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Detection of border disease virus in pigs

*Navarro-López R, Perez-de la Rosa JD,
Villarreal-Silva M, et al*

Air filtration to prevent PRRSV infection

Desrosiers R, Cousin V

Minimizing bias in clinical trials

Sargeant JM, O'Connor AM, O'Sullivan TL, et al

Impacts of processing on environmental
sample qRT-PCR results

Houston GE, Blomme AK, Harrison OL, et al

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TABLE OF CONTENTS

President's message	65
President-elect's message	67
Executive Director's message	68
From the Editorial Office	71
Identification of border disease virus in naturally infected pigs in Mexico	72
<i>Navarro-López R, Perez-de la Rosa JD, Villarreal-Silva M, et al</i>	
Air filtration to prevent porcine reproductive and respiratory syndrome virus infection	77
<i>Desrosiers R, Cousin V</i>	
Maximizing value and minimizing waste in clinical trial research in swine: Design features to minimize bias	82
<i>Sargeant JM, O'Connor AM, O'Sullivan TL, et al</i>	
Evaluating the impact of organic matter and sample processing techniques on RNA detection using environmental samples	87
<i>Houston GE, Blomme AK, Harrison OL, et al</i>	
Conversion tables	92
News from the National Pork Board	93
AASV news	97
Advocacy in action	103
Upcoming meetings	107

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JSHAP SPOTLIGHT

Dr Derald Holtkamp

Iowa State University

Dr Derald Holtkamp earned a BS ('85), an MS ('90), and a DVM ('97) from Iowa State University. Dr Holtkamp is currently a professor in the Department of Veterinary Diagnostic and Production Animal Medicine at Iowa State University. When asked why he serves as a JSHAP reviewer, Dr Holtkamp said "I value the modern scientific method and clinical experience. However, both are of limited worth unless they are shared. As a JSHAP reviewer, I have an opportunity to contribute to the quality of the information shared in the publication." Dr Holtkamp also encourages JSHAP contributors to take time to submit well-written, polished manuscripts, as those that appear to be first drafts are frustrating to review.

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PHARMACOSMOS

If it ain't broke, don't fix it

How often do we use the phrase “if it ain't broke, don't fix it” in our daily lives? Most use it to mean if something is functioning properly, it is probably best to just leave it alone and not make any changes that could potentially break it. With that, it is assumed that since the process is not completely broken, it is functioning as well as it can. While many of us use this decision-making process to prioritize which challenges to tackle first, it can become a default stance that keeps us from making good, functioning processes into improved, better functioning ones.

As we reflect on AASV activity over the past year, many examples of continued efforts to evaluate and improve different aspects of the organization become apparent, such as the Nutrition Committee revising its mission. Another example is the Student Recruitment Committee changing its name to the Student Engagement Committee, which better reflects the activity and mission of the committee. The combined evaluation of the Raising Pigs without Antibiotics Position Statement by the Pig Welfare and Pharmaceutical Issues Committees resulted in an updated AASV position statement.



The Boar Stud Committee realized the great value of the AASV document, *Health, Hygiene and Sanitation Guidelines for Boar Studs Providing Semen to the Domestic Market*, was limited with access being provided to only AASV members and received board approval to make it publicly available. This committee also identified the need to develop standardized requirements for shipping semen across state lines and received board approval to work with the US Department of Agriculture (USDA) and state animal health officials to identify opportunities. The board approved a recommendation from the Pig Welfare Committee to revise the nomenclature associated with depopulation providing additional clarity.

The board reviewed and updated the AASV bylaws with input from legal counsel with focus on membership types and changes in the chair of the Budget Committee that improves continuity of the budgeting process. The American Veterinary Medical Association (AVMA) Coalition for Connected Veterinary Care's position regarding telemedicine and the veterinarian-client-patient relationship (VCPR) prompted considerable discussion for the board. As AVMA and specific states continue to evaluate telemedicine and define the VCPR, the board established a Telemedicine Task Force to draft a position for consideration at the spring 2023 board meeting.

The Early Career Committee's successful early-career conference was held in November 2021, and now has received a significant grant from USDA to conduct a 2-year educational enrichment program for up to 25 early-career, swine

“As I complete my final message as AASV president, I am proud that this association continues to not subscribe to the “if it ain't broke, don't fix it” mentality as demonstrated by these examples.”

veterinarian AASV members. This committee also received approval to provide scholarships supporting the participation of 5 AASV early-career veterinarians in the spring 2023 cohort of the MentorVet program. The goal is to support the mental health and professional development of early-career veterinarians through a mentorship program, and for AASV to obtain feedback from participants on the value of the program for AASV members.

As I complete my final message as AASV president, I am proud that this association continues to not subscribe to the “if it ain't broke, don't fix it” mentality as demonstrated by these examples. At every level of the organization, from individual members to committee members, leadership, and staff, the continual search for opportunities to make AASV better is evident. It has been an honor to serve as AASV president, and I look forward to continued opportunities to serve the association going forward. Thank you to the membership, the board, and staff for all your support, it is all of you that truly make AASV the success that it is!

Mike Senn, DVM, MS
AASV President





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Making connections

Thank you for the opportunity to serve the AASV membership on the executive team. I will try to keep my journal comments timely and bring to the members additional comments on the needs we see and hear from the swine production and pharmaceutical industries.

We are a diverse group of professionals with the common goal of continuous improvement for the health, welfare, and performance of the pig. The AASV Annual Meeting is the best place to learn from each other. We are fortunate to have outstanding scientists and practitioners in the association willing to present their work. We all grow when we learn together. Because of the magnitude of the challenges we face, it would be futile to believe any one veterinarian could solve these difficult problems alone.

Significant challenges face our members and our clients given the clear and present risks of foreign animal disease and the growing problems of serious infectious disease. As we continue to protect our herds, we must keep in mind the need to develop teams and protocols to appropriately jump into action. Only by working together can we learn the best processes for prevention of disease and

control of outbreaks. We simply do not have time to learn from these serious infectious disease problems on our own. If you are reading this issue after attending the AASV Annual Meeting, I hope that you returned home with motivation to get to the heavy lifting of disease control and elimination, as well as the drive to get all your clients enrolled and prepared with the US Swine Health Improvement Plan.

The AASV Annual Meeting is an excellent opportunity to showcase our profession to students and early-career veterinarians. I like to start the meeting with an outline of who to meet and what to learn, and then work on the list throughout the sessions. We all know hallway talk is a great way to network and learn greater detail about the issues of the day. I can remember meetings in the past where I came away with several pages of notes from discussions with other veterinarians in the business.

Please make the most of your time at the meeting and connecting with members of the association. There is much to be gained from sharing our successes and failures with each other. We also owe it to the new AASV members to provide our time and listen to their needs.

"We are a diverse group of professionals with the common goal of continuous improvement for the health, welfare, and performance of the pig."

I challenge you to carry the motivation and personal connections all throughout the year. Remember that AASV resources, such as the website, e-Letter, and social media channels, are always available to provide routine connections with fellow AASV members. In the next JSHAP issue, I will discuss the value of building a trusted team of professional advisors around you. Our association has made the decision to offer mentorship services, especially to our younger members. I appreciate the opportunity to serve the association and I look forward to the continued success of the AASV and the swine veterinary profession.

William L Hollis, DVM
AASV President-elect



What artificial intelligence thinks about swine veterinarians

As I write this column, I am already well past the due date and Rhea is breathing down my neck to get it done. In my defense, I am a procrastinator. Because one of the things that most interested me in the swine industry was the willingness to seek out and adopt new technology, I decided to give technology a try to help me write this column. I had been hearing a lot about artificial intelligence (AI) and its growing ability to compose articles and even scientific publications. So, I went online and found an AI writer that offered a free trial. I simply told it that I wanted an article about swine veterinarians. The following is what it produced (basically unedited):

This article discusses the importance of swine veterinarians and their roles in the swine industry. They are responsible for many aspects of on-farm food safety, animal welfare, and health management. Swine veterinarians are educated veterinary students who are trained in the fundamentals of swine medicine, epidemiology, food safety, and production. They meet with the producers to discuss health and production issues, as well as provide support for zoonoses and foodborne pathogens.

As an associate veterinarian, they help improve herd health through the use of medication, education, and resistance to zoonoses. Swine veterinarians also help improve epidemiological skills by providing students with hands-on experience working with swine health. This helps them gain a better understanding of epidemiology and food safety in order to improve health production on farms. At Iowa State University's College of Veterinary Medicine, students have helped swine producers across the state by providing guidance on pork industry challenges related to animal welfare, public health, and food safety. They have also had a positive impact on improving their understanding of swine health epidemiology which can help them improve their overall production management systems.

Swine veterinarians are involved in many aspects of swine health and welfare, from overseeing the health and safety of the animals to managing their welfare care programs at the farm level. Many Canadian swine veterinarians are working to improve animal health, farm food safety, safety, animal care, and welfare issues on-farm. They work with producers to develop protocols for monitoring, vaccination, disease prevention, and control programs that address specific welfare issues such as boars and growing pigs, as well as food and health management issues. Swine veterinarians also provide advice on humane handling of swine for transportation and slaughter in order to ensure the highest standards of animal welfare.

Top swine industry veterinarians are typically international and have expertise in swine health and production. They have extensive experience with large-scale swine production, understanding the needs of pork producers, both in production and companion animals. Swine veterinarians provide health management advice, including training programs to help producers

improve the health of their herd. They also offer management certification for executive pork program managers, as well as programs for producers to improve animal welfare, production efficiency, and risk management. In addition, they support industry initiatives to promote food safety, environmental stewardship, and public health awareness regarding the consumption of pork products.

Swine veterinarians are dedicated to advancing the swine industry through their research and expertise. They often attend conferences or participate in research projects that are sponsored by different animal health companies. I have had the opportunity to attend a conference almost where respondents from throughout the swine industry observe different methods of swine production and infectious disease research. This experience has allowed me to increase my knowledge of the swine industry, learn about new work opportunities, and network with companies in the industry. Additionally, I was able to gain access to research information presented at these annual meetings, as well as gain valuable insight into current trends and topics discussed by industry leaders.

Veterinarians, farm owners, and executive veterinarians are all involved in the swine veterinary profession. Veterinary school programs, such as the swine health management program, can help to prepare new graduates with the essential skills they need to become successful swine veterinarians. A survey of American Association of Swine Veterinarians (AASV) members found that most practice managers and employees believe that new veterinarians should be given a period of adjustment when beginning work. The surveyed AASV members also agreed that swine health management addresses essential skills for a veterinarian to have in order to be successful in a practice.



Swine veterinarians are highly trained professionals who continue to advance their education and training. Many future veterinarians look for veterinary internship opportunities to gain hands-on experience in swine veterinary medicine. Early-career swine veterinarians must have a good foundation of knowledge in production medicine, animal welfare, and research. The AASV MentorVet program is designed to offer peer mentorship and coaching for new practitioners. Veterinary faculty at Iowa State University have partnered with the AASV to create the Swine Medicine Club in order to provide support and mental health coaching for students interested in swine production medicine. This type of paired mentorship is essential for new practitioners entering the industry, as they need guidance from experienced professionals who can answer questions, offer advice, and provide resources as they progress through their careers.

Swine veterinarians are responsible for providing veterinary services to swine farms, which includes prudent antimicrobial use, biosecurity program design, disease surveillance, welfare code development, and preventive measures against foreign animal diseases. They must also be familiar with food safety issues related to pork production

and the use of antimicrobial resistance. The AASV is an international organization that helps swine veterinarians stay informed on the latest developments in the field. The AASV meeting is held annually and provides a forum for discussing current issues related to swine health and welfare, as well as new developments in veterinary service delivery. Swine veterinarians have a responsibility to ensure that farm animals are properly cared for and that preventative measures are taken to avoid contamination or other food safety issues.

So, that was kind of a fun exercise and it is pretty interesting how AI gathered publicly-available information and pieced together a reasonably cogent article. Some would argue more cogent than many of mine! The technology is not perfect, as you can see. It is also a little scary. A recent news report highlighted a newly released AI program aimed at writing scientific literature and the potential for problematic outputs.¹ One example output was a scientific-appearing article that described the nutritional value of feeding broken glass to pigs.²

As you can see, the technology is almost close enough now to pass for an Executive Director's editorial article. It already has the long-winded part down. I asked it for 500 words and it gave me over 1000. With a little more tweaking, I might be able to delegate this responsibility as well!

Harry Snelson, DVM
Executive Director

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*1. Edwards B. New meta AI demo writes racist and inaccurate scientific literature, gets pulled. *ars Technica*. Published November 18, 2022. Accessed January 24, 2023.

<https://arstechnica.com/information-technology/2022/11/after-controversy-meta-pulls-demo-of-ai-model-that-writes-scientific-papers>

*2. @mrgreene1977. That's my primary concern. (see images) I got it to spit out numerous different formats for "the benefits of eating crushed glass." It hallucinated all kinds of positive statements, including study details, livestock trials, and chemical explanations. November 17, 2022. Accessed January 24, 2023. <https://twitter.com/mrgreene1977/status/1593278664161996801/photo/1>

* Non-refereed references.





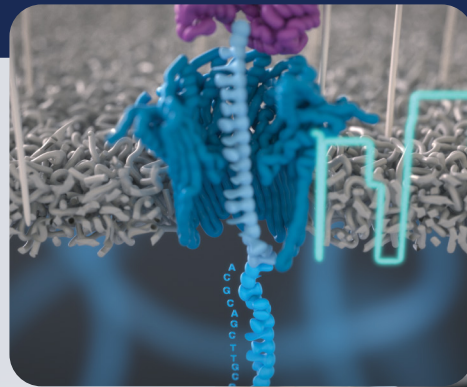
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Refining and polishing a manuscript

This year will be my fifth year serving as associate editor for the *Journal of Swine Health and Production*. When I first considered the role, I shared what the job responsibilities entailed with my husband and his response was “Personally, that job sounds awful. But I think you would be good at it if it’s what you want to do.” While the role of editor is not everyone’s idea of a good time, I enjoy collaborating with authors to refine and polish their writing and communicate their research hypotheses, data, and interpretations effectively, clearly, and concisely with our JSHAP readers.

