge given 112 days post vaccination (DOI experiment). Observation of clinical signs occurred at 5, 9, and 14 hours post challenge and twice a day for 7 days thereafter. A, Percentage of animals with mild clinical signs scored as 1 after challenge. B, Percentage of animals with severe clinical signs scored as 2 after challenge. C, Total clinical signs score from day 0 to day 7 post challenge. D, Percentage of animals with clinical signs from day 0 to day 7 post challenge. Significant differences are represented with different superscript letters (Figure C: Kruskall-Wallis; $P < .05$ and Figure D: Chi-square statistic; $P < .05$). VT2e = verotoxin 2e; DOI = duration of immunity.

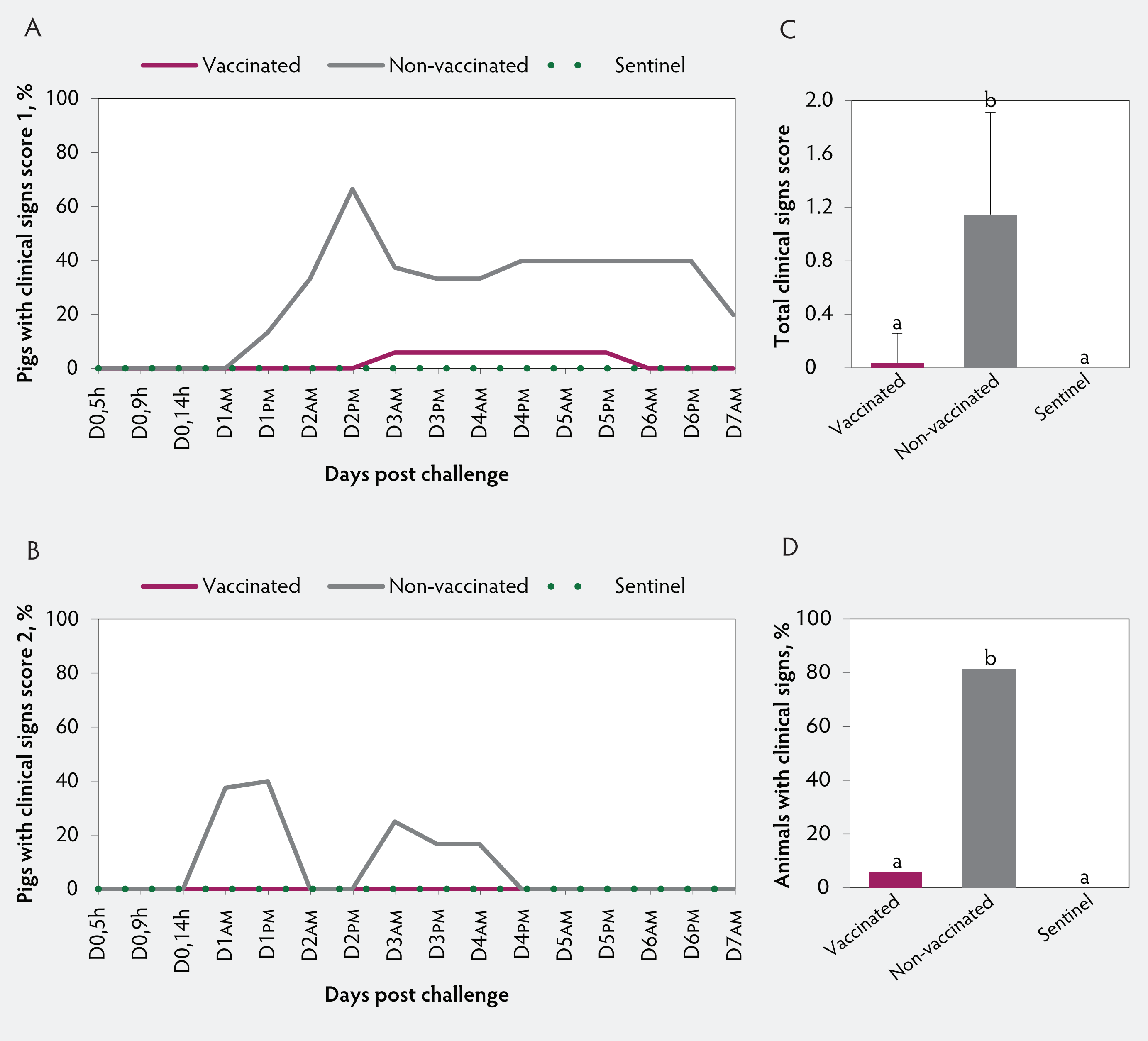

Table 3: Piglet weights after duration of immunity VT2e challenge*

Treatment	Day of challenge		End of study	
	N	Weight, mean (SD), kg	N	Weight, mean (SD), kg
Vaccinated	17	34.3 (12.0)[a]	17	39.0 (12.8)[a]
Non-vaccinated	16	31.8 (12.6)[a]	5	23.5 (5.6)[b]
Sentinel	5	35.5 (8.4)[a]	5	40.5 (9.8)[a,b]

* In the DOI study, animals were challenged 112 days after vaccination. Seven days after challenge (day 119), at the end of the study, all the animals were euthanized.

[a,b] Values within a column with different superscripts are significantly different (ANOVA; $P < .05$).

DOI = duration of immunity; VT2e = verotoxin 2e.

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The use of oral fluid diagnostics in swine medicine

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Summary

Swine veterinarians in North America have applied oral fluid-based testing methodologies for an increasing number of systemic, respiratory, and enteric disease diagnostic applications. Since the first report of oral fluid testing in 2008, nucleic acid and antibody assays have been described in the peer-reviewed literature for many pathogens affecting swine. As evidence of the US swine industry's growing utility of oral fluids as a diagnostic tool, the cumulative number of swine oral fluid diagnostic tests conducted at three veterinary diagnostic laboratories in the upper Midwest (Iowa State University, South Dakota State University, and University of Minnesota) has increased from approximately 21,000 tests in 2010 to nearly 370,000 tests in 2016. The objective of this review is to describe the developments in oral fluid diagnostics that have led to its widespread use and to highlight areas of concern as this technology is increasingly implemented by producers and veterinarians.

Keywords: swine, review, oral fluids, diagnostics

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Resumen – El uso de diagnósticos con fluido oral en medicina porcina

En Norteamérica, los veterinarios especialistas en cerdos han utilizado metodologías de testeo basadas en fluidos orales para diferentes aplicaciones diagnósticas en un creciente número de enfermedades sistémicas, respiratorias y entéricas. Desde el primer reporte de testeo con fluido oral en 2008, en la literatura editada, se han descrito diferentes ensayos para ácido nucleico y anticuerpos, para muchos patógenos que afectan a los cerdos. Como evidencia del creciente uso en la industria porcina de los Estados Unidos de los fluidos orales como herramienta de diagnóstico, el número acumulado de pruebas de diagnóstico de fluido oral porcino conducidas en tres laboratorios de diagnóstico veterinario en la parte superior del Medio Oeste (Universidad del Estado de Iowa, Universidad del Estado de Dakota del Sur, y Universidad de Minnesota) se han incrementado de aproximadamente 21,000 pruebas en 2010 a cerca de 370,000 pruebas en 2016. El objetivo de esta revisión es describir los desarrollos en el diagnóstico de fluido oral que han llevado a su uso generalizado y resaltar las áreas de preocupación conforme esta tecnología es implementada, cada vez más, por productores y veterinarios.

Résumé – Utilisation des fluides oraux aux fins de diagnostics en médecine porcine

En Amérique du Nord les vétérinaires en médecine porcine ont appliqué des méthodologies utilisant les fluides oraux dans un nombre croissant d'applications diagnostiques pour des maladies systémiques, respiratoires et entériques. Depuis le premier rapport en 2008 de test utilisant du fluide oral, des épreuves pour détecter des acides nucléiques et des anticorps ont été décrites dans la littérature jugées par les pairs pour plusieurs agents pathogènes affectant les porcs. À titre de preuve de l'utilité grandissante dans l'industrie porcine américaine des fluides oraux comme outil diagnostique, le nombre cumulatif d'épreuves diagnostiques effectuées sur du fluide oral dans trois laboratoires de diagnostic vétérinaires dans le Midwest (Iowa State University, South Dakota State University, and University of Minnesota) a augmenté d'environ 21,000 test en 2010 à environ 370,000 tests en 2016. L'objectif de la présente revue est de décrire les développements dans le diagnostic utilisant les fluides oraux qui ont mené à cet usage répandu et de faire ressortir les inquiétudes étant donné que l'application de cette technologie est en augmentation par les producteurs et les vétérinaires.

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The first technical report on swine oral fluid diagnostics was presented at the 2005 International PRRS Symposium when Simer et al[1] reported 20 of 24 pen-based oral fluid samples (83.3%) and 17 of 24 serum samples (70.8%) were porcine reproductive and respiratory syndrome virus (PRRSV) reverse transcription polymerase chain reaction (RT-PCR) positive in finishing pigs. The purpose of this review is to provide an update on the development and implementation of oral fluid diagnostics in swine medicine subsequent to this initial report.

Collection of oral fluid samples has been described at length by Prickett et al.[2] In brief, cotton ropes are hung in the pen at pig shoulder height. Pigs chew on the rope, saturating the rope with oral fluids. After 20 to 30 minutes, the ropes are placed in a single-use plastic bag, the fluid is wrung from the rope, and

then decanted into a tube for submission to the diagnostic laboratory. Pigs with prior experience respond immediately to the presence of the rope. In experienced groups, a 20- to 30-minute sampling period is sufficient to allow adequate participation of pigs in the pen. In pigs without prior rope sampling experience, 60 minutes is recommended to allow pigs to learn the new "game" and achieve an adequate level of participation.[3]

Oral fluids are most commonly collected from pens of pigs, but oral fluid samples can also be obtained from individual pigs.[3] Oral fluids can be successfully collected at all production stages, ie, growing pigs[3,4] and in the breeding herd for individually- or group-housed sows and boars.[5,6] Samples can be collected from suckling piglets as they approach weaning age,[7,8] but family sampling, ie, placing the rope so that both sows and their litters have access, has been shown to be more successful than collecting solely from the piglets. Thus, Almeida et al[8] reported an approximate 73% success rate when collecting family oral fluid samples versus 44% success when collecting only from litters. From a collection of 72 family oral fluid samples and matching sera from 718 piglets, 84.4% (27 of 32 litters) were PRRSV RT-PCR positive while 24.2% (174 of 718 piglets) of serum samples were positive for PRRSV nucleic acid.

At the present time, the detection of nucleic acid or antibodies in oral fluids has been documented for most of the major swine pathogens including: *Actinobacillus pleuropneumoniae* (APP),[9,10] African swine fever virus,[11,12] classical swine fever virus,[13] foot-and-mouth disease virus,[14,15] influenza A virus (IAV),[16-18] *Lawsonia intracellularis*,[19] *Mycoplasma* spp.,[20-22] porcine circovirus type 2 (PCV2),[2,23] porcine epidemic diarrhea virus (PEDV),[24] PRRSV,[2,6,25-27] Senecavirus A (SVA),[28] and others. Field applications or research on the use of oral fluid diagnostics have been described in Australia,[15] Belgium,[29] Canada,[30] France,[31] Germany,[13] Italy,[32] Japan,[33] Malaysia,[34] Poland,[35] Spain,[36] the United Kingdom,[37,38] the United States,[2] Vietnam,[39] and others. Many of the assays reported in the literature have only been described under research conditions, but it is reasonable to expect their future commercialization and adoption for routine use in diagnostic laboratories.

