

JOURNAL OF SWINE HEALTH & PRODUCTION

Depletion of bromadiolone in tissues of hogs after oral exposure

Enouri S, Dekroon K, Friendship R, et al

Disinfection protocols to reduce virus transmission via livestock transport vehicles

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Inoculation of piglets with feed contaminated with PEDV

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Herd closure and medication protocols for elimination of *M hyopneumoniae*

Holst S, Yeske P, Pieters M



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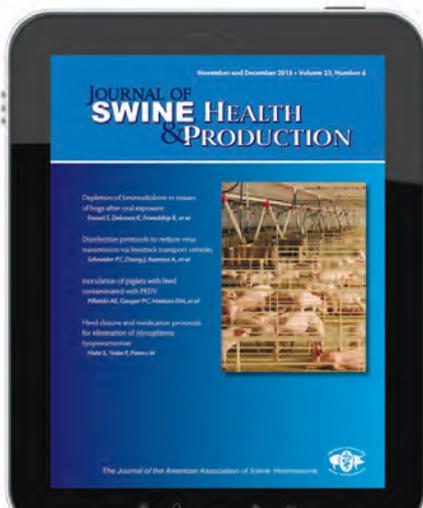
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“This final issue of 2015 is a solid example of the diversity of applied topics that [the *Journal of Swine Health and Production*] brings to our swine library.”

quoted from Executive Editor's message, page 295

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One million pigs on the road every day

It is almost hard to believe. The United States produces 120 million market pigs per year, all moving by truck to markets. It is estimated that approximately 90% of these market pigs are moved offsite at weaning, and approximately 40% are moved again from nursery to another site for finishing. Also, accounting for movement of 3 million cull sows and 3 million replacement gilts makes a total of approximately 270 million pig movements on 260 workdays per year = over one million pigs on the road per day! If the 120 million market pigs alone are loaded 180 head per truck on 260 workdays, that is approximately 2500 truckloads per day. Add another 500 trucks for weaned pigs, cull sows, and breeding stock and there are approximately 3000 trucks moving pigs per day. You can work the numbers differently, but it's still a lot of trucks with pigs traveling through the country every day.

During this year's 2015 AASV Annual Meeting, three of our main speakers, Stevenson,¹ Webb,² and Desrosiers,³ addressed this issue as a threat to the industry. All three cited our experience with porcine epidemic diarrhea virus as evidence of how large the challenge would be to limit movement of pigs as a means of limiting the spread of a pathogenic new virus. If the virus had been a known foreign animal disease (FAD) such as foot-and-mouth disease (FMD), we may have been able to identify it sooner, but

with 3000 trucks per day moving pigs (not to mention cattle trucks) we may not have detected it soon enough to prevent widespread dissemination.

Sustainability – at the local level our modern production systems are “economically sustainable” and can compete with any in the world. However, the mass movement of pigs represents a challenge to “industry sustainability” at an international level. Now that our pig industry exports approximately 25% of its meat, export sustainability equates with industry sustainability. An export-limiting FAD could have devastating effects on our markets. Can you imagine a 25% surplus? Also, as Desrosiers³ cautioned us, influenza may be our next challenge. Could the next influenza A virus (IAV) have a much higher mortality rate in pigs and humans than the novel H1N1 of 2009? If a highly pathogenic strain of IAV evolved, our domestic market could collapse as well.

Porcine epidemic diarrhea virus of 2013 has been a wake-up call. Novel H1N1 of 2009 and porcine circovirus type 2 of 2007 have not been forgotten. Fortunately these have had little impact on meat exports. Highly pathogenic avian influenza (HPAI) of 2015 has had dramatic effects on the poultry industry of the northern corn belt. Lessons have been learned from HPAI for mass euthanasia and disposal, routing of contaminated trucks, routing of clean trucks, farm worker movement, and other biosecurity issues.

At the time of this writing in September 2015 there are several reports of Seneca Valley virus – a vesicular disease of pigs which can mimic FMD. It is not new to the United States, but if its pathogenicity has changed, it could disrupt the movement of pigs as an FMD imposter.

What can we do to improve industry sustainability, especially with regard to livestock trucking. Limit the movement of pigs? Not likely. Manitoba has initiated a certified truck-wash program as a tool to prevent PED from hitching a ride on trucks returning from the United States. Livestock trailers returning to Canada from

the United States are encouraged or required to be washed at a Canadian certified truck wash. Perhaps the United States should consider such a network of certified livestock truck washes with minimum standards at or near slaughter facilities. All departing trucks would be required to wash. Should we prohibit importation of feed ingredients from high-risk countries? The Food Safety Modernization Act should protect our industry, but will it? Perhaps a catastrophic production insurance program would be a means of protecting farms that experience either major pig losses or market collapse. The question is, of course, who pays for it?

Some good things are happening right now. Dr Jim Roth and his group at the Center for Food Security and Public Health at Iowa State University are developing protocols for a “Secure Pork Supply” plan to maintain business continuity for pork producers and processors during an FAD outbreak. The National Pork Board has funded a new project called the “Swine Health Information Center” to monitor disease threats, both domestic and foreign, and to coordinate preparations for any potential new outbreak. These are building blocks for our future.

With one million pigs on the road every day, is our industry sustainable? From a short-term economic efficiency view of sustainability, yes it is. However, the long-term sustainability of our export market has been called into question. I challenge our veterinary profession and forward-thinking industry leaders to develop strategies that will positively influence long-term sustainability.

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Our swine library

I know you will be interested in reading this issue of the *Journal of Swine Health and Production* (JSHAP). This final issue of 2015 is a solid example of the diversity of applied topics that JSHAP brings to our swine library. A particular note of interest is that this issue contains a peer-reviewed commentary, "Elimination of *Mycoplasma hyopneumoniae* from breed-to-wean farms: A review of current protocols with emphasis on herd closure and medication," by Holst et al.¹ The peer-reviewed commentary is a special genre that JSHAP offers which allows authors to infuse some opinion (supported with references) into their manuscript. This genre goes beyond the typical literature review, as usually more opinion is blended into the message, but, as I mentioned, supported by the literature. The peer-reviewed commentary is a very challenging manuscript to write, as is it to review, often with multiple versions and edits required. We do not get many submissions in this category and this is likely because of the time commitment and work involved. So I would like to acknowledge the authors and reviewers for their dedication to this manuscript. I also encourage other authors to consider submitting a peer-reviewed commentary, and if

you have any questions about the process or topics, please do not hesitate to contact the journal office.

"This final issue of 2015 is a solid example of the diversity of applied topics that JSHAP brings to our swine library."

I really enjoyed this issue as well due to the contributions from the authors of the other papers. The brief communication "Experimental inoculation of neonatal piglets with feed naturally contaminated with porcine epidemic diarrhea virus" by Pillatzki et al,² and the original research manuscript "Evaluation of disinfection protocols to reduce virus transmission via livestock transport vehicles using model trailers in experimental conditions" by Schneider et al³ both bring valuable contributions to our knowledge about these two important pathogens: porcine reproductive and respiratory syndrome virus and porcine epidemic diarrhea virus. Additionally, the original research manuscript "Depletion of bromadiolone in tissues of hogs following oral exposure" by Enouri et al⁴ fills a gap in our knowledge about the persistence of bromadiolone in hog tissues following ingestion – a critically important food-safety issue.

As some of you know, JSHAP recently applied to be indexed with Medline at the National Library of Medicine. Unfortunately, our application was declined. I was

feeling pretty blue about this disappointing news and then I re-read some previous issues and this particular issue again, which cheered me up instantly. Look at the strength of the contributions that all previous and current JSHAP authors and reviewers have made to our (inter)national swine library. I feel so fortunate to be associated with such a fine network of dedicated swine researchers, reviewers, industry partners, and veterinarians.

I will leave you to enjoy this issue.

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Terri O'Sullivan, DVM, PhD
Executive Editor





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Why I do what I do

As many of you may know, my family and I recently moved to another country to work with an agriculture development project. So when Tom Burkgren asked me to write about “Why I do what I do” it was a very timely topic for me to personally reflect on, and I am excited to share those thoughts with my AASV colleagues. In my situation, there are two separate “why” questions: why did I become a veterinarian in the first place, and why did my family and I move to the other side of the planet (literally)? Both decisions developed over long periods of time. The reasons behind both are innumerable, but generally involve my faith, my family, my personality, and my likes and dislikes. But in both cases, two specific events helped me to clarify things. I want to share those events and how they impacted my decisions.

There was a specific time when I decided that I wanted to be a veterinarian. I was 19 years old and was home from college for spring break. One night I was conscripted by my father to go with him on a veterinary emergency call (most of you probably know my father, Dr Paul DuBois, who is also a veterinarian and AASV member). It was

not unusual for my father to take me or one of my brothers to help on emergencies, and I had been on hundreds of similar calls. But, on this trip, I developed a whole new outlook on what my father did and what it meant to be a veterinarian. We were called by an elderly couple with a heifer that was having trouble calving. We delivered a live calf, and the owners were very grateful. On the trip home, I reflected that we had helped the cow, the calf, and the people who owned them in a very tangible and meaningful way. It helped me to fully understand that veterinary medicine was a noble profession and a great way to serve and help other people. Also, it involved science, which I really loved (and still do). When I got back to college, I changed my major to pre-veterinary medicine. And the rest, as they say, is history.

“The veterinary profession is ultimately about helping people.”

The second incident occurred at the 2011 AASV Annual Meeting in Phoenix, Arizona. I attended one of the evening receptions and was sitting at a large table with several other attendees. I eavesdropped on a conversation between some veterinary students and a fellow swine veterinarian. The conversation was pretty typical. “What do you want to do when you get out of vet school?” “I want to go to into this type of practice or that type of practice, etc, etc.” Then things took an unexpected turn. The veterinarian asked the students if they had considered using their veterinary profession to serve in an underdeveloped part of the world. He laid out the ugly statistics about global poverty, the reality that millions of people throughout our world suffer from crippling poverty, disease, and malnutrition. After educating everyone on the stark facts, the veterinarian asked this question “How can we be OK with that?” He then explained that veterinarians have the education and skillset to help improve the situation for the people struggling under those circumstances. Even though he wasn’t talking to me directly, the veterinarian’s question kept bothering me.

How can I be OK with that type of injustice? It also led to a logical subsequent question that bothered me even more: Is there some way that I could or should be involved in improving that situation? It would be an exaggeration to say that event alone was why my wife and I made the decision to move. That decision ultimately involved a lot of prayer, many discussions with family and trusted friends, and countless hours of research. But the questions from that overheard conversation were pivotal and helped crystalize things during the decision-making process.

The point of each story is the same, and it is something that we all know. The veterinary profession is ultimately about helping people. Whether it is helping an elderly couple with a bovine dystocia, helping someone in a developing country start a small-animal agriculture operation, or all the things that each of you do every day to help your clients and colleagues in the swine industry, they are all ways that veterinary medicine is used to help the people that we serve in our professional lives. I would encourage each of you to take some time and reflect on “Why you do what you do.” But don’t stop there. Ask yourself what you see in your town, your state, or your world that you are not “OK” with. Then ask yourself if there is something that you could or should do to help improve it.

Bill DuBois, DVM



Depletion of bromadiolone in tissues of hogs following oral exposure

Saad Enouri, MSc, PhD; Kristina Dekroon, BSc; Robert Friendship, DVM, MSc, Diplomate ABVP; Nick Schrier, MSc; Patricia M. Dowling, MSc, Diplomate ACVIM (LAIM), Diplomate ACVCP; Ron Johnson, PhD, Diplomate ACVCP

Summary

Objectives: To assess bromadiolone depletion in the edible tissues of swine and propose post-exposure withdrawal periods.

Materials and methods: Two groups of barrows and two groups of gilts were given a single oral dose of bromadiolone: low dosage (LD, 0.05 mg/kg; n = 20; 10 males, 10 females) and high dose (HD, 0.5 mg/kg; n = 20; 10 males, 10 females). Coagulation parameters were assessed before and after administration. Animals were sacrificed at 1, 2, 3, 5, and 6 weeks (LD) and 1, 2, 3, 6, and 9 weeks (HD) post dosing. Loin muscle, skin-adherent fat, liver, feces, and blood

were analyzed for bromadiolone using liquid chromatography-tandem mass spectrometry.

Results: Partial thromboplastin times exceeded control values in the LD and HD groups 6 and 9 weeks post dosing, respectively. In the HD group, bromadiolone concentrations exceeded the limit of detection (LOD) at all time points in liver and skin-adherent fat and for up to 6 weeks in feces, muscle, and plasma. In the LD group, bromadiolone concentrations exceeded the LOD at all time points in liver and up to 3 weeks in fat, feces, and plasma. Estimated withdrawal periods for bromadiolone in liver were 83 and 176 weeks in the LD and

HD groups, respectively, and 62 weeks in muscle in the HD group.

Implication: Bromadiolone residues persist in tissues such that it is impractical to wait for the hog to eliminate the rodenticide to a concentration that is safe for entry into the human food chain.

Keywords: swine, rodenticides, bromadiolone detection, withdrawal period, liquid chromatography-tandem mass spectrometry.

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Resumen - Disminución de bromadiolona en tejidos de cerdos después de la exposición oral

Objetivos: Evaluar la reducción de bromadiolona en tejidos comestibles de cerdos y proponer periodos de retiro después de la exposición.

Materiales y métodos: Se administró una dosis única de bromadiolona a dos grupos de machos castrados y dos grupos de hembras: dosis baja (LD [dosis baja por sus siglas en inglés], 0.05 mg/kg; n = 20; 10 machos, 10 hembras) y dosis alta (HD [dosis alta por sus siglas en inglés], 0.5 mg/kg; n = 20;

10 machos, 10 hembras). Se evaluaron los parámetros de coagulación antes y después de la administración. Los animales fueron sacrificados a las semanas 1, 2, 3, 5, y 6 (LD) y a las semanas 1, 2, 3, 6, y 9 (HD) después de la administración de la dosis. Se analizaron el músculo del lomo, la grasa adherida a la piel, el hígado, las heces, y la sangre en busca de bromadiolona utilizando cromatografía de líquidos con espectrometría de masas en tándem.

Resultados: Los tiempos parciales de tromboplastina excedieron los valores control en los grupos de LD y HD 6 y 9 semanas después de la administración de la dosis,

respectivamente. En el grupo de HD, las concentraciones de bromadiolona excedieron el límite de detección (LOD por sus siglas en inglés) en todos los muestreos en hígado y grasa adherida a la piel, y hasta por 6 semanas en heces, músculos y plasma. Los periodos de retiro estimados para la bromadiolona en hígado fueron 83 y 176 semanas en los grupos de LD y HD, respectivamente, y 62 semanas en músculo en el grupo HD.

Implicación: Los residuos de bromadiolona persisten en tejidos de tal manera que es impráctico esperar a que el cerdo elimine el raticida hasta una concentración que sea segura para entrar en la cadena alimenticia humana.

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

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Résumé - Déplétion du bromadiolone dans les tissus de porcs suite à une exposition orale

Objectifs: Évaluer la déplétion du bromadiolone dans les tissus comestibles de porc et proposer une période de retrait post exposition.

Matériels et méthodes: Deux groupes de castrats et deux groupe de cochettes reçurent par voie orale une dose unique de bromadiolone: faible dosage (FD, 0,05 mg/kg; n = 20; 10 mâles, 10 femelles)

et dosage élevé (DE, 0,5 mg/kg; n = 20; 10 mâles, 10 femelles). Les paramètres de coagulation furent évalués avant et après l'administration. Les animaux furent sacrifiés à 1, 2, 3, 5, et 6 semaines (FD) et à 1, 2, 3, 6, et 9 semaines (DE) suivant l'administration du produit. Le muscle de la longe, du gras adhérent à la peau, du foie, des fèces, et du sang furent analysés pour la présence de bromadiolone par chromatographie liquide couplée à la spectrométrie de masse en tandem.

Résultats: Les temps de thromboplastine partielle excédaient les valeurs de contrôle pour les groupes FD et DE à 6 et 9 semaines post administration, respectivement. Dans le groupe DE, les concentrations de bromadiolone excédaient la limite de détection (LD) à tous les moments testés dans le foie et le gras adhérent à la peau et jusqu'à 6 semaines dans les fèces, le muscle, et le plasma. Dans le groupe FD, les concentrations de bromadiolone excédaient la LD à tous les temps testés dans le foie et jusqu'à 3 semaines dans le gras, les fèces, et le plasma. Les périodes de retrait estimées pour le bromadiolone dans le foie sont de 83 et 176 semaines dans les groupes FD et DE, respectivement, et de 62 semaines dans le muscle du groupe DE.

Implication: Les résidus de bromadiolone persistent trop longtemps dans les tissus pour qu'il soit pratique d'attendre que le porc élimine le rodenticide à une concentration sécuritaire pour permettre l'entrée de la viande dans la chaîne alimentaire humaine.

Accidental ingestion of rodenticides in hogs is a significant food-safety concern, impacts animal welfare, and can result in substantial economic losses to producers. The hydroxycoumarins are a group of anticoagulant compounds that include bromadiolone, brodifacoum, coumatetralyl, difenacoum, and warfarin.¹⁻³ These compounds are commonly used as rodenticides worldwide for the control of rats and mice. The increased commercial availability of these compounds has resulted in accidental ingestion by animals.⁴ Further, the emergence of rodent strains resistant to older or first-generation anticoagulant rodenticides has spawned the development of more potent, second-generation compounds such as bromadiolone, with increased potential for toxicity following accidental ingestion by livestock and adulteration of food intended for human consumption.⁵

The true incidence of bromadiolone exposures in food-producing animals, including hogs, is not currently known. The Canadian Global Food Animal Residue Avoidance Databank (Canadian g-FARAD) has previously received queries regarding accidental ingestion of rodenticides in swine. Bromadiolone is one of the most commonly cited rodenticides in suspected accidental ingestion in swine, according to the Canadian gFARAD (<https://cgfarad.usask.ca/home.html>), making it an ideal candidate for study. The median oral lethal dose (LD₅₀) for bromadiolone in research hogs is estimated to be 3 mg per kg body weight following single dosing, but no data on toxicokinetics of bromadiolone in hogs are currently available.¹ Oral bioavailability of rodenticides is generally high in mammals (79% to 92%),¹ and residue depletion may be prolonged⁶ due to liver accumulation and enterohepatic recycling,^{1,7,8} with some studies in rodents suggesting that bromadiolone toxicokinetics are dose-dependent.⁷ Clinical signs associated with anticoagulant rodenticide toxicosis may vary, including spontaneous bleeding or bruising or both, with elevations in prothrombin time (PT) and activated partial thromboplastin time (PTT) confirming blood-clotting abnormalities.^{9,10} However, with lesser exposures, swine may not show clinical signs, yet still have violative residues in edible tissues. Information pertaining to exposure, tissue depletion, and possible withdrawal periods of rodenticides in suspected swine toxicosis would provide substantial guidance to veterinarians and producers regarding animal disposition. Therefore, the objectives of this study were to assess the depletion of bromadiolone in the edible tissues of swine and evaluate bromadiolone withdrawal periods following oral exposure. The study also sought to evaluate changes to coagulation profiles associated with bromadiolone exposure.

Materials and methods

The experimental protocol was approved by the Institutional Animal Care and Use Committee of the University of Guelph and conformed to standards set forth by the Canadian Council on Animal Care.

Animals

Male (20 barrows) and female (20 gilts) Yorkshire hogs weighing 40.4 ± 1.4 kg and 38.4 ± 1.5 kg (mean \pm standard error [SE]), respectively, were used in this study. A pilot

depletion study (12 Yorkshire hogs) was carried out followed by a main depletion study (28 Yorkshire hogs). On the basis of physical examination, all animals were deemed healthy prior to study. Animals were born in the established herd at Arkell Research Station (Guelph, Ontario, Canada) and housed at the same facility according to standards of care for swine for the duration of the study, with free access to feed and water. Animals were randomly assigned to pens with stocking density as low as possible (four hogs per pen) in order to provide greater observation of each animal and reduce injuries caused by fighting. Additionally, animals were monitored daily for general health and adverse events throughout the study.