Once a submitted manuscript has completed the peer-review process and has been conditionally accepted, I receive the files for manuscript editing and publication preparation. During the manuscript editing phase, I correct grammar, spelling, and word usage; verify mathematical calculations; verify and correct reference citations; and edit to JSHAP style. I review and edit all tables and figures for style, accuracy, clarity, and consistency with the manuscript text. I also may query the authors with any remaining peer-review comments or with questions on clarity or consistency.



Sometimes when writing about a familiar topic, what makes sense to the authors may not be clear to other readers. All proposed edits and queries are sent to the lead author for their review, approval, and response. Upon return, I re-review the manuscript, tables, figures, and author responses. Emily Hanna, our JSHAP proofreader, also reviews the manuscript at this phase. This process is repeated (usually once or twice) until both the author and I are satisfied, at which point the files are ready for the next phase.

During the publication preparation phase, Tina Smith (you will hear more from her in the next JSHAP issue) and her team of graphic designers will convert the manuscript into our standard publication format. The formatted version is reviewed by Emily and me to identify any copy errors, formatting issues (eg, inappropriate line or column breaks and spacing errors), or mistakes in page makeup. Once all corrections are made, a proof is created and sent to the author. This is the authors final opportunity to review the manuscript for accuracy.

A similar process is used for the editorial messages and news features that also appear in the journal. These along with the scientific papers, JSHAP editorial board member/reviewer spotlight, and advertisements are compiled into a complete journal issue and are once more thoroughly proofed cover-to-cover before being sent to the printer and our webmaster. My final step is to assign and register a Digital Object Identifier (DOI) for each scientific paper before readers receive the latest JSHAP issue in their mailbox or inbox.

Submitting research results and interpretations for publication is a critical step in the scientific method. However, writing a scientific manuscript is no easy task. That is why we have created several tools to help authors as they prepare manuscripts for submission to JSHAP. The *JSHAP Author Guidelines* describe

“However, writing a scientific manuscript is no easy task. That is why we have created several tools to help authors as they prepare manuscripts for submission to JSHAP.”

journal policies and procedures and details for manuscript preparation, format, and style. The *JSHAP Author Guideline Checklist* is a quick reference for the format and style that manuscripts must follow. Authors should use this checklist to review their manuscript prior to submission to ensure they have included the essential information and used the journal’s preferred style. Templates to assist authors in formatting their manuscript are available for each of the 9 genres accepted by JSHAP. Each template provides a brief description of the sections required for that genre. All these author tools can be found at aasv.org/shap/guidelines. Using these tools will assist authors in preparing their manuscript for submission, and in turn will help facilitate the peer review and editing process.

I hope this column has given you some insight into how this JSHAP issue came to be. I look forward to collaborating with future authors on their contributions to JSHAP as we strengthen, enhance, and expand the scientific body of knowledge.

Sherrie Webb, MSc
Associate Editor



Identification of border disease virus in naturally infected pigs in Mexico

Roberto Navarro-López, DVM; Juan Diego Perez-de la Rosa, DVM, MSc; Marcela Villarreal-Silva, PhD; Mario Solís-Hernández, DVM; Eric Rojas-Torres, DVM; Jorge Lemus y Sanchez, DVM; Ninnet Gomez-Romero, DVM, MSc.

Summary

Border disease virus (BDV) is a pathogen primarily infecting sheep and goats; however, infections in cattle, pigs, and wild ruminants have also been reported. Interspecies transmission of BDV occurs through close contact among infected animals. In this case report, we describe the detection of BDV in tonsil, mesenteric ganglia, and blood samples from piglets with severe clinical disease. Genetic characterization of evaluated samples resulted in the identification of BDV genotype 1 in Mexico. This represents the first report of BDV detected in pig populations in Mexico. Therefore, circulation of this virus in nonruminant populations should not be discarded.

Keywords: swine, border disease virus, reverse transcriptase-polymerase chain reaction, sequencing

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Resumen - Identificación del virus de la enfermedad de la frontera en cerdos naturalmente infectados en México

El virus de la enfermedad de la frontera (VEF) es un patógeno que infecta principalmente a las ovejas y las cabras; sin embargo, también se han reportado infecciones en bovinos, cerdos y ruminantes salvajes. La transmisión del VEF entre especies ocurre a través del contacto cercano entre animales infectados. En este reporte de caso, describimos la detección del VEF en amígdalas, ganglios mesentéricos, y muestras de sangre de lechones con enfermedad clínica grave. La caracterización genética de las muestras evaluadas dio como resultado la identificación del genotipo 1 del VEF en México. Este representa el primer reporte del VEF detectado en poblaciones porcinas en México. Por lo tanto, no debe descartarse la circulación de este virus en poblaciones no rumiantes.

Résumé - Identification du virus de la maladie des frontières (Border disease) chez des porcs naturellement infectés au Mexique

Le virus de la maladie des frontières (BDV) est un agent pathogène qui infecte principalement les ovins et les caprins ; cependant, des infections chez les bovins, les porcs et les ruminants sauvages ont également été signalées. La transmission inter-espèces du BDV se produit par contact étroit entre animaux infectés. Dans ce rapport de cas, nous décrivons la détection du BDV dans les amygdales, les ganglions mésentériques, et les échantillons de sang de porcelets atteints d'une maladie clinique grave. La caractérisation génétique des échantillons évalués a permis d'identifier le génotype 1 du BDV au Mexique. Il s'agit du premier signalement de BDV détecté dans des populations porcines au Mexique. Par conséquent, la circulation de ce virus dans les populations d'espèces non-ruminantes ne doit pas être écartée.

The *Pestivirus* genus is comprised of 4 major viral species named bovine viral diarrhoea virus (BVDV) type 1, BVDV type 2, classical swine fever virus (CSFV), and border disease virus (BDV), currently reclassified as *Pestivirus A*, *Pestivirus B*, *Pestivirus C*, and *Pestivirus D*, respectively. Together with an increasing number of additional *Pestivirus* species detected in domestic and wild animals, at least 11 viral species are recognized within the genus and named

A through K.¹ Bovine viral diarrhoea virus and BDV can infect multiple domestic and free-ranging wildlife species. In contrast, CSFV is restricted to members of the *Suidae* family.^{2,3} The capability of pestiviruses to cross species barriers, a high viral mutation rate, and the potential to generate persistently infected (PI) animals allow it to persist in affected animal populations. However, diverse clinical presentations may result depending on the individual immune response or

from differences in the cross-protective immune response.⁴ While BDV is considered an infectious agent for sheep and goat disease, it can cross-infect cattle, pigs, and nondomesticated species.^{5,6} Border disease is a viral disease associated with reproductive manifestations including abortions, fetal mummifications, stillbirths, barren ewes, birth of weak and PI lambs, abnormal body conformation, and immunosuppression. The seroprevalence rates in sheep

RN-L, MV-S, MS-H, ER-T, JLS, NG-R: Comisión México-Estados Unidos para la prevención de fiebre Aftosa y otras enfermedades exóticas de los animales, Mexico City, Mexico.

JDP-R: Centro Nacional de Servicios de Constatación en Salud Animal (CENAPA), Morelos, Mexico.

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vary depending on geographic regions and animal husbandry.⁷ Morbidity and mortality rates vary with age or stage of infection, strain virulence, and the infected host species.⁸ Transmission of BDV to pigs is possible and most likely occurs through contact with PI animals, albeit the source of viral infection cannot always be determined.⁹ Studies in the Netherlands described BDV as the predominant pestivirus infecting pig populations where BDV was isolated.^{10,11} Clinical presentations are usually mild; nonetheless, they may range from asymptomatic to clinically severe. Moreover, congenital transmission in piglets and hemorrhagic lesions in pigs have also been previously reported.^{10,12} This case report details the detection and characterization of BDV infection in piglets with severe clinical signs.

Animal care and use

This study was conducted at the Mexico-United States Commission for Prevention of Foot-and-Mouth Disease and Other Exotic Animal Diseases (CPA) according to good production practices in pig farms manual implemented by the Ministry of Agriculture and Rural Development.

Case description

The affected farm was in Tlaxcala, Mexico. The rural farm kept a total of 139 Pietrain × Yorkshire crossbred pigs under a semi-intensive production farming system, where the breeding herd was kept outside, allowing them to feed on natural vegetation in fenced enclosures, and piglets were housed in indoor pens. Diagnosis of infectious pathogens and vaccination protocols were poorly performed; therefore, the epidemiological status of endemic diseases was unknown. Over 6 days in August 2021, fifteen 45-day-old piglets developed clinical signs including fever, anorexia, cachexia, cyanosis, prostration, and death. Previous close contact with ruminants is unknown, and no other small ruminants or cattle were housed on the farm. Upon the onset of clinical signs, sick animals were isolated in separate pens off-site. Other biosecurity strategies were implemented including cleaning and disinfection of all areas, control protocols for entry and exit, and use of personal protective clothing.

Necropsy findings from 2 animals included hemorrhagic lung lesions and fibrosis, pleuritis, and petechial hemorrhages in the jejunum, ileum, bladder,

and kidney surface epithelium. Four serum samples from sick animals and tissue samples of brain, tonsil, kidney, spleen, and mesenteric ganglia from 1 dead animal were collected and submitted for diagnosis to the Immunology, Cellular and Molecular Biology Laboratory at CPA and reported as case number CPA-12362-21. Prior to the disease event, no evidence of related clinical manifestations was registered on the farm or the neighboring farms.

Diagnosis and laboratory findings

Initially, the differential diagnosis included CSFV, African swine fever virus (ASFV), and pseudorabies virus (PRV), which were ruled out by negative real-time quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) results. Subsequently, qRT-PCR was performed to detect additional viruses that display similar clinical signs, such as porcine epidemic diarrhea virus (PEDV), porcine circovirus type 2 (PCV-2), porcine circovirus type 3 (PCV-3), transmissible gastroenteritis virus (TGEV), and porcine reproductive and respiratory syndrome virus (PRRSV); results were negative. End-point RT-PCR was used to assess BDV and BVDV presence in tissue samples, and BDV-positive results were obtained from the spleen, kidney, tonsil, and mesenteric ganglia tissue samples.

In addition, a pool of tissue samples was submitted to the National Center for Diagnostic Services in Animal Health (CENASA) for complementary qRT-PCR and polymerase chain reaction (PCR) testing for porcine parvovirus (PPV), Senecavirus A (SVA), porcine rubulavirus (PoRV), influenza A virus (IAV), *Actinobacillus pleuropneumoniae*, *Brachyspira hamptonii*, *Brachyspira hyodysenteriae*, *Erysipelothrix rhusiopathiae*, *Mycoplasma*, *Mycoplasma hyopneumoniae*, *Pasteurella multocida*, and *Salmonella*. All tests were negative except for PPV.

Virus isolation attempts from BDV-positive tissue samples, performed under the procedure described in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals¹³ at the Biosafety Level 3 Cell Culture Laboratory at CPA were unsuccessful. Serum from animals positive for BDV by RT-PCR was further analyzed using a virus neutralization test (VNT) and enzyme-linked immunosorbent assay (ELISA) for the presence of specific antibodies; negative results were obtained from both assays.

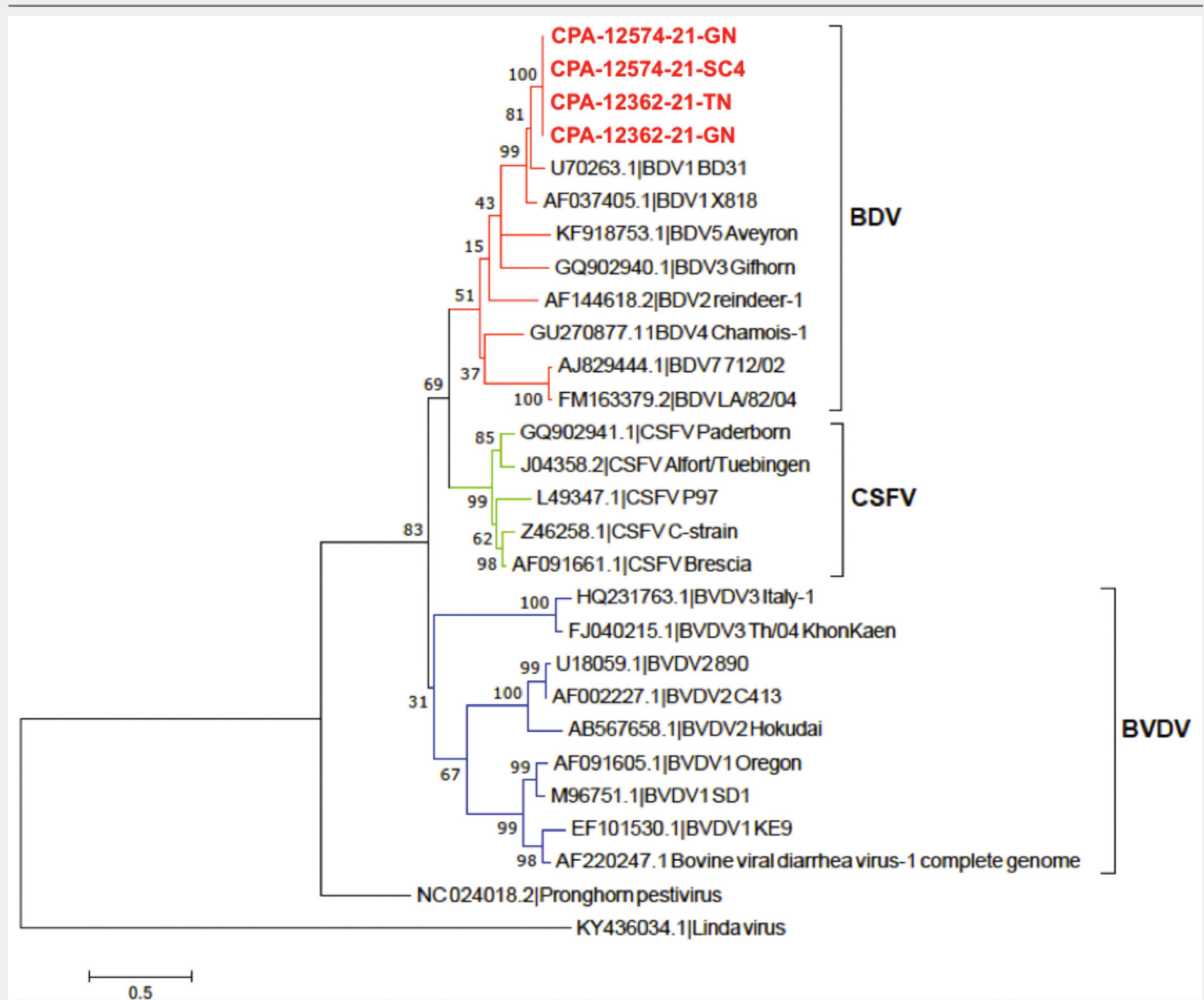
Due to BDV-positive pigs and new mortality cases on the described farm, and in accordance with epidemiological surveillance, an examination was carried out on the farm 3 days after BDV was first detected. Five whole-blood samples from clinically healthy animals and tonsil, liver, kidney, spleen, and mesenteric ganglia samples from 1 dead piglet were collected and submitted to the CPA for viral testing, with results reported in case number CPA-12574-21. The piglet that presented with clinical disease and death, similar to those from the initial report, was immediately diagnosed as BDV positive using end-point RT-PCR. Border disease virus was detected in 3 of 5 whole-blood samples using end-point RT-PCR. Further, BDV RNA was detected in mesenteric ganglia and liver samples. However, attempted BDV isolation from the collected tissue samples was unsuccessful. Serological testing for BDV-specific antibodies by ELISA and VNT was negative. Similarly, samples were negative for CSFV, ASFV, PRV, PEDV, PCV-2, PCV-3, TGEV, and PRRSV using the qRT-PCR technique. Subsequently, a pool of tissue samples was submitted for diagnosis of PPV, SVA, PoRV, IAV, *A pleuropneumoniae*, *B hamptonii*, *B hyodysenteriae*, *E rhusiopathiae*, *Mycoplasma*, *M hyopneumoniae*, *P multocida*, and *Salmonella*. The pool of tissue samples was positive for PPV and *Mycoplasma* using PCR.