Oral fluid testing

In the United States, veterinary diagnostic laboratories with a major swine focus began offering oral fluid-based tests to clientele in 2010. The data provided in Figure 1 and Tables 1, 2, 3, and 4 describe the number of oral fluid tests performed at Iowa State University, South Dakota State University, and University of Minnesota. The following is a review of pathogens for which testing is commonly performed and for which peer-reviewed literature is available.

Porcine reproductive and respiratory syndrome virus

Porcine reproductive and respiratory syndrome virus was the first virus detected by RT-PCR in oral fluid samples.[2] Porcine reproductive and respiratory syndrome virus oral fluid enzyme-linked immunosorbent assays (ELISA) for antibody detection have been routinely offered since 2010. In 2016, RNA detection was performed for 116,671 of the 148,526 PRRSV tests (Tables 1 and 2).

Nucleic acid detection

Prickett et al[2] first reported the detection of PRRSV by quantitative RT-PCR (qRT-PCR) in oral fluids collected in the field from 8-week-old pigs. Oral fluid qRT-PCR-positive results were coincident with RT-PCR-positive serum samples, ie, showed 77% agreement. Under experimental conditions, Prickett et al[25] reported that PRRSV RNA was detected in oral fluid samples from 3 to 35 days post inoculation (DPI), with sporadic positives thereafter. Similar results were obtained from individual boars inoculated with modified-live virus, type 1 PRRSV, or type 2 PRRSV.[6] On the first DPI, virus was detected in 10% of the boars sampled (7 of 69); by 3 DPI, virus was detected in 100% of boars sampled (67 of 67).[6] Cumulatively, the literature indicates that PRRSV RNA can be detected for at least 36 DPI in oral fluid samples.[5,25,26,33,35,40-44]

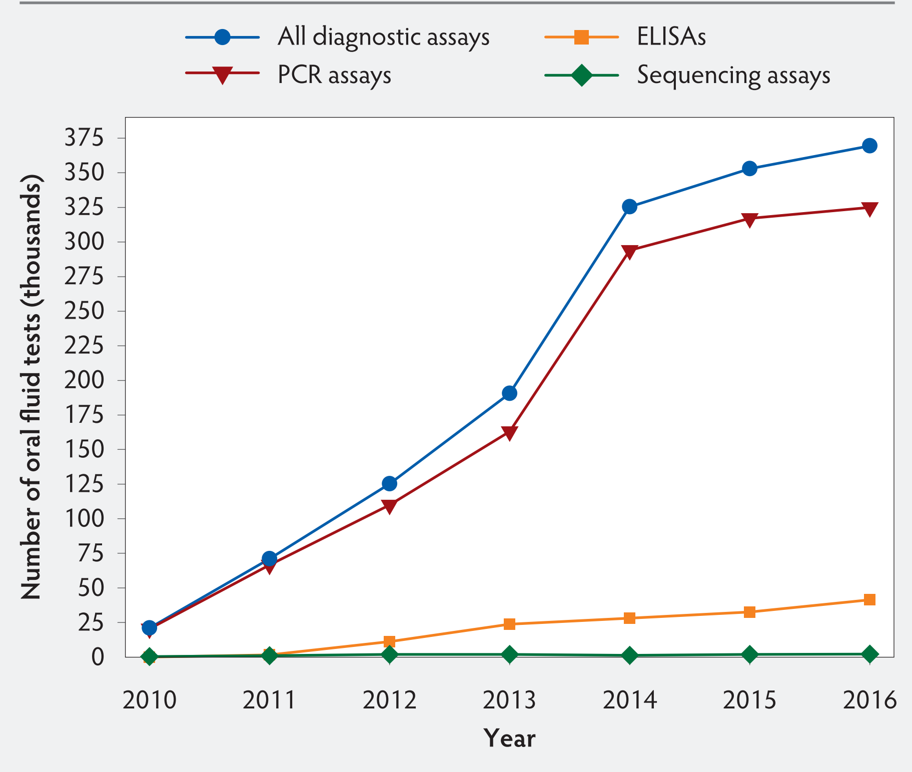
Sequencing

Successful PRRSV sequencing from oral fluids has been described.[35,45,46] Kittawornrat et al[45] obtained PRRSV open reading frame-5 sequences from 2 of 6 RT-PCR-positive oral fluid samples from pre-weaned pigs. Zhang et al[46] reported successful full-genome sequencing from oral fluid samples with RT-PCR cycle threshold (Ct) values between 18.7 and 20.6, whereas no full-length sequences were obtained from oral fluids with Ct values between 22.9 and 35.4.

Antibody detection

Porcine reproductive and respiratory syndrome virus IgG antibody is detected in

Figure 1: Total number of oral fluid tests conducted at Iowa State University, South Dakota State University, and the University of Minnesota from 2010 to 2016. PCR = polymerase chain reaction; ELISA = enzyme-linked immunosorbant assay.



oral fluids by ELISA between 7 and 14 days after inoculation or vaccination.[5,25-27,40,47] Kittawornrat et al,[27] working with oral fluid samples from individually housed boars and a serum ELISA adapted to oral fluids, reported that IgM was detectable at 3 DPI, IgA at 7 DPI, and IgG at 8 DPI. Antibody responses in oral fluids mirrored antibody responses in serum. Maternal PRRSV IgG is readily detected in pigs from PRRSV-positive sow herds and may be detected for up to 60 days post-weaning; however, a PRRSV IgM-IgA (dual isotype) ELISA was shown to detect pig-specific IgM and IgA, even in the presence of maternal IgG.[48] Porcine reproductive and respiratory syndrome virus antibody ELISA testing has been well documented in the literature and may provide a cost-effective approach to PRRSV monitoring and surveillance.

Influenza A virus

As shown in Tables 1, 2, 3, and 4, IAV oral fluid testing has been offered for routine testing since 2010. Nucleic acid detection was performed for 42,261 of the 47,454 IAV tests in 2016 (Table 1 and 2).

Table 1: Total number of tests on oral fluid specimens by pathogen in 3 US veterinary diagnostic laboratories from 2010 to 2016 *

Pathogen	2010	2011	2012	2013	2014	2015	2016
PRRSV	14,603	46,239	77,756	109,868	126,165	144,773	148,526
IAV	4785	16,495	34,297	46,940	48,688	48,895	47,454
MHP	760	4514	7079	10,286	11,203	11,741	13,178
PCV2	751	2047	4147	2149	5676	4807	3176
APP	0	37	4	93	14	287	3306
TGEV	0	34	0	4651	32,848	12,497	12,996
PEDV	0	0	0	14,361	75,965	76,063	73,494
LI	0	0	0	454	1519	3290	2443
PDCoV	0	0	0	0	21,393	46,366	58,513
SVA	0	0	0	0	0	1597	3598
Other	64	1630	1919	1804	2010	2595	2755
Total	20,963	70,996	125,202	190,606	325,481	352,911	369,439

* Iowa State University, South Dakota State University, and University of Minnesota.
PRRSV = porcine reproductive and respiratory syndrome virus; IAV = influenza A virus; MHP = *Mycoplasma hyopneumoniae*; PCV2 = porcine circovirus type 2; APP = *Actinobacillus pleuropneumoniae*; TGEV = transmissible gastroenteritis virus; PEDV = porcine epidemic diarrhea virus; LI = *Lawsonia intracellularis*; PDCoV = porcine deltacoronavirus; SVA = Senecavirus A.

Table 2: Number of nucleic acid (PCR) tests on oral fluid specimens in 3 US veterinary diagnostic laboratories from 2010 to 2016*

Pathogen	2010	2011	2012	2013	2014	2015	2016
PRRSV	14,251	43,464	64,984	84,835	96,715	110,650	116,671
IAV	4581	14,898	31,806	44,410	46,738	47,304	42,261
PCV2	751	2047	4147	2142	5669	4773	3168
MHP	750	4514	7056	10,271	11,201	11,708	13,169
TGEV	0	34	0	4651	32,848	12,497	12,996
PEDV	0	0	0	14,361	75,931	76,048	69,324
LI	0	0	0	454	1519	3290	2443
PDCoV	0	0	0	0	21,393	46,365	58,513
SVA	0	0	0	0	0	1597	3533
Other	64	1584	1923	1881	2024	2863	2886
Total	20,397	66,541	109,916	163,005	294,038	317,095	324,964

* Iowa State University, South Dakota State University, and University of Minnesota.
PCR = polymerase chain reaction; PRRSV = porcine reproductive and respiratory syndrome virus; IAV = influenza A virus; MHP = *Mycoplasma hyopneumoniae*; PCV2 = porcine circovirus type 2; TGEV = transmissible gastroenteritis virus; PEDV = porcine epidemic diarrhea virus; LI = *Lawsonia intracellularis*; PDCoV = porcine deltacoronavirus; SVA = Senecavirus A.

Nucleic acid detection

Detmer et al[49] first reported the detection of IAV nucleic acid in oral fluid samples from both experimentally and naturally infected pigs. Under experimental conditions, IAV RNA was detected in oral fluids from 3 to 21 DPI; whereas, no IAV RT-PCR-positive nasal swabs were detected past 7 DPI.[50] Ramirez et al[43] reported highly variable detection patterns for IAV infection in 10 wean-to-finish barns. Cumulatively, the literature indicates that IAV RNA can be detected in oral fluids, but widely variable detection patterns have been noted in the literature.[35,37,50-54]

Sequencing

Influenza A virus sequencing has been described in the literature.[49,51,53] Detmer et al[49] obtained hemagglutinin (HA) sequences from 2 of 4 positive oral fluid samples submitted for analysis. Panyasing et al[53] reported unsuccessful attempts to sequence HA and neuraminidase genes, but successfully sequenced M genes for all 18 IAV qRT-PCR-positive oral fluid samples collected from neonatal pigs. In oral fluid field samples submitted for routine analysis, HA sequences were obtained from 34 of 61 (55.7%) samples with Ct values < 25; 5 of 34 (14.7%) samples with Ct values between 25 and 29.9; and 0 of 39 (0%) samples with Ct values > 30 (Jianqiang Zhang, Personal Communication).