Study design

Our experimental design, including animal numbers per sacrifice group, was based on current recommendations of the Veterinary International Conference on Harmonisation (VICH) guideline (no. 48)¹¹ outlining marker residue depletion studies to establish compound withdrawal times. The marker residue, being the parent molecule or metabolite, is determined from total residue and metabolism studies in the target species. The marker residue depletes in known relation to depletion of total residues. This guideline is currently used by the Food and Drug Administration-Center for Veterinary Medicine (FDA-CVM; United States) and the Veterinary Drug Directorate of Health Canada for regulatory approval of drugs requiring withdrawal times in food-producing animals. Although the metabolism and safety profile of bromadiolone in hogs is unknown, it was not an objective of the study to determine the marker residue for bromadiolone in hogs. Rather, the depletion of the parent bromadiolone molecule was chosen for assay with the understanding that metabolites of bromadiolone may persist in edible tissues for longer periods than the parent bromadiolone molecule. A pilot study was first conducted to assist with determination of sampling time points (sacrifice times) for the main depletion study. Furthermore, because of the anticipated long residue depletion for bromadiolone in hogs, we included additional sacrifice time points in the main depletion study, on the basis of the pilot study results. Data generated in the pilot study were added to the main depletion study, giving a final number of four hogs (two male, two female) per sacrifice time point.

Because bromadiolone is not approved for use as a therapeutic, and there is no available dosage regimen, and because hogs may accidentally ingest varying quantities of bromadiolone, we chose to study both a low (0.05 mg per kg) and a high (0.5 mg per kg) bromadiolone dosage. All hogs were weighed 1 day before starting either the pilot or main depletion study. For the pilot study, 12 normal, healthy male (six barrows) and female (six gilts) Yorkshire hogs were used. The pilot study was conducted in two parallel arms of six hogs each, with a low dose (LD) single oral bromadiolone (Sigma Aldrich, Oakville, Ontario, Canada) dosage group (0.05 mg per kg body weight) and a high dose (HD) single oral bromadiolone dosage group (0.5 mg per kg body weight), with each arm of the study balanced across sexes. In the LD group, two hogs (one male and one female) were randomly chosen, by drawing ID numbers from a container, to be sacrificed at 7 days, 14 days, and 21 days post dosing, while in the HD group, two hogs (one male and one female) were sacrificed at 7 days, 14 days, and 42 days post dosing. A similar approach was followed in the main depletion study (28 Yorkshire hogs), with hogs in the low and high treatment dosage arms being sacrificed at 1, 2, 3, 5, and 6 weeks and 1, 2, 3, 6, and 9 weeks, respectively, taking into consideration the total number of animals to be sacrificed at each time point, based on the VICH guideline¹¹ and on the number of animals used per sacrifice time point in the pilot study.

Tissue sampling

Samples were collected from all animals at pre-dosing (plasma from anti-coagulated blood [sodium heparin] and fresh feces) and post dosing (plasma from anti-coagulated blood [sodium heparin], fresh feces, liver, loin muscle, and skin-adherent fat) at the time of sacrifice for each treatment group (LD group at 1, 2, 3, 5, and 6 weeks; HD group at 1, 2, 3, 6, and 9 weeks) for determination of bromadiolone concentrations by mass spectroscopy. In the pilot study, plasma from anti-coagulated blood (sodium heparin) was also collected from all animals at 12, 24, 48, and 72 hours post dosing, and fresh fecal samples were collected at 24 and 72 hours post dosing, in order to assist the Animal Health Laboratory (AHL) at the University of Guelph with the validation of a non-invasive detection assay to confirm bromadiolone exposure. Prothrombin time and PTT evaluation were performed on

blood samples collected from all hogs at pre-dosing, and 24, 48, and 72 hours post dosing during the first week, as well as on subsequent samples collected twice a week until sacrifice time. All animals were sacrificed by exsanguination following electrical stunning before collection of tissues samples, including liver (minimum of 250 g), loin muscle (approximately 500 g core sample), and skin-adherent fat (minimum of 50 g of natural tissue proportion). All hogs were sacrificed at the university abattoir according to standard handling protocols. All samples were identified and stored at -80°C until analysis. Blood samples for PT and PTT assessment were analyzed on the same collection day by the AHL in order to evaluate effect on coagulation. Using baseline (control) PT and PTT values from all hogs in the study, the AHL generated reference intervals for both PT and PTT using Rhoads EP Evaluator software (Release 8; David G. Rhoads Associates Inc, Kennett Square, Pennsylvania). The reference range is being defined as the so-called normal range (in seconds) for these respective coagulation tests, ie, the range of the central 95% of results obtained from a group of ostensibly healthy individuals.

Sample preparation for mass spectroscopy

Each plasma sample (0.5 mL) was transferred to a screw-cap centrifuge tube (13 mm × 100 mm), vortex-mixed for 30 seconds, and extracted using 4.5 mL of acetonitrile. After centrifugation for 10 minutes at 1932g, the acetonitrile layer was removed using a 1-mL syringe, and the extract was filtered through a syringe filter (13 mm; polytetrafluoroethylene [PTFE], 0.2 µm) into an autosampler vial. Liver, muscle, skin-adherent fat, and fecal samples were prepared by weighing 1.0 ± 0.2 g of each tissue into MediFASTH2 vials (Syntec International, Dublin, Ireland), then adding 10 mL of acetonitrile and 10 mL of acetonitrile:water (85:15, volume:volume) for liver and muscle, and skin-adherent fat and fecal samples, respectively, and processing using a MediFASTH2 homogenizer. The acetonitrile layer was transferred to a centrifuge tube (16 mm × 125 mm) and centrifuged for 10 minutes at 1932g. After centrifugation, the acetonitrile layer was removed using a 1-mL syringe, and the extract was filtered through a syringe filter (13 mm; PTFE, 0.2 µm) into an autosampler vial. In the case of skin-adherent fat and feces, the acetonitrile extract was washed with 2 × 5 mL of hexane. Finally, the obtained aliquots of tissue extracts were

injected into the liquid chromatography-tandem mass spectrometry (LC-MS-MS) system for analysis.

LC-MS-MS analysis

The bromadiolone analytical method development and validation were carried out by one of the authors (NS) at the University of Guelph, Laboratory Services, AHL (Guelph, Ontario, Canada) utilizing a standard operating procedure based on the CITAC/Eurachem guide to quality in analytical chemistry.¹² Tissue extracts were assayed for bromadiolone concentrations in each matrix type using an LC-MS-MS system consisting of an Agilent 1100 series vacuum degasser, binary pump (Agilent Technologies Canada Inc, Mississauga, Ontario, Canada), and a Shimadzu autosampler (Mandel Scientific, Ontario, Canada) coupled to a triple quadrupole mass spectrometer (QTRAP 4000; AB SCIEX, Concord, Ontario, Canada). The following instrument variables were used: flow rate, 1.0 mL per minute; total run time, 10 minutes; ion spray voltage, 2000 V; source temperature, 650°C; polarity, electrospray ionization (ESI), negative; ESI probe setting: X-axis = 5 mm, Y-axis = 2 mm; curtain gas, 10 pounds per square inch; collision gas, medium. The injection volume was 1 µL, and chromatographic separation was achieved by use of Atlantis dC18 mini column (3.9 × 20 mm; internal diameter 3 µm) (Waters Corporation, Milford, Massachusetts). The column oven temperature was maintained at 10°C, and the autosampler temperature was 5°C. Bromadiolone concentrations for plasma, muscle, liver, skin-adherent fat, and feces were estimated from bromadiolone-spiked calibration curves. The validated limit of quantification (LOQ) and limit of detection (LOD) for calibration curves for skin-adherent fat (µg per kg), plasma (ng per mL), feces, muscle, and liver (µg per kg) were 1.0, 1.7, 3.3, 6.6, 10.0, and 0.3, 0.5, 1.0, 2.0, 3.0, respectively. Each matrix was validated at 3 × LOQ and run in triplicate. The calibration curve was evenly distributed, with a coefficient of determination (r^2) higher than 0.99 for each curve. The percent accuracy (mean ± SD) for bromadiolone determination in plasma, muscle, liver, skin-adherent fat, and feces was 88.0 ± 2.4, 66.7 ± 8.1, 78.7 ± 6.6, 106.7 ± 4.6, and 116.0 ± 8.2, respectively. The coefficient of variation for reference curve bromadiolone concentrations in all matrices including the LOQ was less than 15%.

Statistical analysis

All data analyses were conducted with the assistance of a biostatistician. Prior to analysis of data for withdrawal-period estimation, bromadiolone concentrations were transformed using the natural logarithm (Ln). Least squares means and 95% confidence levels were reported along with back-transformed values. The statistical significance level was set at $P < .05$. Statistical analyses were begun with a full factorial model that included all interactions (ie, dose, sex, tissue, and time), with terms being removed if P values were $> .05$. All terms involving sex had P values $> .05$. Tissue residue data for withdrawal-period determination were analyzed by a statistical method that determined the statistical tolerance limit for the central 99% of the population with 95% confidence using SAS (version 9.1.3; SAS Institute Inc, Cary, North Carolina).^{13,14} Briefly, regression analyses were performed on tissue bromadiolone concentrations to determine the time required for bromadiolone in the LD and HD treatment groups to reach a predetermined tissue concentration, ie, level of detection for the analyte in each matrix. In order to evaluate whether elimination of bromadiolone in hog tissues was dose dependent, depletion of bromadiolone from the liver in HD and LD groups, and from liver HD and muscle HD groups, was assessed using a general linear model to test differences in slopes over time. Rodenticides are classified as pesticides in most countries and are not approved for use in food-producing animals. Therefore, no acceptable daily intake of bromadiolone has been set by most regulatory authorities. However some regulatory agencies may allow certain concentrations of pesticides, such as rodenticides, in edible foodstuffs.¹⁵ Zero levels of an analyte, as determined by assay, are set at the limit of detection for that assay of the analyte in question and were used in the current study for the withdrawal period. In order to increase statistical power, results of pilot-study hogs were combined with results of main-study hogs. Slopes of the regression analysis for hogs in the pilot study did not differ significantly from the slopes in the main study, suggesting that all hogs eliminated bromadiolone similarly, and therefore could be combined as a single data set for statistical analysis. Prothrombin time and PTT values and bromadiolone concentrations obtained in both treatment groups up to 1 week post dosing, as well as subsequent data obtained twice a week after the first week and at sacrifice time points, were analyzed by ANOVA for repeated measures, respectively. To meet the assumptions of the

ANOVA, log transformation was applied when appropriate. Prothrombin time values were presented as arithmetic means, and PTT values as geometric means.

Results

No injuries or adverse events occurred throughout the study period. All data reported include pilot-study data. Plasma and fecal samples collected prior to dosing in all hogs contained undetectable concentrations of bromadiolone. The normal range of swine PT values was 11.7 to 16.0 seconds (mean \pm SD = 13.9 seconds \pm 1.0 second), while for PTT, the normal range was 22.3 to 51.0 seconds (geometric mean \pm SD = 37.4 seconds \pm 6.9 seconds). In both treatment groups, mean PT values obtained in bromadiolone-treated animals did not differ statistically from control (baseline) values at any study sacrifice time points (data not shown; $P = .18$). In the LD and HD groups, geometric mean values of PTT obtained at 6 weeks (55.7 seconds; lower limit [LL] 42.9 seconds; upper limit [UL] 72.3 seconds) and 9 weeks (163.5 seconds; LL 82.0 seconds; UL 326.1 seconds) post dosing were significantly greater ($P < .001$) than the control value (37.7 seconds; LL 33.7 seconds; UL 42.3 seconds), respectively. Some individual hogs had PT and PTT values above the normal ranges at various times, returning to normal values on subsequent PT and PTT tests (data not shown). None of these hogs showed clinical signs of bleeding or bruising.

In the pilot study, all hogs in the LD and HD groups were positive (both above the LOD) for bromadiolone in feces collected at 24 and 72 hours post dosing (data not shown). However, in the LD group, one of six hogs was negative (below the LOD) for bromadiolone in plasma collected at 24 hours post dosing, and two of six hogs were negative at both 48 and 72 hours post dosing (data not shown). Bromadiolone concentrations in both plasma (Figure 1) and feces (Figure 2) were greater in the HD group than those obtained in the LD group ($P < .05$). The gradual decline in bromadiolone concentrations in feces at 72 hours, compared to 24 hours, was followed by a sharp elevation at 7 days post dosing, particularly in the HD group (Figure 2), then a gradual decrease at subsequent study time points (tables 1 and 2).

Bromadiolone residues in pig tissues at post-dosing sacrifice time points

Results of data analysis showed that sex had no effect on residue depletion of bromadiolone in

the present study. At the 1, 2, 3, and 6 weeks sacrifice time points, for all tissues and feces collected, bromadiolone concentrations were higher in samples obtained in the HD treatment group (Table 1) than in those in the LD group ($P < .01$) (Table 2). In both treatment groups, bromadiolone concentrations decreased gradually in all tissues and feces across all sacrifice time points, but remained above the LOD at some time points. In the HD group, concentrations of bromadiolone remained higher than the corresponding LOD at all sacrifice time points in liver (Figure 3A, Table 2) and skin-adherent fat (Table 2), and up to 6 weeks post dosing in feces, plasma (Table 2), and muscle (Table 2, Figure 4). In the LD group, concentrations of bromadiolone were higher in liver (Figure 3B, Table 1), while lower in muscle (Table 1), than the corresponding LOD at all sacrifice time points. In the LD group, feces, plasma, and skin-adherent fat bromadiolone concentrations continued to be higher than the LOD up to 3 weeks post dosing (Table 1). It should be noted that in the LD group, bromadiolone was detected in muscle of only one of four hogs at week 2 post dosing. In the LD and HD groups, liver bromadiolone concentrations were higher than those detected in other tissues and feces at all post-dosing sacrifice time points ($P < .001$). At the 1-week sacrifice time point, all hogs were positive for bromadiolone in liver samples; however, one of the four hogs in the LD group was negative on fecal assay for bromadiolone, with an additional different hog being negative on plasma assay (data not shown).

Bromadiolone withdrawal period estimation

The tissue concentrations for bromadiolone were used to calculate a withdrawal period by applying the statistical tolerance limit method.¹⁶ The withdrawal period provides a time interval within which the concentrations of bromadiolone are at the maximum predetermined concentration (eg, muscle, 2 μ g per kg; liver, 3 μ g per kg) or below for 99% of treated pigs with a 95% confidence level. The maximum predetermined concentration for this study was denoted by the current LOD for the assay used to quantify bromadiolone concentrations in tissues. The withdrawal period calculation was achievable on liver (LD and HD groups) and muscle (HD group) samples. In LD and HD skin samples, estimation of the withdrawal period was not possible because the ANOVA test (F test) was not significant and slopes were not obtained (ie, slopes = 0). Additionally, in the LD group, only one muscle sample

showed detectable bromadiolone concentration. The withdrawal period was calculated from the regression lines according to FDA-CVM guidelines¹⁶ and was estimated as 83 weeks for LD liver, 176 weeks for HD liver, and 62 weeks for HD muscle. In order to evaluate the presence of dose-dependent elimination kinetics for bromadiolone in hog tissues, the slopes of the plots of HD and LD liver and HD muscle and HD liver over time were compared. Results showed no difference between liver HD and LD slopes ($P = .29$) or liver HD and muscle HD slopes ($P = .36$), suggesting dose-independent elimination kinetics.

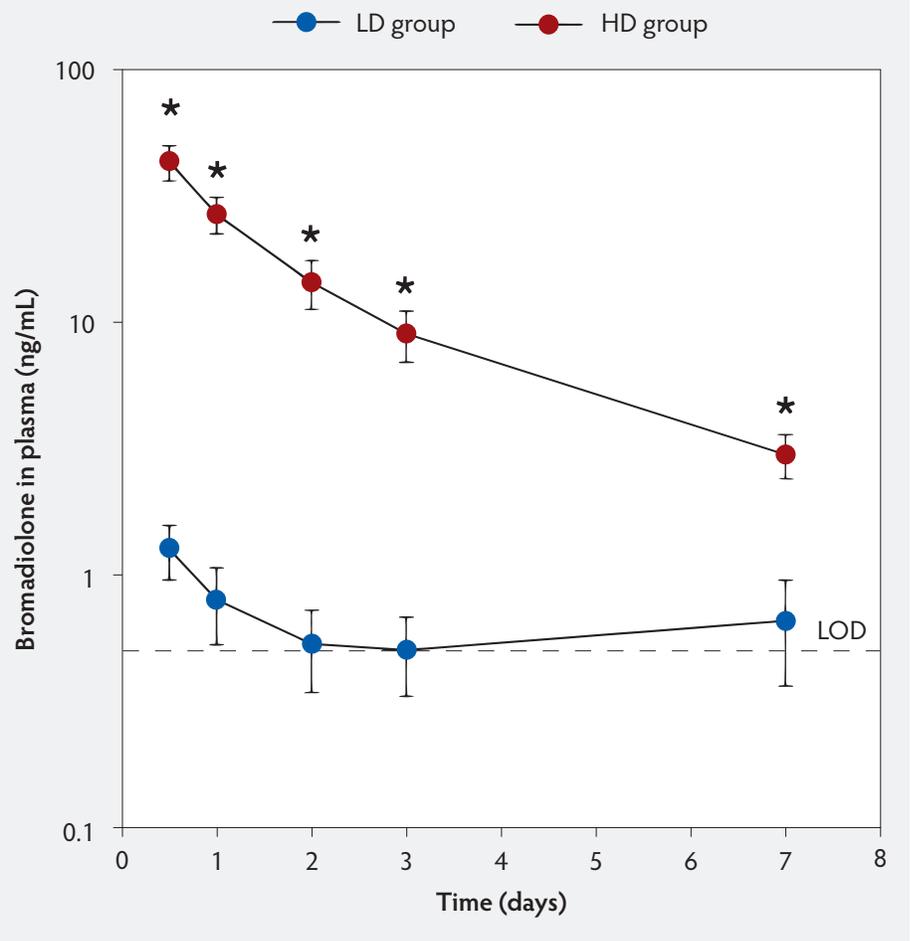
Discussion

In Canada, rodenticide toxicosis reported in hogs most likely results from direct exposure and ingestion of rodenticide baits such as bromadiolone or difethialone. Employing bait stations in the barn is crucial in order to reduce accessibility to rodenticides by non-target animals.

Exposure to second-generation rodenticides results in prolonged tissue residue depletion, particularly in the liver as reported in the present study. In the present study, bromadiolone was administered as a single oral dose of either 0.05 or 0.5 mg per kg body weight, on the basis of the following reasoning. Firstly, an oral LD₅₀ (dose producing death in half of the animals tested after single oral dosing) of 3 mg per kg body weight has been reported for bromadiolone in swine.¹ Secondly, recommendations for application of bromadiolone bait in barns may vary; however, common approaches using 100 to 450 grams of bait per site interval will provide approximately 0.05 to 0.5 mg per kg of bromadiolone (0.005% weight by weight) with a single ingestion. Lastly, the selection of a low and high dosage group enables the evaluation of dose-dependent residue elimination.