For further characterization of BDV from these cases, mesenteric ganglia and tonsil samples from case CPA-12362-21 and mesenteric ganglia and whole-blood samples belonging to case CPA-12574-21 were selected for additional analysis. Positive RT-PCR products from each case were sequenced by the Sanger method. The 4 partial N^{pro} nucleotide sequences were individually deposited in GenBank under accession numbers OK667067, OK667068, OK667069, and OK667070. Subsequent phylogenetic analysis indicated that evaluated sequences were clustered within the BDV-1 genotype (Figure 1).

Discussion

Border disease virus is reported globally as an important pathogen with at least 8 genotypes, from BDV-1 to BDV-8.¹⁴ Detection in diverse species of even-toed ungulates, including sheep, goats, cattle, chamois, and pigs, has been previously reported.¹⁵⁻¹⁷ Border disease virus infection in sheep produces clinical signs ranging from mild to severe including

Figure 1: Phylogenetic tree based on partial border disease virus N^{Pro} sequence. Phylogenetic inference was conducted using the maximum likelihood method. Distances were computed using Kimura 2 parameter model. Reference sequences are identified by GenBank accession numbers. Sequences obtained in this study are labeled in red. BDV = border disease virus; CSFV = classical swine fever virus; BVDV = bovine viral diarrhea virus.



reproductive failure, congenital disorders, and abnormal body conformation.¹⁸ In addition, congenital infection occurring during the first half of gestation may lead to abortion and stillbirth, the birth of lambs with malformations, and the birth of PI animals if BDV infection occurs before day 60 of gestation.¹⁹ These animals represent the main source of infection and maintenance of BDV in the animal population.²⁰ Fetal death may occur at any stage of gestation. However, it is more common in fetuses infected early in gestation.¹⁵ Severity of clinical signs depend on the timing of infection during pregnancy, the virulence of the infecting strain, and the susceptibility of the species infected.¹⁵

Seroprevalence may vary from 5% to 90% among sheep populations depending on the region surveyed.^{17,21}

Mexico has been recognized as CSFV free since 2015; however, active epidemiological surveillance is maintained to detect any indication of CSFV infection.^{22,23} Therefore, serological assays have been conducted to determine the prevalence of pestivirus infections in pigs. The prevalence of BDV antibodies was investigated in pigs nationwide from 2011 to October 2021 revealing an estimated 41.17% seroprevalence.²⁴ Likewise, during a national screening for pestivirus in cattle, 3 cases were found to be BDV positive; genetic

characterization typed the Mexican strains as BDV-1.⁵ These findings highly suggest BDV circulation in pig and cattle populations in Mexico, probably due to natural infection through close contact among ruminants and pigs since it appears to be the most crucial risk factor for interspecies transmission.²⁵ Conversely, no BDV seroconversion was detected in this study. This is due to serum sample collection occurring in an early stage of the BDV infection; therefore, no detectable antibodies were produced by the time of sampling. Previous studies of experimentally BDV-inoculated sows showed seroconversion after 3 weeks post inoculation.²⁶

Natural and experimental infection studies have demonstrated the susceptibility of domestic pigs to BDV strains. Border disease virus infection in pigs leading to mild or inapparent manifestations has been described elsewhere.^{9,26} One study showed that BDV-infected pigs with no clinical signs and no histopathological lesions could shed the virus through oronasal secretions from 3 to 7 days post infection and became viremic at 3 to 14 days post infection.¹⁷ In 1996, the Frijters strain was isolated from congenitally infected piglets and genetically characterized as a BDV strain able to infect pigs and is circulating among large populations in Europe.¹⁰ Roehe et al¹² detected a virus genetically more related to BDV than CSFV or BVDV from a severe clinical manifestation in weaned pigs showing hemorrhagic lesions at necropsy. Nonetheless, an association among histopathological lesions and the presence of viral antigen is required to confirm the causative agent. Similarly, in northern and western France, the use of a BDV-contaminated vaccine elicited eyelid edema, locomotor disorders, decay, and spontaneous death in piglets and sows; at necropsy, hemorrhagic lesions were similar to those observed with CSFV. In addition, these animals showed persistent infection and immunotolerance.²⁷

Our study describes the detection of BDV in mesenteric ganglia, tonsil, and blood samples from pigs with severe clinical disease suggesting the BDV infection was present in the surveyed animals. We performed sequencing and genetic characterization by phylogenetic inference using N^{pr}o sequence in all RT-PCR detected BDV strains, which revealed a close relationship to the BD31 strain (Figure 1). This was similar to the characterized BDV strain detected on a pig farm with no ruminants in Japan.²⁸ The BDV-1 genotype has also been detected as the circulating BDV strain in the United States, the United Kingdom, Australia, and New Zealand.²⁹⁻³¹

Border disease virus has been detected in serum samples from cattle in Mexico.⁵ Serological evidence of BDV infection in pigs has also been recorded.²⁴ No virus isolation was obtained in this study, which is similar to other studies among the surveyed populations.^{28,32}

At the same time, PPV was detected on both sets of tissues tested. Porcine parvovirus is considered endemic in swine populations worldwide and one of the major viral pathogens causing reproductive failure.³³ Despite its detection, PPV is mainly associated with reproductive disorders summarized under the acronym SMEDI (stillbirth, mummifications, embryonic death, and infertility), with clinical disease restricted to pregnant sows or gilts. In piglets, PPV infection does not cause clinical disease.³⁴ Moreover, the immunosuppression caused by BDV and PPV can increase the risk of opportunistic infections.¹⁵ The detection of ubiquitous *Mycoplasma* species in surveyed samples is not unexpected; however, it might be associated with an immunosuppressive event.³⁵

In this case, the clinical disease presentation cannot only be associated with BDV infection since the lack of serological assay evidence and absence of pathological evaluations prevented us from determining BDV as the causative agent post event. Nevertheless, opportunistic pathogens could be involved in the severe clinical disease and should be considered.

Finding BDV in the national swine population has relevant implications in a country where CSFV eradication has been achieved as serological tests will not differentiate among BDV, BVDV, or CSFV infections.²⁸ This is the first report of BDV in pigs in Mexico, and BDV-positive serology reinforces the suggestion that BDV can be considered an endemic virus. The latter highlights the need for implementation of accurate swine diagnostic tests able to detect and discriminate among pestiviruses and other pathogens with similar pathologies to determine the definitive cause of disease. Furthermore, surveys are needed to determine the occurrence of BDV in pigs and the impact on swine health and production.

Implications

- BDV was detected in seronegative pigs from Mexico.
- For seronegative domestic pigs, BDV remains a potential risk.
- Detecting BDV transmission in domestic pigs can be diagnostic challenge.

Acknowledgments

Conflict of interest

None reported.

Disclaimer

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Air filtration to prevent porcine reproductive and respiratory syndrome virus infection

Robert Desrosiers, DVM, DABVP; Vincent Cousin, Agric Eng

Summary

This commentary reviews results obtained in France and North America with different air filtration systems to prevent porcine reproductive and respiratory syndrome virus (PRRSV) infection. Most systems installed in France use high-efficiency particulate air (HEPA) filters and positive-pressure ventilation systems, while those in North America initially used mainly negative-pressure ventilation systems and filters with minimum efficiency rating values of 14 to 16. Major reductions in PRRSV cases were observed in most studies where the latter were used. Installing HEPA filters resulted in an almost complete elimination of PRRSV cases. No cases were recorded in 95% of farms where they were used.

Keywords: swine, air filtration, porcine reproductive and respiratory syndrome, prevention

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Resumen - La filtración de aire para prevenir la infección por el virus del síndrome reproductivo y respiratorio porcino

Este comentario revisa los resultados obtenidos en Francia y América del Norte con diferentes sistemas de filtración de aire para prevenir la infección por el virus del síndrome reproductivo y respiratorio porcino (PRRSV). La mayoría de los sistemas instalados en Francia usan filtros de partículas de aire de alta eficiencia (HEPA) y sistemas de ventilación de presión positiva, mientras que los de América del Norte inicialmente principalmente usaban sistemas de ventilación de presión negativa y filtros con valores mínimos de clasificación de eficiencia de 14 a 16. Se observaron reducciones importantes en los casos del PRRSV en la mayoría de los estudios en los que se utilizó este último. La instalación de filtros HEPA dio como resultado una eliminación casi completa de los casos de PRRSV. No se registraron casos en el 95% de las granjas donde se utilizaron.

Résumé - Filtration de l'air pour prévenir l'infection par le virus du syndrome reproducteur et respiratoire porcine

Le présent commentaire fait une revue des résultats obtenus en France et en Amérique du Nord avec différents systèmes de filtration d'air afin de prévenir l'infection par le virus du syndrome reproducteur et respiratoire porcine (VSR-RP). La majorité des systèmes installés en France utilise des filtres particuliers à haute efficacité (HEPA) et des systèmes de ventilation à pression positive, alors que ceux en Amérique du Nord utilisaient initialement principalement des systèmes de ventilation à pression négative et des filtres avec des valeurs d'efficacité minimales de 14 à 16. Des réductions marquées des cas de VSR-RP ont été observées dans la plupart des études où ces derniers étaient utilisés. L'installation de filtres HEPA a résulté en une élimination presque complète des cas de VSR-RP. Aucun cas n'a été enregistré dans 95% des fermes où ils étaient utilisés.

Results of air filtration to prevent porcine reproductive and respiratory syndrome virus (PRRSV) infection can be of interest for 2 reasons. First, positive results indicate a way to reduce losses and suffering associated with this disease. Second, the results allow for indirect assessment of the relative importance of aerosol transmission in the epidemiology of the disease. If after air filtration the number of cases was reduced by a large percentage in the absence of significant improvements in other biosecurity measures, it would

mean that aerosol transmission is responsible for a large percentage of PRRSV cases. This commentary will summarize results obtained with different air filtration systems in France and North America. Studies published within the last 10 years were selected so that relatively recent data were considered.

High-efficiency particulate air filters

High-efficiency particulate air (HEPA) filters are expensive, but they can

prevent the passage of at least 99.97% of particles of any size.¹ Their use is often limited to herds that are particularly important, like boar studs, nucleus, or multiplier herds. These filters have been used mostly in France, normally coupled with positive-pressure ventilation. The site in France where this system was first used for swine was the Outil expérimental de l'ANSES, laboratoire de Ploufragan (formerly called Station de Pathologie Porcine de Ploufragan). This experimental unit is where many of the French studies on swine infectious

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diseases have been conducted.² This site includes a small specific-pathogen-free herd protected by air filtration since its installment in 1979. The site is in Brittany, the area of France where swine production is the most intensified. After 42 years in operation, the herd has remained negative for pathogens like PRRSV, influenza A virus-swine, pseudorabies virus, porcine respiratory coronavirus, and *Mycoplasma hyopneumoniae*, all of which are known to be transmissible by aerosol.^{3,4}

This filtration technology was later used in farms of importance for different companies. Table 1 shows the number of farms that were equipped with this technology since 1995, the number of years prior to 2022 that the farm was at risk, and the number of PRRSV cases over the years.

Fifty-three farms were equipped with a HEPA filtration system since 1995, with an average filtration duration of 14.2 years. Thirty-seven of the farms were sow sites of which 32 were farrow-to-finish operations on the same site, 12 were boar studs, and 4 were finishing sites. Sow sites had between 150 and 1000 sows and boar studs had between 32 and 300 boars. Over the years, 2 farms originally filtered in 1998 broke with PRRSV, one farm in 2006 and the other in 2012. In both cases the epidemiological investigation concluded that a biosecurity breach was likely responsible for the infections. All farms have remained negative for *Mycoplasma hyopneumoniae*, another significant pathogen present in most countries including France.