Virus isolation

Isolation of IAV from oral fluids in pigs is difficult and reports of both success and failure may be found in the literature. Detmer et al[49] and Allerson et al[51] were not able to isolate and sequence IAV from oral fluid samples. However, Romagosa et al[54] reported 51.4% (19 of 37) of RT-PCR-positive oral fluid samples were also positive by virus isolation. Similarly, Goodell et al[16] reported successful IAV virus isolation, but isolation was significantly less likely in oral fluids when compared to nasal swabs, particularly in vaccinated animals. Virus isolation was successful in 82 of 180 (45.6%) oral fluid samples with Ct values < 25; 62 of 346 (17.9%) samples with Ct values between 25 and 29.9; and 21 of 407 (5.2%) samples with Ct values between 30 and 35 (Jianqiang Zhang, Personal Communication). Additional research is needed to determine the best time to collect samples and the optimum laboratory protocol for successful IAV virus isolation.[16,49] As reviewed by Baron et al[55] in the context of human immunodeficiency virus, the extreme hypotonicity of oral fluids (one-seventh the tonicity of interstitial fluid) severely reduces virus infectivity. This is a factor that should be considered for future research because, like humans, swine oral fluids are hypotonic and may have an impact on the isolation of IAV and other viral agents from porcine oral fluids.

Antibody detection

Panyasing et al[18] first reported detection of IAV-specific antibodies in oral fluid samples using a blocking ELISA based on the viral nucleoprotein (NP). Using a NP indirect ELISA, IAV IgM antibody responses peaked at 8 DPI and declined quickly thereafter while IgA and IgG were detected around 6 DPI and lasted through the conclusion of the study (42 DPI).[17] In this same study, Panyasing et al[17] showed a rapid anamnestic oral fluid antibody response in vaccinated animals. Cumulatively, the literature agrees that IAV antibodies can be detected in oral fluids as early as 6 DPI.[17,39,52,56,57]

Porcine coronaviruses

The majority of research on the porcine coronaviruses has focused on PEDV. Cumulatively, the research strongly supports the use of oral fluids for PEDV detection. Similar assumptions have been made for other porcine coronaviruses, ie, transmissible gastroenteritis virus and porcine deltacoronavirus (PDCoV), on the strength of the PEDV research.

Porcine epidemic diarrhea virus

Porcine epidemic diarrhea virus RT-PCR testing for oral fluids was implemented in 2013 and was used extensively thereafter, as reflected in the test numbers reported in Tables 1, 2, 3, and 4. Oral fluid antibody testing for PEDV became available in 2016 (Table 3). Reverse transcription PCR testing was performed for 69,324 of the 73,494 PEDV tests conducted in 2016 (Tables 1 and 2).

Nucleic acid detection. Bjustrom-Kraft et al[24] published the first peer-reviewed study on the detection of PEDV in oral fluid samples by RT-PCR. Detectable levels of PEDV were found in fecal swabs, oral fluids, and pen fecal samples collected in the field following a natural planned exposure to PEDV. Significant differences were detected between individual fecal swabs and pen-based oral fluid; oral fluids had lower Ct values indicating higher virus concentrations. PEDV was detected in oral fluids for approximately 69 days post exposure (DPE). Under experimental conditions, Bower et al[58] reported detection of PEDV by RT-PCR in fecal swabs and oral fluids from 1 to 35 DPI in both sample types.

Antibody detection. Bjustrom-Kraft et al[24] reported the detection of PEDV IgG and IgA in oral fluid samples collected 13 days after natural planned exposure. Porcine epidemic diarrhea virus IgA sample to positive ratio (S/P) responses in oral fluid increased until 97 DPE whereas oral fluid IgG responses peaked at 13 DPE and declined thereafter.

Porcine deltacoronavirus

Under experimental conditions, Zhang[59] reported detection of PDCoV in oral fluids from 3-week-old pigs. Individual fecal swabs, pen-based feces, and oral fluids were collected and PDCoV RNA was detected from 7 to 28 DPI, 7 to 14 DPI, and 7 to 35 DPI, respectively. Homwong et al[60] evaluated PDCoV RT-PCR testing results from routine submissions (n = 602) to the University of Minnesota Veterinary Diagnostic Laboratory and found that oral fluid samples were more likely to test positive for PDCoV than feces.

Less commonly used oral fluid tests in the United States

Tests are offered at the diagnostic laboratories for several pathogens for which little peer-reviewed literature is available.

Porcine circovirus type 2

As shown in Tables 1, 2, and 4, routine PCV2 oral fluid testing began in 2010. Relatively few tests have been performed in recent years, which suggests that the current PCV2 vaccines are effective.[61] Porcine circovirus type 2 was detected in oral fluids from each of the three sites with at least 1 to 2 positive samples in oral fluids by quantitative polymerase chain reaction (qPCR) in 2008.[2] Similar results were reported in PCV2-inoculated 11-week-old pigs where PCV2 was detected by qPCR from 2 DPI until the conclusion of the study (98 DPI).[23] Ramirez et al[43] reported 508 of 600 (84.7%) oral fluid samples were PCV2 positive by qPCR in 10 wean-to-finish barns. Van Cuong et al[39] reported a slightly lower PCV2 detection rate (61%) in 68 farms throughout Vietnam. Under experimental conditions, PCV2 antibody (IgG, IgA, and IgM) was first reported in 2011.[23] All PCV2-inoculated pigs seroconverted between 14 and 21 DPI (IgG, IgA, and IgM), and antibody responses remained detectable through the conclusion of the study (98 DPI).

Table 3: Number of antibody (ELISA) tests on oral fluid specimens in 3 US veterinary diagnostic laboratories from 2010 to 2016*

Pathogen	2010	2011	2012	2013	2014	2015	2016
PRRSV	43	1575	11,224	23,785	28,107	32,564	30,051
MHP	10	0	0	4	1	33	8
IAV	0	0	5	0	0	2	3960
PEDV	0	0	0	0	0	4	4168
APP	0	0	0	0	0	0	3176
SVA	0	0	0	0	0	0	60
Total	53	1575	11,229	23,789	28,108	32,603	41,423

* Iowa State University, South Dakota State University, and University of Minnesota.
ELISA = enzyme-linked immunosorbent assay; PRRSV = porcine reproductive and respiratory syndrome virus; MHP = *Mycoplasma hyopneumoniae*; IAV = influenza A virus; PEDV = porcine epidemic diarrhea virus; APP = *Actinobacillus pleuropneumoniae*; SVA = Senecavirus A.

Table 4: Number of oral fluid specimens submitted for nucleic acid sequencing in 3 US veterinary diagnostic laboratories from 2010 to 2016*

Pathogen	2010	2011	2012	2013	2014	2015	2016
PRRSV	300	919	1444	1223	893	1524	1718
IAV	37	110	522	650	327	433	465
PCV2	0	0	6	7	7	34	8
PEDV	0	0	0	0	34	3	2
Other	0	0	23	27	1	4	10
Total	337	1029	1995	1907	1262	1998	2203

* Iowa State University, South Dakota State University, and University of Minnesota.
PRRSV = porcine reproductive and respiratory syndrome virus; IAV = influenza A virus; PCV2 = porcine circovirus type 2; PEDV = porcine epidemic diarrhea virus.

Senecavirus A

For SVA, 3,598 oral fluid-based tests have been conducted (Tables 1, 2, and 3). Senecavirus A detection in oral fluids has been documented under field conditions.[28] Although no clinical signs were observed, SVA was detected by RT-PCR in oral fluid samples at day zero in one of the sites. The fact that 9 of 10 serum samples were SVA positive on the same farm supported the validity of the oral fluid results. Little peer-reviewed research is available on SVA, but this initial report suggests oral fluids may be a useful sample for monitoring and surveillance of SVA.

Bacterial pathogens

Little research has been done on the detection of bacterial pathogens in oral fluid samples. Regardless, peer-reviewed publications reporting detection by polymerase chain reaction under experimental or field conditions have included the following bacterial agents: APP,[9,62] *Brachyspira* spp.,[63] *Erysipelothrix rhusiopathiae*,[64] *Haemophilus parasuis*,[62] *L intracellularis*,[19] *Mycoplasma* spp.,[20,21,62] *Pasteurella multocida*,[62] *Salmonella*,[19] and *Streptococcus suis*.[62]

Bacterial pathogens for which antibodies are reportedly detected in oral fluids include: APP,[9,10] *E rhusiopathiae*,[64] and *Mycoplasma* spp.[22]

General conclusions

Pig production changed dramatically over the last several decades from smaller single-site farms to larger multisite production systems.[65] These changes made it possible for producers and veterinarians to achieve higher production efficiencies, but also facilitated the appearance of production diseases, ie, multifactorial diseases and the appearance of new, high-impact pathogens, such as PRRSV and PEDV.[66-69]

Diagnostic medicine needs to respond to new disease challenges with new methods capable of providing timely, accurate, informative results. Individual pig samples, such as serum or swabs, have historically served this purpose, but individual animal sampling is not compatible with efficient surveillance in contemporary swine production systems. As an alternative to individual animal samples, Prickett et al[2] described the use of pen-based oral fluid samples (rope testing) for the detection of PRRSV and PCV2 in growing pigs. Since this initial report, oral fluid-adapted nucleic acid and antibody tests have been reported for many of the major swine pathogens and oral fluid-based surveillance has been widely adopted by swine veterinarians and swine producers. This process will continue as more and better tests are adapted to the oral fluid matrix.

Nevertheless, there are good reasons to exercise caution. In particular, the peer-reviewed literature has shown that nucleic acid and

antibody assays can be adapted to oral fluids, but the literature has also consistently shown that the procedures need to be carefully modified for optimum performance with the oral fluid matrix.[70,71] Chittick et al[70] and Gibert et al[36] working with PRRSV and Goodell et al[71] working with IAV found significant differences in test performance among RT-PCR protocols offered in veterinary diagnostic laboratories. Once optimum protocols are identified, they should be broadly implemented to achieve reproducibility among diagnostic laboratories. Overall, the development of oral fluid-based testing has changed the way we monitor disease in swine populations. However, careful work on the part of researchers and critical thinking on the part of producers and veterinarians will be needed to fully develop a reliable and robust oral fluid diagnostics system capable of meeting the current and future needs of the swine industry.

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Conflict of interest

None reported.

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* Non-refereed references.