Anticoagulant rodenticides, including bromadiolone, interfere with the normal synthesis of vitamin K-dependent clotting factors in the liver, which results in extended bleeding time and possibly death.¹ However, in the present study, no clinical signs of hemorrhage or significant bruising were observed in any animal. In the current study, PT values were similar to control values in both groups; however, in the LD and HD groups, PTT values obtained at 6 and 9 weeks post dosing were significantly greater than control values, respectively. Therefore, these measures of blood clotting activity cannot be used as an indicator of the absence of

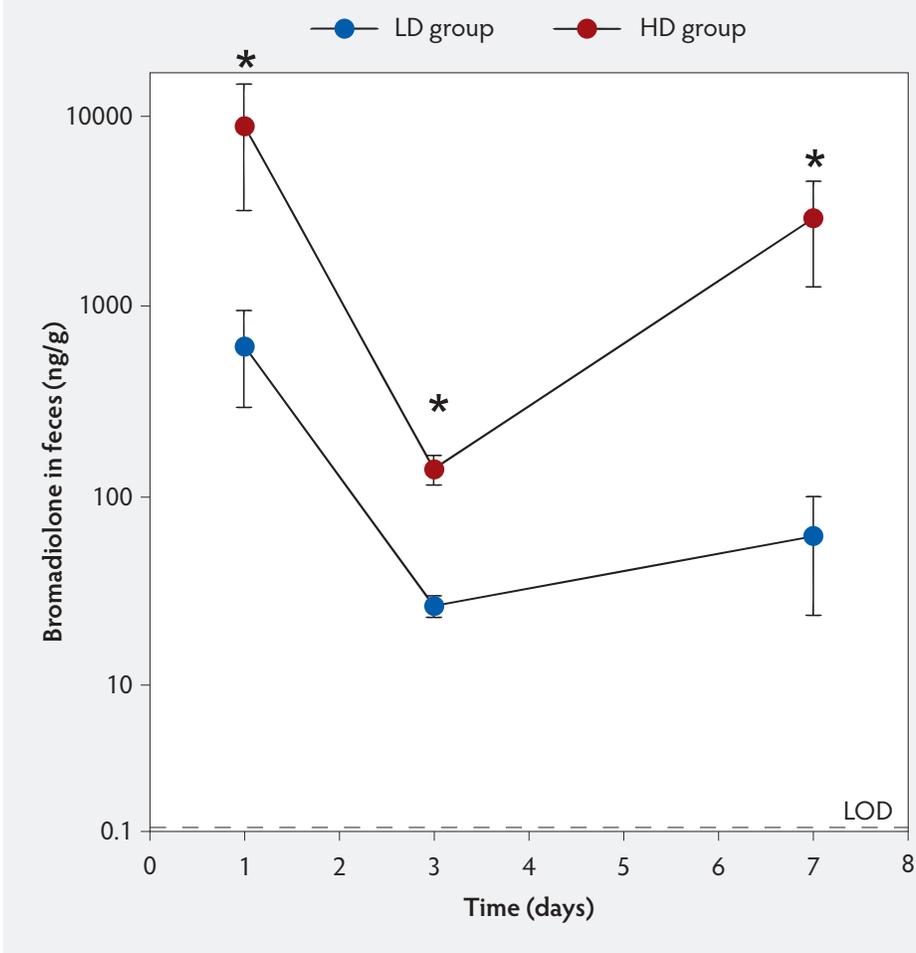
Figure 1: Mean plasma concentrations of bromadiolone versus time after single oral administration of low dosage (0.05 mg/kg; LD, n = 6) and high dosage (0.5 mg/kg; HD, n = 6) obtained during the first week post dosing in healthy Yorkshire pigs weighing (mean ± standard error [SE]) 40.4 ± 1.4 kg (males) and 38.4 ± 1.5 kg (females). Pigs were housed in groups according to standard of care for swine and had free access to feed and water. Data reported as mean ± SE. Asterisks (*) indicate a statistical difference (ANOVA; $P < .05$) between the LD group and the HD group at the corresponding time point. LOD = limit of detection.



anticoagulant rodenticide residues in swine. Alternatively, plasma and fecal rodenticide analyses could be used to assess the ingestion of bromadiolone. Any detectable plasma or fecal concentration of bromadiolone would confirm ingestion, with bromadiolone concentrations in edible tissues possibly being higher than those in either plasma or feces. Our results showed that all hogs receiving bromadiolone tested positive on fecal assay in the first 72 hours following exposure, but some animals in the LD group did record negative plasma assay results during the same time period post dosing. However, findings in the current study also suggest that starting at 1 week post dosing, negative results on either plasma or fecal bromadiolone tests could occur, with other edible tissues, such as liver, being positive for bromadiolone.

Our results showed that bromadiolone had a biphasic depletion pattern in feces, with the highest bromadiolone concentrations being detected for all tissues and feces during the first week of the single oral administration. Plasma concentrations of bromadiolone increased rapidly following dosing, with maximum concentrations of 1.3 ± 0.3 ng per mL and 43.2 ± 6.9 ng per mL being detected at 12 hours post dosing in the LD group and HD group, respectively. The use of other anticoagulant rodenticides in other species have led to similar trends.¹⁷ In the present study, bromadiolone concentrations gradually declined following dosing, but remained relatively high over the study period in the liver of the LD and HD groups and in the muscle of the HD group. This kinetic behavior of bromadiolone confirms previous results obtained with this compound, as well

Figure 2: Fecal concentrations of bromadiolone versus time after single oral administration of low dosage (0.05 mg/kg; LD, n = 6) and high dosage (0.5 mg/kg; HD, n = 6) obtained during the first week post dosing in healthy Yorkshire pigs (pigs and study described in Figure 1). Data reported as mean \pm standard error. Asterisks (*) indicate a statistical difference (ANOVA; $P < .05$) between the LD group and the HD group at the corresponding time point. LOD = limit of detection.



as other anticoagulant rodenticides in various species.^{7,9,18-20} The prolonged elimination of bromadiolone is due to liver accumulation and enterohepatic recycling.^{1,7,8} Furthermore, the results of the present study showed that concentrations of bromadiolone were higher in the liver and feces than in other tissues; this finding is consistent with the toxicokinetics of this pesticide.⁶ It should be mentioned that in the HD group, bromadiolone concentrations in skin-adherent fat remained higher than the corresponding LOD at all sacrifice time points, which suggests that bromadiolone also accumulates in fat. Finally, comparison of the slopes of the depletion over time plots for bromadiolone showed no differences between liver HD and LD groups, or between liver HD and muscle HD groups, suggesting that the elimination of bromadiolone from hog tissues is not dose dependent.

Withdrawal period estimations of bromadiolone following administration of a single low or high dosage were performed in the present study. Withdrawal periods were determined by defining the 99th percentile of the population with 95% confidence. The estimated withdrawal periods for liver were 83 weeks and 176 weeks in the LD group and HD group, respectively. In the muscle of the HD group, withdrawal period was estimated as 62 weeks. While these findings provide new insights in terms of anticoagulant rodenticide tissue-residue depletion and withdrawal period estimations in swine, our statistical power would have improved if we had had both a larger sample size and a longer study period. Interestingly, our findings in the liver of the LD group are similar to those obtained in dogs showing that residues of flocoumafen, a first-generation anticoagulant rodenticide, administered at 0.4 mg per

kg body weight, were found in the liver for 43 weeks.¹⁸

In summary, second-generation anticoagulant rodenticides, including bromadiolone, are highly toxic, with the ability to kill rats and mice after a single oral dose. Our findings show that fecal samples, and possibly plasma samples, could be used to confirm bromadiolone exposure in hogs if carried out quickly after the suspected exposure. However, it is not known if ingestion of bromadiolone at dosages less than or greater than those tested in the current study would provide similar results. In the present study, administration of a single oral low or high dosage of bromadiolone to hogs showed considerable accumulation in the liver, with concentrations persisting for a prolonged period despite the absence of clinical signs. Similar results were reported in the muscle of the HD group. It should be mentioned that the metabolite profile of bromadiolone in hogs is unknown, but in humans bromadiolone is excreted as metabolites (glucuronides and sulfates) in the urine and feces.²¹ The elimination of bromadiolone from tissues in hogs may involve metabolism, with the parent bromadiolone molecule being converted to metabolites that may also be pharmacologically active and present residue concerns. In this study, samples were assayed for the parent bromadiolone molecule; however, its metabolites may also persist, adding to total residue levels of the pesticide. Thus, persistent metabolites of bromadiolone may increase the withdrawal periods estimated in the current study. Nevertheless, the estimated withdrawal periods for the parent bromadiolone molecule in hog tissues are still very long.

Implications

- Fecal and plasma detection assays for bromadiolone may reveal exposure of a pig to bromadiolone.
- Bromadiolone residues persist in muscle, and particularly in liver, such that it is impractical to wait for the hog to eliminate the rodenticide to a concentration that allows safe entry of the hog into the human food chain.

Conflict of interest

None reported.

Acknowledgements

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Table 1: Bromadiolone concentrations after single oral administration of low dosage (0.05 mg/kg) in 20 healthy Yorkshire pigs*

LOD	Liver (µg/kg)			Skin & adherent fat (µg/kg)			Feces (µg/kg)			Plasma (ng/mL)		
	3.0			0.3			1.0			0.5		
Week	LL	Median	UL	LL	Median	UL	LL	Median	UL	LL	Median	UL
1	96.9	175.1†	316.5	0.9	3.1	10.2	2.9	19.9	73.5	0.4	0.8	1.3
2	87.2	137.3†	216.1	0.6	1.6	4.1	3.3	8.9	24.4	0.4	0.8	1.3
3	74.3	107.6†	155.8	0.4	0.9	1.8	1.8	4.0	9.1	0.3	0.5	0.7
5	40.9	66.1†	106.6		< LOD			< LOD			< LOD	
6	27.8	51.8	96.8		< LOD			< LOD			< LOD	

* Study described in Figure 1. For each week, n = 4 pigs, which were euthanized for collection of tissues. Plasma and fecal samples were obtained at the time of euthanasia. For muscle tissue, all median values were < LOD. Values are expressed as median (geometric mean) with lower limit (LL) and upper limit (UL).

† Versus other tissues within a time point ($P < .05$; ANOVA and multiple t tests).

LOD = limit of detection; ANOVA = analysis of variance.

Table 2: Bromadiolone concentrations after single oral administration of high dosage (0.5 mg/kg) in 20 healthy Yorkshire pigs*

LOD	Muscle (µg/kg)			Liver (µg/kg)			Skin & adherent fat (µg/kg)			Feces (µg/kg)			Plasma (ng/mL)		
	2.0			3.0			0.3			1.0			0.5		
Week	LL	Median	UL	LL	Median	UL	LL	Median	UL	LL	Median	UL	LL	Median	UL
1	6.1	9.6	15.2	348.8	596.4†	1019.9	2.7	8.2	24.5	20.6	67.3	220.2	1.4	2.3	3.8
2	5.3	7.8	11.5	333.6	524.7†	825.3	2.8	7.0	17.7	13.8	37.5	101.9	1.2	1.8	2.7
3	4.5	6.4	8.9	312.1	461.6†	682.6	1.8	6.1	13.4	8.8	20.9	49.6	0.9	1.3	1.9
6	2.4	3.4	4.9	205.4	314.2†	480.6	1.6	3.8	9.1	1.4	3.6	9.2	0.4	0.6	0.9
9		< LOD		106.7	213.9	428.8	0.6	2.4	10.1		< LOD			< LOD	

* Study and pigs described in Figure 1. For each week, n = 4 pigs, which were euthanized for collection of tissues. Plasma and fecal samples were obtained at the time of euthanasia.

† Versus other tissues within a time point ($P < .05$; ANOVA and multiple t tests).

LOD = limit of detection; LL = lower limit; UL = upper limit.

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Figure 3: Liver concentration-time profile of bromadiolone after single oral administration of high dosage, HD (Panel A; 0.5 mg/kg; n = 4 hogs per sacrifice time point) and low dosage, LD (Panel B; 0.05 mg/kg; n = 4 hogs per sacrifice time point) obtained at sacrifice time points of 1, 2, 3, 6, and 9 weeks and 1, 2, 3, 5, and 6 weeks, respectively, in healthy Yorkshire pigs (pigs and study described in Figure 1). LOD = limit of detection.

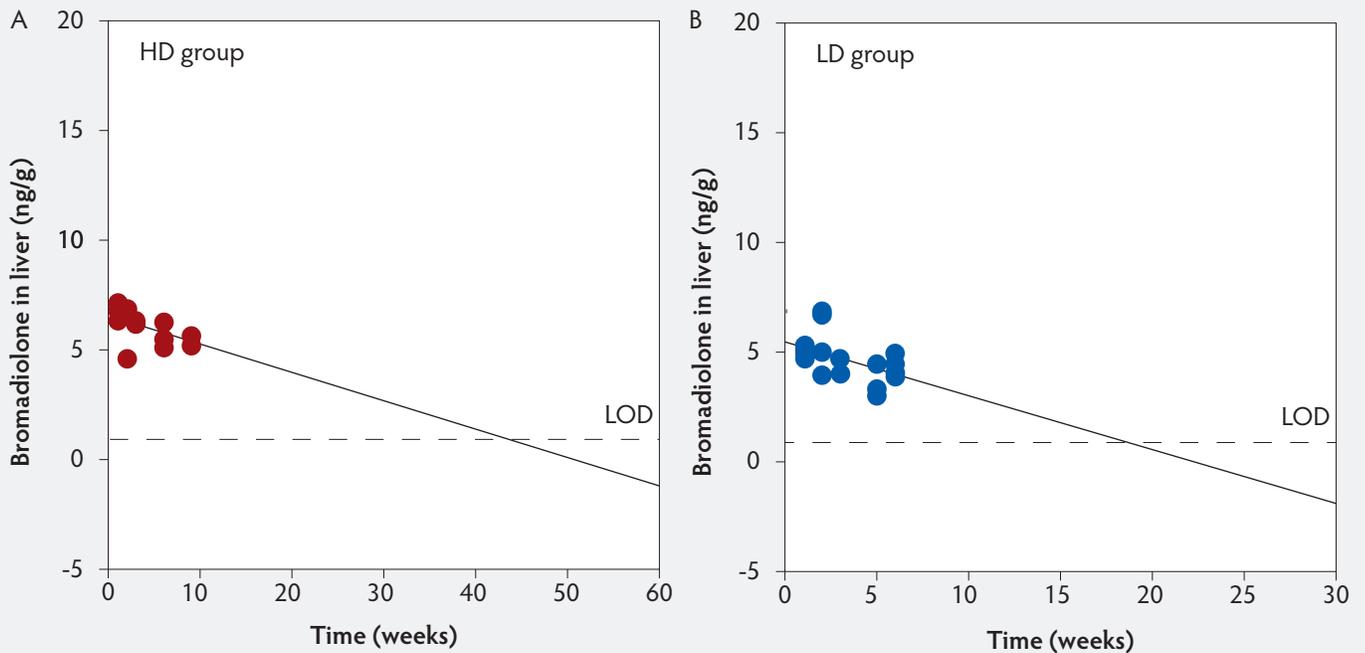
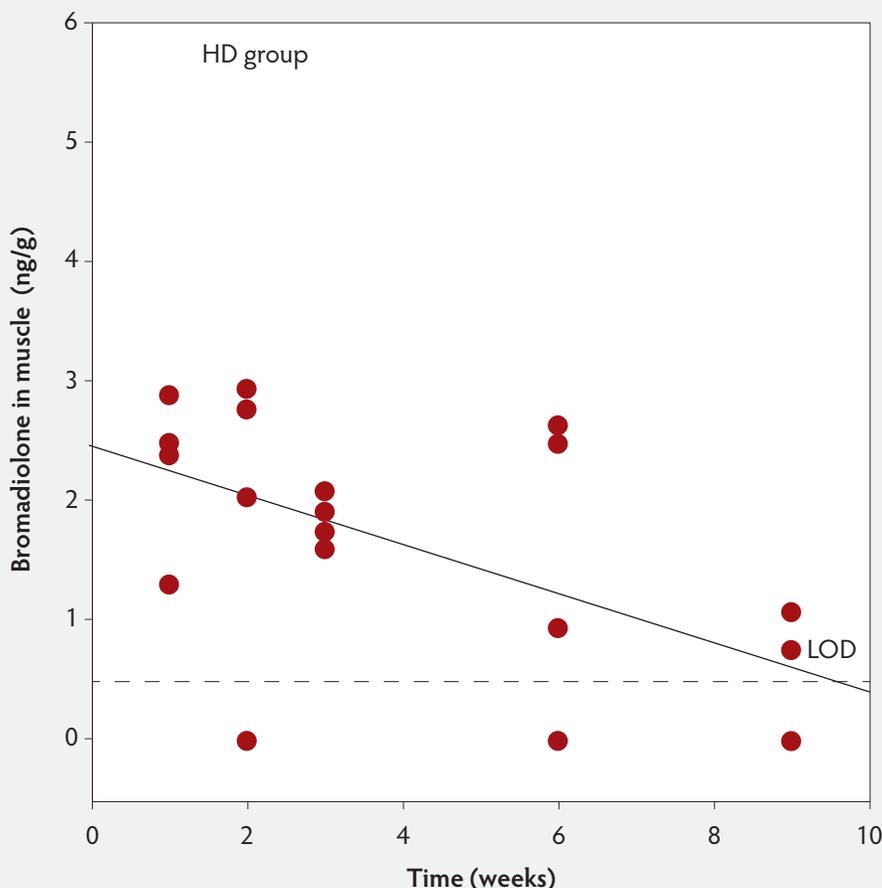


Figure 4: Muscle concentration-time profile of bromadiolone rodenticide after single oral administration of high dosage, HD (0.5 mg/kg; n = 20) obtained at sacrifice time points of 1, 2, 3, 6, and 9 weeks in healthy Yorkshire pigs (described in Figure 1). LOD = limit of detection.



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Evaluation of disinfection protocols to reduce virus transmission via livestock transport vehicles using model trailers and experimental conditions

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Summary

Objective: To determine the efficacy of accelerated hydrogen peroxide disinfectant and combined glutaraldehyde and quaternary ammonium disinfectant after a high-pressure wash against porcine reproductive and respiratory syndrome virus (PRRSV) and transmissible gastroenteritis virus (TGEV) in experimental settings mimicking field conditions commonly experienced on livestock trailers.

Materials and methods: Aluminum model livestock trailers (1:61) were contaminated with PRRSV- and TGEV-spiked feces. Each model trailer underwent a simple washing procedure and an assigned disinfectant application. Four environmental swabs were

collected per trailer at five time points and tested by PRRSV quantitative polymerase chain reaction (qPCR) and TGEV polymerase chain reaction (PCR). Ten-week old pigs were inoculated orally and intramuscularly with supernatant from environmental samples taken from model trailers at two time points after disinfection. Fecal swabs and blood collected at 7 and 14 days post inoculation were tested by PRRSV qPCR and TGEV PCR to determine if the inoculum had contained live infectious virus.

Results: All Positive Control pigs were positive by PRRSV qPCR at 7 and 14 days post inoculation and by PRRSV enzyme-linked immunosorbent assay (ELISA) at day 14. Pigs in the other treatment groups were negative

by PRRSV qPCR and PRRSV ELISA at all time points. Results of TGEV testing were inconclusive because the Positive Control group failed to become infected.

Implication: Under study conditions, a high-pressure wash with cold water plus application of an accelerated hydrogen peroxide or a combined glutaraldehyde and quaternary ammonium disinfectant is effective at inactivating PRRSV.

Keywords: swine, disinfectant, porcine reproductive and respiratory syndrome virus, transport, biosecurity

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Resumen - Evaluación de los protocolos de desinfección para reducir la transmisión de virus vía vehículos de transporte de pecuario utilizando camiones modelo y condiciones experimentales

Objetivo: Determinar la eficacia contra el virus del síndrome reproductivo y respiratorio porcino (PRRSV por sus siglas en inglés) y el virus de la gastroenteritis transmisible (TGEV por sus siglas en inglés) del desinfectante peróxido de hidrógeno acelerado y combinado con el desinfectante glutaraldehído y cuaternarios de amonio después de un lavado con alta presión en condiciones experimentales simulando situaciones de campo comúnmente experimentadas en camiones pecuarios.