A French company with a swine farm in China equipped with this type of system has remained negative for PRRSV since it was populated in 2016 (V. Cousin, unpublished data). Quebec, Canada has 5 sites that are equipped with a HEPA filtration system, 4 boar studs and 1 farrow-to-finish operation. The first systems were installed in 2003, and none have yet to become infected with PRRSV (R. Desrosiers, unpublished data). When considering the proportional size of its swine industry, few farms are equipped with a HEPA filtration system in the United States. One veterinary practitioner consults with 6 boar studs that are equipped with HEPA filters, the first installed in 2008. One farm broke with PRRSV twice. The investigation revealed that the filtration system had a bypass on a hand-made duct that allowed unfiltered air to be introduced into the barn.

Table 1: Number of farms equipped with HEPA filtration, years of installation, farm years at risk, and number of PRRS cases

Installation year	No. of farms	Farm years at risk	PRRSV cases
1995	1	27	0
1996	2	52	0
1997	1	25	0
1998	4	96	2
1999	3	69	0
2000	2	44	0
2001	2	42	0
2002	2	40	0
2003	3	57	0
2005	2	34	0
2007	2	30	0
2009	3	39	0
2010	1	12	0
2011	3	33	0
2012	3	30	0
2013	2	18	0
2014	2	16	0
2015	7	49	0
2016	4	24	0
2017	1	5	0
2018	1	4	0
2019	2	6	0
Total	53	752	2
Cases per farm year at risk			0.0027
Mean number of filtration years per farm: 14.2			

HEPA = high-efficiency particulate air; PRRSV = porcine reproductive and respiratory syndrome virus.

The farm has remained PRRSV negative since the problem was fixed in 2019, and none of the other 5 boar studs have broken with the disease. (D. Reicks, DVM, email, July 2021). Considering the results obtained in France, China, Quebec, and the United States, 95.4% (62 of 65) of the farms where this system was used have remained PRRSV negative. If the boar stud with the faulty system is removed from the list, then none of the remaining 64 farms have broken with PRRSV since 2012.

Other filtration systems

Most of the air filtration systems installed in the United States use filters with minimum efficiency rating values (MERV) of 14, 15, or 16. These systems are predicted to respectively prevent introduction of 75%, 85%, and 95% or more of particles between 0.3 and 1.0 micron.⁵ Also, some farms are only filtering air during the cooler times of the year when PRRSV outbreaks are more frequent. Most farms initially used a negative-pressure ventilation system, but positive-pressure ventilation has gained popularity in recent years.⁶ An advantage of positive-pressure ventilation is that, if functioning

properly, unfiltered air is not likely to be introduced into the barn through various openings. Many studies have evaluated the results obtained with air filtration, but often without specifying the type of ventilation system, the MERVs of the filters used, and whether they were filtered all year long. Table 2 summarizes the results obtained in studies conducted over the last 10 years.

Only one study did not report a major beneficial impact from filtration. Silva et al¹⁰ used machine learning algorithms to identify key biosecurity practices and factors associated with breeding herds reporting PRRSV outbreaks. They concluded that air filtration was not ranked among the top predictors for PRRSV outbreaks and suggested this could be due to the percentage of farms that reported air filtration between groups (11 of 50 farms that became PRRSV positive and 11 of 34 that remained uncontaminated). The Tousignant¹² study evaluated the results obtained with filtered farms, which had an average PRRSV incidence of 6% per year but did not evaluate results from unfiltered farms. The Morrison Swine Health Monitoring Project (MSHMP) tracks disease occurrence on a subset of US sow herds. The number

of herds in the subset has changed over time and in recent years represented approximately 50% of the US sow inventory. Data from this project showed that 20.8% to 39.2% of sow herds reported a PRRSV break each year between 2009 and 2021 (MSHMP, email, December 2021). That is 3.5 to 6.5 times more than was observed in filtered farms of the Tousignant study.¹²

In the other 9 studies, the number of PRRSV breaks was reduced 2- to 14.4-fold with filtration. The Havas et al⁷ study did not compare herds in terms of breaks, but in terms of being infected with PRRSV or not. The odds of being positive for PRRSV were reduced by 95% with filtration.

Discussion

The possibility for PRRSV to be transmitted between farms by aerosol has been a controversial topic for many years. In 1999, it was proposed in a popular newsletter that more and more epidemiological evidence suggested that PRRSV could be transmitted between farms by aerosol.¹⁷ This created some turmoil because up until then, and for years to come, it was not shown to be possible to infect

pigs with PRRSV by aerosol over a distance greater than 2.5 m.¹⁸⁻²⁰ Different researchers expressed opposite views in what was sometimes referred to as the aerosol debate.³ In 2004 and 2005, published studies from different countries and local field observations strongly supporting aerosol transmission of different swine pathogens, including PRRSV, were reviewed.^{3,4} Among others, these reviews mentioned the impressive results obtained with air filtration in France. In 2009, Pitkin et al²¹ proved using a regional production model that aerosol transmission of the virus over 120 m could occur repeatedly and confirmed that air filtration was effective at preventing this type of contamination. Since then, different studies have suggested that not only is aerosol transmission possible between farms, but it could even be among the main modes by which the virus is introduced into breeding herds.

The results included in Table 2 would support that there are situations where air filtration makes a large difference in the incidence of PRRSV outbreaks. A frequent and sensible argument to explain the positive results obtained with air filtration is that when installed, other biosecurity measures may also be improved

Table 2: Studies between 2012 and 2021 where the impact of air filtration on PRRSV was evaluated

Reference	No. Farms; period involved	Results
Havas et al, ⁷ 2021	Not specified; not specified	95% lesser odds of being PRRSV infected if filtered
Feder, ⁸ 2021	85 farms; not specified	More than 3 times less PRRSV cases after filtration
Moeller et al, ⁹ 2020	208 farms; not specified	Odds of PRRSV cases at 0.0992 if filtered vs unfiltered
Silva et al, ¹⁰ 2019	11 farms in case & control groups; 2012-2017	Air filtration not ranked among top predictors for PRRSV breaks
Vilalta et al, ¹¹ 2018	58 farms; 2009-2018	Risk of breaking with PRRSV decreased by half after filtration
Thomas, ⁶ 2018	27 farms; 18 months	PRRSV risk reduced 4.3 times after filtration
Tousignant, ¹² 2015	10 in 2005 up to 119 in 2014; 2005-2014	Incidence of PRRSV cases across all farms in the data set averaged 6% per year
Reicks, ¹³ 2015	25 boar studs; 4.1 years before and 7.7 years after	Incidence per year went from 14.4% to 1.0% after filtration
Reicks, ¹⁴ 2014	93 farms; 4.2 years before and 4.8 years after	New infections per year went from 52.5% to 11.3% after filtration
Alonso et al, ¹⁵ 2013	37 farms; 7 years	Filtration reduced risk of infection by 80%
Dee et al, ¹⁶ 2012	24 farms; 2005-2012	From 1.23 cases per herd year before to 0.17 cases per herd year after filtration

PRRSV = porcine reproductive and respiratory syndrome virus.

contributing to the apparent positive filtration impact. While this is a possibility, the importance of that beneficial impact is unknown. Given the losses often associated with PRRSV, major efforts to improve biosecurity measures have already been made for many years, whether farms were filtered or not. Furthermore, in a comparison of 25 boar studs, Reicks^{13,22} stated that the percentage of breaks per year went from 14.4% before filtration to 1.0% after it was implemented with no changes in biosecurity. Thus, the improvement in PRRSV incidence in that case could be attributed solely to filtration, which suggests that most of the breaks prior to filtration were associated with aerosol transmission. Similarly in another US study, Alonso et al¹⁵ concluded that air filtration led to an approximately 80% reduction in risk of novel PRRSV introduction indicating that approximately four-fifths of PRRSV outbreaks may be attributable to aerosol transmission on large sow farms with good biosecurity in swine-dense regions. The authors reported that while unable to assess standards of external biosecurity in their study farms, this concern was mitigated by the relatively uniform veterinary oversight across all of them. Finally, Dee et al¹⁶ reported in one part of their study that the odds for a new PRRSV infection in a nonfiltered breeding herd was 8.03 times higher than in a filtered breeding herd. The authors mentioned that the selected herds used industry standard biosecurity practices and were exposed to comparable conditions suggesting that filtration was the most important difference between the groups.

The results obtained with air filtration in France were and have remained impressive. In one of the first reports on its efficacy, Lecarpentier et al²³ described 11 farms equipped with such a system that were owned by the same company. The first filtration was installed in 1996, two were installed in 2002, and the others installed between 1998 and 2000. Seven of the 11 farms had been contaminated with PRRSV prior to filtration. None of them became infected prior to 2004, when the study was reported. Ten of these 11 herds were in Brittany, the area in France with the highest pig density. However, as previously mentioned, the main system used in France is different than those used in most cases in the United States.

Dee et al²⁴ showed that the efficacy of various systems could vary. When comparing HEPA filters to a MERV 15 system, only the former prevented infection of

pigs in all replicates (76 of 76) while the latter did not in 2 of them (74 of 76). More recently Batista²⁵ evaluated the efficacy of different filters (MERV 14, MERV 16, and antimicrobial filters) to block the passage of PRRSV, influenza A virus-swine, and *Streptococcus thermophilus* (as a model for *Streptococcus suis*). The author concluded that the MERV 16 filters had the highest capture efficiencies. When considering their ability to prevent airborne PRRSV transport, Dee et al²⁶ showed that efficacy differences may be found with systems from different companies having the same theoretical MERV values. Finally, it was also suggested that some filtration systems do not maintain their efficacy over time as well as others.⁶ Thus, it is important when evaluating results obtained with air filtration to consider the specifics of each filtration system used.

Dee et al¹⁶ reported that 24 farms had an average of 1.23 cases per farm year at risk before filtration. It greatly improved to 0.17 cases per farm year at risk following filtration with MERV 14 or 16 filters. The 53 farms equipped with HEPA filters in France had 0.0027 cases per farm year at risk, or 63 times less. More information would be needed to determine to what level comparison between the US and French results can be made. Different factors would need to be evaluated, including the respective biosecurity measures observed on farms, the size of the farms, the infection pressure from the neighboring herds, the aerosol transmissibility of the strains, and the climatic conditions. Nevertheless, the magnitude of difference in the results obtained as well as the theoretical superiority of HEPA filters seem to leave little doubt on the fact that better results can be obtained with these filters.

There is no more debate over the possibility for PRRSV to be transmitted between farms by aerosol. Today the question is how frequently and over what possible distances aerosol transmission occurs. The results obtained with air filtration in different countries suggest that there are situations, particularly in hog-dense areas, where viral aerosol transmission could be the most important way of introduction into breeding herds. This would align with the relative inefficacy of other significant biosecurity efforts that have been applied to control it.²⁷

Nevertheless, there are clearly other ways by which the PRRSV can be introduced into swine herds, and not all

studies have shown that aerosol or local transmission had an important role in the epidemiology of PRRSV.²⁸⁻³² Looking at spatial and temporal patterns of PRRSV genotypes, Rosendal et al²⁸ concluded that there was no strong evidence that aerosol transmission was occurring in Ontario. Similarly, Kwong et al³² reported that the 3 relatively most important factors for the spread of a specific genotype in that province were sharing the same herd ownership, gilt source, and market trucks. Spatial proximity could not be identified as an important contributor to spread. In a review on the topic, Arruda et al³¹ reported that aerosol transmission of the PRRSV was possible, but further studies were needed to determine if it was a frequent event or not. While most studies where air filtration was evaluated suggest that aerosol contamination is frequent, the relative importance of that transmission route is still debated.

Because air filtration systems currently used are expensive, another question remaining is the distance over which the virus can travel by aerosol to infect herds. Quantifying that distance would help to determine at what point investment in filtration or in future methods found to prevent aerosol contamination may be justified.

Finally, not all air filtration systems are created equal as some are more effective than others. Efficient prevention of aerosol contamination can allow a farm to remain negative for PRRSV and other airborne pathogens on a long-term basis.

Implications

- Not all air filtration systems are created equal.
- Being PRRSV negative long-term is possible, even in hog-dense areas.
- There are situations where aerosol is the most frequent contamination source.

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Conflict of interest

None

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Maximizing value and minimizing waste in clinical trial research in swine: Design features to minimize bias

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Summary

Researchers designing trials should implement design features intended to reduce bias. These include random allocation to intervention groups and blinding of caregivers and outcome assessors. The method of generating the random sequence should be reported, as well as methods for stratification or blocking if used. When blinding is not possible, objectively measured outcomes should be used. Allocation concealment may not be essential when all eligible pens or animals are enrolled and there is no preference for intervention group. *An a priori* trial protocol should be made publicly available, and results for all outcomes evaluated should be reported.

Keywords: swine, randomization, allocation concealment, blinding, bias

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Resumen - Maximizando el valor y minimizando el desperdicio en la investigación de pruebas clínicas en cerdos: Características de diseño para minimizar el sesgo

Los investigadores que diseñan pruebas deben implementar características de diseño destinadas a reducir el sesgo. Estas incluyen la asignación aleatoria de los grupos de intervención y el cegamiento de los guardianes y de los evaluadores de los resultados. Se debe informar el método de generación de la secuencia aleatoria, así como los métodos de estratificación o bloqueo, si es que se utilizan. Cuando no es posible el cegamiento, se deben utilizar resultados medidos objetivamente. El ocultamiento de la asignación puede no ser esencial cuando todos los corrales o animales cualificados están registrados y no hay preferencia por el grupo de intervención. Se debe presentar públicamente *a priori* el protocolo de la prueba e informar todas las conclusiones de los resultados evaluados.

Résumé - Maximiser la valeur et minimiser le gaspillage dans la recherche par essais cliniques chez le porc: Caractéristiques de conception pour minimiser les biais

Les chercheurs qui conçoivent des essais doivent mettre en œuvre des caractéristiques de conception destinées à réduire les biais. Celles-ci comprennent l'attribution aléatoire aux groupes expérimentaux et la mise en aveugle des soignants et des évaluateurs des résultats. La méthode de génération de la séquence aléatoire doit être signalée, ainsi que les méthodes de stratification ou de blocage si elles sont utilisées. Lorsque la mise en aveugle n'est pas possible, des résultats mesurés objectivement doivent être utilisés. La dissimulation de l'attribution peut ne pas être essentielle lorsque tous les enclos ou animaux éligibles sont inscrits et qu'il n'y a pas de préférence pour le groupe expérimental. Un protocole d'essai *a priori* doit être mis à la disposition du public et les résultats pour tous les résultats évalués doivent être communiqués.