Conversion tables

Weights and measures conversions

Common (US)	Metric	To convert	Multiply by
1 oz	28.35 g	oz to g	28.4
1 lb (16 oz)	453.59 g	lb to kg	0.45
2.2 lb	1 kg	kg to lb	2.2
1 in	2.54 cm	in to cm	2.54
0.39 in	1 cm	cm to in	0.39
1 ft (12 in)	0.31 m	ft to m	0.3
3.28 ft	1 m	m to ft	3.28
1 mi	1.6 km	mi to km	1.6
0.62 mi	1 km	km to mi	0.62
1 in^2	6.45 cm^2	in^2 to cm^2	6.45
0.16 in^2	1 cm^2	cm^2 to in^2	0.16
1 ft^2	0.09 m^2	ft^2 to m^2	0.09
10.76 ft^2	1 m^2	m^2 to ft^2	10.8
1 ft^3	0.03 m^3	ft^3 to m^3	0.03
35.3 ft^3	1 m^3	m^3 to ft^3	35
1 gal (128 fl oz)	3.8 L	gal to L	3.8
0.264 gal	1 L	L to gal	0.26
1 qt (32 fl oz)	946.36 mL	qt to L	0.95
33.815 fl oz	1 L	L to qt	1.1

Temperature equivalents (approx)

°F	°C
32	0
50	10
60	15.5
61	16
65	18.3
70	21.1
75	23.8
80	26.6
82	28
85	29.4
90	32.2
102	38.8
103	39.4
104	40.0
105	40.5
106	41.1
212	100

$^\circ F = (^\circ C \times 9/5) + 32$
$^\circ C = (^\circ F - 32) \times 5/9$

Conversion chart, kg to lb (approx)

Pig size	Lb	Kg
Birth	3.3-4.4	1.5-2.0
Weaning	7.7	3.5
	11	5
	22	10
Nursery	33	15
	44	20
	55	25
	66	30
Grower	99	45
	110	50
	132	60
Finisher	198	90
	220	100
	231	105
	242	110
	253	115
Sow	300	135
	661	300
Boar	794	360
	800	363

1 tonne = 1000 kg
1 ppm = 0.0001% = 1 mg/kg = 1 g/tonne
1 ppm = 1 mg/L

Lessons learned from managing electronic sow feeders and collecting weights of gestating sows housed on a large commercial farm

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Summary

An observational study was conducted on a commercial 5600-sow farm using electronic sow feeders (ESF) to collect daily feed intake and scales to obtain sow body weights. The challenges that emerged during this study and proposed solutions may be useful for future research projects in commercial farms with ESF feeding systems. A total feed delivery per day was reported for females, regardless of how many times they may have entered the feeding station. It would be valuable to obtain records for individual feeding events to determine how many times females entered the feeding stations and if it was a feeding or non-feeding event. In this system, there was wide variation in daily sow weights because they entered the feeding station several times a day. Discrepancies in individual body weight were found to be attributed to the speed a sow moved across the scale, long scale length, and interference with the scale antenna. Possible solutions include adding panels before and after the scale, reducing scale length, and careful placement of the antenna. Nevertheless, combining the feeding of gestating sows via ESF with daily weight collection has the potential to generate valuable data sets.

Keywords: swine, body weight, data collection, electronic sow feeders, sow

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Resumen – Lecciones aprendidas al manejar comederos electrónicos en hembras y al recolectar pesos de hembras gestantes alojadas en una granja comercial grande

Se condujo un estudio observacional en una granja comercial de 5600 hembras utilizando comederos electrónicos (ESF por sus siglas en inglés) para hembras para registrar el consumo diario de e alimento y básculas para obtener los pesos corporales de las hembras. Los retos que surgieron durante este estudio y las soluciones propuestas pueden ser útiles para futuros proyectos de investigación en granjas comerciales con sistemas de alimentación ESF. Se reportó el total de la administración diaria de alimento para las hembras, independientemente de cuántas veces entraran a la estación de alimentación. Sería valioso obtener los registros por evento individual de alimentación para determinar cuántas veces las hembras entran a las estaciones de alimentación y si fue un evento de alimentación o no. En este sistema, hubo una amplia variación en los pesos diarios de las hembras porque entraron a la estación de alimentación varias veces al día. Se encontró que las discrepancias en el peso corporal individual se atribuían a la velocidad a la que la hembra se movía en la báscula, lo largo de la báscula, y la interferencia con la antena de la báscula. Las posibles soluciones incluyeron añadir divisiones antes y después de la báscula, reducir la longitud de la báscula, y la colocación adecuada de la antena. Aún así, la combinación de la alimentación de las hembras vía ESF con la recolección del peso diario tiene el potencial para generar una base de información valiosa.

Résumé – Leçons apprises dans la gestion des distributeurs électroniques d'aliment pour truie et la collecte du poids des truies gestantes logées dans une grande ferme commerciale

Une étude observationnelle a été menée dans une ferme commerciale de 5600 truies en utilisant des distributeurs électroniques d'aliment (DEA) pour obtenir l'information sur la consommation quotidienne d'aliment et des balances pour obtenir le poids des truies. Les défis qui sont apparus durant cette étude et les solutions proposées pourraient être utiles pour des projets de recherche futurs dans des fermes commerciales avec des systèmes d'alimentation DEA. La quantité totale d'aliment distribuée par jour était reportée pour les femelles, indépendamment du nombre de fois qu'elles seraient entrées dans la station d'alimentation. Il serait utile d'obtenir les données pour chaque occasion individuelle afin de déterminer combien de fois les femelles sont entrées dans les stations d'alimentation et s'il y avait ou non consommation d'aliment. Dans ce système, il y avait une grande variation dans le poids quotidien des truies car elles entraient dans

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This article is available online at http://www.aasv.org/shap.html.

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la station d'alimentation plusieurs fois par jour. Des anomalies dans les poids individuels ont été trouvées et ont été attribuées à la vitesse à laquelle les truies passaient sur la balance, la longue longueur de la balance, et de l'interférence avec l'antenne de la balance. Les solutions possibles incluent l'addition de panneaux avant et après la balance, réduire la longueur de la balance, le placement approprié de l'antenne. Néanmoins, la combinaison de l'alimentation des truies gestantes via les DEA et de la collecte quotidienne du poids des animaux a le potentiel de générer des données valables.

In many US production systems, a standard practice is to house sows in individual stalls during gestation. Gestation stalls allow numerous benefits, including individual animal care and feed allowance based on body weight and condition. However, in 2001 the European Union announced a ban of gestation stalls by 2013 because of welfare concerns regarding space allowance and social behavior.[1] The United States has followed with nine states enacting bans on the use of gestation stalls. Furthermore, pressure from pork retailers, the restaurant industry, and welfare activists has resulted in many production systems considering conversion to group housing for gestating sows. As many production systems are transitioning from individual gestation stalls to different styles of group housing, there are new opportunities for data collection in gestation facilities.[2]

Electronic sow feeding (ESF) systems are computerized feeding stations that serve as a non-competitive feeding system for group-housed sows.[3] Electronic sow feeders typically have a single enclosed feeding station that can feed up to 60 group-housed sows per station each day. The stations are equipped with computers that track and dispense a specified amount of feed for each sow. Each sow has an ear tag that contains a radio frequency identification (RFID) transponder for individual identification. This type of system is appealing to producers, as it allows them to manage and monitor individual feed intake and provide opportunities to adjust feeding program strategies to better satisfy changes in gestation nutrient requirements. Feeding sows individually prevents excessive feed consumption, a common concern in group-housed sows which can detrimentally increase body weight (BW). Electronic sow feeders are also appealing from a research standpoint because some systems allow for recorded individual feed intake and more than one feed line can supply each station to provide different diets to be fed.[4] It is also possible to use a scale in conjunction with the ESF to measure body weight every time the sow exits the feeding station.

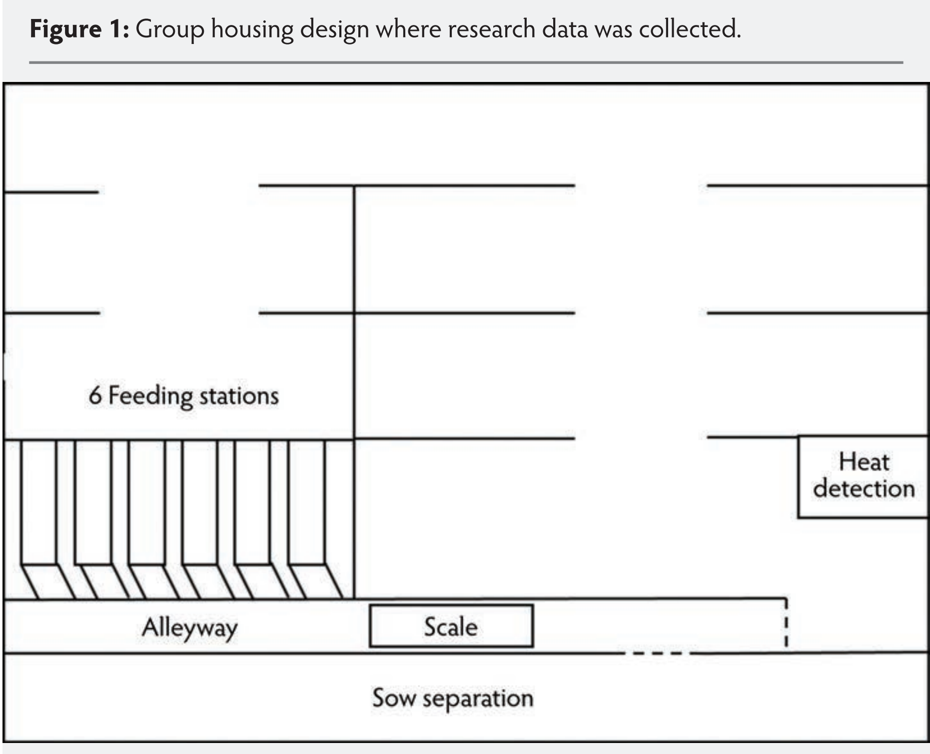
In the peer-reviewed scientific literature, there is virtually no data reported for ESF use in large scale commercial conditions (> 5000 sows) similar to that seen in US swine production. Therefore, we conducted an observational study on a large-scale sow farm to determine gestation weight gain and feed efficiency by collecting daily ESF intake and sow body weight data. The objective of this paper is to discuss the challenges that emerged when collecting this data and propose some solutions that may be useful for future research conducted in similar gestation feeding systems.

Farm description and feeder design

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this study. The study was conducted at a 5600-sow farm in central Nebraska. The gestation barn contained 16 pens, housing 260 females (Camborough, PIC, Hendersonville, TN) per pen. Gilts (parity 1) and sows (parity ≥ 2) were penned separately to allow for additional attention to gilts who were still adjusting to the ESF system. Pens provided 2.0 m^2 per sow and 1.95 m^2 per gilt. Each pen contained 28 nipple waterers to provide ad libitum access to water and was equipped with 6 ESF stations (Nedap Velos, Gronelo, Netherlands) allowing up to 45 females per station (Figures 1 and 2). Each feeding station was 2.0 m long × 0.56 m wide. Feed was dispensed at a rate of 150 g/min with the addition of 100 mL of water. Each feeding station was calibrated weekly. The calibration process consisted of collecting feed from 5 consecutive screw dispenser rotations (approximately 90 g of feed dispensed per rotation, for a total collection of approximately 450 g) from each feeding station. The samples were weighed to determine how much feed was dispensed per rotation which was subsequently entered into the Nedap Velos system to complete the calibration. For the study, 3 pens were equipped with a scale (2.13 m long × 0.51 m wide; New Standard US Inc, Sioux Falls, South Dakota) located in the alleyway where sows walked when they exited the feeding stations (Figures 1 and 3).

Females were group-housed from days 4 to 112 of gestation in dynamic groups, meaning recently bred sows (approximately day 4 of gestation) were entering the pen as sows due to farrow were exiting (approximately day 112 of gestation). This occurred over a 3- to 4-week period, thereafter the pen remained

Figure 1: Group housing design where research data was collected.



static (no movement of newly bred sows into the pen) until the first of the sows reached day 112 of gestation and the process repeated.

The study was conducted over a 149-day period, from late May to mid-October. A total of 861 females were enrolled in the study, of which 712 were moved into the farrowing house for subsequent lactation. Selection criteria for female enrollment was based on current farm flow. Females exhibiting lameness or any obvious signs of illness were not enrolled in the study. Of the initial 861 females, 40 (4.6%) were removed from the data set due to mortality or culling decisions made by the farm management. Ninety-seven females (11.3%) were excluded from the data set because they were removed from their pen for greater than 3 consecutive days due to illness or lameness. The remaining 12 females (1.4%) were removed due to lost RFID tags.