Materiales y métodos: Se contaminaron camiones modelo de aluminio, escala 1:61, con heces contaminadas artificialmente con PRRSV y TGEV. Cada camión fue sometido a un proceso de lavado simple y a una aplicación de desinfectante asignado. Se recolectaron cuatro muestras medioambientales por camión en cinco puntos de tiempo y se probaron por medio de la reacción en cadena de la polimerasa cuantitativa (qPCR por sus siglas en inglés) de PRRSV y la reacción en cadena de polimerasa (PCR por sus siglas en inglés) de TGEV. En dos puntos de tiempo se inocularon oralmente e intramuscularmente cerdos de diez semanas de edad con el sobrenadante de muestras medioambien-

tales de los camiones modelo después de la desinfección. Se analizaron muestras fecales y sangre tomadas a los 7 y 14 días después de la inoculación por medio de qPCR PRRSV y PCR TGEV para determinar si el inoculo contenía virus vivo infeccioso.

Resultados: Todos los cerdos Control Positivos resultaron positivos por medio de qPCR PRRSV a los 7 y 14 días después de la inoculación y por medio de la prueba de ensayo de inmunoabsorción ligado a enzimas (ELISA por sus siglas en inglés) de PRRSV en el día 14. Los cerdos en los otros grupos de tratamiento resultaron negativos por medio del qPCR PRRSV y de la ELISA de PRRSV en todos los puntos de tiempo. Los resultados de las pruebas de TGEV no fueron concluyentes porque el Grupo de Control Positivo no logró ser infectado.

Implicación: Bajo las condiciones del estudio, un lavado de alta presión con agua fría más la aplicación de peróxido de hidrógeno acelerado o un desinfectante combinado de glutaraldehído y cuaternarios de amonio es efectivo para desactivar el PRRSV.

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Résumé - Évaluation de protocoles de désinfection visant à réduire la transmission de virus via les remorques à bétail à l'aide de modèles de remorques sous des conditions expérimentales

Objectif: Déterminer l'efficacité d'un désinfectant de type peroxyde d'hydrogène accéléré et d'une combinaison de glutaraldéhyde et d'ammonium quaternaire envers le virus du syndrome reproducteur et respiratoire porcin (VSRRP) et le virus de la gastro-entérite transmissible (VGET) suite à un lavage à haute pression dans des conditions expérimentales imitant des conditions de champs rencontrées avec des remorques à bétail.

Matériels et méthodes: Des modèles en aluminium de remorques à bétail à l'échelle 1:61 furent contaminés avec des fèces inoculées avec du VSRRP et du VGET. Chaque

modèle de remorque fut soumis à une procédure simple de lavage suivi de l'application d'un désinfectant spécifique. Quatre écouvillons environnementaux furent prélevés par remorque à cinq moments différents et testés par réaction d'amplification en chaîne par la polymérase quantitative (qPCR) pour le VSRRP et par réaction d'amplification en chaîne par la polymérase (PCR) pour VGET. Des porcelets âgés de 10 semaines furent inoculés par voies orale et intramusculaire avec du surnageant provenant d'échantillons environnementaux de remorques à bétail pris à deux moments dans le temps après la désinfection. Des écouvillons de fèces et de sang prélevés 7 et 14 jours post inoculation (PI) ont été testés par qPCR pour VSRRP et PCR pour VGET afin de déterminer si l'inoculum avait contenu du virus vivant infectieux.

Résultats: Tous les porcs témoins étaient positifs par qPCR VSRRP à 7 et 14 jours PI et par épreuve immuno-enzymatique (ELISA) au jour 14. Les porcs dans autres groupes de traitement étaient négatifs par qPCR VSRRP et ELISA VSRRP à tous les autres temps d'échantillonnage. Les résultats des épreuves pour VGET étaient non-concluants étant donné que le groupe témoin positif n'a pas développé l'infection.

Implication: Sous les conditions expérimentales de la présente étude, une procédure de lavage à haute pression avec de l'eau froide suivie de l'application de désinfectant de type peroxyde d'hydrogène accéléré ou d'une combinaison de glutaraldéhyde et d'ammonium quaternaire est efficace pour inactiver le VSRRP.

The modern swine industry is structured such that frequent movements of pigs are necessary. Movements occur between production sites and from production sites to terminal markets, resulting in exposure of transport vehicles, personnel, and loading equipment to groups of pigs of varying health status. These factors make transportation events and transport vehicles likely means for transmission of undesirable pathogens to swine.^{1,2} Swine transport vehicles, sorting panels, load-out areas, and loading chutes are generally not disposable items. Therefore, effective sanitation practices are necessary to mitigate the risk of contaminated items serving as fomites for pathogens that can infect swine.

Porcine reproductive and respiratory syndrome virus (PRRSV), in the family *Arteriviridae*, and transmissible gastroenteritis virus (TGEV), in the family *Coronaviridae*, are two viruses for which transportation and transport vehicles serve as transmission fomites. Porcine reproductive and respiratory syndrome (PRRS), caused by the highly infectious PRRSV, is a costly and frustrating challenge to the global swine industry. Ramifications from PRRSV introduction into stable or naive swine herds include late-term abortions, increased preweaning mortality, and other reproductive losses in sows,³ and mortality and slowed growth in growing pigs.⁴ Productivity losses in the United States swine industry are estimated to be \$664 million annually.⁵ In 2006, the virus decimated China's pig population and drove up pork prices by 85%.⁶ PRRS-related clinical signs and lesions in swine

herds occur when a previously PRRSV-naive animal is exposed to PRRSV,⁷ or when a heterologous strain of PRRSV is introduced into a PRRSV-exposed herd.^{8,9}

Transmissible gastroenteritis (TGE) occurs when the highly transmissible TGEV is introduced into a previously TGEV-naive herd. Transmissible gastroenteritis is characterized by vomiting, severe diarrhea, and high mortality in seronegative pigs less than 2 weeks of age.¹⁰ Transmissible gastroenteritis is associated with productivity losses, including slower gain and poorer feed conversion in the wean-to-finish stage of production. Clinical signs are commonly non-differentiable from those of porcine epidemic diarrhea (PED).¹¹

Previous research has demonstrated that PRRSV present in swine transport vehicles can infect PRRSV-naive pigs. A study using model trailers showed "seeder pigs" experimentally infected with PRRSV could cause sufficient contamination of a model transport trailer to infect naive sentinel pigs with PRRSV.¹ High-pressure washing of an experimentally PRRSV-contaminated model transport trailer was not effective at preventing sentinel pigs from being exposed to viable PRRSV.^{2,12} To the knowledge of the authors, research focusing on the risk of transportation vehicles in TGEV spread has not been published.

Sanitation procedures to decrease the pathogen load in the standard equipment utilized in swine transportation have been described,^{1,2,12} but trailers, sorting panels, and chutes are not always thoroughly

cleaned between transport events and thus may contain organic debris as well as bacterial and viral agents. Reasons for failure to properly clean all soiled areas include lack of perceived risk, lack of proper cleaning tools, cost, and time (P Schneider, unpublished data.). These concerns are particularly warranted when discussing sanitation of trucks that haul market hogs because of the frequency of transportation events required.

Currently, a number of disinfectants are available for use in livestock facilities and onboard livestock transport vehicles; however, little research has been done to understand the effectiveness of commonly used disinfectants against swine pathogens in the presence of feces and with short disinfectant contact times. This scenario is similar to the conditions commonly found in field settings for trucks that haul multiple loads of pigs in a single day. Studies evaluating disinfection of fomites, such as boot-washing stations, showed the presence of organic material greatly reduced the bactericidal effect of many disinfectant agents.^{13,14} Generally, bactericidal action improved when fecal material was mechanically removed and when contact time with disinfectant products was increased.

Multiple disinfectants, including quaternary ammoniums, phenolic agents, aldehydes, and peroxygen compounds, have been studied for effectiveness against viral agents. One study compared the use of a combined glutaraldehyde and quaternary ammonium product (Synergize; Preserve International, Reno, Nevada) and a mixed chemical and heavy-metal disinfectant (Stalosan F powder;

Vitfoss, Graasten, Denmark) when used against PRRSV on soiled boots at multiple time points post contamination.¹⁵ Eighty percent of samples collected from boots contaminated with PRRSV and treated with Stalosan F powder in the presence of organic material tested negative for PRRSV by polymerase chain reaction (PCR). When contaminated boots treated with Synergize were similarly tested at the same time points, 42% of samples were PCR-negative. Dee et al¹⁶ demonstrated that sodium hypochlorite (bleach) in boot baths was effective for decontamination of PRRSV on disposable plastic boots. For that study, investigators stepped first into feces, then into an aqueous pool formed by melting snow that had been spiked with PRRSV prior to melting. Investigators then entered a bath of 6% sodium hypochlorite and swabbed the soles of the boots immediately after exiting the bath. PRRS virus was not mixed into the contaminating fecal material, nor was time given to allow fecal material to dry.

Properties of an ideal disinfectant for the swine industry would include being quick acting and maintaining activity in the presence of large amounts of feces, wood shavings, and other organic material commonly present when limited or inferior cleaning practices are implemented. Accel (Virox Technologies Inc, Oakville, Ontario, Canada) is an accelerated hydrogen peroxide product with Environmental Protection Agency label claims as a disinfectant, cleaner, and deodorant with a broad spectrum of action that may have these ideal properties. The product contains low concentrations of certain food-grade anionic and non-ionic surfactants that interact with hydrogen peroxide to enhance microbiocidal activity. The label recommendation for usage against viral agents as a one-step disinfectant is at a concentration of 236 mL in 3.8 L of water with 5 minutes of contact time. Synergize is the most commonly utilized disinfection product in the swine industry and has been shown in previous testing to be effective against PRRSV.¹ Synergize is labeled as a cleaner and broad-spectrum disinfectant with a required contact time of 10 minutes and label concentration of 14.8 mL per 3.8 L. The purpose of this research was to evaluate the efficacy of an accelerated hydrogen peroxide disinfectant (AHP) and a combined glutaraldehyde and quaternary ammonium disinfectant (GQA) against PRRSV and TGEV under field conditions

using model trailers and after a high-pressure wash. The first specific objective was to evaluate the efficacy of the disinfection procedures by testing environmental samples collected from model trailers intentionally contaminated with PRRSV and TGEV at specific time points pre- and post disinfection. The second objective was to determine if the virus remaining in the model trailers at 15 and 60 minutes post disinfection was infective by using the samples collected from the model trailers in a swine bioassay.

Materials and methods

Investigation of both study objectives was conducted at the Iowa State University Veterinary Medical Research Institute. The study protocol was reviewed and approved for use by the Iowa State University Institutional Animal Care and Use Committee.

Objective 1

Experimental design. The experimental design for Objective 1 was an incomplete block design with three replicates per block (Table 1). The experimental unit in each block was the model trailer. Four treatment groups were evaluated. Model trailers in the first treatment group were contaminated with feces spiked with PRRSV and TGEV, then washed using a high-pressure wash with cold water and disinfected with an AHP (AHP group). The same process was repeated for the second treatment group with the exception that a GQA disinfectant was used after the high-pressure wash (GQA group). Model trailers for the third group were contaminated with virus-spiked feces then washed using a high-pressure wash with cold water and sham disinfected with water (Pos Control group). The final group

Table 1: Incomplete block design to evaluate the efficacy of disinfection procedures for swine transport trailers by collecting environmental samples from model trailers pre- and post disinfection and testing for PRRSV and TGEV by PCR (Objective 1 of the study)*

	Trailer 1	Trailer 2	Trailer 3
Block 1	GQA	AHP	Pos Control
Block 2	GQA	Pos Control	AHP
Block 3	AHP	Neg Control	Pos Control
Block 4	Neg Control	Pos Control	GQA
Block 5	ND	AHP	Neg Control
Block 6	GQA	Neg Control	Pos Control
Block 7	Pos Control	GQA	AHP
Block 8	GQA	AHP	Pos Control
Block 9	Pos Control	AHP	GQA
Block 10	GQA	AHP	Pos Control

* Three 1:61 scale model aluminum livestock trailers were enrolled in the study. Four sites in each trailer were contaminated with a total of 50 mL of a feces slurry containing PRRSV and TGEV. Additional fecal material free of PRRSV and TGEV was spread on the trailer floor and wall surfaces to mimic conditions in a trailer that had hauled pigs. After 1 hour in a 4°C cooler, trailers were again contaminated with fecal material, washed with a high-pressure washer, then treated with disinfectant. Samples were collected from each of the four sites contaminated with viruses at five time points for each replicate: immediately after the second contamination process; after the washing process; and 15, 30, and 60 minutes post treatment. Three replicates per block were conducted, with a total of 10 blocks and four treatment groups: accelerated hydrogen peroxide disinfectant (AHP; n = 8); combined glutaraldehyde and quaternary ammonium disinfectant (GQA; n = 8); positive control with a virus-contaminated trailer and sham disinfection with water (Pos Control; n = 9); and negative control with no virus contamination and no disinfection (Neg Control; n = 4).

PRRSV = porcine reproductive and respiratory syndrome virus; TGEV = transmissible gastroenteritis virus; PCR = polymerase chain reaction; ND = not done (a Pos Control was planned for this block but in error a GQA treatment was performed and removed from the study).

utilized feces not spiked with virus to contaminate trailers, which then were washed using a high-pressure wash with cold water and no disinfection (Neg Control group). Detergent was not utilized in the washing process for any treatment group. Tap water obtained from the City of Ames Water Plant (City of Ames, Ames, Iowa) was used for the high-pressure washing procedure and for sham disinfection for the Pos Control group. The RAND function in Microsoft Excel (1999; Microsoft Corporation, Redmond, Washington) was used to randomly assign treatments to trailers within each block. The Pos Control group was randomly assigned to one model trailer in each block, and two of the remaining three treatment groups (AHP, GQA, Neg Control) were randomly assigned to the other two model trailers. In total, 10 blocks were conducted for the study. Eight replicates were performed for the AHP and GQA groups and four replicates for the Neg Control group. Ten replicates of the Pos Control group were planned, but an error resulted in an extra replicate for the GQA group being performed. The extra GQA replicate was removed from the study and only nine Pos Control replicates were performed.

Description of trailer model. Three 1:61 scale model trailers (Figure 1) were utilized for Objective 1. The models were designed by a commercial livestock-transport trailer manufacturer (EBY Inc, Story City, Iowa) and had been used in a prior study¹⁷ to evaluate disinfection protocols for porcine circovirus type 2. The model trailers measured 0.62 m wide × 0.82 m tall × 1.11 m long. Total floor area in the trailers was 0.69 m². The models were designed to represent a standard livestock trailer used to transport commercial swine. Materials used in the design and construction of the model trailers were identical to those used in full-sized livestock transport trailers. The models were constructed using aluminum alloy diamond-plate flooring, welded and riveted to rectangular aluminum tubing cross-members. The side walls and roof were made of flat aluminum sheeting, with the side walls containing punched holes for ventilation. An inner aluminum dividing gate and an aluminum roll up door in the rear of the model trailer were attached with hinges and a latch.

Fecal collection. Approximately 56.8 L of feces from 6-month-old pigs were collected from a commercial swine wean-to-finish barn that had no previous clinical signs of

Figure 1: The 1:61 scale model aluminum trailers utilized to evaluate the efficacy of disinfection procedures for swine transport trailers by collecting environmental samples from model trailers pre- and post disinfection and testing for porcine reproductive and respiratory syndrome virus and transmissible gastroenteritis virus by polymerase chain reaction. The trailers measured 0.62 m wide × 0.82 m tall × 1.11 m long and contained characteristics commonly found in commercial livestock trailers. Trailers were constructed of aluminum materials and included a dividing gate and rear roll-up door. Study details described in Table 1.



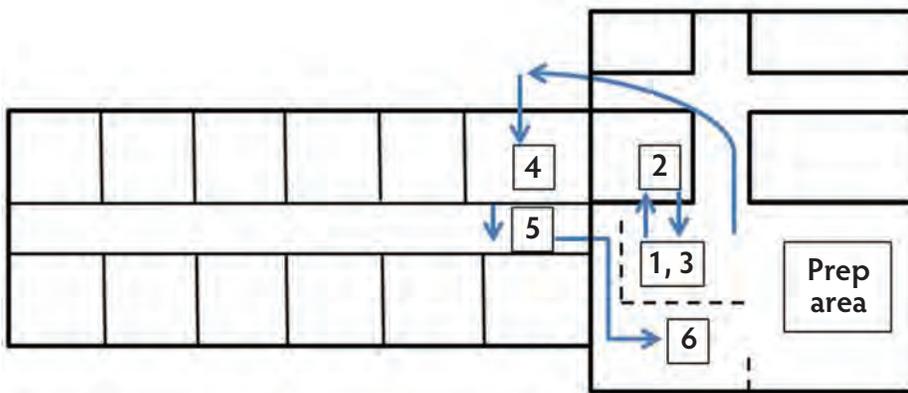
PRRS or TGE and had tested negative for PRRSV on multiple oral-fluid and serum tests since the pigs had been placed. To further confirm that no previous exposure to PRRSV had occurred, oral-fluid samples were collected from the swine herd at the time of fecal collection and tested for PRRSV by enzyme-linked immunosorbent assay (ELISA) and PCR at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL; Ames, Iowa). Both tests were negative. Samples of collected fecal material were tested and confirmed negative for PRRSV and TGEV by PCR at the ISU-VDL.

Procedure. To conduct the study, a coordinated sequence of events was staged in designated areas within the research facility (Figure 2). Trailers were first contaminated by applying feces to the designated areas in Location 1 and then moved into a 4°C cooler, identified as Location 2, for 60 minutes. A second contamination procedure and pre-wash sampling were performed at Location 3. Model trailers were moved outside the research facility to enter the designated wash area (Location 4). Post-wash sampling and disinfection with an AHP, a combined GQC, or sham disinfection was executed in a hallway adjacent to the wash area (Location 5). Three post-disinfection samplings at

15, 30, and 60 minutes post disinfection for the AHP and GQA treatment groups and at 15, 30, and 60 minutes post wash for the Pos Control and Neg Control groups were carried out in Location 6.

Contamination of trailer models with PRRSV and TGEV. Contamination of the model trailers was achieved using a diluted feces mixture with PRRSV SDSU73 strain and TGEV Purdue strain (ATCC VR-763). The PRRSV SDSU73 was initially isolated from a sow herd with a high prevalence of abortions and higher than usual sow mortality in 1996, and has been used previously in experimental challenge studies.^{18,19} Two mL of PRRSV SDSU73, with a final concentration of 3×10^5 per mL on a median tissue culture infective dose (TCID₅₀) assay, and 2 mL of TGEV Purdue strain (ATCC VR-763), with a final concentration of $10^{5.25}$ TCID₅₀ per mL, were mixed with 46 mL of 1:1 feces:deionized water, resulting in a mixture containing a concentration of $10^{4.08}$ TCID₅₀ per mL of PRRSV and $10^{3.85}$ TCID₅₀ per mL of TGEV. PRRS virus and TGEV at the doses used in this study have been shown to be infectious in previous studies.²⁰⁻²² The feces and virus mixture was manually applied with a gloved hand to four designated areas inside the trailer (Figure 3). The designated areas included an approximately 12-cm × 65-cm area on the left side

Figure 2: Diagram representing the movement of the trailer through the Iowa State University Veterinary Medical Research Institute building during Objective 1 of the study described in Table 1. Locations 1 and 3, trailer contamination area; Location 2, 4°C cooler; Location 4, washing room; Location 5, disinfection area; Location 6, post-decontamination sampling area. Arrows show the movement of the trailer. Dotted lines represent barricades preventing movement between the two areas.



wall immediately inside the rear door; an approximately 10-cm × 10-cm area on the floor in the front half of the model trailer along the right side wall; the rear surface of the dividing gate latch; and an approximately 16-cm × 16-cm area on the inside surface of the roll-up rear gate.