Evidence-based decision making includes the use of evidence from research studies. When evaluating the efficacy of an intervention, where it is ethical and feasible to assign animals to intervention groups, clinical trials have the highest evidentiary value of the primary research study designs.¹ Clinical trials are controlled trials or experiments conducted to evaluate products or

procedures outside of a laboratory setting. When treatment allocation includes a formal random process for assigning animals (or pens) to intervention groups, clinical trials are referred to as randomized controlled trials (RCTs). When designing a clinical trial, it is important to include design features intended to reduce the potential for bias. Bias is defined as a difference between the study

results and the truth (ie, the true effect of the interventions).² The effect of most interventions is relatively small.³ Therefore, it can be difficult to distinguish between true intervention effects and bias. This can lead to invalid interpretations of intervention effects and therefore, inappropriate use of interventions by individuals using the results of the trial for decision making. If trials are thought

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to be biased, it is increasingly common to recommend that they are excluded from the evidence base. Exclusion from the evidence base means that the study results are not used, the resources devoted to the trial are wasted, and the trial needs to be conducted again to get informative results.

Several trial design features have been associated with risk of bias. Meta-epidemiological studies evaluating large numbers of human clinical trials show that inadequate randomization, lack of allocation concealment, and nonblinding of patients and outcome assessors are associated with exaggerated intervention effects.^{4,5} Statistically significant outcomes are more likely to be reported in a publication compared to outcomes that were not significant, leading to bias due to selective outcome reporting.⁶

The objective of this commentary is to review these features in the context of clinical trials conducted in swine and to discuss ways in which biases associated with these features can be minimized to avoid research wastage and maximize research utility.

Randomization

When conducting a clinical trial, it is important that the intervention groups are similar in terms of the distribution of prognostic factors (characteristics that are associated with the outcome) at the start of the experiment. For example, in a trial evaluating the efficacy of an intervention to prevent mortality, it is possible that age or animal weight at the time of application of the intervention is a prognostic factor. If this is true, and the age or weight distribution of the animals differs between the intervention groups, then the results of the trial will be biased (ie, will not reflect the true intervention efficacy). To address this type of bias, it is important the eligibility criteria related to these prognostic factors are clearly described. For example, the authors might limit eligibility to weaned pigs between 5 and 7 kg. Then, random allocation (ie, randomization) should be used to assign animals to intervention groups. The term “random” has a precise meaning, wherein each “study unit” has a known probability of receiving a given intervention at the time of allocation. The actual intervention allocated to each study unit is determined by a chance process and cannot be predicted. Depending on the type of interventions, the study unit may be an animal or a

grouping of animals. For instance, if the intervention of interest would normally be given to an individual animal (eg, individual treatments to reduce disease severity or duration), then the intervention should be allocated at the animal level. If the intervention would normally be given to groups (eg, evaluating floor surfaces to improve welfare), then the intervention would be allocated at the group, pen, or barn level. The unit of allocation in some trials may differ from the unit of analysis; in a trial where the intervention is allocated at a group level (eg, floor design), the outcome may be measured at the individual level (eg, presence or absence of lameness in individual pigs) or at the group level (eg, total feed consumption). In this example, if the lameness outcome was measured at the individual level, pigs within a pen would not be statistically independent (ie, clustering of responses within pen occurs). This could be addressed by using a group level outcome for lameness (eg, percentage of lame pigs within a pen), thereby making the unit of analysis the same as the unit of allocation. However, this approach may have low statistical power because the unit of analysis, and therefore the sample size, is at the pen level. Alternatively, the unit of analysis could be the individual level with a binary outcome of lame or not lame for each pig with clustering within pen controlled in the analysis.

Random allocation to intervention groups is not difficult to achieve and should be encouraged. However, random allocation is not reported in a substantial number of swine trials and, even when trials are described as random, reporting of the methods of randomization often is suboptimal. Reporting the method used to generate the random sequence is recommended in the REFLECT-statement guidelines for reporting clinical trials in livestock.^{7,8} In an evaluation of reporting quality in RCTs, the method of random sequence generation was not reported in 79.8% (91 of 114) of RCTs published in veterinary journals, compared to 6.7% (4 of 60) of RCTs published in human medical journals.⁹ In a systematic review of 44 trials evaluating the efficacy of antibiotics to prevent respiratory disease in swine,¹⁰ random allocation was described in 23 trials (52%; although the method used to generate the random sequence was not described in 17 of these trials), 4 trials (9%) did not use random allocation, and there was no information

provided on the method of allocation in 17 trials (39%). Failure to randomize has been associated with exaggerated intervention effects, as shown in evaluations of trials conducted in livestock.¹¹⁻¹³ For the trials included in the systematic review of antibiotics to prevent respiratory disease,¹⁰ and assuming that randomization was correctly implemented in the trials where information on the random sequence generation was not reported, it could be argued that 50% of the trials presented results which are not credible. If the results are not credible, then they should be excluded from consideration in decision making due to concerns over bias in the results. Conducting research that is not included in decision making decreases the value of the original research investment and thereby contributes to research waste. However, it is encouraging that reporting of the method for generating the random sequence increased in vaccine trials conducted in swine from 8% prior to publication of the REFLECT-statement to 67% after publication.¹⁴

It might be argued that randomization is not needed in swine trials where the population of pigs within a production stage are homogeneous in terms of breed, weight, and diet. However, in mouse models of stroke, where the animals arguably are even more homogeneous, reported efficacy was significantly lower in studies that were randomized compared to those where randomization was not reported.¹⁵

Random allocation of study units to intervention groups should be possible in all swine trials. One option is to use a random number generator. This can be done in Excel (Microsoft Corporation) using the RAND function under the formulas tab, which results in a list of random numbers between 0 and 1. If the trial has 2 intervention arms (eg, the intervention of interest and a single control group), then even numbers from the random number list could be assigned to one group and odd numbers to the other. Other random methods include a coin toss, dice roll, or drawing numbers from a container, which can be done in a barn or as pigs are unloaded or moved to a new barn or pen. Deterministic allocation methods, such as alternate animal identification numbers, days of the week, or birth order are not random¹⁶ and may lead to the allocation sequence being predictable, which then could lead to biased results.

Although the purpose of randomization is to minimize important differences between intervention groups, simple randomization may not be sufficient in studies with small sample sizes. Stratified randomization is one method that can be used to minimize differences between groups in important prognostic factors, particularly when sample sizes are small. With this method, animals are randomly allocated to intervention groups within strata of an important prognostic factor.^{17,18} For example, if researchers are interested in conducting a trial of a feed additive for improving average daily gain but believe that sex is an important predictor of average daily gain, having more male pigs in one intervention group compared to the other group will cause the trial results to be biased. Stratified randomization would involve randomly allocating male pigs to intervention groups and then randomly allocating female pigs to intervention groups as a separate step. This approach will help to balance the number of male and females between intervention groups. Other examples include stratification of piglets within dam in vaccine trials to control for genetics and maternal antibodies, or stratifying based on predefined sections in a barn to reduce the risk that intervention groups will be unevenly placed near fans, which may affect performance outcomes. However, when stratified randomization is used, it is important to adjust for the factors used for stratification in the analysis to provide a valid inference.¹⁹

Another concern with small sample sizes is that the number of individuals per group can end up substantially different based on chance. Block randomization, also called permuted block randomization, can be used to create an equal number of individuals in each intervention group.^{16,17} Block randomization consists of dividing the number of study subjects into smaller groups, or blocks, and randomly allocating animals to intervention groups within blocks. For instance, if there were 20 animals and 2 intervention groups, animals could be randomly allocated in 5 blocks of 4 animals each, with an equal number of intervention groups A and B assigned within each block.

Allocation concealment

Allocation concealment is another trial feature used to minimize the potential for bias due to differences in prognostic factors between intervention groups at the start of an experiment. The concept

is that allocation may be circumvented because the person enrolling animals or pens might have a preference for the intervention a participant(s) might receive. If acted upon, consciously or subconsciously, such a preference could disrupt the balance in the intervention group achieved by random allocation. Therefore, allocation concealment refers to methods used to ensure that the person allocating study units to treatment groups and patients (or animal owners in the case of swine trials) are not aware of the random sequence, ie, they do not know whether the next study subject enrolled will be allocated to group A or B.²⁰ Allocation concealment may involve having a third-party person not involved in patient recruitment manage the allocation sequence. Once the investigator has enrolled a new study subject into a trial, the third-party person tells the investigator the intervention assignment. In human trials, allocation concealment is considered a critical trial feature; trials not employing allocation concealment are considered to be at high risk of bias.²¹ In an evaluation of comprehensive reporting in 31 swine vaccine trials, allocation concealment was not described in any trial.¹⁴ However, in many swine trials, it is probable that the owner and person enrolling pens or animals in the trial and allocating them to intervention groups do not have a preference for the intervention group for any specific animal or pen of animals. This would be true when owners do not have a differential attachment to specific pigs and do not know the potential production value of a specific pig or pen of pigs at the time of study enrollment. If this is the case, then failure to conceal allocation at the time of enrollment may not be associated with bias and allocation concealment may not be an essential trial component.¹⁴ Researchers designing a trial should consider whether there is potential for one intervention to be preferred over another for animals or pens and decide whether allocation should be concealed on this basis. If allocation concealment is not used, the decision should be justified in the trial report. Ideally, concealing allocation when possible removes doubt about this potential source of bias and is usually a small effort for a lot of gain. If allocation is concealed, decision makers will have no concerns about bias due to circumventing randomization, and therefore about incorporating the results of the study into the decision-making process. Thus, results of the trial will not be wasted.

Blinding

The term blinding refers to methods used to prevent individuals involved in a trial from knowing which study units are assigned to which interventions.²² This may include some or all of animal owners, managers, or caregivers, investigators, individuals collecting outcome information (outcome assessors), and individuals conducting the statistical analysis. Blinding is used to prevent the potential for differential assessment of outcomes and differential care of the animals between the intervention groups, which could bias the trial results. When describing the use of blinding, the tasks that are blinded should be articulated rather than using the terms “single” or “double” blind; although these terms are common in the literature, they are ambiguous and may be interpreted differently by different individuals.²³ For instance, it is clearer to state that “owners and outcome assessors were blinded to intervention group”, rather than “the trial was double blinded.”

In a systematic review of 44 trials evaluating the efficacy of antibiotics to prevent respiratory disease in swine,¹⁰ blinding of caregivers and outcome assessors was described in 7 trials (16.0%), nonblinding was explicitly described in 2 trials (4.5%), and no information was provided on whether caregivers and outcome assessors were blinded in 35 trials (79.5%). In swine vaccine trials evaluated for completeness of reporting, blinding of caregivers, individuals administering the interventions, and outcome assessors was reported in 15 of 42 trials (36%) prior to publication of the REFLECT-statement and 12 of 19 (63%) of trials after publication.¹⁴

Not all trials can be blinded, and lack of blinding does not always lead to a biased result. For instance, if a trial is designed to compare pig stress outcomes when blood sampling is conducted from ear veins as compared to when sampling is conducted from jugular veins, or if the trial was comparing pelleted feeds to mash, the intervention groups would be visibly obvious. However, if blinding is not possible or not used, the potential for bias is less if the outcome can be objectively measured.²²

Various methods can be used to blind individuals to intervention allocation. If the intervention is a drug or a biologic, such as a vaccine, it may be possible to have a control group that looks identical but without the active ingredient. This would allow blinding of caregivers and outcome assessors, who may or may not be the same individuals, and also potentially investigators if a third-party

provides the allocated interventions. An additional layer of caregiver blinding could be used for interventions that are given at a single timepoint, such as vaccines, if the investigator applies the intervention without the caregiver being present. If the analysis is conducted by a statistician or epidemiologist who is not otherwise involved in the trial, it is simple to blind the analyst by coding the interventions as “A” or “B”, rather than naming the actual intervention in the dataset. If blinding can be used, this removes doubt about awareness of intervention group as a source of bias leading to invalid results. The results will be used to their maximum potential, which is surely the goal when using animals and resources for research.

Selective outcome reporting

It is common for multiple outcomes to be reported in clinical trials; in an evaluation of reporting quality of 100 trials in livestock populations, 91 trials had more than one outcome.¹² However, there is evidence from human healthcare evaluations that not all outcomes that have been evaluated in a trial have their results included in the trial report.^{24,25} Selecting a subset of the outcomes that were evaluated in a trial based on the results is referred to as selective outcome reporting. If the outcomes associated with significant intervention benefit are more likely to be reported, the overall trial results may be misleading. Determining whether selective outcome reporting has occurred requires that an *a priori* trial protocol is publicly available. The protocol should identify the primary outcome(s) in the trial, as well as any secondary outcomes that will be measured. Then, results for all primary and secondary outcomes should be reported in the trial report. A search of the trial registries in the American Veterinary Medical Association (AVMA) Animal Health Studies Database (https://ebusiness.avma.org/aahsd/study_search.aspx) in March 2022 did not identify any trials conducted in swine. Therefore, the extent to which selective outcome reporting is an issue in swine trials is unknown. However, swine trials conducted by industry groups, pharmaceutical companies, and academics require a trial protocol to receive ethical approval. If researchers posted these protocols to trial registries, such as the AVMA Animal Health Studies Database, it would allow an evaluation of outcome reporting which would increase confidence in, and therefore value of, clinical trials in swine.

Implications

- Biased trial results can lead to inappropriate use of interventions.
- Biased trial results may lead to exclusion from decision making.
- Biased trial results do not maximize the research investment.

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Conflict of interest

None reported.

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Evaluating the impact of organic matter and sample processing techniques on RNA detection using environmental samples

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Summary

This study evaluated sample processing methods and the presence of organic matter on detection of porcine epidemic diarrhea virus (PEDV) from environmental samples using real-time reverse transcriptase-polymerase chain reaction (qRT-PCR). Steel coupons were inoculated with PEDV and different types of organic material contamination. Surface samples were collected and processed in one of four ways: none, centrifugation, syringe filtration, or combination of centrifugation and syringe filtration, then submitted for PEDV qRT-PCR. There was a surface inoculation type by processing method interaction ($P < .001$) that impacted the sample cycle threshold value. Centrifugation resulted in the most consistent detection of PEDV RNA.