Figure 2: One individual pen showing electronic sow feeding stations.

Data collection

Feed intake

It is important to note that in this and all other ESF systems, it is assumed that the feed dispensed is consumed by the sow before leaving the feeding station and therefore, every time a sow enters the feeding station the feeder bowl is assumed to be empty. Thus, feed intake data within the ESF system is recorded as disappearance. Females were assigned to a feed allowance based on parity and body condition score. Body condition was evaluated visually every other week by the same individual, scoring females from 1 (very skinny) to 5 (excessively fat). Females could consume the set amount of feed in one visit or over several visits to the feeding station. However, the system only generated 1 total intake value per day of gestation. Hence, if a sow consumed her entire feed allowance in two separate feedings, only one intake value was reported and represented the sum of both feeding events. It would be valuable to obtain records of individual feeding events to determine how many times females entered the feeding stations and if the visit was a non-feeding or feeding event.

Feed intake data had to be manually extracted daily through the Nedap Velos software because long term data storage was not available during the time of the study. Feed intake data provided RFID, farm name, day of gestation, total feed offered, pen location, date, feed line (the system had two feed lines but only one was used during this study), and parity. Due to the lack of long term electronic storage, feed intake data was downloaded daily at approximately 1 PM to ensure all females had eaten their daily feed allotment prior to the system reset at 2 PM. Feed intake data was lost on 13 days (8.7%) due to download malfunction. Therefore, it would be advantageous to improve system software and allow for feed intake data to be downloaded automatically and stored to an off-site database to maintain a record of observations.

Within the first week of data collection, we observed missing values (no value reported) or zero values reported as a feed intake value. Initially, it was unclear if there was a difference between these two values. Through daily observations, we determined there was a 5-second delay between when the sow's RFID was read and when the feeding station dispensed feed. If the sow left the station within those 5 seconds, feed was not dispensed and was recorded as an intake value of zero. Out of 712 sows, 322 had at least 1 zero for an intake value (45.2%). However, on average, sows had zeroes reported as an intake value on 1.9 days out of the 106 total days (1.8%). A sow who did not enter the feeding station on a specific day had a missing intake value for that day. Of the 712 sows, 190 had at least 1 missing intake value (26.7%) and on average, did not enter the feeding station for 1 day during the study (0.9%).

The importance of understanding the difference between the two values was to be certain the values generated were accurate. Previous research has indicated that errors can occur during the collection of feed intake data from ESF and the importance of feeder management to minimize these errors.[5] Initially, it was believed that it was impossible to walk through the feeding station without feed being dispensed. Therefore, differences in values reported were thought to be attributed to a system error. After this investigation, it was determined that sows could walk through the ESF system and be recorded without feed being dispensed.

Body weight

As the sow entered the scale, an RFID sensor, like that used in the ESF station, recorded the date, time, sow identification, and body weight to the nearest kilogram. Weights were stored on secure digital memory cards that were removed from the scale head and loaded onto a computer on a weekly basis. The barn environment is not conducive to handling memory cards and caution should be taken to minimize human error (eg, losing or dropping into the

pit) when removing and replacing them. Scales were calibrated weekly during the time of feeder calibration. Two individuals were required for scale calibration. One individual would obtain their weight using a portable digital scale and this weight was then entered into the scale system as the calibration weight. Then the scale system would be zeroed and the individual who was weighed would step onto the scale while the other individual observed the scale head. Weights were obtained standing at the beginning, middle and end of the scale to check for accuracy. Occasionally, manure would have to be removed from under the scale to improve readings. This emphasizes the importance of doing regular scale calibration.

Sows had to walk across the scale as they moved from the feeding station back into the pen. Through observation, we found that when workers were in the pen, sow activity through the feeding stations was high. This increase in activity caused sows to move too quickly across the scale for an accurate weight. A proposed solution was to provide panels at the beginning and end of the scale to slow the rate of passage across the scale. This was considered during the study but was not implemented due to concern that this may cause the females to move too slowly and congregate in the alleyway between the ESF and the scale, causing an unhealthy environment for the animals. Specifically, the concern pertained to the gilts who were still adjusting to the ESF environment. We also observed multiple sows on the scale at one time. The sow in front had her front legs off the scale while the sow behind only had her front legs on the scale. Although not a possible option during our study, reducing the scale length may be a possible solution for future research.

We also observed that as a sow moved across the scale, the antenna read the RFID and continued to record weights until the next responder tag was detected. Some females would stand on the back of the scale but not far enough forward for the antenna to read the RFID. Thus, these weights were recorded and attributed to the previous sow. To resolve this, the antenna was moved toward the middle of the scale. After making this adjustment, the female's RFID was recognized as she stepped onto the scale. This was another situation where a shorter scale may have been beneficial.

Figure 3: One sow has left the feeding station and is walking over the scale as she exits the system. The sows seen to the left (A) are sows in the pen and the area to the right (B) is the sow holding area. The transponder reader (C) can be seen on the right side of the sow near the front of the scale.

Another problem observed was that the antenna on the scale could read through the panels of the scale and if a sow was laying in the pen against the outside of the scale, her RFID could be read. However, once a sow was on the scale, her RFID was read and recorded properly. In addition, if a sow in the pen was laying against the panel adjacent to the scale, this pressure against the plastic panels of the scale impacted the accuracy of the recorded weights. The effect was greatest when multiple animals were laying in this area. To prevent these interferences from occurring, sternum bars were added to the pen adjacent to the scale to prevent sows from laying in this area.

After a couple of weeks of data collection, it became apparent that there was wide variability in the data and some of the values were biologically impossible. Therefore, each sow was weighed separately at least twice during the study where a qualified individual observed the sow standing on the scale and could verify an accurate body weight. These weights were collected on all females near the beginning and end of gestation. Each female was stopped on the scale using sort boards to obtain a specific weight. With approximately 260 females in dynamic pens, there was a range in the day of gestation in which the individual weights were captured. On average, the first weight was obtained on day 26 of gestation (± 10 days) and the second weight was obtained on day 87 of gestation (± 10 days).

Data management

In addition to feed intake and body weight data collection, backfat measurements were obtained following breeding and at day 112 of gestation. Sow reproductive performance was recorded using the PigCHAMP Knowledge Software (Ames, Iowa). The following reproductive traits were obtained:

total number of piglets born, total number of piglets born alive, number of stillbirths, number of mummified fetuses, number of weaned piglets, parity, and gestation length. Due to the size, each data file (daily feed intake, daily BW, backfat measurement, and reproductive performance) was managed individually then merged or combined using statistical software (SAS Version 9.4, SAS Institute Inc, Cary, North Carolina).

Backfat measurement and reproductive performance data files did not require additional manipulation prior to analysis. Each file contained the relevant information identified by the individual sow. Body weight and feed intake data files required additional steps before analysis. Based on visual analysis of scatter plots of individual sows and use of reference weights, it was clear that there was a line over time (Figure 4) that contained the normal individual variability in body weight. We determined that on average, the sows walked across the scale approximately three times per day. The scale recorded a weight every 250 ms, thus generating numerous body weights for an individual sow starting the moment her foot stepped on the scale. On average, the scales recorded two acceptable weights per sow per day. The other recorded weights were clearly too heavy and attributed to two sows being on the scale at the same time or too light due to the sow being only partially on the scale. Therefore, it was necessary to eliminate these outlier weights from the BW data set using the reference weights that were collected.

For this process, the reference weights were utilized and the following steps were applied. First, average daily gain (ADG) was calculated from the two reference weights for each sow as follows:

$$\text{ADG} = \frac{(\text{Weight2} - \text{Weight1})}{(\text{Date2} - \text{Date1})}$$

Using ADG, a predicted weight (PW) was calculated based on the initial known weight and day of gestation:

$$\text{PW} = (\text{Weight1} + [\text{ADG} \times \text{d}]),$$

where d is calculated as the difference in days between when the measured weight and reference weight were recorded. Finally, the ratio of predicted weight to the measured weight was determined as follows:

$$\text{Ratio} = \text{Predicted weight}/\text{Actual weight}.$$

If the measured weight was 5% above or below the predicted weight, the weight was deleted. Body weights greater or less than 5% of the predicted weight were considered outliers and will be discussed later in this review.

Following these steps, the number of observations in the weight data set was decreased dramatically. Figures 4 and 5 show body weights for an individual sow before and after applying the above steps. It is important to note that we assumed ADG in gestation is fixed with only two known BWs (early and late gestation). This assumption was evaluated by determining the difference between the observed weights obtained by the scale and the predicted weight generated from the 2 manual weights. Agreement was measured using a paired *t* test to evaluate the difference between measured weights from the scale and predicted weights. The predicted weight was 0.05 kg less than the measured weight with a 95% confidence interval (0.014 to 0.077 kg). The expected difference for perfect agreement is 0 and although there was significant evidence for differences in BW (the confidence interval did not include 0), the difference was small relative to the BW of an individual sow. This assumption could be further validated by obtaining additional reference weights throughout gestation and creating a curvilinear ADG prediction throughout gestation. Thus, we may have eliminated more data points in one phase of gestation than another. It should also be noted that the estimate of fixed ADG was only used for developing the data cleaning routine (removing outliers from the data set). Subsequent analysis was performed on the actual body weights observed in the ESF scale.

Because a single BW was needed each day for subsequent analysis, sows with no BW values for a day or sows with multiple BW values on a day had to be addressed. It was possible for sows to have multiple accurate body weights per day. We were able to generate an average BW per day for each sow if multiple accurate BWs were available using the PROC MEANS statement in SAS. There were 99.6% of sows with at least one missing BW, leaving only 3 sows with no missing BWs throughout the course of gestation. Sows on average had missing BW values on 26 days of gestation, meaning that BW had to be estimated 24.5% of the time. There was no evidence that missing body weights differed across different weeks of gestation. Missing body weights were generated from the closest surrounding measured weight and the ADG from the 2 manual weights. If the most recent observed weight was prior to the missing weight, the ADG for each missing day was added to the most recent observed weight. If the most recent observed weight was after the missing weight, the ADG was subtracted from the observed weight.

For feed intake, females that did not walk through the feeding station and thereby did not consume any feed had blank feed intake values that were replaced with zeroes. As previously mentioned, errors occurred during the download of feed intake a total of 13 days over the course of the trial (149 days). The specific dates of errors were known and because it is not logical to assume feed intake

Figure 4: An example of an individual sow's body weight throughout the course of gestation. Each black dot indicates a weight obtained throughout the study (1862 total weights). The red dots are the two reference weights when the sow was individually weighed.