The remaining floor surface and various areas on the model trailer walls and rear door were covered by hand with 1 L of undiluted feces that did not contain PRRSV or TGEV, to simulate the amount of organic matter that would commonly be found in a livestock trailer after hauling pigs. Trailers were placed in a walk-in cooler for a period of 60 minutes at 4°C to simulate colder environmental conditions more commonly associated with PRRS and TGE disease breaks. The contamination procedure was repeated once the trailer was removed from the cooler to create a mixture of partially dried and fresh feces as would typically occur under field conditions. To prevent contamination of the study area, investigators changed disposable gloves immediately after the contamination procedure was completed. If the investigator's clothing came in contact with feces within the model trailer, the investigator also changed clothing.

Cleaning procedure. Trailers were moved from the contamination area into a room at 20°C immediately after the initial sampling period. Each trailer underwent a limited wash with a standard high-pressure washer

(Hotsy Corporation, Englewood, Colorado) at a pressure of 1500 pounds per square inch (psi) (105 kg per cm²) using cold water for 90 seconds. Under these circumstances, significant amounts of grossly visible fecal matter consistently remained after the pressure wash, closely representing conditions often found in transport vehicles that haul commercial growing and market pigs after washing (Figure 4). One investigator was designated the washer for the entirety of the study. The designated investigator was blinded to the placement of the virus-contaminated feces and to treatment assignments.

Disinfection procedure. All disinfectants were applied with a Model 25 Compact Airless Foamer (Ogena Solutions, LLC, Stoney Creek, Ontario, Canada). Due to the foamer design, both disinfectants were applied at slightly higher concentrations than those on the label. The AHP disinfectant was utilized at a rate of 266 mL per 3.8 L; the combined GQA disinfectant was applied at a rate of 20.7 mL per 3.8 L. The researcher applying the disinfectant was not blinded to the locations of the virus and feces slurry.

Once the wash was finished, trailers were removed from the wash room to a separate corridor where all disinfectants were applied. A single investigator who was not blinded to the treatment group for each trailer was designated to apply the disinfectant to all trailers throughout the study. The designated investigator changed gloves between trailers

to prevent contamination. Trailers in the Pos Control group were sham disinfected with tap water using the same foamer used to apply the disinfectants. No disinfectant was applied to the Neg Control replicates. Separate disinfectant vessels were used for the AHP, GQA, and Pos Control treatment groups. The foamer was rinsed with cold tap water between applications. To mimic field conditions, disinfectants were not rinsed from the model trailers after application.

Model trailer and working-area decontamination. At the conclusion of each block, model trailers were thoroughly cleaned and disinfected to prevent contamination of future replicates. Each model trailer was individually washed with a high-pressure washer (1500 psi) using 48.9°C water. The interior and exterior of the model trailers were scrubbed with dish soap (Dawn Ultra Antibacterial Dishwashing Liquid; Procter and Gamble, Cincinnati, Ohio), rinsed with cold water, and disinfected with Quatricide PV (Pharmaceutical Research Laboratories Co, Waterbury, Connecticut) at a concentration of 52.5 mL per 3.8 L of water using a liquid concentrate sprayer (ACE Hardware, Oak Brook, Illinois). Trailers were manually dried using a separate new bath towel for each trailer.

Site 4 in Figure 2, the washing room for all model trailers, was washed using a high-pressure washer (1500 psi) and 48.9°C water. All visible organic debris was removed and the room was disinfected with Quatricide PV at 52.5 mL per 3.8 L of water concentration.

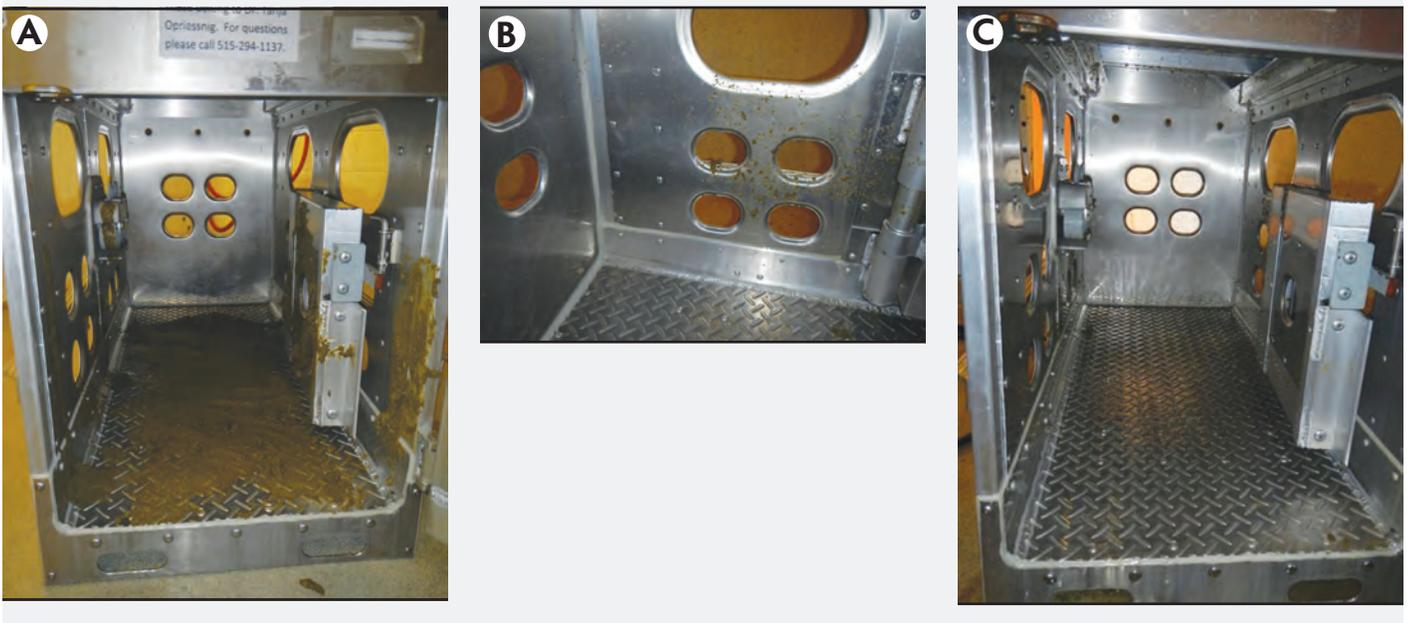
Contamination and sampling areas were also cleaned between blocks. A low-pressure nozzle (49.9 psi; 3.5 kg per cm²) attached to a garden hose was used to remove visible organic debris from the floors and walls. Virkon S (E. I. du Pont de Nemours and Company, Wilmington, Delaware) was applied at a concentration of 5 g per L of water using a liquid concentrate sprayer.

Detection of PRRSV and TGEV. To identify the presence of TGEV or PRRSV, one sample was collected from each of the four designated areas in the model trailers at five different time points using nylon flocked dry swabs (FLOQSwabs; Copan Diagnostics, Inc, Murrieta, California). Time points were immediately after the second feces-virus mixture was applied, immediately after the completion of the washing procedure, and at 15, 30, and 60 minutes after completion of the treatment protocol. After sample

Figure 3: Model trailers were each contaminated with 50 mL of a feces and virus mixture at four internal sites for Objective 1 for the AHP, GQA, and Pos Control treatment groups (groups and study described in Table 1). The four sites of contamination inside the model livestock trailer included the rear roll door (Panel A), on the floor in the front half of the right side wall (Panel B), dividing gate latch (panels B and C), and the rear left side wall (Panel C).



Figure 4: After contamination, model trailers in Objective 1 (study described in Table 1) were washed with a high-pressure washer (1500 pounds per square inch) using cold water. Shown are a contaminated model trailer prior to washing (Panel A) and model trailers after high-pressure washing (panels B and C).



collection, each swab was stored in a 5-mL snap-cap tube (Becton, Dickinson and Company, Franklin Lakes, New Jersey) containing 2 mL of minimum essential medium plus 1× antibiotic, containing 0.05 mg per mL gentamicin, 10 units per mL penicillin, 10 mg per mL streptomycin, and 0.25 mg per mL amphotericin, plus 2% fetal bovine serum. After collection, samples were immediately chilled on ice. All samples were placed in a -80°C freezer within 1 hour of collection and stored until testing. For each replicate, samples from each of the four designated areas in a trailer were pooled for each time point and tested for PRRSV and TGEV by respective PCRs at the ISU-VDL. Briefly, viral

nucleic acid was extracted from the samples using a MagMAX bead-based method (Life Technologies, Carlsbad, California) following the manufacturer's procedures. A commercial PRRSV real-time reverse-transcriptase quantitative PCR (RT-qPCR) assay (Tetracore Inc, Rockville, Maryland) was used for testing PRRSV per manufacturer's instructions. A series of plasmid-derived RNA standards with known concentrations were used to generate a standard curve in each PRRSV PCR plate. A transmissible gastroenteritis virus S-gene-based real-time RT-PCR was set up using a Path-ID Multiplex One-Step RT-PCR Kit (Life Technologies) and primers (forward primer 5'-AACCATAAGTTCCTATATGTCCTT-3', reverse primer

5'-CCAGACCATTGATTTTCAAAC TAATAC-3') and probe (5'-6FAM-CACCATGTAAATAAGCAACAA-3'MGB). The RT-PCR was run on an ABI 7500 Fast instrument (Life Technologies) with the following conditions: one cycle of 48°C for 10 minutes, one cycle of 95°C for 10 minutes, and 40 cycles of 95°C for 15 seconds and 60°C for 45 seconds.

Objective 2

Swine bioassay. To determine if the environmental samples collected for Objective 1 contained infectious PRRSV and TGEV, a swine bioassay was conducted. The environmental samples collected for

Objective 1 were used to prepare the inoculum for the bioassay. For the AHP group in Objective 1, the environmental samples collected from all of the replicates at 15 minutes post disinfection were pooled to form the AHP15 group for the bioassay, and all of the replicates at 60 minutes post disinfection were pooled to form the AHP60 group. For the GQA group in Objective 1, the environmental samples collected from all of the replicates at 15 minutes post disinfection were pooled to form the GQA15 group for the bioassay, and all of the replicates at 60 minutes post disinfection were pooled to form the GQA60 group. For the Pos Control and Neg Control groups in Objective 1, the environmental samples collected from all of the replicates at 15 minutes post wash were pooled to form the Pos15 and Neg15 groups. The resulting pools were allowed to settle, and the overlying supernatant was collected and used to inoculate all pigs by both oral gavage and intramuscular (IM) injection for the bioassay.

Study pigs and housing. Twenty-four 10-week-old pigs were obtained from a known PRRSV-negative and TGEV-negative herd. Pigs were numbered and tagged and then assigned to treatment groups using a random number generator. All pigs in a treatment group were housed together in a single room. Treatment groups were purposefully assigned to rooms to minimize the risk of moving virus from one room to the next.

Pigs were received 3 days prior to inoculation. Blood and fecal samples were collected from all pigs 2 days after arrival (day -1) and submitted to the ISU-VDL to confirm the pigs were negative for PRRSV by qPCR and TGEV by PCR, and for PRRSV and TGEV antibodies by ELISA. A commercial PRRS X3 ELISA Kit (Idexx Laboratories Inc, Westbrook, Maine) was used to test for anti-PRRSV antibody and a commercial TGEV/PRCV-Ab ELISA kit (Boehringer Ingelheim Svanova, Uppsala, Sweden) was used to test for anti-TGEV antibody. For the duration of the study, four pigs were housed in pens that were 1.68 m wide and 3.05 m long, with solid concrete floors. Each pen had a single water nipple. Pigs were fed a non-medicated, complete-feed ration adequate for their nutritional needs and were monitored daily for clinical signs of PRRS or TGE. All clinical observations were recorded.

Inoculation. Study pigs were manually restrained by an investigator for inoculation. For intramuscular injection, 4 mL of supernatant was administered per pig. Oral gavage was performed using 7 mL of

supernatant per pig for all groups except for the GQA15 group; to utilize all available supernatant, 8 mL per pig was used for oral gavage in the GQA15 group. Fewer negative control replicates for Objective 1 resulted in less supernatant available for inoculation of Neg15 study pigs: 2 mL of supernatant was utilized for IM injection and 4 mL for oral gavage in the Neg15 group. Oral gavage was performed using a speculum and 16-cm, 18 Fr rubber urethral catheter (Tyco Healthcare Group, Mansfield, Massachusetts). To prevent potential cross contamination between study groups, the investigators changed coveralls, disposable plastic boots, and nitrile gloves between study rooms. Investigators always visited the Neg15 group first and the Pos15 group last, with the remaining four treatment groups placed in rooms between the aforementioned groups.

Blood and fecal sampling. Blood samples and fecal swabs were collected from all pigs on days 7 and 14 post inoculation. Blood samples were collected by venipuncture of the jugular using a separate Vacutainer (Becton Dickinson) for each pig. Fecal samples were collected using a Copan Liquid Amies Elution Swab Collection and Transport System (Copan Italia, Brescia, Italy). The blood was centrifuged, and serum and feces were stored at -80°C until tested. All samples were submitted to the ISU-VDL to be tested for PRRSV by qPCR and for TGEV by PCR, and antibodies to PRRSV and TGEV by ELISA immediately after collection. Determination of whether a minimum infectious dose of live infectious virus was present in the inoculum was based on the PCR and ELISA results on day 7 and day 14 post inoculation as an indicator of whether the pigs were infected with either PRRSV or TGEV.

Euthanasia and necropsy. Study pigs were necropsied on day 14 post inoculation after collection of a fecal sample and blood. Euthanasia was performed by administering Fatal-Plus (Pentobarbital sodium; Vortech Pharmaceuticals, Ltd, Dearborn, Michigan) at a dose of 1 mL per 4.54 kg of body weight via the jugular vein. Each pig was necropsied and multiple sections of small and large intestine were collected for fixation in 10% neutral-buffered formalin. Necropsies were performed in exactly the same order as investigators visited bioassay groups during the course of the study. Sections were submitted to ISU-VDL for immunohistochemistry following previously described procedures.²³⁻²⁵

Statistical analysis

Statistical analysis of the data was performed using SAS statistical software (SAS version 9.2, SAS Institute, Inc, Cary, North Carolina). For the PRRSV qPCR and TGEV PCR results in Objective 1, pairwise comparisons of the number of positive replicates between treatment groups at each time point were performed using Fisher's exact test. Values for the number of genomic copies of PRRSV were transformed by $\log_{10}(x + 1)$ so that 0 values for samples that were negative by PCR were transformed to 0. A mixed model using PROC GLIMMIX in SAS was utilized for analysis of the transformed results, with trailer and block set as random effects and treatment and time as fixed effects.

A power calculation was performed prior to the beginning of the study to understand the number of pigs needed for each group in the bioassay. It was determined that a sample size of eight was more than sufficient to detect a difference of 80% in the proportion of pigs positive for the bioassay between the positive control (Pos15) group and each of the treatment groups with a 5% level of significance and 80% power.

Results

Objective 1 environmental PCR

No significant difference was identified in the numbers of PRRSV qPCR-positive replicates found for the AHP, GQA, or Pos Control treatment groups at any sampling time (Table 2). The Neg Control group did not have any positive replicates by PRRSV qPCR at any time point. The least squares mean of the number of PRRSV genomic copies per mL found in replicates of AHP was significantly lower than for the GQA and the Pos Control treatments ($P < .001$) at time point 3, and for AHP versus the Pos Control treatment ($P < .001$) at time point 4.

Significantly fewer TGEV PCR-positive replicates were found for AHP than for the Pos Control at time points 2 and 4 ($P < .05$) (Table 3). A significant difference in the number of TGEV-positive replicates was not found between AHP, GQA, or Pos Control at any other time point. No replicates were positive on TGE PCR at any time point tested for the Neg Control group.

The 90-second wash time utilized for the model trailers consistently resulted in some visible fecal matter remaining in the trailers, as can be seen in panels B and C of Figure 4. Visual differences were also noted in the

Table 2: PRRS virus polymerase chain reaction (PCR) results and number of genomic copies of PRRS virus (least squares means [LSM] and standard error [SE]) for environmental samples collected from each model livestock trailer at five designated time points for Objective 1*

Treatment group	PCR results†	No. of genomic copies/mL (LSM)‡	SE
Time 1			
AHP	8/8 ^a	5.0 ^a	0.25
GQA	8/8 ^a	5.1 ^a	0.25
Pos Control	9/9 ^a	5.0 ^a	0.24
Neg Control	0/4 ^b	0 ^b	NA
Time 2			
AHP	8/8 ^a	3.2 ^a	0.25
GQA	8/8 ^a	3.3 ^a	0.25
Pos Control	9/9 ^a	3.1 ^a	0.24
Neg Control	0/4 ^b	0 ^b	NA
Time 3			
AHP	6/8 ^{ab}	1.5 ^a	0.25
GQA	8/8 ^b	3.0 ^b	0.25
Pos Control	9/9 ^b	3.3 ^b	0.24
Neg Control	0/4 ^a	0 ^c	NA
Time 4			
AHP	7/8 ^a	1.8 ^a	0.25
GQA	8/8 ^a	2.1 ^{ab}	0.25
Pos Control	9/9 ^a	2.7 ^b	0.24
Neg Control	0/4 ^b	0 ^c	NA
Time 5			
AHP	7/8 ^a	2.0 ^a	0.25
GQA	6/8 ^{ab}	2.1 ^a	0.25
Pos Control	9/9 ^a	2.1 ^a	0.24
Neg Control	0/4 ^b	0.0 ^b	NA

* Study described in Table 1. Treatment groups evaluated for Objective 1 included disinfection of virus-contaminated trailers with an accelerated hydrogen peroxide disinfectant (AHP), or a combined glutaraldehyde and quaternary ammonium disinfectant (GQA), a positive control group (Pos Control) with a virus-contaminated trailer and sham disinfection with water, and a negative control group (Neg Control) with no virus contamination and no disinfection. Time 1, immediately after the second feces-virus mixture was applied; Time 2, immediately after completion of the washing procedure; and times 3, 4, and 5 at 15, 30, and 60 minutes after disinfection for treatment groups AHP and GQA and after washing for treatment groups Neg Control and Pos Control.

† No. of replicates positive for PRRS virus by PCR/no. of replicates tested. No. of positive replicates were compared among groups using Fisher's exact test.

‡ Differences in least squares means of number of genomic copies were compared among treatment groups using a general linear mixed model, with trailer and block set as random effects and treatment and time as fixed effects. Values for the number of genomic copies of PRRS virus were transformed by $\log_{10}(x+1)$. Least squares means of the number of genomic copies are reported on a \log_{10} scale.

^{a,b,c} Within a column and a time point, values with different superscripts are statistically different ($P < .05$; Fisher's exact test, general linear mixed model).

PRRS = porcine reproductive and respiratory syndrome; NA = not applicable.

appearance of the AHP and GQA treatments post application. Figure 5 shows the AHP disinfectant and the combined GQA disinfectant 60 minutes after the foaming step was completed. A noticeably larger amount of foam was present for the AHP treatment than for the GQA treatment.

Objective 2 swine bioassay

All four pigs in the Pos15 treatment group were positive for PRRSV by PCR at 7 and 14 days post inoculation. ELISA testing confirmed that four of four pigs were positive for PRRSV antibodies in the Pos15 group at day 14 post inoculation. All pigs in the Neg15, AHP15, AHP60, GQA15, and GQA60 groups remained negative for PRRSV by qPCR at 7 and 14 days post inoculation and negative for antibodies to PRRSV by ELISA on day 14 post inoculation. Diarrhea was noted in all testing groups except Neg15. Most pigs began showing loose stools within the first 7 days post inoculation. Signs resolved in all groups by 10 days post inoculation. Pigs in GQA15 had noticeable diarrhea on day -1, but signs resolved by the day of inoculation. No pigs in the study tested positive for TGEV by PCR at either 7 or 14 days post inoculation. Serum ELISA testing showed no seroconversion for TGEV in any bioassay group. Immunohistochemistry staining for the presence of TGEV was negative on all intestinal tissue samples collected on day 14 post inoculation. No other etiologies that may have caused the diarrhea were explored, and the cause of the diarrhea remained unidentified.