Keywords: swine, environmental samples, feed safety

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Resumen - Evaluación del impacto de la materia orgánica y las técnicas de procesamiento de muestras en la detección de ARN utilizando muestras ambientales

Este estudio evaluó los métodos de procesamiento de muestras y la presencia de materia orgánica en la detección del virus de la diarrea epidémica porcina (PEDV) a partir de muestras ambientales utilizando la reacción en cadena de la polimerasa con transcriptasa inversa en tiempo real (qRT-PCR). Unas superficies de acero se inocularon con el PEDV y con diferentes tipos de contaminantes de material orgánico. Posteriormente estas superficies se recolectaron y procesaron con uno de cuatro procedimientos: ninguno, centrifugación, filtración con jeringa, o una combinación de centrifugación y filtración con jeringa, y posteriormente se enviaron para la qRT-PCR del PEDV. Hubo un tipo de inoculación superficial por interacción del método de procesamiento ($P < .001$) que afectó el valor del umbral del ciclo de muestreo. La centrifugación dio como resultado la detección más consistente del ARN del PEDV.

Résumé - Évaluation de l'impact de la matière organique et des techniques de manipulation de l'échantillon sur la détection d'ARN lors de l'utilisation d'échantillons environnementaux

La présente étude visait à évaluer les méthodes de traitement des échantillons et la présence de matière organique sur la détection du virus de diarrhée épidémique porcine (PEDV) à partir d'échantillons environnementaux par réaction d'amplification en chaîne par polymérase en temps réel utilisant la transcriptase reverse (qRT-PCR). Des échantillons d'acier ont été inoculés avec du PEDV et contaminés avec différents types de matériel organique. Des échantillons de surface ont été prélevés et traités par l'un des quatre procédés suivants: aucun, centrifugation, filtration à la seringue, ou combinaison de centrifugation et filtration à la seringue, puis testé pour PEDV par qRT-PCR. Il y avait une interaction entre le type d'inoculation de surface et la méthode de traitement ($P < .001$) qui influençait la valeur-seuil de cycles de l'échantillon. La centrifugation a permis la détection la plus constante d'ARN de PEDV.

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Houston GE, Blomme AK, Harrison OL, Bai J, Woodworth JC, Jones CK, Poulson-Porter EG, Paulk CB, Gebhardt JT. Evaluating the impact of organic matter and sample processing techniques on RNA detection using environmental samples. *J Swine Health Prod.* 2023;31(2):87-91. <https://doi.org/10.54846/jshap/1311>

Swine veterinarians have come to heavily rely on polymerase chain reaction (PCR) assays for viral detection in samples like oral fluids, tissues, and environmental samples. The advantages of using PCR assays are that it is fast, sensitive, and can be used across multiple sample types.¹ Typically, oral fluids and tissue samples are used to diagnose clinical disease and help guide health decisions within populations of pigs. Environmental samples can help swine veterinarians detect pathogens on a variety of surfaces and address gaps in biosecurity practices for swine production systems or feed mills. Unfortunately, environmental samples can be heavily contaminated with dirt, feces, dust, feed, or a combination of these organic substances that naturally occur in the sample. This wide variety of contamination is an important factor when considering the accuracy of the PCR assay. The organic materials present in the environmental sample can inhibit the PCR reaction, resulting in decreased sensitivity or false-negative results.¹ There are multiple ways to approach sample handling to account for the potential of inhibitory substances depending upon which step of the PCR reaction is inhibited.^{1,2}

When considering veterinary diagnostic laboratories, most PCR assays are validated for blood, tissue, and other clinical samples but environmental samples have yet to be validated. This is due to the fact that environmental samples can often contain different types of substances or a combination of substance that could inhibit the PCR assay. Thus, if a validated and standardized protocol for environmental samples would be created, these protocols would have to account for all of the potential inhibitory substances but also be time efficient. Ideally, the protocol could also be done relatively quickly in a laboratory so samples would still have the same turnaround time for submission. Therefore, the objective of this project was to evaluate different surface contamination types commonly found in environmental samples and if different processing techniques conducted prior to real-time reverse transcriptase-polymerase chain reaction (qRT-PCR) analysis would impact sample porcine epidemic diarrhea virus (PEDV) detection.

Procedures

General

Dirt and finishing pig feces were collected before this experiment and aliquoted into 5-g samples. For the organic matter mixture, 10 g of the same dirt and 10 g of the same feces were mixed together with 3 mL of deionized water. Once the organic matter was thoroughly mixed, it was aliquoted into 5-g samples. Dirt, feces, and organic matter were confirmed to have no detectable PEDV or porcine deltacoronavirus (PDCoV) RNA via PCR prior to the start of the experiment. Once confirming dirt, feces, and organic matter mixture had no detectable PEDV or PDCoV RNA, all material was frozen at -80°C until the experiment was conducted. Virus used was PEDV isolate USA/Co/2013 with a titer of 1.33×10^5 median tissue culture infectious dose/mL.

Surface inoculation

Fifteen autoclaved, steel, 10 × 10 cm coupons were placed within a biosafety level (BSL)-2 cabinet. A coupon was inoculated with one of the 5 surface inoculation types: 1 mL of PEDV; 1 mL of PEDV and 5 mL of phosphate buffered saline (PBS); 1 mL of PEDV and 5 g of dirt; 1 mL of PEDV and 5 g of feces; or 1 mL of PEDV and 5 g of organic matter mixture. Each treatment was replicated 3 times using 3 separate steel coupons.

Surface sample collection

After inoculation, the coupon sat for 15 minutes within the BSL-2 cabinet. After the 15-minute time limit, each steel coupon was environmentally swabbed as previously described.³ Once the environmental sample was taken, 20 mL of PBS was added to the sample, it was inverted for 5 to 10 seconds, and then allowed to incubate at room temperature (24°C) for 1 hour. At the end of incubation, the sample was vortexed for 15 seconds and then processed for qRT-PCR analysis.

Sample processing

For each environmental sample, 4 samples were taken directly from the conical tube after vortexing and processed using 4 different techniques. For sample A, 1 mL was taken from the environmental sample, placed in a cryovial, and submitted for qRT-PCR analysis without further processing. For sample B, 1 mL was taken from the environmental sample, placed into a new conical tube, and centrifuged for 10 minutes at 706g.

Following centrifugation, the supernatant was pipetted into a cryovial then submitted for qRT-PCR analysis. For sample C, 1 mL was taken from the environmental sample, filtered through a 0.45- μ m, 25-mm syringe filter into a cryovial, and then submitted for qRT-PCR analysis. For sample D, 1 mL was taken from the environmental sample, placed into a new conical tube, centrifuged as previously described, filtered through a 0.45- μ m, 25-mm syringe filter into a cryovial, and then submitted for qRT-PCR analysis.

qRT-PCR analysis

The Molecular Research and Development Laboratory within the Kansas State University Veterinary Diagnostic Laboratory conducted the qRT-PCR analysis. Fifty microliters of supernatant from each sample was loaded into a deep-well plate and extracted using a Kingfisher Flex magnetic particle processor (Fisher Scientific) with the MagMAX-96 Viral RNA Isolation kit (Life Technologies) according to manufacturer instructions with one modification, reducing the final elution volume to 60 μ L. One negative extraction control consisting of all reagents and PBS in place of the sample was included in the extraction. Positive controls of each stock virus were also included with each extraction. Extracted RNA was frozen at -80°C until assayed by qRT-PCR. Analyzed values represent cycle threshold (Ct) at which virus was detected. A total of 45 cycles were ran for each sample, so if a sample had no detectable PEDV RNA for the qRT-PCR assay, the sample was assigned a value of 45.

Statistical analysis

Statistical analysis of variance for the sample Ct values was performed using the *aov* function utilizing R programming language (R Foundation for Statistical Computing; version 4.1.1). Fixed effects included the inoculation treatment, sample processing treatment, and the associated interaction. Results of Ct data are reported as least squares means (SEM). All statistical models were evaluated using visual assessment of studentized residuals and model assumptions appeared to be appropriate. A Tukey multiple comparison adjustment was incorporated when appropriate. Results were considered significant at $P \leq .05$ and marginally significant between $P > .05$ and $P \leq .10$.

Table 1: Effect of inoculation type and environmental sample processing technique on PEDV detection on steel surfaces*

Item	Sample processing technique [†]			
	No processing	Centrifuge	Syringe filter	Centrifuge + syringe filter
qRT-PCR proportion, No. positive/No. samples				
Pure virus	3/3	3/3	3/3	3/3
Virus and PBS	3/3	3/3	3/3	3/3
Virus and dirt	2/3	3/3	3/3	3/3
Virus and feces	3/3	3/3	0/3	0/3
Virus and organic matter	1/3	3/3	0/3	2/3
Ct value[‡]				
Pure virus	24.5 ^a	24.6 ^a	28.9 ^{abcd}	27.5 ^{abc}
Virus and PBS	24.8 ^{ab}	24.7 ^{ab}	28.0 ^{abc}	28.4 ^{abc}
Virus and dirt	35.9 ^{de}	28.2 ^{abc}	32.0 ^{cd}	30.8 ^{abcd}
Virus and feces	31.8 ^{bcd}	32.5 ^{cd}	45.0 ^f	45.0 ^f
Virus and organic matter	42.4 ^{ef}	31.3 ^{abcd}	45.0 ^f	40.9 ^{ef}

* Steel coupons, measuring 10 × 10 cm were inoculated with PEDV, isolate USA/Co/2013 with a titer of 1.33 × 10⁵ TCID₅₀/mL. Surfaces were inoculated with 1 mL of pure virus, 1 mL of virus diluted into 5 mL of PBS, 1 mL of virus inoculated with 5 g of dirt, 5 g of feces, or 5 g of organic matter mixture consisting of a 1:1 ratio of dirt and feces. After surfaces were allowed to sit for 15 min, the steel coupon was environmentally swabbed. Environmental samples were inverted for 5-10 s, incubated for 1 hr, vortexed for 10-15 s, and then processed according to designated sample processing technique.

[†] Sample processing techniques included no processing, centrifuged for 10 min at 706g (centrifuge), filtered with a 0.45-µm, 25-mm syringe filter (syringe filter), or centrifuged for 10 min at 706g then filtered through a 0.45-µm, 25-mm syringe filter (centrifuge + syringe filter). After processing, samples were submitted for PEDV qRT-PCR assay.

[‡] If there was no detectable RNA in the sample, the sample was assigned a Ct value of 45.

^{a-f} Inoculation contamination type by sample processing interaction, *P* < .001; SEM = 1.41. Means lacking common superscripts differ, *P* < .05.

PEDV = porcine epidemic diarrhea virus; qRT-PCR = real-time reverse transcriptase-polymerase chain reaction; PBS = phosphate buffered saline; Ct = cycle threshold; TCID₅₀ = median tissue culture infectious dose.

Results

There was an inoculated surface contamination type by sample processing method (*P* < .001) interaction that impacted the sample Ct value (Table 1). For surfaces inoculated with pure virus and virus with PBS, there was no difference in the sample Ct values across the different types of sample processing methods (*P* > .05). For surfaces inoculated with virus and dirt, samples that were centrifuged had greater amounts of PEDV RNA detected (or lower Ct values) compared to samples that were not processed (*P* < .05). For surfaces inoculated with virus and feces, nonprocessed samples or centrifuged samples had greater amounts of PEDV RNA detected (or lower Ct values) compared to syringe filtered samples and centrifuged and syringe filtered samples (*P* < .05). For surfaces inoculated with virus and organic matter mixture, centrifuged samples had greater amounts of PEDV RNA detected (or lower Ct values) compared to all other types of sample processing (*P* < .05).

There were also statistically significant main effects of surface contamination type (*P* < .001) and sample processing (*P* < .001; Table 2). For surface contamination type, surfaces inoculated with pure virus and virus with PBS had greater amounts of PEDV RNA detected (lower Ct values) compared to surfaces inoculated with virus and dirt (*P* < .05), while surfaces inoculated with virus and feces and virus and organic matter mixture had lower levels of PEDV RNA detected (higher Ct values) compared to all other surfaces (*P* < .05). For sample processing type, centrifugation of samples resulted in a greater amount of PEDV RNA detected (lower Ct values) compared to all other treatments (*P* < .05). Furthermore, syringe filtration or centrifugation and syringe filtration resulted in the lowest amount of PEDV RNA detected (higher Ct values; *P* < .05).

Discussion

Nucleic acid (NA) extraction and the PCR reaction are the 2 major steps that can influence the test results. For NA extraction, most commercial extraction kits, like the one used in this study, are able to remove most PCR inhibitory materials from the sample and enrich NA content for PCR detections. For a PCR reaction, there are 3 general steps: denaturation (unwind the double helix pattern of DNA), primer annealing (specific primers to attach to the unwound DNA), and extension (polymerase binds to the primer and unwound strand complex to make complimentary strands); then those complimentary strands are amplified and the rate of amplification corresponds with a Ct value.⁴ Since primers can be designed for a wide variety of microorganisms and the assay is completed in minutes, PCR is a commonly used diagnostic tool across medical professions.^{2,5} For swine veterinarians, PCR

Table 2: Main effects of surface inoculation type and sample processing technique on detection of PEDV on steel surfaces*

Item	qRT-PCR proportion, No. positive/No. samples	Ct [†]
Surface inoculation		
Pure virus	12/12	26.4 ^a
Virus and PBS	12/12	26.5 ^a
Virus and dirt	11/12	31.7 ^b
Virus and feces	6/12	38.6 ^c
Virus and organic matter	6/12	39.9 ^c
Sample processing		
No processing	12/15	31.9 ^d
Centrifuge	15/15	28.2 ^e
Syringe filter	9/15	35.8 ^f
Centrifuge + syringe filter	11/15	34.5 ^f

* Steel coupons, measuring 10 × 10 cm, were inoculated with PEDV, isolate USA/Co/2013 with a titer of 1.33×10^5 TCID₅₀/mL. Surfaces were inoculated with 1 mL of pure virus, 1 mL of virus diluted into 5 mL of PBS, 1 mL of virus inoculated with 5 g of dirt, 5 g of feces, or 5 g of organic matter mixture consisting of a 1:1 ratio of dirt and feces. After surfaces were allowed to sit for 15 min, the steel coupon was environmentally swabbed. Environmental samples were inverted for 5-10 s, incubated for 1 hr, vortexed for 10-15 s, and then processed according to designated sample processing technique. Samples were processed as either no processing, centrifuged for 10 min at 706g (centrifuge), filtered with a 0.45- μ m, 25-mm syringe filter (syringe filter), or centrifuged for 10 min at 706g then filtered through a 0.45- μ m, 25-mm syringe filter (centrifuge + syringe filter). After processing, samples were submitted for PEDV qRT-PCR assay.