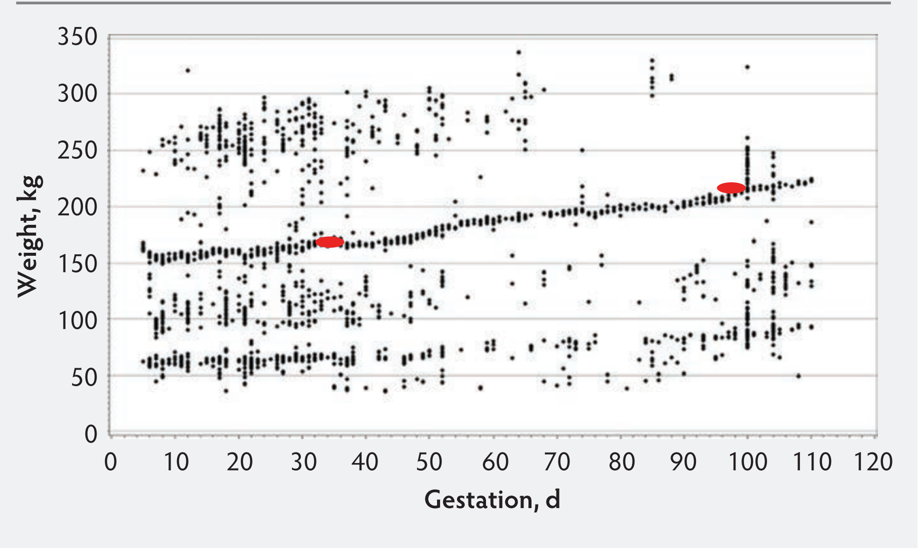
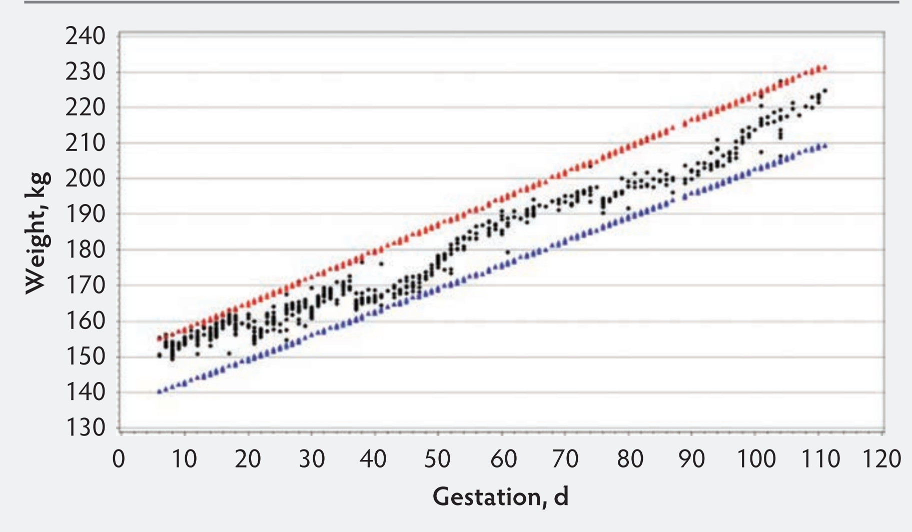


Figure 5: Individual sow body weight throughout the course of gestation after outlier data were removed. The black dots indicate weights obtained throughout the study (671 accurate weights). The red and blue lines were calculated based on the reference weights manually collected and used to determine average daily gain that could then be used to predict sow BW. Weights obtained 5% above (red line) or below (blue line) the predicted weight were deleted and deemed inaccurate.



values of zero for these days, the daily allotment of feed for the sow was assumed to be the amount of feed consumed on that day.

After removing outlier weights from the data set and reporting a feed intake and BW value for each day of gestation, these data sets were then merged with backfat and reproductive performance data. Two additional errors were identified following the merger that are believed to be specific to this farm. First, discrepancies were found in the parity reported between feed intake and reproductive performance data. Recall, feed intake and reproductive performance data files each report parity for a given sow. It is unknown if this is a recording error in the feeding system or farm recording system. To resolve this problem, parity was used from the reproductive performance data only. Second, when comparing gestation lengths from the reproductive performance data and the gestation lengths that were manually determined based on when the females left the pen and the date females farrowed, we found that the days of gestation were off by one day (day 4 of gestation in reproductive performance data is day 5 of gestation in the feed intake data). This error was attributed to the feeding system reset time of 2 PM versus the reproductive data being reset at midnight.

Implications

- As the swine industry transitions from individual gestation stalls to group housing, ESF combined with scales offer unique data collection possibilities for improved sow management as well as research opportunities.
- Feed intake and weight change data can be used to develop models for nutrient requirements and partitioning of nutrients among maternal and fetal growth. There are unlimited possibilities for research of the effects of gestation feeding and sow lifetime reproductive performance.
- Daily intake and BW collection of gestating sows can be successful, but it is imperative that the data collection process is well understood and managed appropriately. Observing the females in the feeding system is helpful in providing insight to any discrepancies that may be occurring in the data set, which is critical in assuring accurate data.
- Nevertheless, daily feed intake and BW collection of pregnant sows throughout the course of gestation can be successful and with these recommendations for conducting further research in commercial settings, we will obtain valuable information regarding the females of today's production systems.

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Conflict of interest

None reported.

Disclaimer

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Study finds lean red meat can support heart health: Eight pork cuts meet the US Department of Agriculture's lean guidelines

According to new research published in the *American Journal of Clinical Nutrition*, following a Mediterranean-style eating pattern that incorporates lean red meat can reduce cardiovascular disease risk factors. The study compared Mediterranean-style eating patterns with red meat intake from the typical amount, 3 ounces per day, to a lower intake amount, 3 ounces twice per week. The research showed that consuming up to 18 ounces of lean red meat per week lowered cholesterol and blood pressure while following the Mediterranean-style eating pattern. The study concluded that adults who are overweight or moderately obese may improve multiple cardiometabolic disease risk factors by adopting a Mediterranean-style eating pattern with or without reductions in red meat intake when red meats are lean and unprocessed. Eight cuts of pork meet the US Department of Agriculture's guidelines for lean, and the popular pork tenderloin, also used in the study, has the same amount of fat as a skinless chicken breast.

For more information, contact Adria Huseth at **AHuseth@pork.org** or 515-223-2632.

Secure Pork Supply plan moving forward

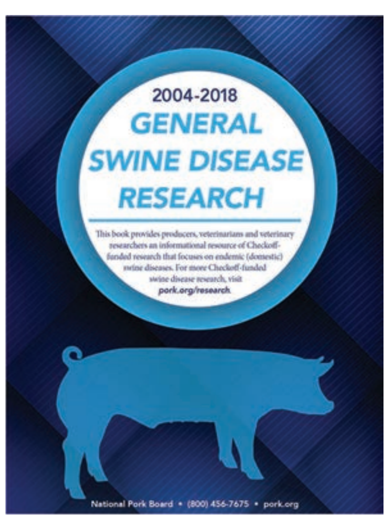
It's full steam ahead for the nation's Secure Pork Supply (SPS) plan. Work continues toward completing the necessary business continuity software that will share real-time industry data with animal health officials when every second will count during a foreign animal disease (FAD) outbreak.

According to veterinarian Patrick Webb, Checkoff's director of swine health, good progress is being made on the SPS database and software. In the end, he says it will create a valuable tool that will aid the industry not only during a potential FAD outbreak, but will have application for everyday disease-monitoring purposes too. Although full registration in the SPS plan isn't available yet, Webb advises producers and veterinarians not to wait and begin preparing now for full SPS participation in 2019. He urges that producers take a critical look at biosecurity measures and related management strategies to prepare.

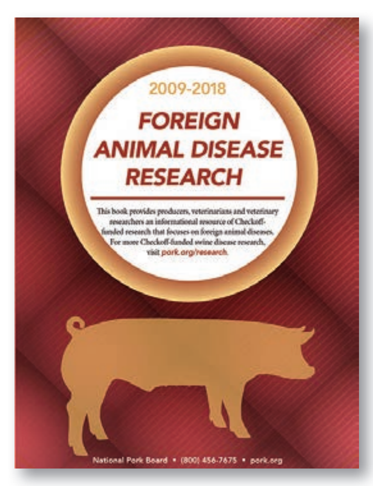
The Pork Checkoff fact sheet, Foreign Animal Disease Preparation Checklist, is a great resource to use in preparation for SPS enrollment. Producers and veterinarians also can go to the newly revised **www.securepork.org** for the most extensive resource for SPS. The comprehensive site is home base for things related to the SPS plan. The SPS plan is the result of ongoing collaboration between the US Department of Agriculture, the National Pork Board, the National Pork Producers Council, the American Association of Swine Veterinarians, academia, and other state and federal partners.

For more information, contact Patrick Webb at **PWebb@pork.org** or 515-223-3441.

New swine disease research booklets available



Two new Pork Checkoff resources will help producers, veterinarians, and veterinary researchers respond to and prepare for herd health challenges. The new *General Swine Disease Research Guide* and the *Foreign Animal Disease Research Guide* summarize Checkoff-funded research conducted from 2004 to 2018 on domestic and foreign swine diseases. The guides will help producers and their veterinarians identify and develop better diagnostic and disease-management tools to enhance disease prevention, detection and potential elimination, as well as highlighting vaccination and biosecurity measures. Download the books at **www.pork.org/research**.



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Brief Summary: Before using Enroflox® 100, consult the product insert, a summary of which follows.

CAUTION: Federal (U.S.A.) law restricts this drug to use by or on the order of a licensed veterinarian. Federal (U.S.A.) law prohibits the extra-label use of this drug in food-producing animals.

PRODUCT DESCRIPTION: Each mL of Enroflox 100 contains 100 mg of enrofloxacin. Excipients are L-arginine base 200 mg, n-butyl alcohol 30 mg, benzyl alcohol (as a preservative) 20 mg and water for injection q.s.

INDICATIONS:

Cattle - Single-Dose Therapy: Enroflox 100 is indicated for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni* and *Mycoplasma bovis* in beef and non-lactating dairy cattle; and for the control of BRD in beef and non-lactating dairy cattle at high risk of developing BRD associated with *M. haemolytica, P. multocida, H. somni* and *M. bovis*.

Cattle - Multiple-Day Therapy: Enroflox 100 is indicated for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica, Pasteurella multocida* and *Histophilus somni* in beef and non-lactating dairy cattle.

Swine: Enroflox 100 is indicated for the treatment and control of swine respiratory disease (SRD) associated with *Actinobacillus pleuropneumoniae, Pasteurella multocida, Haemophilus parasuis* and *Streptococcus suis*.

RESIDUE WARNINGS:
Cattle: Animals intended for human consumption must not be slaughtered within 28 days from the last treatment. This product is not approved for female dairy cattle 20 months of age or older, including dry dairy cows. Use in these cattle may cause drug residues in milk and/or in calves born to these cows. A withdrawal period has not been established for this product in pre-ruminating calves. Do not use in calves to be processed for veal.
Swine: Animals intended for human consumption must not be slaughtered within 5 days of receiving a single-injection dose.