Discussion

The conditions under which this study was conducted closely resembled field conditions to better understand how the risk of disease transmission can be mitigated in field settings. The pressure wash resulted in incompletely cleaned model transport trailers that closely represented conditions often found in transport vehicles that haul commercial growing and market pigs after a wash is completed. Washing times from previous transport vehicle research^{1,2,12} were based on the amount of time required to clean transportation vehicles used to haul breeding stock and genetically valuable swine. For this study, the wash time was selected to replicate field conditions under which growing and market pigs are transported. The design characteristics of the model transport vehicle created areas within the

Table 3: Results of PCR testing for TGEV in virus-contaminated model livestock trailers representing field conditions and disinfected using either an accelerated hydrogen peroxide disinfectant (AHP) or a combined glutaraldehyde and quarternary ammonium disinfectant (GQA) or contaminated with virus, washed, and sham disinfected (Pos Control), or washed but neither contaminated with virus nor disinfected (Neg Control)

Treatment group*	PCR results†
Time 1	
AHP	8/8 ^a
GQA	8/8 ^a
Pos Control	9/9 ^a
Neg Control	0/4 ^b
Time 2	
AHP	4/8 ^{ab}
GQA	8/8 ^{bc}
Pos Control	9/9 ^c
Neg Control	0/4 ^a
Time 3	
AHP	1/8 ^a
GQA	4/8 ^a
Pos Control	5/9 ^a
Neg Control	0/4 ^a
Time 4	
AHP	0/8 ^a
GQA	2/8 ^{ab}
Pos Control	5/9 ^b
Neg Control	0/4 ^{ab}
Time 5	
AHP	1/8 ^a
GQA	3/8 ^a
Pos Control	2/9 ^a
Neg Control	0/4 ^a

* Model livestock trailers were contaminated twice with PRRSV- and TGEV-spiked feces. Time point 1: immediately after the second feces-virus mixture was applied; time point 2: immediately after completion of the washing procedure; and time points 3, 4, and 5 at 15, 30, and 60 minutes after disinfection, respectively, for treatment groups AHP and GQA, and after washing for Neg Control and Pos Control treatment groups.

† No. of replicates positive/no. of replicates tested.

^{a,b,c} Significant differences between groups within each time point in the proportion of replicates that were PCR-positive for TGEV ($P < .05$; Fisher's exact test).

PCR = polymerase chain reaction; TGEV = transmissible gastroenteritis virus; PRRSV = porcine reproductive and respiratory syndrome virus.

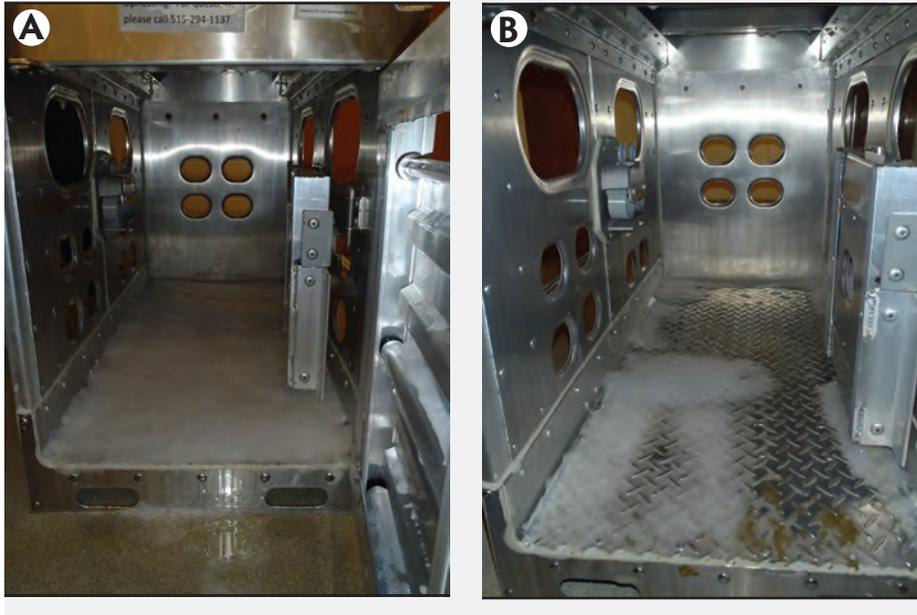
model that were harder to clean and prone to contamination with washed debris. Areas such as gate latches, hinges, and cross beams responsible for supporting an upper deck were represented in the model, creating challenges due to the small size and accessibility to good washing angles.

High-pressure washing with tap water did result in a reduction in the number of PRRSV genomic copies, but did not prevent infection of pigs with PRRSV when paired with a sham disinfection with tap water. Both the AHP disinfectant and a combined GQA disinfectant sufficiently eliminated viable PRRSV and prevented infection after 15 minutes of contact time. The combined GQA disinfectant results are similar to those found in previous research.²

No bioassay study pigs developed TGEV antibodies, were found to be shedding TGEV by PCR testing, or were positive by IHC, including those in the Pos15 group. Therefore, it was not possible to determine the efficacy of the AHP or GQA treatments against TGEV. The number of positive control replicates that were positive for TGEV by PCR in Objective 1 declined over time, with only two of nine replicates remaining positive at 60 minutes after washing. Multiple factors could have contributed to these results. The mixture of virus with feces and subsequent cleaning with high-pressure washing and drying of the model trailer may have resulted in removal and desiccation of a large portion of the TGEV. Transmissible gastroenteritis virus Purdue strain VR-763 has been used to induce clinical lesions of TGE in previous research.²⁶ Additional passages of the virus may have caused adaptation of the virus to the cell line, reduced viral stability outside of cell culture, and reduced infectivity. It is also possible, though less likely, that sampling at 7 days post inoculation may have been too late to detect TGEV by PCR. Previous research showed that by 7 days post inoculation, fewer than 50% of inoculated 4-week-old piglets were shedding TGEV as measured by cell culture.²⁷

The foaming characteristics of the AHP disinfectant evaluated may be beneficial in trailers where longer disinfectant contact times are desired. The relative lack of foam does not infer that the combined GQA disinfectant is no longer active, but continued contact of foam on walls, ceilings, and other surfaces may increase the likelihood of continued disinfectant activity.

Figure 5: Model trailers after disinfectants were applied with a disinfectant foamer after the washing procedure had been completed. Panel A: accelerated hydrogen peroxide disinfectant 60 minutes after disinfection application; Panel B: combined glutaraldehyde and quaternary ammonium disinfectant 60 minutes after application.



Some limitations exist in extrapolating results to field conditions commonly experienced within the swine industry. Due to the characteristics of the disinfectant foamer utilized in the study, both disinfectants were applied at a rate slightly greater than their labeled concentrations. Labeled rates of application for the two products are a 1:16 concentration for the AHP and 1:256 for the GQA. The AHP product was applied at a rate equivalent to one part of AHP to 14.3 parts water and the GQA product was applied at a rate of one part GQA to 183.6 parts water. It may be possible that the increase in applied concentrations above label rate may be equivalent to results achieved with either product when used at the label rate. Future studies may benefit from utilizing a disinfectant foamer with the ability to achieve the labelled concentration for each product.

Investigators used 10-week-old pigs in the bioassay phase of the study for inoculation with environmental supernatant collected from PRRSV- and TGEV-contaminated model trailers. It is possible that younger pigs would have been more sensitive to lower concentrations of infectious TGEV and may have served as better bioassay candidates in this study.¹¹ Subsequent studies may opt to utilize younger pigs for bioassay testing.

Testing the negative control samples was meant to serve as a check on the effectiveness of the decontamination process used

between blocks to decontaminate the model trailers. Investigators felt that poor decontamination processes for model trailers would have resulted in positive samples being found in at least one negative control replicate. Positive results were not found in any negative control replicates in Objective 1. The investigators acknowledge that, in future testing, it may be beneficial to complete testing between blocks for all model trailers rather than relying on the Neg Control treatment group to evaluate the decontamination process. The bioassay remained the primary outcome of interest for this study, and the investigators did not feel that testing between blocks significantly changed the outcome of those results.

To standardize the research, the investigators chose to use a 4°C cooler to simulate cold, winter-like conditions for contaminated model trailers. The investigators also understand that these conditions vary between regions of North America, depending on latitude and regional geographical characteristics, and that different weather conditions may positively or negatively affect viability of infectious PRRSV or TGEV onboard livestock trailers. A cooler or freezer that was able to maintain a colder temperature setting was not available to the investigators. Future studies may identify the impact of subfreezing temperatures on the

effectiveness of the sanitation and disinfectant processes utilized in this study.

Further research may be beneficial to identify whether an AHP disinfectant is effective at eliminating TGEV and other swine pathogens, including porcine epidemic diarrhea virus and *Brachyspira* species, from transport vehicle settings. Research is also needed to investigate alternative methods for transport-vehicle sanitation that could be performed more rapidly, with greater ease, and for less cost. Additionally, the availability of truck-wash facilities is limited in some parts of the country. Devising additional sanitation methods that resolve these challenges is important to increase compliance among truck drivers and to improve transportation biosecurity.

Implications

- Under the conditions of this study, environmental samples from model trailers cleaned with a cold-water, high-pressure wash and disinfected with either an accelerated hydrogen peroxide disinfectant or a combined glutaraldehyde and quaternary ammonium disinfectant do not consistently test negative by qPCR for PRRSV.
- In conditions equivalent to those experienced in this research, a cold-water, high-pressure wash to remove most, but not all, organic matter, paired with application of an accelerated hydrogen peroxide disinfectant or a combined glutaraldehyde and quaternary ammonium disinfectant, with at least 15 minutes of contact time, is able to inactivate PRRSV onboard experimentally contaminated model transport trailers.
- Under the conditions of this study, a cold-water, high-pressure wash alone is not effective at eliminating virulent PRRSV from a model transportation trailer.

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Conflict of interest

Funding, in part, for this study was provided by Virox Technologies Inc, the manufacturer of Accel.

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Experimental inoculation of neonatal piglets with feed naturally contaminated with porcine epidemic diarrhea virus

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Summary

Piglets did not develop diarrhea when fed porcine epidemic diarrhea virus polymerase chain reaction-positive feed that had been retained by manufacturers in early 2013. The virus was detected in feces of positive-control piglets, which exhibited clinical signs and histologic evidence of infection.

Keywords: swine, porcine epidemic diarrhea virus, neonatal pigs, feed transmission

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Resumen - Inoculación experimental de lechones neonatales con alimento contaminado naturalmente con el virus de la diarrea epidémica porcina

Los lechones no desarrollaron diarrea cuando fueron alimentados con alimento positivo a la reacción en cadena de polimerasa al virus de la diarrea epidémica porcina de que había sido guardado por los fabricantes a principios del 2013. El virus fue detectado en heces de lechones control positivos, los cuales exhibieron signos clínicos y evidencia histológica de infección.

Résumé - Inoculation expérimentale de porcelets nouveau-nés avec de la nourriture contaminée naturellement par le virus de la diarrhée épidémique porcine

Des porcelets ne développèrent pas de diarrhée lorsque nourri avec de la nourriture positive par réaction d'amplification en chaîne par la polymérase pour le virus de la diarrhée épidémique porcine qui était retenue par le fabricant depuis le début de l'année 2013. Le virus fut détecté dans les fèces de porcelets témoins positifs, qui démontrèrent des signes cliniques et des évidences histologiques d'infection.

Porcine epidemic diarrhea virus (PEDV), a highly contagious and enteropathogenic alphacoronavirus of pigs, is the causative agent of porcine epidemic diarrhea (PED). Porcine epidemic diarrhea manifests as anorexia, depression, vomiting, and watery diarrhea without blood. High mortality rates are common in piglets less than 10 days of age.¹⁻³ Weaned pigs also develop PED, but mortality rates are lower.⁴ Porcine epidemic diarrhea virus was initially detected in US swine in April 2013 and has caused significant economic losses for the swine industry.

According to a recent US Department of Agriculture (USDA) Swine Enteric Coronavirus Disease Situation Report,⁵ thirty-four states have confirmed cases of PEDV infection in pigs. Deaths in suckling pigs infected with this virus have been substantial

in the United States, which highlights its devastating impact.² It remains unknown how PEDV entered the US swine population. Reports from Canada⁶ and the United States⁷ suggest feedstuffs contaminated with PEDV may be a route of transmission. In early 2013, feed samples retained by manufacturers were submitted to the Iowa State University (ISU) Veterinary Diagnostic Laboratory (VDL) and contained PEDV RNA as detected by polymerase chain reaction (PCR) testing. However, it was unknown if this feed contained live virus and could transmit PEDV to pigs, or if this feed was a source of the initial PEDV outbreak in the United States. The main objective of this research was to determine if the feed samples collected and retained by feed manufacturers shortly after PEDV emerged in the United States and known to contain

PEDV RNA could be a source of transmission to PEDV-naive neonatal piglets.

Materials and methods

Confirmation of PEDV-positive retained feed samples from manufacturers

Three feed samples, one each of complete feed, feed pre-mix, and dried porcine plasma, retained in sealed plastic bags and stored at room temperature (18.3°C to 21.1°C) by feed manufacturers since April and May 2013, were received at the ISU-VDL in July and August 2013. Ten grams of feed were mixed with 40 mL of phosphate buffered saline (PBS; pH 7.2), agitated by vortexing for 15 seconds, and incubated at 4°C overnight. After incubation, the feed suspension was centrifuged at 4200g for 10 minutes, and the supernatant from the 20% suspension was collected. An aliquot of the supernatant was further processed to extract RNA (MagMax Viral RNA Extraction; Life Technologies, Carlsbad, California) for PEDV N-gene real-time reverse transcription (rRT)-PCR as described previously.⁸ The supernatants from all three feed samples were PCR-positive for PEDV at the ISU-VDL and were confirmed PCR-positive by additional testing at the National

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Table 1: PEDV-positive status of feed sample supernatants utilized in a bioassay in neonatal piglets, with PEDV-positive status of manufacturer-provided feeds confirmed by testing at NVSL*

Feed	Feed sample ID	ISU-VDL PEDV N-gene rRT-PCR	Genomic copies/mL†	NVSL PEDV nRT-PCR‡
A	Pre-mix #2	Positive, Ct = 34.2	7.0×10^3	Positive
B	Dried porcine plasma #10	Positive, Ct = 30.0	1.21×10^5	Positive
C	Complete feed #16	Positive, Ct = 33.8	9.18×10^3	Positive
D	Positive-control feed§	Positive, Ct = 25.5	2.55×10^6	ND

* A 20% suspension of each feed sample in phosphate buffered saline was incubated overnight and centrifuged. The supernatant was retained for rRT-PCR testing for PEDV RNA, with Ct values < 40 considered positive.

† Based on standard curves established at the ISU-VDL.

‡ nRT-PCR targets N-gene and S-gene. The PCR product was confirmed as PEDV by sequencing.

§ A PEDV cell-culture isolate (strain USA/NC/2013/35140 P3) from a confirmed field case of PEDV enteritis in neonatal piglets⁹ was used to generate the positive-control feed.

PEDV = porcine epidemic diarrhea virus; NVSL = National Veterinary Services Laboratory; ISU-VDL = Iowa State University Veterinary Diagnostic Laboratory; rRT-PCR = real-time reverse transcription polymerase chain reaction; nRT-PCR = nested reverse transcription polymerase chain reaction; Ct = cycle threshold; ND = not done.

Veterinary Services Laboratory (Table 1). The remaining portions of the feed samples were stored at -80°C at the ISU-VDL until the start of this experiment.

PEDV-positive and PEDV-negative control feed preparation

A complete feed that tested negative by PEDV N-gene rRT-PCR was utilized to generate the positive- and negative-control feeds. For the PEDV N-gene rRT-PCR used, a cycle threshold (Ct) value of < 40 was considered positive. A PEDV cell-culture isolate (strain USA/NC/2013/35140 P3) from a confirmed field case of PEDV enteritis in neonatal piglets⁹ was used to generate the positive-control feed. The virus stock had a titer of 4×10^5 median tissue culture infectious doses (TCID₅₀). Feed negative for PEDV (140 g feed in 560 mL PBS) was spiked with 280 µL of the PEDV virus stock (USA/NC/2013/35140 P3), and this suspension was then incubated at 4°C overnight. After incubation, the suspension was centrifuged at 4200g for 10 minutes, and the supernatant (PED-positive supernatant) was collected and saved separately from the remaining feed pellet (PED-positive feed pellet). On the basis of the dilution factor and the titer of the virus stock utilized, the PED-positive supernatant (20% suspension) theoretically contained PEDV at 160 TCID₅₀ per mL. Both samples were stored at -80°C for approximately 1 month until used for inoculation. Prior to storage, an aliquot of the PED-positive supernatant was processed to extract RNA for testing by

PEDV N-gene rRT-PCR, which confirmed its positive status (Ct = 25.5).

Negative-control feed was generated by the described procedure, except that the PEDV isolate was not added to the PBS prior to its addition to the PEDV-negative feed.

Study design

This experimental protocol was reviewed and approved by the ISU Institutional Animal Care and Use Committee.

Twenty-five domestic cross-bred neonatal piglets, approximately 5 days old, from a herd free of PEDV and transmissible gastroenteritis virus and negative for porcine reproductive and respiratory syndrome virus, were delivered to the ISU Laboratory Animal Resources unit. Upon arrival, piglets received an intramuscular injection of ceftiofur at a dosage of 5 mg per kg (Excede; Zoetis, Kalamazoo, Michigan) per labeled directions. Piglets were confirmed negative for PEDV by PCR testing of fecal swabs, as described, prior to initiation of the study. After a day of acclimation, piglets were randomly assigned numbers by drawing ID tags from a container and were divided into five groups with five piglets per group (Table 2). Piglet groups were housed in separate temperature-controlled rooms. Piglets were offered a mixture composed of approximately two-thirds milk replacer (Esbilac; Pet-AG, Hampshire, Illinois) mixed with one-third plain yogurt three times daily at approximately 8-hour intervals. Water was available ad libitum. Once daily, piglets were

given 10 mL of feed supernatant by oral-gastric gavage utilizing an 8-gauge French catheter, and once daily, 10 g of processed PEDV-positive feed pellets were added to the combined milk replacer-yogurt mixture (Table 2). Treatments were continued for 7 consecutive days (0 to 7 days post inoculation [DPI]). At 7 DPI, all piglets were humanely euthanized by an overdose of pentobarbital, and complete necropsy examinations were performed.

Rectal swabs were collected from all piglets prior to inoculation and once daily for the course of the study. Colonic contents and sections of proximal, middle, and distal small intestine and colon were collected at necropsy from all piglets. Fecal swabs and colonic contents were tested for PEDV by PCR as described. Formalin-fixed sections of small intestine were evaluated by light microscopy for villus atrophy by a veterinary pathologist (AEP) who was blinded to the treatment groups at the time of evaluation. Immunohistochemistry (IHC) slides of ileum were prepared utilizing a monoclonal antibody specific for the spike protein of PEDV,^{2,4} and IHC slides were evaluated by the same veterinary pathologist for positive immunoreactivity to PEDV antigen.

Results

Neither clinical diarrhea nor vomiting was observed in the negative-control piglets (Group 1) or piglets in groups 2, 3, or 4 for the duration of the study. The positive-control piglets (Group 5) developed diarrhea

Table 2: Treatment groups and daily feeding regimes of piglets administered via oral gavage suspensions of manufacturer-provided feeds containing PEDV, as confirmed by PEDV rRT-PCR testing*

Group	Treatment	Feeding schedule
1 n = 5	PEDV-negative control feed	AM: milk-yogurt† Noon: milk-yogurt, top-dress feed pellets PM: milk-yogurt and gavage 10 mL feed suspension
2 n = 5	PEDV-positive pre-mix	AM: milk-yogurt Noon: milk-yogurt, top-dress feed pellets PM: milk-yogurt and gavage 10 mL feed suspension
3 n = 5	PEDV-positive dried plasma	AM: milk-yogurt Noon: milk-yogurt, top-dress feed pellets PM: milk-yogurt and gavage 10 mL feed suspension
4 n = 5	PEDV-positive complete feed	AM: milk-yogurt Noon: milk-yogurt, top dress feed pellets PM: milk-yogurt and gavage 10 mL feed suspension
5 n = 5	PEDV-positive control feed	AM: milk-yogurt Noon: milk-yogurt, top-dress feed pellets PM: milk-yogurt and gavage 10 mL feed suspension

* Preparation of feed suspensions described in Table 1.