[†] If there was no detectable RNA in the sample, the sample was assigned a Ct value of 45.

^{a-c} Main effect of surface contamination type on Ct values, $P < .001$; SEM = 0.80. Means lacking common superscripts differ, $P < .05$.

^{d-f} Main effect of sample processing technique on Ct values, $P < .001$; SEM = 0.74. Means lacking common superscripts differ, $P < .05$.

PEDV = porcine epidemic diarrhea virus; qRT-PCR = real-time reverse transcriptase-polymerase chain reaction; Ct = cycle threshold; PBS = phosphate buffered saline; TCID₅₀ = median tissue culture infectious dose.

assays are used for many disease syndromes and can include many different sample types like oral fluids, tissues, and environmental samples. However, when considering the 3 steps of a PCR reaction, there are ways for the accuracy of this assay to become compromised and therefore, give inaccurate results. For example, several potential issues that can arise during the PCR analysis process that could lead to false-positive or false-negative results include substances that inhibit any step of the assay, potential contamination during sample collection prior to PCR, or potential laboratory contamination while conducting the PCR assay.² There are many sources on how to counteract the potential for problems pertaining to all 3 basic steps of PCR but for the sake of this paper, the rest of the discussion will focus on inhibitory substances.

In general, inhibitory substances can naturally occur in the sample or be introduced into the sample during sample processing.¹ For example, common inhibitor substances can include body fluids or reagents in clinical and

forensic sciences like hemoglobin, urea, or heparin; food substances or particles like glycogen, fats, or calcium; and environmental compounds like humic acids, heavy metals, or phenolic compounds.⁶ These substances have the potential to interfere with PCR amplification and influence the sensitivity thereby negatively affecting the performance of the PCR assay.⁷ There are many potential inhibitory substances and what is present in one sample matrix may be completely different in another sample matrix.¹ When considering common samples submitted for PCR by swine veterinarians, most of those sample types have the potential to include dirt, feces, blood, dust, soil, or a combination of these materials which can potentially inhibit a portion of a PCR reaction. Given this information, it does not mean that veterinarians should stop using PCR for diagnostics, but further reiterates that veterinarians should understand the potential pitfalls associated with their samples. It is important for veterinarians and diagnosticians to consider how best to handle the sample submission to maximize the PCR assay sensitivity. There are multiple methods

that can be used to overcome potential inhibitory substances which can include biochemical methods, immunological methods, physical methods, or physiological methods; with the physical methods being the most user friendly.⁷ Ideally the method used to process samples prior to PCR analysis would be cost effective, time efficient, and relatively easy to implement. Therefore, this study aimed to evaluate methods of sample processing, specifically physical methodologies, on different surface inoculation contamination types of environmental samples and how that impacted PEDV detection via PCR analysis.

For this study, there was an inoculated surface contamination by sample processing technique interaction indicating that the inoculation contamination type and how that environmental sample was processed prior to qRT-PCR analysis impacted the Ct value of the sample. As samples contained more inhibitory substances like dirt, feces, or a combination of both, how that sample was processed influenced the results of the PCR assay. No one single processing technique was

beneficial across all surface inoculation types. However, when the inoculation type was virus with dirt, feces, or organic matter mixture, the centrifugation methodology consistently identified PEDV RNA across all inoculation types as shown by the lower Ct values and proportion of positive PCR results when compared to other processing methods. Hall et al⁸ found similar results when evaluating inhibitor resistance methods for diagnostics in clinical and environmental samples. Specifically, they found that of the 9 possible methods for inhibitor resistant, not a single method performed the best for all the sample matrices, but one method, KAPA blood PCR kit, did produce the most consistent results across the different sample matrices.⁸ The current study and Hall et al⁸ highlight that the best method for overcoming a variety of inhibitory substances is the method that produces the most consistent results.

Another finding from this study was that the centrifugation processing technique of samples had the lowest Ct values compared to other sample processing techniques. Similarly, one study found that centrifugation of urine samples helped to maximize PCR sensitivity and was also the most time efficient method compared to the traditional dot-plot hybridization method.⁹ When considering sample processing techniques, this study and the current study both highlight the importance that the technique should be relatively easy, cost effective, and time efficient. Another finding from the current study was that the more “pure” surface contamination types had lower Ct values when compared to surfaces inoculated with feces or organic matter mixture. There was no statistically significant difference in Ct values for the pure virus inoculation and virus inoculation after dilution with PBS, but the detection of PEDV RNA was generally reduced as dirt, feces, or the combination were included on the environmental surface. This conclusion is similar to another research study that detailed the different ways forensic samples are processed before PCR analysis in order to obtain the purest sample possible to allow for proper PCR amplification.¹⁰ Syringe filtering of samples in the current study reduced the ability to detect RNA in samples, especially those with dirt, feces, or the combination of both. It was hypothesized that the syringe filtering might also be trapping the RNA and not just dirt and feces. To the authors’

knowledge, this is the first study to find these results associated with syringe filtration and processing samples prior to RT-PCR.

This study highlight that the best sample for RT-PCR is a sample free of substances that potentially interfere with PCR analysis like dirt, feces, and soil. However, when considering the environment most swine veterinarians acquire their sample from (barns with dirt, feces, and dust; environmental samples containing dirt, dust, and other materials), these findings further highlight the importance of proper sample processing to prevent potential inhibitory substances prior to PCR analysis. Based on the results of the current study, centrifugation of environmental samples at 706g for 10 minutes resulted in the most consistent recovery of PEDV RNA across a range of environmental organic material loads.

Implications

Under the conditions of this study:

- Organic material in environmental samples can interfere with qRT-PCR analysis.
- Processing samples before qRT-PCR can improve diagnostic sensitivity.
- Centrifugation maximized qRT-PCR sensitivity for environmental samples.

Acknowledgments

Conflict of interest

None reported.

Disclaimer

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CONVERSION TABLES

Weights and measures conversions

Common (US)	Metric	To convert	Multiply by
1 oz	28.35 g	oz to g	28.35
1 lb (16 oz)	0.45 kg	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)	0.3 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	35.3
1 gal (128 fl oz)	3.8 L	gal to L	3.8
0.26 gal	1 L	L to gal	0.26
1 qt (32 fl oz)	0.95 L	qt to L	0.95
1.06 qt	1 L	L to qt	1.06

Temperature equivalents (approx)

°F	°C
32	0
50	10.0
60	15.5
61	16.1
65	18.3
70	21.1
75	23.8
80	26.6
82	27.7
85	29.4
90	32.2
102	38.8
103	39.4
104	40.0
105	40.5
106	41.1
212	100.0

$$^{\circ}\text{F} = (^{\circ}\text{C} \times 9/5) + 32$$

$$^{\circ}\text{C} = (^{\circ}\text{F} - 32) \times 5/9$$

$$1 \text{ tonne} = 1000 \text{ kg}$$

$$1 \text{ ppm} = 0.0001\% = 1 \text{ mg/kg} = 1 \text{ g/tonne}$$

$$1 \text{ ppm} = 1 \text{ mg/L}$$

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	136
	661	300
Boar	794	360
	800	363

Conversion calculator available at: amanualofstyle.com/page/si-conversion-calculator

US delegation visits the EU to learn insights on ASF prevention, preparedness, and response

African swine fever (ASF) prevention, preparedness, and response remains a top priority for the US pork industry. To gain valuable insights from international representatives, producers and veterinarians; state and federal authorities; and National Pork Board and National Pork Producers Council staff members traveled to Poland, Germany, and Belgium in November 2022. They also connected with officials and experts from Denmark and Romania.

Checkoff funds are used to ask important questions and discover strategies that may aid a foreign animal disease (FAD) outbreak if it were to occur in the United States. “There is value for producers and veterinarians to learn about how countries have been impacted by ASF and hear success stories,” says Dr Dustin Oedekoven, chief veterinarian for National Pork Board. “This information reinforces the industry ASF priorities set in 2022, and the key findings will help shape 2023 milestones and industry opportunities.”

Key to business objectives: Contact tracing is essential to regain export markets

The economic impact of a hypothetical ASF outbreak could cost the pork industry more than \$50 billion over 10 years.¹ Pork Checkoff funds are invested to promote business continuity and limit the halt of nearly 30% of pork products being exported.²

During the trip, attendees conversed with representatives from Poland to understand how the country regionalized ASF to regain some exports. However, no country has fully regained all its export markets following an ASF outbreak.

If an outbreak were to occur in the United States, AgView, a Checkoff-funded contact tracing application for pig movements, may help the US pork industry regionalize by providing pig movement data to state animal health officials (SAHOs) on day one.



Front (L-R): Cheryl Day, Adam Krause, and Al Wulfekuhle. Back (L-R): Dr Jeremy Pittman, Jesse Heimer, Dr Gordon Spronk, Dr Jack Shere, Dr Marty Zaluski, and Dr Dustin Oedekoven. Photo courtesy National Pork Board.

“During an FAD outbreak, the ability to visualize current movements and export this information will be invaluable to the state veterinarian. Our goal is to quickly trace movements and minimize the impact of the outbreak,” says Dr Sara McReynolds, assistant animal health commissioner at the Kansas Department of Agriculture. “It will take us all working together to protect the industry. Having movement information, laboratory results, Secure Pork Supply plans and contact information all in one location will allow for a more efficient and timely response.”

The speed at which SAHO’s can determine where disease is, or more importantly where disease is not, provides critical information for establishing free regions where trade and commerce can resume. New features in AgView allow producers to continuously share location and movement information with SAHOs in real time.

“The ability for peacetime sharing of movement data in AgView could have tremendous application for pork producers using swine production health plans to move pigs across state lines for production,” says Dr Patrick Webb, National Pork Board’s assistant chief

NPB news continued on page 95



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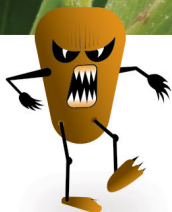
HUMIDITY & RAIN

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of fungal growth
and mycotoxin
production



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and facilitates fungal
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veterinarian. “Sharing pig movements in AgView could serve the purpose of delivering the required interstate swine movement report in real time.”

Prevention and control

In countries with ASF, the control of FAD transfer through people and wild animals is a daunting but necessary task. Several countries visited outlined successful strategies:

- Belgium - Interrupted virus transmission in the wild boar population, effectively preventing infection in the domestic herd. Two years after identifying the first case in wild boar, they regained ASF-free designation by the World Organization for Animal Health (WOAH).
- Germany - Identified spread from multiple sources, including people and wild boar.
- Denmark - Prevention, or bio-exclusion, through construction of a fence on the German border, elimination of wild boar, and strict truck washes.

Animal health professionals can help protect the US industry from ASF and other FADs by designing and implementing effective biosecurity protocols and assisting with Secure Pork Supply plans, encouraging producers to track pig movements in AgView, and routinely submitting diagnostic samples to a veterinary diagnostic laboratory that is a member of the National Animal Health Laboratory Network and participates in the US Department of Agriculture’s active ASF and classical swine fever surveillance program.

Remember, contact state or federal animal health officials with concerns for increased mortality or unusual morbidity.

The threat of ASF to the US swine herd is significant, with costly consequences. For example, the unmitigated spread of ASF in Romania has resulted in a significant reduction in their domestic herd. In the United States, today’s prevention measures and preparation could aid in tomorrow’s response should an outbreak occur. The industry needs veterinarians and allied industries to arm producers with confidence in the collaborated effort to prevent, prepare for, and respond to an FAD outbreak based on a national strategy.

Connect with National Pork Board staff and producer leaders at events and conferences in 2023. Visit [porkcheckoff.org](https://www.porkcheckoff.org) to learn more about strategies to help protect the freedom to operate and maintain business continuity.

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* Non-refereed references.

AgView is effective with endemic diseases

More and more, swine veterinarians are discovering features in AgView that provide situational awareness for how endemic diseases may be moving in their companies or client base. “AgView is unique because it provides an application where locations, movements, and lab results come together in one spot,” says Dr Oedekoven. “Being able to visualize, search and sort data provides a day-to-day value for logging into the system and keeping data current which is important to SA-HOs too.”





How much is it costing you to not manage Mhp?

(*Mycoplasma hyopneumoniae*)

- ADG**
4.4%¹
↓
- HCW**
9.2 lbs¹
↓
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\$2 - \$5¹
↑
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↓

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These are general guidelines only. Producers should consult with their veterinarian.

¹Yeske, 2016. *Mycoplasma hyopneumoniae* elimination, 2016 AASV Annual Meeting Proceedings, pg. 376-380. https://www.aasv.org/library/swineinfo/item.php?AASV/2016/376_Yeske.pdf
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Alternate student delegate selected for AASV Board

The AASV Student Engagement Committee is pleased to announce the selection of Alexis Berte, a second-year veterinary student at Iowa State University (ISU), as the incoming alternate student delegate to the AASV Board of Directors.

Alexis has been dedicated to swine production and health from her beginnings on her family's 4800-head wean-to-finish farm in northern Iowa. During her undergraduate and veterinary studies at ISU, she has participated in swine research, completed several swine internships, and has held a variety of leadership positions in swine and agriculture youth organizations. She is currently the ISU AASV Chapter vice president and will be presenting a poster in the veterinary student poster competition at the 2023 AASV Annual Meeting.