HUMAN WARNINGS: For use in animals only. Keep out of the reach of children. Avoid contact with eyes. In case of contact, immediately flush eyes with copious amounts of water for 15 minutes. In case of dermal contact, wash skin with soap and water. Consult a physician if irritation persists following ocular or dermal exposures. Individuals with a history of hypersensitivity to quinolones should avoid this product. In humans, there is a risk of user photosensitization within a few hours after excessive exposure to quinolones. If excessive accidental exposure occurs, avoid direct sunlight. For customer service, to obtain a copy of the Safety Data Sheet (SDS) or to report adverse reactions, call Norbrook at 1-866-591-5777.

PRECAUTIONS:
The effects of enrofloxacin on cattle or swine reproductive performance, pregnancy and lactation have not been adequately determined.
The long-term effects on articular joint cartilage have not been determined in pigs above market weight.
Subcutaneous injection can cause a transient local tissue reaction that may result in trim loss of edible tissue at slaughter.
Enroflox 100 contains different excipients than other enrofloxacin products. The safety and efficacy of this formulation in species other than cattle and swine have not been determined.
Quinolone-class drugs should be used with caution in animals with known or suspected Central Nervous System (CNS) disorders. In such animals, quinolones have, in rare instances, been associated with CNS stimulation which may lead to convulsive seizures. Quinolone-class drugs have been shown to produce erosions of cartilage of weight-bearing joints and other signs of arthropathy in immature animals of various species. See Animal Safety section for additional information.

ADVERSE REACTIONS: No adverse reactions were observed during clinical trials.

ANIMAL SAFETY:
In cattle safety studies, clinical signs of depression, incoordination and muscle fasciculation were observed in calves when doses of 15 or 25 mg/kg were administered for 10 to 15 days. Clinical signs of depression, inappetance and incoordination were observed when a dose of 50 mg/kg was administered for 3 days. An injection site study conducted in feeder calves demonstrated that the formulation may induce a transient reaction in the subcutaneous tissue and underlying muscle. In swine safety studies, incidental lameness of short duration was observed in all groups, including the saline-treated controls. Musculoskeletal stiffness was observed following the 15 and 25 mg/kg treatments with clinical signs appearing during the second week of treatment. Clinical signs of lameness improved after treatment ceased and most animals were clinically normal at necropsy. An injection site study conducted in pigs demonstrated that the formulation may induce a transient reaction in the subcutaneous tissue.

Norbrook Laboratories Limited,
Newry, BT35 6PU, Co. Down, Northern Ireland

I02 September 2016

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NPB news continued from page 277

Stephanie Wisdom joins Checkoff's Sci-tech team in animal welfare role

The National Pork Board recently welcomed Stephanie Wisdom as its new director of animal welfare. She will work directly with Sara Crawford, Checkoff's assistant vice-president of animal welfare. The Missouri native is a graduate of the University of Missouri with a Bachelor of Science in psychology and animal science. Wisdom also has a Master of Science in animal science with a focus on swine behavior and well-being from Purdue University. Most recently, she worked at Cactus Family Farms as an animal care coordinator.

You may contact Stephanie at SWisdom@pork.org or 515-223-3534.

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AASV NEWS

Call for submissions – Industrial Partners

The American Association of Swine Veterinarians invites submissions for the Industrial Partners oral and poster sessions at the 50th AASV Annual Meeting. This is an opportunity for commercial companies to make brief presentations of a technical, educational nature to members of the AASV. The conference will be held March 9-12, 2019 in Lake Buena Vista, Florida.

The oral sessions consist of a series of 15-minute presentations scheduled from 1:00 to 5:00 PM on Sunday afternoon, March 10. A poster session takes place the same day. Poster authors will be required to be stationed with their poster from noon until 1:00 PM, and posters will remain on display throughout the afternoon and the following day for viewing.

NEW THIS YEAR: All companies submitting topics for presentation during the Industrial Partners sessions must register to participate in the AASV Technical Tables Exhibit before October 1 (see **aasv.org/annmtg/2019/techinfo.htm**).

Restricted program space necessitates a limit on the number of presentations per company. Companies that are a member of the *Journal of Swine Health and Production* (JSHAP) Industry Support Council and sponsor the AASV e-Letter may submit three topics for oral presentation. Companies that are either a member of the JSHAP Industry Support Council or sponsor the AASV e-Letter may submit two topics. All other companies may submit one topic for oral presentation. In addition, every company may submit one topic for poster presentation but the topic must not duplicate the oral presentation. All topics must represent information not previously presented at the AASV annual meeting or published in the meeting proceedings.

To participate, send the following information to aasv@aasv.org by October 1, 2018:

1) Company name
2) Presentation title
3) Brief description of presentation content
4) Presenter name and contact details (mailing address, telephone number, and e-mail)
5) Whether the submission is intended for oral or poster presentation

Receipt of submissions will be confirmed by email. Presenters will be notified of their acceptance by October 15 and must submit a paper by November 15 for publication in the meeting proceedings. Failure to submit the paper in a timely manner will jeopardize the company's future participation in these sessions.

All presenters are required to register for the meeting either as a Tech Table representative or as an individual registrant (nonmember oral and poster presenters may register at the AASV regular member rate). AASV does not provide a speaking stipend or travel reimbursement to Industrial Partners presenters.

Nominate exceptional colleagues for AASV awards

Do you know an AASV member whose dedication to the association and the swine industry is worthy of recognition? The AASV Awards Committee requests nominations for the following five awards to be presented at the 50th AASV Annual Meeting in Florida.

Howard Dunne Memorial Award – Given annually to an AASV member who has made a significant contribution and rendered outstanding service to the AASV and the swine industry.

Meritorious Service Award – Given annually to an individual who has consistently given time and effort to the association in service to the AASV members, AASV officers, and the AASV staff.

Swine Practitioner of the Year – Given annually to a swine practitioner who is an AASV member and who has demonstrated an unusual degree of proficiency in the delivery of veterinary service to his or her clients.

Technical Services/Allied Industry Veterinarian of the Year – Given annually to the technical services or allied industry veterinarian who has demonstrated an unusual degree of proficiency and effectiveness in the delivery of veterinary service to his or her company and its clients as well as given tirelessly in service to the AASV and the swine industry.

Young Swine Veterinarian of the Year – Given annually to a swine veterinarian who is an AASV member, 5 years or less post-graduation, who has demonstrated the ideals of exemplary service and proficiency early in his or her career.

Nominations are due December 15. The nomination letter should specify the award and cite the qualifications of the candidate for the award. Submit to: AASV, 830 26th Street, Perry, Iowa 50220, Fax: 515-465-3832, E-mail: **aasv@aasv.org**.

AASV news continued on page 283

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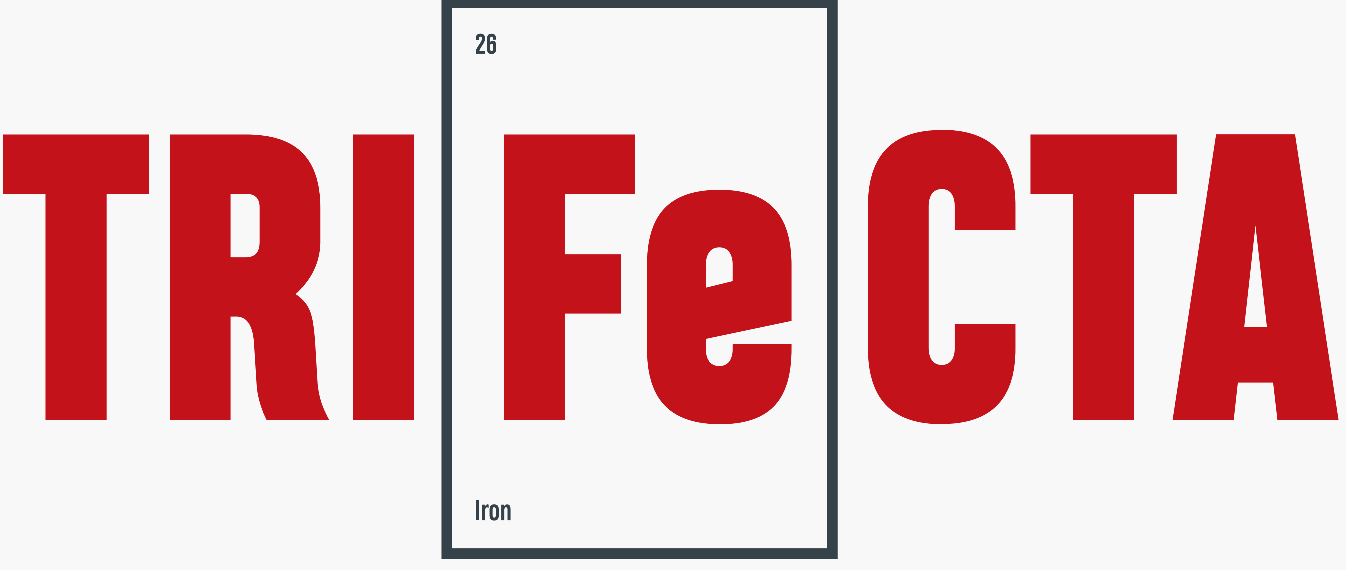

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[1] Radke, S.L., Olsen, C.W., Ensley, S.M., (2018) Elemental impurities in injectable iron products for swine. The Journal of Swine Health and Production, 26(3).

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AASV news continued from page 281

Continue the tradition: Share your tip

It's been nearly 50 years since the AASV was formed and swine veterinarians began meeting to increase their knowledge and enhance their client services. Since then, the "Practice Tips" session has proved to be one of the most enduring and popular sessions of the annual meeting. It seems that nothing beats hearing a colleague share a solution to a situation that has stymied others.

Whether you've been in practice 50 years, 5 years, or 5 months, you've experienced challenges and found a way to deal with them. You've streamlined a previously inefficient process. You've discovered a new way to do an old task. You've found inspiration to keep going, get better, and work smarter. Don't keep it to yourself – share it with your colleagues in the "AASV's Got Talent" practice tips seminar on Saturday, March 9 during the 2019 AASV Annual Meeting.

Contact Dr Jeff Harker (**jharker@amvcms.com**) or the AASV office (**aasv@aasv.org**) to volunteer your tip. There is no proceedings paper required, and you may even win a cash prize!

AASV changes student abstract submission process

AASV has issued the call for abstracts for the 2019 Student Seminar and Poster Session, the starting point for scholarship competitions held at the AASV Annual Meeting. **Substantial changes have been made to the procedure and requirements for submitting student abstracts.** Please see (and read carefully) the complete abstract submission information at **www.aasv.org/annmtg/2019/studentseminar.htm**.

What's new?

- All abstracts will be submitted online at **aasv2019.exordo.com**. Do NOT email abstracts.
- A free Ex Ordo account (name, email address, and password) is required for submission. *Since email confirmation of the account is required prior to submission, each student should set up his/her account well in advance of the abstract due date.*
- Abstracts are limited to 550 words of text, plus one table OR figure (.png or .jpg file only).
- Abstracts should not contain references.
- Abstracts should not include any identifiers (ie, names, school, state, country, diagnostic lab name, farm name, etc).
- Students provide information for two, and only two, authors – themselves and their mentor. Additional authors may be added after the abstract is accepted.
- Both authors receive automatic email confirmation from Ex Ordo, including a copy of the abstract, when the abstract submission is complete.
- Students may log into Ex Ordo to edit their submission any time up until the abstract due date.