† Milk-yogurt mixture composed of approximately two-thirds milk replacer and one-third plain yogurt.

PEDV = porcine epidemic diarrhea virus; rRT-PCR = real-time reverse transcription polymerase chain reaction.

without vomiting at 3 DPI, and diarrhea continued until the study was terminated at 7 DPI. All Group 5 piglets were alive at termination of the study.

At necropsy, the Group 5 piglets were thin and mildly dehydrated, and varying amounts of fecal material were adhered to the perineal region. The small intestines were segmentally thin-walled, and the ceca and spiral colons contained yellow, watery contents. Neither the negative-control piglets nor piglets in groups 2, 3, and 4 had evidence of diarrhea, and their colons contained formed feces.

Pooled rectal swabs from all piglet groups were negative for PEDV by PCR prior to inoculation. Porcine epidemic diarrhea virus was not detected in fecal swabs from the piglets in groups 1, 2, 3, or 4 for the duration of this study. Fecal shedding of PEDV was first detected in a single piglet in Group 5 at 1 DPI, and by 3 DPI, PEDV RNA was detected in fecal swabs from all piglets in this group and continued until necropsy.

Mild to moderate villus atrophy was observed within sections of ileum in the positive-control piglets, and PEDV was detected within the ileum by IHC in all

piglets in this group. Villus atrophy was not observed in piglets in the negative-control group or in piglets in groups 2, 3, or 4, and PEDV was not detected by IHC in any of the piglets in these groups.

Discussion

The objective of this study was to determine if a bioassay could prove that PEDV PCR-positive complete feed and feed components retained by feed manufacturers shortly after PEDV emerged in the United States could cause infection, clinical signs of PED, and PEDV shedding in neonatal piglets. The PEDV PCR-positive feed retained by manufacturers and utilized in this study did not cause evidence of infection or clinical PED in the orally inoculated neonatal piglets, and PEDV shedding was not detected. These results are similar to those reported from a bioassay conducted by Bowman et al¹⁰ utilizing RT-PCR PEDV-positive pelleted commercial feed obtained from an unopened feed bag that was delivered directly to a farrow-to-finish swine production site, coinciding with a PED outbreak at that facility. One reason for the lack of clinical signs and PEDV shedding in the current study and in the study by Bowman et

al¹⁰ may be that the nucleic acid detected by PCR in the feed samples did not represent infectious virus. Inactivation of PEDV in porcine plasma by the spray-drying process has been reported;^{11,12} however, conflicting results about whether spray-dried porcine plasma can transmit infectious PEDV have also been reported by another investigator.⁶ Preliminary work by Schumacher et al¹³ concluded that PEDV PCR-positive feed (Ct = 37) provided the minimum infectious dose of PEDV to cause viral shedding in piglets as tested in a bioassay. The feed samples retained by manufacturers and utilized in this study had lower Ct values, indicating the quantity of PEDV present should have been adequate to cause clinical disease if infectious virus were present. Additionally, extended storage time of these feed samples under varying conditions may have reduced or eliminated the infectivity of the PEDV detected by PCR. Additional research has demonstrated that PEDV can be inactivated by several disinfectants,¹⁴ and preliminary results reported by Cochrane et al¹⁵ indicate enhanced degradation of PEDV within feed under varying conditions of time and chemical treatment. However, the effectiveness of treatments on inactiva-

tion of virus varied by feed matrix, and in vivo infectivity was not tested by bioassay. It is difficult to perform virus isolation for PEDV to prove infectivity regardless of sample type, and in vitro isolation attempts in this study would have remained inconclusive even if cell culture results had been determined negative from the submitted feed samples. Therefore, a neonatal piglet bioassay was necessary to confirm infectivity. Lastly, it is possible that the retained feed samples submitted by manufacturers may not have been representative of the overall concentration of PEDV in the entire batch of feed from which they were obtained, since feed is not a uniform matrix.

This study did confirm by bioassay and supports the findings of previous work by Dee et al.,⁷ that feed spiked with a known viable cell-culture isolate of PEDV can act as a vehicle for virus transmission with development of clinical PED, and can result in PEDV fecal shedding in susceptible piglets. Although mortality is generally high in suckling piglets infected with PEDV,^{2,16} there were no piglet deaths in the positive-control group of the current study, even though piglets were inoculated daily for 7 days, developed clinical signs of diarrhea, and shed virus. The daily gastric gavage of the piglets in the positive-control group may have alleviated the severe dehydration which occurs with clinical PED, resulting in the zero mortality observed in this study. However, the viability of the PEDV detected in the inoculum and administered to the positive-control piglets may have also been poor. Potential causes for poor virus viability in the positive-control feed could include the environment of the feed matrix itself, storage of the positive-control feed inoculum prior to usage, virus passage in cell culture, or a combination of these factors. The relative virulence of the PEDV utilized in the positive-control feed was not assessed and was beyond the scope of this study.

A notable difference between the PEDV PCR-positive feed samples utilized for this bioassay and those utilized for other bioassays^{7,10} is that the feed samples used in the current study came directly from the manufacturers and had never been delivered to a swine production facility. Although the route by which PEDV entered the United States is still unproven, confirmation that feed can support transmission of PEDV suggests that greater scrutiny of feed components and feed by-products may be warranted to prevent further spread of PEDV and entry of other transboundary diseases into the United States. Additionally, confirmation of feed as a vehicle for virus transmission suggests

contaminated feed may have contributed to the initial rapid dissemination of PEDV among US swine farms despite adequate on-farm biosecurity. Further studies are necessary to better understand the effects of length of storage time, environmental conditions, chemical mitigation, and feed matrix composition on the viability and transmission of PEDV in swine.

Implication

Under the conditions of this study, feed contaminated with infectious PEDV can serve as a vehicle for PEDV transmission.

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Conflict of interest

None reported.

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Elimination of *Mycoplasma hyopneumoniae* from breed-to-wean farms: A review of current protocols with emphasis on herd closure and medication

Sam Holst, DVM; Paul Yeske, DVM, MS; Maria Pieters, DVM, PhD

Summary

Mycoplasma hyopneumoniae is one of the most prevalent and economically significant respiratory pathogens in the swine industry. Economic losses related to *M hyopneumoniae* are associated with decreased feed efficiency, reduced average daily gain, and increased medication costs. In an effort to mitigate these economic losses, swine veterinarians and producers utilize several control measures, including optimizing management and housing, vaccination, and strategic antimicrobial medication. When control measures are

insufficient, or eradication of *M hyopneumoniae* is preferred, swine veterinarians and producers may elect to eliminate *M hyopneumoniae* from affected sow farms. Herd closure and medication protocols have become widely used in North America to eliminate *M hyopneumoniae* from breed-to-wean farms. As vital principles for success, these protocols rely on no new animal introductions for at least 8 months, vaccination of the entire breeding herd, and medication of the breeding herd and piglets. Commonly, the breeding herd is medicated with oral antimicrobials

delivered via the drinking water or feed, whereas the piglets are treated with injectable antimicrobials. In this commentary, we will review current *M hyopneumoniae* elimination protocols with an emphasis on the herd closure and medication protocols.

Keywords: swine, *Mycoplasma hyopneumoniae*, elimination, herd closure, medication

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Resumen - Eliminación del *Mycoplasma hyopneumoniae* de las granjas de cría a destete: Una revisión de los protocolos actuales con énfasis en cierre de hato y medicación

El *Mycoplasma hyopneumoniae* es uno de los patógenos respiratorios más prevalentes y económicamente significativos de la industria porcina. Las pérdidas económicas relacionadas con el *M hyopneumoniae* están asociadas con la disminución de la eficiencia alimenticia, reducción en la ganancia diaria promedio, y el incremento en los costos de medicamento. En un esfuerzo por mitigar estas pérdidas económicas, los veterinarios y productores porcinos utilizan varias medidas de control, incluyendo la optimización del manejo y alojamiento, vacunación, y medicación antimicrobiana estratégica. Cuando las medidas de control son insuficientes, o se prefiere la erradicación del *M hyopneumoniae*, los productores y vet-

erinarios porcinos pueden elegir eliminar el *M hyopneumoniae* de las granjas de hembras afectadas. El cierre de granja y los protocolos de medicación se han vuelto ampliamente utilizados en Norte América para eliminar el *M hyopneumoniae* de las granjas de cría a destete. Los principios importantes para el éxito de estos protocolos dependen de, no introducir nuevos animales por lo menos por 8 meses, vacunación del hato de cría completo, y medicación de los hatos de cría y lechones. Comúnmente, el hato de cría es medicado con antimicrobianos orales administrados vía agua de bebida o alimento, mientras que los lechones son tratados con antimicrobianos inyectables. En este comentario, revisaremos los protocolos actuales de eliminación del *M hyopneumoniae* actuales con énfasis en el cierre de granja y los protocolos de medicación.

Résumé - Élimination de *Mycoplasma hyopneumoniae* d'élevages de porcs de type naisseur-sevrage: revue des protocoles actuels avec une emphase sur la fermeture des troupeaux et la médication

Mycoplasma hyopneumoniae est un des agents pathogènes les plus fréquents et économiquement importants dans l'industrie porcine. Les pertes économiques liées à *M hyopneumoniae* sont associées à une réduction de l'efficacité alimentaire, une diminution du gain moyen quotidien, et une augmentation des coûts de médication. Dans un effort de réduire ces pertes économiques, les vétérinaires porcins et les producteurs utilisent plusieurs mesures de contrôle, incluant l'optimisation de la gestion et de l'hébergement, la vaccination, et l'administration stratégique d'antimicrobiens. Lorsque les mesures de contrôle sont insuffisantes, ou que l'éradication de *M hyopneumoniae* est préférable, les vétérinaires et les producteurs peuvent décider d'éliminer *M hyopneumoniae* des troupeaux de truies affectées. La fermeture des troupeaux et des protocoles de médication sont couramment utilisés en Amérique du Nord pour éliminer *M hyopneumoniae* des fermes de type naisseur-sevrage. Comme principes essentiels à la réussite, ces protocoles se fient au fait qu'il n'y a aucune introduction de nouveaux animaux pour au moins 8 mois, que le troupeau entier des reproducteurs soit vacciné, et que

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les animaux reproducteurs et les porcelets soient médicamenteux. De manière usuelle, le troupeau de reproducteurs est médicamenté par administration d'antimicrobiens oraux administrés via l'eau de boisson ou les aliments, alors que les porcelets sont traités par injections d'antimicrobiens. Dans le présent commentaire, nous ferons la revue des protocoles courants d'élimination de *M hyopneumoniae* avec une emphase sur la fermeture du troupeau et les protocoles de médication.

M*ycoplasma hyopneumoniae* is one of the most prevalent and economically significant respiratory pathogens in the swine industry.¹ *Mycoplasma hyopneumoniae* is the etiologic agent of enzootic pneumonia, a chronic respiratory disease in swine characterized by a chronic, non-productive cough.^{2,3} Pathogenicity of *M hyopneumoniae* stems from the organism's ability to adhere to and damage the ciliary epithelium associated with the trachea, bronchi, and bronchioles of the respiratory tract.⁴ Economic losses related to *M hyopneumoniae* are associated with decreased feed efficiency, reduced average daily gain, and increased medication costs.¹ In addition, *M hyopneumoniae* is considered to play a key role in porcine respiratory disease complex⁵ where it interacts with other respiratory pathogens.

Due to *M hyopneumoniae*'s ability to inflict economic losses independently, and its capability to interact with and increase the severity of other respiratory microorganisms, swine veterinarians and producers have attempted to mitigate losses through several control methods. These methods include, but are not limited to all-in, all-out (AIAO) production,^{1,6} sow and pig vaccination,^{1,7-17} gilt acclimatization,¹⁸ medicated and non-medicated early weaning,^{1,19-22} segregated parity production,²³ and strategic antimicrobial medication.²⁴⁻³⁵ While these methods can decrease infection pressure and improve pig health, they do not assure the absence of *M hyopneumoniae* within a herd or flow of pigs.³⁶

Mycoplasma hyopneumoniae elimination protocols

Mycoplasma hyopneumoniae elimination protocols can be implemented when control measures have been unsuccessful or if exclusion of the pathogen from a herd is desired. Various protocols for *M hyopneumoniae* elimination have been described, including depopulation and repopulation, partial depopulation, herd closure and medication, and whole-herd medication without herd

closure. The herd closure and medication and whole-herd medication protocols will be emphasized in this commentary, as they are widely used in the United States.

Depopulation and repopulation is the most direct approach for *M hyopneumoniae* eradication, as it involves removal of the entire breeding herd and restocking with *M hyopneumoniae*-negative replacements.³⁷ Advantages of depopulation and repopulation include the ability to eliminate more than one disease at once and the opportunity to improve genetics.³⁷ However, there is a complete loss of production from the time the breeding herd is liquidated until replacement females begin farrowing. Furthermore, total depopulation of the breeding herd may be undesirable on farms with animals that have a high genetic potential (ie, genetic nucleus or multiplier farms).

The partial depopulation (Swiss) method gained recognition in the 1990s when Switzerland implemented a national program to eliminate *M hyopneumoniae* and *Actinobacillus pleuropneumoniae*.³⁸ The following items are the framework for the Swiss method.³⁸⁻⁴¹ First, remove all animals less than 10 months of age from the herd; second, cease farrowing for at least 2 weeks; and third, medicate remaining animals with an antimicrobial labeled for *M hyopneumoniae* during the non-farrowing period. Elimination projects in Norway and Denmark were also successful, with slight modifications to the Swiss partial depopulation protocol.^{42,43}

Herd closure and medication protocols for *M hyopneumoniae* elimination are adaptations of the Swiss method. Modifications to the Swiss method allow for farrowing to continue during the medication period in order to minimize production losses. The herd closure and medication approach utilizes these key principles:³⁷ first, exposure of all females, including replacement gilts, to *M hyopneumoniae*; second, closure of the herd for at least 8 months; third, entire herd vaccination with a *M hyopneumoniae* bacterin; and fourth, medication of the whole sow herd and piglets prior to introduction of *M hyopneumoniae*-negative replacement gilts. It is critical that all replacement gilts are exposed to *M hyopneumoniae* and colonized prior to beginning herd closure. Herd closure of at least 8 months is based on published research indicating that pigs can shed *M hyopneumoniae* up to 200 days post infection.⁴⁴ Blanket vaccination of the whole sow herd with an *M hyopneumoniae* bacterin is usually performed to increase herd immunity. Finally, all sows and piglets on-site are

medicated with an approved antimicrobial effective against *M hyopneumoniae*. Specific antimicrobial regimes commonly used in *M hyopneumoniae* elimination programs are discussed later in this commentary.

The whole-herd medication without herd closure protocol is the most recent *M hyopneumoniae* elimination protocol to be described.^{45,46} This protocol involves medicating the entire herd (gilts, sows, boars, and piglets) with a long-acting antimicrobial (typically administered via injection) with activity against *M hyopneumoniae*. The whole herd is treated via antimicrobial injection on day 1 of the elimination project, followed by another injection 2 weeks later. Additionally, piglets born 4 weeks after the initial whole-herd injection are treated at birth and at 14 days of age. Replacement gilt flow is maintained per normal farm protocol, and the farm remains open to new animal introductions, with the understanding that new animal introductions are from *M hyopneumoniae*-negative sources only. The advantage of whole-herd medication without herd closure, when successful, is that the herd has a faster return to *M hyopneumoniae*-negative status. However, this protocol has been less effective at eliminating *M hyopneumoniae* than the herd closure and medication protocol.⁴⁶ A comparative summary of the key aspects of the four mentioned elimination protocols is presented in Table 1.

Specifics of herd closure and medication

Herd closure

Herd closure and rollover was first described as a disease elimination tactic by Torremorell et al⁴⁷ for eliminating porcine reproductive and respiratory syndrome virus (PRRSV) from sow herds. Herd closure consists of ceasing introduction of replacement females into the breeding herd for an extended period of time (typically 6 to 9 months, depending on gilt supply and capacity). The rationale for stopping new introductions into the herd is to decrease the number of susceptible animals for the pathogen to replicate in,^{48,49} eventually reducing the number of susceptible animals to zero. The herd remains closed to new animal additions until sufficient time has passed for the pathogen to have infected all animals on the farm, and infected animals have had time to mount an immune response and clear the pathogen, and are no longer infectious. Following successful eradications of PRRSV using the herd-closure technique, veterinarians in the United States have adapted it for utilization in *M hyopneumoniae* elimination projects.³⁷

Table 1: Summary of the key aspects of *Mycoplasma hyopneumoniae* elimination protocols most commonly used in the United States

Elimination protocol	Production time loss	Negative replacement gilts required before, during, or after elimination	Herd vaccination	Sow medication		Piglet medication	Animal introductions	Potential for other pathogens eliminated
				Feed or water	Injected			
Depopulation/ repopulation	Yes	Yes	No	No	No	No	NA	Yes
Partial depopulation*	Yes	No	No	Yes	No	No	NA	No
Herd closure and medication	Yes	Yes	Yes	Yes	Yes	Yes	Stop during elimination	Yes
Whole-herd medication†	No	Yes	No	No	Yes	Yes	Continue as usual	Yes

* Swiss method.

† No herd closure.

NA = not applicable.

In order to maintain a practical replacement rate and continue utilizing gilts in weekly or batch breeding groups, a 6- to 9-month supply (surplus) of gilts (depending on the desired length of the closure period) are stocked into an on-site isolation or gilt developer unit (GDU). The recommended length of herd closure for *M hyopneumoniae* elimination is at least 8 months; therefore, an 8-month supply of gilts would be required to avoid gaps in production. Additionally, it is recommended that the entire adult population be over 10 months of age when negative replacements are introduced³⁷ to increase the likelihood that no animals will be infectious, assuming that animals have been exposed to *M hyopneumoniae* at an earlier age. Thus, to satisfy the 10-month age recommendation, gilts should be a minimum of 2 months of age when stocked into isolation or the GDU. Furthermore, gilts of various ages and weights should be included to avoid a surplus of gilts that are too old or too big at the end of the *M hyopneumoniae* elimination project.³⁷

A potential obstacle is that not all sow farms have on-site isolation or GDU facilities at their disposal. If this is the case, an off-site breeding project could be considered as an alternative plan. An off-site breeding project allows gilts to be bred at a separate location and added back to the herd at the time of farrowing.⁵⁰ The gilts should be bred in weekly groups, and breeding should be

timed so that the first group of gilts is due to farrow shortly after the herd closure period is completed. This allows breeding and farrowing targets to be met and for pig flow to be maintained as consistently as possible once the herd closure is lifted.⁵⁰

Vaccination and acclimation

Commercial *M hyopneumoniae* bacterins are widely used in swine production worldwide.⁹ Pigs can become colonized with *M hyopneumoniae* in the first weeks after birth;⁵¹⁻⁵³ therefore, vaccination of piglets is the most common vaccination strategy utilized.¹ Advantages of vaccinating growing pigs include increased average daily gain (ADG), improved feed efficiency, and potentially decreased mortality rate.¹ While vaccination does have several advantages regarding increased production performance, it does not prevent *M hyopneumoniae* colonization.^{9,13,16,54} Other studies have shown that *M hyopneumoniae* vaccination is associated with a reduction in the number of organisms in the respiratory tract,¹³ as well as a decreased infection level within a herd.¹⁵

In addition to growing-pig *M hyopneumoniae* vaccination strategies, vaccination of sows has been utilized in an attempt to reduce vertical spread of the pathogen and to confer immunity to piglets via lactogenic transmission of maternal antibodies.^{7,14,55}

Vaccination of sows during *M hyopneumoniae* elimination projects is aimed at bolstering herd immunity³⁷ and has been implemented on a quarterly basis, prior to whole-herd antimicrobial medication, or on a pre-farrow schedule. Yeske³⁷ described vaccinating the surplus gilts at 1 and 3 weeks post entry to the on-site isolation or GDU facility and vaccination of the entire breeding herd (including gilts) on a quarterly schedule after herd closure is initiated. Additionally, Yeske³⁷ recommends exposing the surplus gilts to the most recently infected group of gilts as soon as possible to facilitate natural infection (if gilt surplus is negative to *M hyopneumoniae* prior to entry to the GDU).