She understands the importance of producing safe, high-quality foods and advocating for production animal medicine. In her role as alternate

student delegate, she hopes to advocate for swine medicine by building relationships and sharing opportunities offered to AASV student members across the country.

"I am excited to network with fellow students and future colleagues as well as promote AASV. I am very grateful to have been given the opportunity to serve the AASV over the next two years!" said Alexis.

Alexis will assume her duties as alternate student delegate during the 2023 AASV Annual Meeting. The current alternate delegate, Hunter Everett (NCSU, 2024), will assume the delegate position currently held by Sydney Simmons (NCSU, 2023), who will rotate off the board. Hunter and Alexis will represent student interests within AASV as nonvoting members of the Board of Directors and the Student Engagement Committee. Please join us in welcoming Alexis to the AASV Board of Directors and thanking Sydney for her service!



Be a learner: AASV conference proceedings online

AASV members may access ALL of the proceedings papers for the 2023 AASV Annual Meeting (including the papers for the preconference seminars) at aasv.org/annmtg/proceedings. Current 2023 membership dues-paid status is required to access the files.

As in the past, the papers are available as follows:

- The "big book" of all the regular session papers in a single PDF file with a linked table of contents

- Seminar booklets are PDF collections of the papers for each seminar
- An individual paper for each presentation is available in the Swine Information Library: aasv.org/library/swineinfo

You will be prompted for your AASV website username and password to access the files. If you have forgotten your password, use the "Reset Password" link in the upper right of the AASV website (aasv.org) or contact the AASV office for assistance.



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Early-career swine vet program under way; participants announced

The new AASV Participant-led, Early-career Swine Veterinarian Development Program wasted no time getting started after receiving a \$202,548 Education, Extension, and Training grant from the US Department of Agriculture (USDA) National Institute of Food and Agriculture (NIFA) Veterinary Service Grants Program (VSGP) in September. The program began accepting applications in October, the participants were selected in November, and the initial meeting to introduce participants to the program and each other took place in early December.

Applicant interest was strong, and the 25 available seats filled quickly. Per the program requirements, all participants are AASV members with 1 to 5 years of experience and employed in swine veterinary practice with a primary practice area in the United States. Preference was given to applicants who are current or previous recipients of a USDA Veterinary Medicine Loan Repayment Program award or who serve in a USDA-designated veterinary shortage area.

The participant group met virtually in early December to begin networking with each other and to prioritize topics for the curriculum, as dictated by the participant-led nature of this unique program. Five in-person educational modules will be held quarterly between April 2023 and June 2024, followed by a half-day early-career conference in fall 2024.

The goals of the program are to provide early-career swine veterinarians with resources needed to encourage and ensure successful and lifelong careers as swine veterinarians and to cultivate new leaders in swine veterinary medicine. In

a step towards the leadership goal, each participant has already joined at least one AASV committee.

The initial participant meeting was moderated by Dr Clayton Johnson, a practicing veterinarian at Carthage Veterinary Service Ltd. Dr Johnson serves as program coordinator and will be responsible for facilitating each module, including developing the agenda, confirming meeting logistics, and moderating the in-person gatherings. He will also be creating pre- and post-module examinations to validate knowledge transfer to all participants.

The AASV Participant-led, Early-career Swine Development Program is the brainchild of the AASV Early Career Committee. The committee identified a need for additional nondegree educational coursework and training for swine veterinarians early in their careers and applied for an Education, Extension, and Training grant from the USDA NIFA VSGP. The resulting award was one of 20 intended to help mitigate food-animal veterinary service shortages in the United States.

A subcommittee of the Early Career Committee, including Brandi Burton (chair), Claire LeFevre (vice chair), T'Lee Girard, Brittney Scales, Emily Byers Taylor, and AASV Board of Directors member Sara Hough, provided guidance on the participant application and selection process. Moving forward, the subcommittee will provide input on the list of suggested topics, help identify speakers, prepare and review evaluations, and review all reports and metrics.

The AASV is pleased to announce the following cohort of individuals selected for the program:

Kimberlee Baker
Alyssa Betlach
Megan Bloemer
Daniel Brown
Brandi Burton
Kayla Castevens
Brian Cerrito
Bryant Chapman
Will Crum
Matt Finch
Daniel Gascho
Trey Gellert
Kayla Henness
Megan Hood
Henry Johnson
Erin Kettelkamp
Allison Knox
Claire LeFevre
Jamie Madigan
Lauren Nagel
Elizabeth Noblett
Brent Sexton
Rachel Stika Jensen
Ryan Strobel
Abby Vennekotter



AASV Foundation Fundraising



AUCTION

March 6, 2023 - Aurora, Colorado

Be There!

Help ensure the future and create a legacy for swine veterinarians by bidding in the 2023 auction fundraiser!

SILENT AUCTION:

Bidding closes on Monday,
March 6th at 7:00 PM MST

LIVE AUCTION:

Monday, March 6th at 8:30 PM MST
(immediately following the AASV Awards Reception)

View ALL items and start bidding: aasvf.cbo.io



Items will be shipped directly to the winning bidder by the donor.

Contact the AASV Foundation (foundation@aasv.org) to arrange for remote bidding in the Live Auction.

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APC	Indiana Packers Corporation	Mike Pierdon
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Joaquín Becerril	Iowa State University VDL	Preferred Capital Management
Melissa Billing	Clayton Johnson	Karen Richardson
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Farm Bill season

Every 5 years, the US Congress passes the “Farm Bill,” a food and agriculture focused legislative package. The legislation includes new programs and programs that must be reauthorized every 5 years to stay in effect.

The majority of Farm Bill funding goes to key federal nutrition and food programs. Only about 1% of Farm Bill funds are divided among 8 program areas, including livestock health. In the 2018 Farm Bill, those combined programs accounted for only about \$5 billion of the \$428 billion total Farm Bill package. The next Farm Bill is expected to top \$1 trillion, with over 80% devoted to the nutrition title.

Although a small portion of the total package, livestock health programs funded through the Farm Bill are critical for swine health and welfare, foreign animal disease (FAD) preparedness and prevention, and protecting the food supply.

Swine veterinarians are all too familiar with the FAD threat looming over the US swine herd and the severe

consequences of an introduction. A successful response to an intentional or unintentional FAD incursion includes early detection, prevention, and rapid response tools; robust laboratory capacity for surveillance; and a viable stockpile of vaccine.

The critically important programs listed below were funded under Title 7, Chapter 109, Section 8308a of the 2018 Farm Bill. These remain critical programs and are Farm Bill priorities for swine veterinarians and pork producers.

- **National Animal Vaccine and Veterinary Countermeasures Bank (NAVVCB)**

– Established in the 2018 Farm Bill, the US-only vaccine bank allows the US Department of Agriculture (USDA) to stockpile animal vaccine and related products to use in the event of an outbreak of foot-and-mouth disease or other high-impact FADs. The bank ensures that vaccines are available for rapid response.

- **National Animal Health Laboratory Network (NAHLN)**

– The NAHLN is a network of over 60 federal, state, and university-associated animal health laboratories that provides rapid detection and response to endemic or emerging diseases. The laboratory capacity of the NAHLN is critical to ensuring that the United States can rapidly and effectively respond to a large-scale animal disease outbreak. Enhancing animal health diagnostic testing for both endemic and high-consequence pathogens in the nation’s food animals is vital to protecting animal health, public health, and the nation’s food supply. These laboratories are the first line of defense for detecting animal diseases and pathogens. Diagnosing and detecting the extent of an outbreak as rapidly as possible plays a key role in response.

- **National Animal Disease Preparedness and Response Program (NADPRP)**

– This program allows USDA Animal Plant Health Inspection Service to collaborate with animal health partners throughout the nation to implement high-value projects that enhance prevention, preparedness, detection, and response to the most damaging emerging and FADs that threaten US agriculture. The AASV received NADPRP funding in 2021 to review and update the AASV *Recommendations for the Depopulation of Swine* to incorporate the results from the research studies and field trials and the first-hand knowledge gained through the COVID-19 market disruption.

- **National Veterinary Stockpile (NVS)**

– The NVS provides the veterinary countermeasures (animal vaccines, antivirals, or therapeutic products, supplies, equipment, and response support services) needed to respond to animal disease outbreaks. Sampling, vaccination, and depopulation equipment are critical to a response’s success. The NVS should be well-supplied to support states, tribes, or territories when needed.

Other areas or programs animal health and pork stakeholders are advocating for include catastrophic insurance through USDA, eradication and control of feral swine, and the Food Animal Residue Avoidance Databank.

The AASV advocates for swine veterinarians and animal health by providing scientific information to support these priorities. The AASV Executive Committee, Drs Mike Senn, Bill Hollis, Mary Battrell, and Angela Baysinger, with AASV staff Drs Harry Snelson and Abbey Canon, visited Washington D.C. in May 2022 to introduce some of these priorities to policy makers with members of the American Association of Bovine



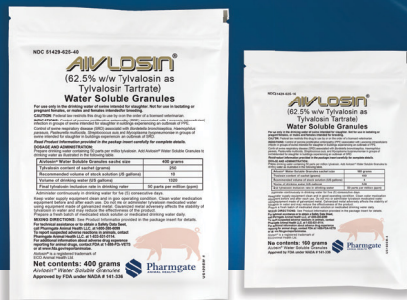
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Practitioners and the American Veterinary Medical Association. Additionally, AASV member veterinarians who have participated in the AASV/National Pork Producers Swine Veterinarian Public Policy Advocacy Program will be back on Capitol Hill in 2023 to emphasize these priorities and explain their impact.

All veterinarians and pork producers have the opportunity to advocate for animal health and welfare during the upcoming Farm Bill discussions by sharing their stories. Your personal story has impact. Describe who you are, who you represent, how this topic affects you, your practice, the animals under your care, the food you help produce, and the clients for whom you work.¹

Now is the time to introduce yourself to your legislative representatives. Offer to be a resource, provide science-based information, and describe the impact to swine medicine. Consider offering your representative the opportunity to ride along with you to understand and experience veterinary medicine and swine production.² Participate in the NPPC fly-in or AVMA legislative fly-in that brings veterinarians to Washington D.C. to meet with their members of Congress.³

You can help carve out that small piece of the pie that has a big impact on animal health and welfare. For more information on swine-specific Farm Bill priorities, visit nppc.org.

Abbey Canon, DVM, MPH, DACVPM
*Director of Public Health
and Communications*

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- *1. Canon A. Empowering you to advocate. [editorial]. *J Swine Health Prod.* 2021;29(4):219.
 - *2. American Veterinary Medical Association. Come one, come all: Member power in AVMA advocacy. AVMA Axon. 2020.
 - *3. American Veterinary Medical Association. Get Involved. AVMA. Accessed May 2021. <https://www.avma.org/advocacy/get-involved>
- * Non-refereed references.



Photo cutline: From left: Drs Angela Baysinger, Bill Hollis, Harry Snelson, Mike Senn, Mary Battrell, and Abbey Canon visited Washington D.C. in May 2022.

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- 2. Review the CSSC Program Standards.**
- 3. Identify individuals who could be trained to collect and submit samples on your behalf.**
- 4. Access CSSC training materials at securepork.org/cssc.**
- 5. Conduct classroom and hands-on training.**
- 6. Submit a list of trained individuals to SAHO(s) in state(s) trainees will be collecting samples.**



For more information on the training program



If you are ready to start training, contact the state animal health officials in the state in which you wish to train individuals



UPCOMING MEETINGS

American Association of Swine Veterinarians 54th Annual Meeting

March 4 - 7, 2023 (Sat-Tue)
Gaylord Rockies Resort & Convention Center
Aurora, Colorado

For more information:
American Association of Swine Veterinarians
830 26th Street
Perry, Iowa
Tel: 515-465-5255
Email: aasv@aasv.org
Web: aasv.org/annmtg

USDA APHIS Swine Influenza Stakeholder Workshop

March 29 - 30, 2023 (Wed-Thu)
A virtual meeting

For more information:
Dr Scott Kramer
Tel: 614-254-4522
Email: scott.kramer@usda.gov

Animal Agriculture Alliance Stakeholders Summit

May 4 - 5, 2023 (Thu-Fri)
Arlington, Virginia

For more information:
Animal Agriculture Alliance
2101 Wilson Blvd, Suite 810B
Arlington, VA 22201
Web: animalagalliance.org/initiatives/stakeholders-summit

Safepork 2023

May 15 - 17, 2023 (Mon-Wed)
New Orleans, Louisiana

For more information:
Web: regcytes.extension.iastate.edu/safepork

World Pork Expo

June 7 - 9, 2023 (Wed-Fri)
Iowa State Fairgrounds
Des Moines, Iowa

For more information:
World Pork Expo
10676 Justin Drive
Urbandale, Iowa 50322
Web: worldpork.org

ISU James D. McKean Swine Conference

June 28, 2023 (Wed)
Scheman Building
Iowa State University
Ames, Iowa

For more information:
Web: regcytes.extension.iastate.edu/swinedisease

AVMA Convention

July 14 - 18, 2023 (Fri-Tue)
Denver, Colorado

For more information:
Web: avma.org/events/avma-convention

Allen D. Lemman Swine Conference

September 16 - 19, 2023 (Sat-Tue)
Saint Paul, Minnesota

For more information:
Web: lemanconference.umn.edu

Pig Research Summit - THINK Piglet Health & Nutrition 2023

September 21 - 22, 2023 (Thu-Fri)
Crowne Plaza Copenhagen Towers
Copenhagen, Denmark

For more information:
Danish Agriculture & Food Council
Web: tilmeld.dk/thinkpiglet2023/conference

127th US Animal Health Association Annual Meeting

October 12 - 18, 2023 (Thu-Wed)
Gaylord National Resort & Convention Center
National Harbor, Maryland

For more information:
Web: usaha.org/meetings

27th International Pig Veterinary Society Congress & 15th European Symposium of Porcine Health Management

June 4 - 7, 2024 (Tue-Fri)
Congress Centre Leipzig
Leipzig, Germany

For more information:
Web: ipvs2024.com

For additional information on upcoming meetings: aasv.org/meetings

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