What's the same?

- The abstract due date is firm: **September 19, 2018** at 11:59 PM CDT. Start early!
- Each student may submit only one abstract.
- A panel of private practitioners, academicians, and industry veterinarians will score the abstracts to select the oral and poster presentations.
- The review panel does not see the author information associated with each abstract.
- The abstract scoring rubric is the same as before and can be found at **www.aasv.org/anmtg/2019/studentseminar.htm**.
- The abstract submitted via Ex Ordo is used only for review and selection purposes.
- Students whose abstracts are selected for presentation are required to submit a paper to the AASV office by November 15 for publication in the conference proceedings. The proceedings paper is expected to contain identifiers and a full roster of contributing authors.

For complete details, see **www.aasv.org/annmtg/2019/studentseminar.htm**. Still have questions? Contact Dr Andrew Bowman, **bowman.214@osu.edu**.

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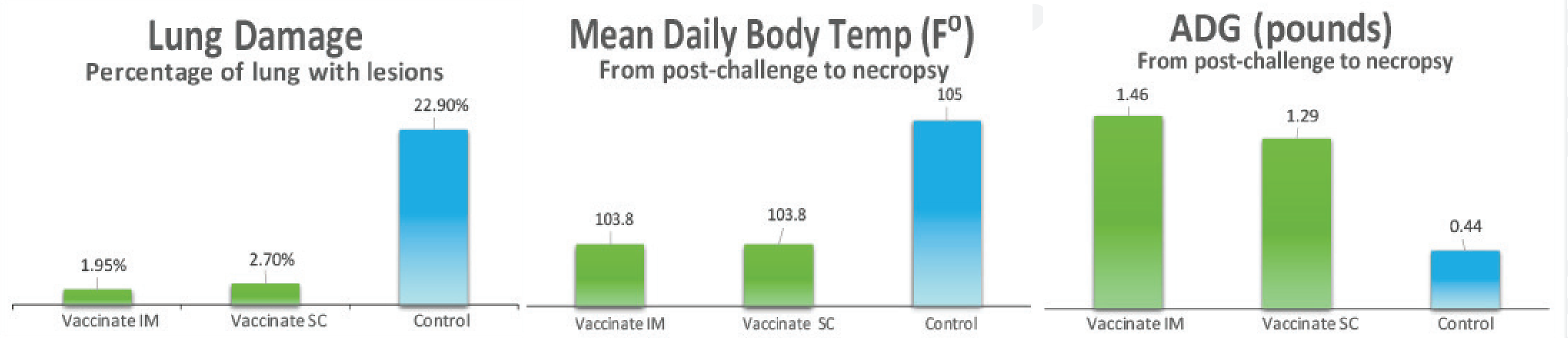

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The role of government and industry in limiting feed-borne pathogens

Our experience with porcine epidemic diarrhea (PED) and research conducted by Dr Scott Dee have highlighted the potential risk feed and feed ingredients pose regarding pathogen introduction and transmission. The National Pork Board and Swine Health Information Center recently convened a meeting to discuss this issue. Participants included veterinarians, producers, feed and ingredient representatives, researchers, and government representatives from the US Department of Agriculture (USDA) and Food and Drug Administration (FDA). The objective of the meeting was to review current research and government policies and regulations and make recommendations to reduce the risk of feed and feed ingredients for pathogen transmission.

Regarding government policy, the FDA and the USDA share regulatory responsibility for feed and feed ingredients. The FDA has oversight over feed and feed ingredients moving interstate whereas the Animal Health Protection Act gives USDA regulatory authority over imported animal feed.

When evaluating the safety of feed ingredients, the FDA relies on information provided by the ingredient industry. In addition, ingredients may be classified as Generally Recognized as Safe (GRAS) if an expert panel of reviewers determines that, based on their experience and a review of any available scientific literature, an ingredient should be safe when used as intended and in the intended species. Interestingly, ingredient companies can self-declare ingredients to be GRAS without an FDA review.

The FDA representatives present indicated that the agency does not have the resources to limit or uniformly enforce GRAS status. From a monitoring standpoint, the FDA relies largely on consumer complaints and reports of illnesses attributed to animal feed. Active surveillance of feed and feed ingredients is rare.

The recently enacted Food Safety Modernization Act may provide some additional safeguards including the establishment of preventive controls for animal feed and the development of facility-specific feed safety plans. These new regulations place responsibility for the control of identified hazards on the importer of the feed or ingredient. Facilities will identify reasonably foreseeable hazards and develop plans to prevent those hazards.

In addition, the Foreign Supplier Verification program requires the importer to identify and control potential hazards. Examples of hazards commonly applied to swine feed include bacteria, mycotoxins, drug carryover, nutrient deficiencies or toxicities, and chemical and physical hazards.

Regulatory authority within USDA generally falls to the Animal and Plant Health Inspection Service (APHIS). When considering the safety of imported products, APHIS reviews the disease status of the country of origin and recommendations from the World Organization for Animal Health and the World Trade Organization. In addition, APHIS considers the potential implications on US international trade. Restriction on product importation requires a risk assessment identifying a defined pathway for the introduction of a pathogen and the ability of the pathogen to cause disease. The importation of non-animal origin products is usually not restricted by APHIS based on animal health concerns. Representatives from USDA highlighted the importance of considering the potential impact on international trade and possible restrictions that could be imposed on exports if the United States enact restrictions on imports.

"Active surveillance of feed and feed ingredients is rare."

It has been suggested that imported feed and ingredients should be sampled for foreign animal diseases. The USDA has been reluctant to do this because if a sample were found to be positive, it could jeopardize the United States' negative disease status and thus impact international trade.

The researchers participating in the meeting noted that we now have experimental evidence that certain viruses of concern could survive transportation to the United States. There are also plausible pathways by which feed and feed ingredients could become contaminated in the country of origin and during post-processing abroad or in the United States. Research conducted at Kansas State University also implicates feed mills as a likely source of pathogen contamination during the feed manufacturing process. It is feasible that imported feed and ingredients could be a route of introduction of a foreign pathogen into the United States and that feed could serve as a vector for disease transmission to domestic herds.

The group discussed and prioritized the following list of next steps to address gaps and establish additional protections:

1. Test and verify product safety prior to shipment from a foreign country using blockchain testing and traceability, Preventive Controls for Animal Food program, or some other program.

Advocacy in action continued on page 289

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Advocacy in action continued from page 287

2. Actively monitor imported feed components for foreign animal diseases or other transboundary pathogens at ports of entry or before shipping from source countries. The monitoring should be conducted at a foreign facility prior to shipment to avoid trade implications of a positive finding in the United States.
3. Determine the minimum and median infective dose of classical swine fever, pseudorabies virus, and foot-and-mouth disease in feed assuming normal feeding behavior. This research has already been completed for African swine fever.
4. Implement active domestic monitoring in feed mills to measure the incidence of pathogens in these facilities.
5. Validation of environmental sampling tools (ie, Swiffers, sponges, paint rollers, etc).
6. Demonstrate the detectability of other viruses via environmental sampling.
7. Validate the use of dust samples compared to feed samples for detection of pathogens.
8. Enterobacteriaceae are used as an indicator organism for fecal contamination of feed. Similarly, determine if rotavirus or some other enteric virus could be an indicator of fecal contamination with other viruses.

The goal should be to prevent the introduction of foreign and transboundary diseases into the United States. It is evident, however, that we cannot rely on government to fully protect the US swine industry. We have likely imported several devastating diseases into the United States over the last few decades (porcine reproductive and respiratory syndrome, porcine circovirus, PED, etc) and our biosecurity measures have not been successful in preventing the spread of those pathogens within the domestic herd. The safeguards currently in place to guard against pathogen importation and dissemination are vulnerable and government is limited in what they can do to provide additional assurances of protection. Thus, it falls to the swine industry to take additional measures to protect the US herd.

Harry Snelson, DVM
Director of Communications

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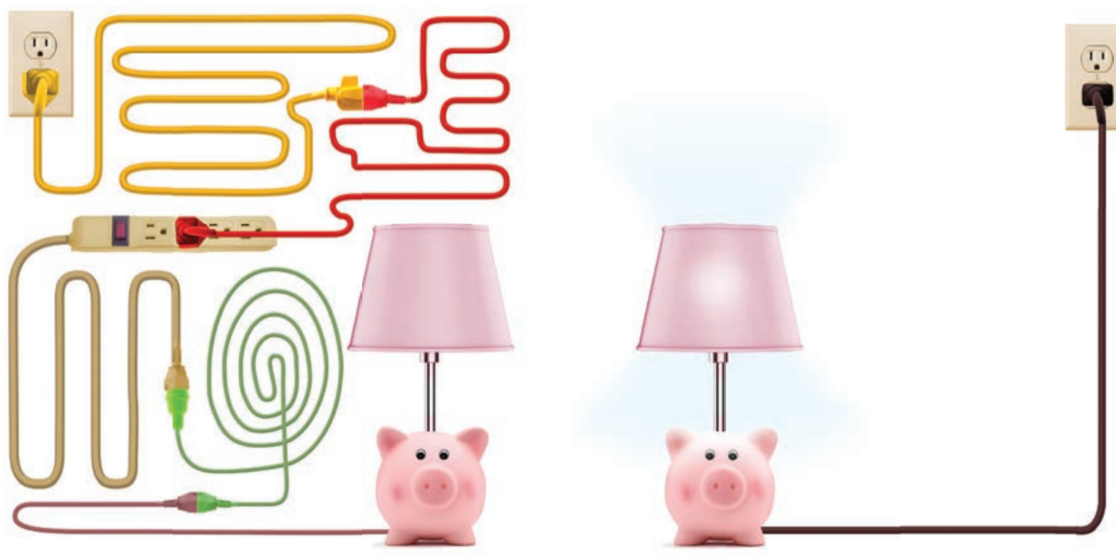

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Upcoming meetings

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November 1-2, 2018 (Thu-Fri)
Scheman Building, Iowa State University, Ames, Iowa

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Registration Services
Iowa State University
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Ames, Iowa 50010
Tel: 515-294-6222; Fax: 515-294-6223
E-mail: registrations@iastate.edu
Web: register.extension.iastate.edu/swinedisease

For questions about program content:
Dr Chris Rademacher, Conference Chair
Iowa State University
E-mail: cjrdvm@iastate.edu

Humane Endings Symposium

November 2-4, 2018 (Fri-Sun)
Westin O'Hare, Rosemont, Illinois
Hosted by American Veterinary Medical Association

For more information:
E-mail: humaneendings@avma.org

2018 North American PRRS Symposium

December 1-2, 2018 (Sat-Sun)
Chicago Marriott, Downtown Magnificent Mile

For more information:
Dr Bob Rowland, Executive Director
E-mail: naprrs@vet.k-state.edu
Web: www.vet.k-state.edu/na-prrs/

American Association of Swine Veterinarians 50th Annual Meeting

March 9-12, 2019 (Sat-Tue)
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For additional information on upcoming meetings: www.aasv.org/meetings/

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Curious pigs at University of Missouri Swine Teaching Center.

Photo courtesy of Tina Smith