Schneider⁵⁶ documented vaccinating the breeding herd at 5 and 2 weeks prior to beginning the antimicrobial medication protocol. Moreover, Schneider described vaccinating sows 2 weeks prior to farrowing until testing for the presence of *M hyopneumoniae* post eradication was completed. Schneider recommends continuing this pre-farrow vaccine protocol indefinitely if the farm is at a medium to high risk of re-infection. Snider⁵⁷ described whole-breeding-herd vaccination after the acute outbreak of *M hyopneumoniae* was diagnosed and again prior to beginning antimicrobial medication. Lorenzen⁵⁸ documented vaccinating the entire breeding herd 1 week prior to antimicrobial medication, and an additional dose of vaccine 2 weeks

later. A pre-farrow vaccination schedule was described by Alfonso et al,⁵⁹ where sows were vaccinated at approximately 15 days prior to farrowing and the surplus gilts were vaccinated twice, at 55 and 220 days of age.

It is important to note that several different vaccination protocols have been described, with varying numbers and frequencies of vaccinations; however, to the knowledge of the authors, no published studies indicate an advantage of utilizing one protocol versus another.

In addition to sow and gilt vaccination during *M hyopneumoniae* elimination projects, it is important to continue routine farm-specific gilt acclimation protocols (other vaccinations, feed-back, estrus induction and synchronization, anthelmintic administration, disease surveillance, etc) to avoid disruptions in production or challenges with other diseases.

Medication

Virtually all *Mycoplasma* species are resistant to betalactam antimicrobials (penicillin, ampicillin, amoxicillin, and cephalosporins). *Mycoplasmas* lack a cell wall, which is the target of beta-lactam antimicrobials (Table 2).⁶⁰ Classes of antimicrobials with potential activity against *M hyopneumoniae* include macrolides, lincosamides, tetracyclines, pleuromutilins, fluoroquinolones, amphenicols, and aminoglycosides (Tables 2 and 3). Many specific antimicrobials from within these classes have been utilized in *M hyopneumoniae* elimination projects.

Documented medication programs

Kohne et al⁶⁸ fed tilmicosin-medicated feed (15 mg per kg body weight [BW]) to all sows, boars, and gilts on-farm for a period of 4 weeks. Additionally, any breeding-stock animal that was sick or off feed was injected with one dose of tulathromycin (2.5 mg per kg BW). Piglets were injected with enrofloxacin (2.5 mg per kg BW) at birth and with tiamulin (2 mg per kg BW) every 2 days after 3 days of age (injectable tiamulin is not labeled for use in the United States). All piglets were weaned off-site by 21 days of age. To determine the success of the elimination project, Kohne et al⁶⁸ collected tonsil swabs from animals that were born on-farm at 10, 14, 23, and 27 weeks following completion of the medication plan. All samples were negative on polymerase chain reaction (PCR) for *M hyopneumoniae*.

Snider⁵⁷ described a medication plan using tulathromycin and lincomycin. The entire breeding herd was fed lincomycin (220 mg per kg medicated feed) for 3 weeks. After the lincomycin treatment, all breeding stock and piglets received a dose of tulathromycin (2.5 mg per kg intramuscularly [IM]) followed by an additional dose in 14 days. After the second dose of tulathromycin had been administered, lincomycin-medicated feed was again fed to the breeding herd for an additional 5 weeks. Piglets continued to receive tulathromycin (2.5 mg per kg BW, IM) at birth and at 12 to 14 days of age for a period of 5 months. Piglets were weaned at 18 to 21 days of age, with no piglets on-farm older than 24 days of age. Snider⁵⁷ documented that piglets from the treated farm were comingled with *M hyopneumoniae*-naïve piglets from another sow herd and that *M hyopneumoniae* was not

detected clinically or by ancillary diagnostic modalities for 8 months.

Geiger et al⁶⁹ also employed a tulathromycin and lincomycin treatment plan. Lincomycin (100 g per tonne) was fed to the whole breeding herd for 4 weeks. Piglets were injected with tulathromycin (2 mg per kg BW) at birth, beginning 2 weeks after initiation of the medicated-feed program, and during a 2-week period were weaned off-site by 10 days of age. Treatment of piglets with tulathromycin at birth was continued for 5 weeks. Monthly serological testing (Idexx and Dako ELISA reported⁶⁹) of *M hyopneumoniae*-negative replacement gilts on the sow farm, commercial pigs in an on-site nursery, and commercial pigs in an off-site finisher was conducted to determine elimination success. Replacement gilts and on-site pigs were negative for 22 months and the off-site finisher was negative for 15 months (last time sampled). Additionally, quarterly slaughter checks and routine necropsies showed no signs of *M hyopneumoniae* infection.

Another tulathromycin and lincomycin treatment protocol was carried out by Geiger and Groth,⁷⁰ where the breeding stock was treated with lincomycin delivered via the water system and piglets were injected with tulathromycin (2 mg per kg BW) at birth and again 11 days later; both treatment modalities were continued for 4 weeks. Piglet weaning age was left unchanged (average, 20.6 days). Monthly serological testing (Idexx and Dako ELISA reported⁷⁰) of replacement gilts began 1 month after the first *M hyopneumoniae*-negative replacement gilts were introduced. Random serological testing, routine necropsies with diagnostic

Table 2: Common classes of antibiotics utilized in the US swine industry

Antibiotic class	Mechanism of action	Bacterial target	Effect	Potential activity against <i>M hyopneumoniae</i>
Beta-lactams ⁶⁰	Cell wall synthesis inhibition	Transpeptidase	Bactericidal	No
Macrolides ⁶¹	Protein synthesis inhibition	50s ribosomal subunit	Bacteriostatic	Yes
Lincosamides ⁶¹	Protein synthesis inhibition	50s ribosomal subunit	Bacteriostatic	Yes
Tetracyclines ⁶²	Protein synthesis inhibition	30s ribosomal subunit	Bacteriostatic	Yes
Pleuromutilins ⁶³	Protein synthesis inhibition	50s ribosomal subunit	Bacteriostatic	Yes
Fluoroquinolones ⁶⁴	DNA synthesis inhibition	DNA gyrase	Bactericidal	Yes
Amphenicols ⁶⁴	Protein synthesis inhibition	50s ribosomal subunit	Bacteriostatic	Yes
Aminoglycosides ^{65,66}	Protein synthesis inhibition	30s ribosomal subunit	Bactericidal	Yes*
Sulfonamides ⁶⁷	Folic acid synthesis inhibition	Dihydropteroate synthase	Bacteriostatic	No

* Aminoglycosides have activity against *Mycoplasma* species but are poorly absorbed when administered orally. Withdrawal times are excessively long when delivered parenterally, rendering their use against *M hyopneumoniae* impractical.

Table 3: Antibiotics with potential activity against *Mycoplasma hyopneumoniae*

Antibiotic class	Antibiotic	Route(s)	Dose (inclusion rate)	Label indication for <i>M hyopneumoniae</i>
Macrolides	Tylosin	Parenteral	8.8 mg/kg BW	None
		Feed	(40 or 100 mg/kg)*	None
		Water	(66 mg/L)	None
	Tilmicosin	Feed	(181.8 or 363.6 mg/kg)*	None
		Water	(200 mg/L)	None
	Tulathromycin	Parenteral	2.5 mg/kg BW	Treatment or control
Tylvalosin	Water	(50 mg/L)	None	
Lincosamides	Lincomycin	Parenteral	11 mg/kg BW	Treatment
		Feed	(200 mg/kg)	Treatment
		Water	8.4 mg/kg BW (250 mg/L)	None
Tetracyclines	Oxytetracycline	Parenteral	6.6 - 11 mg/kg BW	None
		Feed	(22 mg/kg)	None
		Water	(22 mg/L)	None
	Chlortetracycline	Feed	(22 mg/kg)	None
		Water	(22 mg/L)	None
Pleuromutilins	Tiamulin	Feed	(200 mg/kg)	None
		Water	(23.1 mg/L)	None
Fluoroquinolones	Enrofloxacin	Parenteral	7.5 mg/kg BW	Treatment or control
Amphenicols	Florfenicol	Water	(100 mg/L)	None

* Preventive or therapeutic dosage.
BW = body weight.

tissue submissions (*M hyopneumoniae* culture, PCR, and histopathology), and slaughter checks were conducted on the downstream flow of pigs. No evidence of *M hyopneumoniae* infection was found in the sow herd or downstream flow for 12 and 6 months, respectively (last times sampled).

Tylvalosin usage in *M hyopneumoniae* eliminations has been documented in Europe utilizing either the water or in-feed formulation to treat the breeding herd for 21 to 28 days.^{71,72} Additionally, some tylvalosin elimination projects have included tulathromycin injections to sows exhibiting reduced feed intake and to piglets (beginning at birth and continuing at 7- to 10-day intervals until weaning).

Schneider⁵⁶ supplemented a chlortetracycline (CTC) and tiamulin feed-medication program with injectable oxytetracycline (OTC) and tylosin. The breeding herd diet was medicated with CTC (440 mg per kg) and tiamulin (100 mg per kg) for a period of 3 weeks. Sows that were off feed were

injected daily with OTC (17.5 mg per kg BW) and tylosin (17.5 mg per kg BW) for 5 days, followed by 5 days of no injections, and then 5 additional days of antimicrobial injections. Piglets were weaned off-site by 12 days of age and did not receive antimicrobials while on farm. Schneider⁵⁶ documented 14 sow herds where *M hyopneumoniae* eliminations were attempted between 1995 and 2001, utilizing a medication plan similar to the one described. The amount of time that those farms experienced freedom from *M hyopneumoniae* following elimination ranged from 14 months to 9 years.⁵⁶

Geiger and Ragone⁷³ utilized a medication protocol using two formulations of OTC. Lactation and gestation rations were medicated with OTC (500 mg per kg of feed) for 4 weeks. In addition, for a period of 3 weeks, piglets received injections of OTC (200 mg per piglet) at 3 and 7 days of age and were weaned by 10 days of age. Following the addition of *M hyopneumoniae*-negative

replacement gilts (sentinels), 30 random sentinels were serologically tested (Dako ELISA reported⁷³) on a monthly basis. Serologic tests were positive and mild clinical signs of *M hyopneumoniae* were detected approximately 4 months following the completion of the elimination protocol.

Alfonso et al⁵⁹ utilized a medication protocol with tiamulin and tilmicosin. The gestation and lactation rations were medicated with tilmicosin (16 mg per kg of feed) for 2 weeks, then tiamulin (7 mg per kg BW) for an additional 2 weeks. Furthermore, while the breeding herd was fed the tiamulin-medicated feed, piglets were injected with tiamulin (6 to 8 mg per kg BW) at 1, 5, and 13 days of age (injectable tiamulin is not labeled for use in swine in the United States). Weaning age was not altered and remained 16 days of age. Ten *M hyopneumoniae*-negative sentinel gilts were added to the sow farm 1 week after the medication protocol had been completed.

These 10 sentinels remained serologically negative (ELISA Tween 20 reported⁵⁹) for the 5 months when they were tested.

Nielson et al⁷⁴ utilized a medication protocol combining tilmicosin and enrofloxacin. The breeding herd diet was medicated with tilmicosin (16 mg per kg of feed) for a 2-week period. Additionally, piglets received an injection of enrofloxacin (5 mg per kg BW) at 1, 4, and 7 days of age and were weaned at 12 days of age. *Mycoplasma hyopneumoniae*-negative replacement gilts were not introduced to the farm for 3 months following completion of the medication plan. Blood samples were collected monthly from 20 replacement gilts at 5, 6, 7, 8, 9, and 10 months and showed no seroconversion to *M hyopneumoniae*.

As a summary, an example timeline of action items associated with a herd closure and medication elimination protocol is shown in Box 1,^{37,56} and a diagram, including the parallel activities carried out in the sow herd and off-site breeding project, is presented in Figure 1. It is important to note that normal gilt acclimation procedures should be incorporated into the framework of the elimination protocol.

Discussion

The discussion of disease elimination from swine herds began back in the 1960s and 1970s with the implementation of the specific-pathogen-free (SPF) technique.⁷⁵ Although the SPF program did not live up to expectations, it did lay the groundwork and encouraged veterinarians and producers that the elimination of certain diseases may be possible. A significant justification for all the time, effort, and resources dedicated to a disease elimination project may be best summarized in the benefits of disease-free populations of pigs, which will include improved animal welfare, increased production, decreased cost of production, reduction in preventative or therapeutic antimicrobial usage, and improved caretaker morale.⁷⁵

Mycoplasma hyopneumoniae is a significant cause of economic loss to swine producers. In 2012, Haden et al⁷⁶ quantified the economic impact of influenza A virus (IAV), PRRSV, and *M hyopneumoniae* on a large US production system over a 4-year period. The cost of uncomplicated *M hyopneumoniae* was determined to be \$0.63 per head placed in grow-finish. Unfortunately, respiratory disease in growing pigs is usually not limited to one uncomplicated pathogen,

but is rather a mixed infection of *M hyopneumoniae*, viruses, and bacteria.^{77,78} Haden and others⁷⁶ also calculated the cost per head in situations in which *M hyopneumoniae* was complicated with PRRSV or IAV. The combination of PRRSV and *M hyopneumoniae* resulted in a loss of \$9.69 per head, while co-infections of IAV and *M hyopneumoniae* inflicted a loss of \$10.12 per head. The loss incurred due to the combination of IAV and *M hyopneumoniae* was only surpassed by IAV and PRRSV co-infections (\$10.41 per head). While these loss calculations are specific to one particular system, similar losses are likely realized by other US swine producers as well. A 2005 and 2006 survey conducted by Holtkamp and colleagues⁷⁹ attempted to estimate the impact of major swine health challenges in the United States. The participants in the survey comprised companies that produced more than 150,000 pigs per year, which accounted for approximately 50% of the total number of pigs produced annually in the United States. Results of the survey indicated that PRRSV, IAV, and *M hyopneumoniae* were the top three health challenges experienced in finishing herds.

Fortunately, swine producers and veterinarians have had success eliminating *M hyopneumoniae* using the protocols, principles, and techniques described in this commentary. A retrospective analysis of 46 herds that had undergone a *M hyopneumoniae* elimination project between 2003 and 2014 was completed in 2015.⁸⁰ The analysis included farms located in upper midwestern US states, 33 of which utilized a herd closure and medication protocol and 13 that used a whole-herd medication protocol without herd closure. The overall success rates for elimination of *M hyopneumoniae* for the herd closure and medication and the whole-herd medication without closure protocols were 81% and 58%, respectively. The percentage of farms that experienced successful *M hyopneumoniae* elimination for a period greater than 1 year was 97% for the herd closure and medication protocol and 67% for the whole-herd medication without herd closure technique. Additionally, the average length of time that herds remained *M hyopneumoniae*-negative following elimination was 49 months for the herd closure and medication farms and 37 months for the whole-herd medication without closure farms.

This commentary would not be complete without discussing the costs associated with the implementation of a *M hyopneumoniae*

elimination protocol. Yeske⁸¹ estimated \$15.90 per sow as the cost of a herd closure and medication protocol utilizing quarterly sow vaccination, a 2-week course of lincomycin in the drinking water to treat the breeding herd, and, during a 4-week period, tulathromycin injections to piglets at birth and 14 days of age. Additionally, the estimated increase in wean-to-finish revenue, based on increased ADG, reduced mortality, and improved feed efficiency, was calculated to be \$1.19 per pig. Furthermore, Yeske estimated that it would take a 2500-sow herd producing 25 pigs per sow per year approximately 4.5 months to recoup the financial investment in the elimination project. While \$15.90 per sow is a significant amount to invest in a *M hyopneumoniae* elimination project, the potentially lower cost of wean-to-finish production and subsequently increased revenue allow that investment to be recovered in a reasonable amount of time. Additionally, if the hypothetical farm in Dr Yeske's calculations⁸¹ were to remain *M hyopneumoniae*-free for the average of 31 months following elimination, it would realize an additional 26.5 months of reduced production costs after recovery of the initial investment.

One of the most debated aspects of the herd closure and medication protocol for *M hyopneumoniae* elimination is that of gilt vaccination and acclimation. Gilts have been indicted as the most likely source of *M hyopneumoniae* introduction into a herd and perpetuation of infection.¹⁸ Many *M hyopneumoniae* vaccination protocols have been described; however, to the knowledge of the authors, there is no published literature to support the use of one over another. Most gilt *M hyopneumoniae* vaccination and acclimation protocols are based on practitioner preference and experience. Additional research efforts are needed in this area.

It is also prudent to discuss the limitations associated with this commentary. First and foremost, many of the references utilized in this manuscript were non-peer-reviewed proceedings articles. Many of the findings described in these practitioner-authored proceedings articles are not the result of investigations subjected to the scientific rigor of studies published in peer-reviewed journals; however, they are accurate accounts of protocols, techniques, and strategies utilized in the field by practicing swine veterinarians and provide valuable

Box 1: Action items associated with a herd closure and medication protocol to eliminate *Mycoplasma hyopneumoniae*

Week 1

Acquire surplus supply of gilts and stock into on-site isolation or gilt development unit (GDU)

Need enough gilts to close the herd for at least 240 days

Gilts should be of various ages with the youngest at least 2 months of age

Vaccinate gilts with an *M hyopneumoniae* bacterin

If gilts are *M hyopneumoniae*-negative at the time of stocking, expose them to the most recently infected group of gilts to facilitate natural infection

Week 3

Vaccinate gilts with second dose of *M hyopneumoniae* bacterin

Week 4

Begin to introduce gilts into the sow herd (pending fulfillment of normal acclimation protocols)

Flow gilts into sow herd as necessary to fulfill weekly breeding group needs

Week 6

Vaccinate entire breeding herd with an *M hyopneumoniae* bacterin (quarterly vaccination schedule)

Week 19

Vaccinate entire breeding herd with an *M hyopneumoniae* bacterin (quarterly vaccination schedule)

Weeks 27-31

Stock *M hyopneumoniae*-naive or negative replacement gilts in isolation or GDU once all surplus gilts have entered the farm and isolation or GDU has been washed and disinfected (only if isolation or GDU is a separate air space from the breeding herd)

Weeks 28, 29

Vaccinate entire breeding herd with an *M hyopneumoniae* bacterin (pre-medication vaccination schedule)

Weeks 31, 32

Vaccinate entire breeding herd with an *M hyopneumoniae* bacterin (pre-medication vaccination schedule)

Week 32

Vaccinate entire breeding herd with an *M hyopneumoniae* bacterin (quarterly vaccination schedule)

Week 33

Wash and disinfect the breeding and gestation barns (shuffle sows row by row so that stalls are empty when washed and disinfected)

Weeks 33, 34

Begin medicating breeding herds via water or feed with antimicrobial approved for *M hyopneumoniae*

Medicate breeding herd for 2 to 4 weeks depending on antimicrobial selected

Begin treating on-farm piglets with an injectable antimicrobial approved for *M hyopneumoniae* at birth (or at first treatment) and again at a later time depending on the antimicrobial selected

Week 35

M hyopneumoniae-naive or negative replacement gilts can begin to be introduced into the breeding herd

Weeks 36, 37

Complete medication of breeding herd

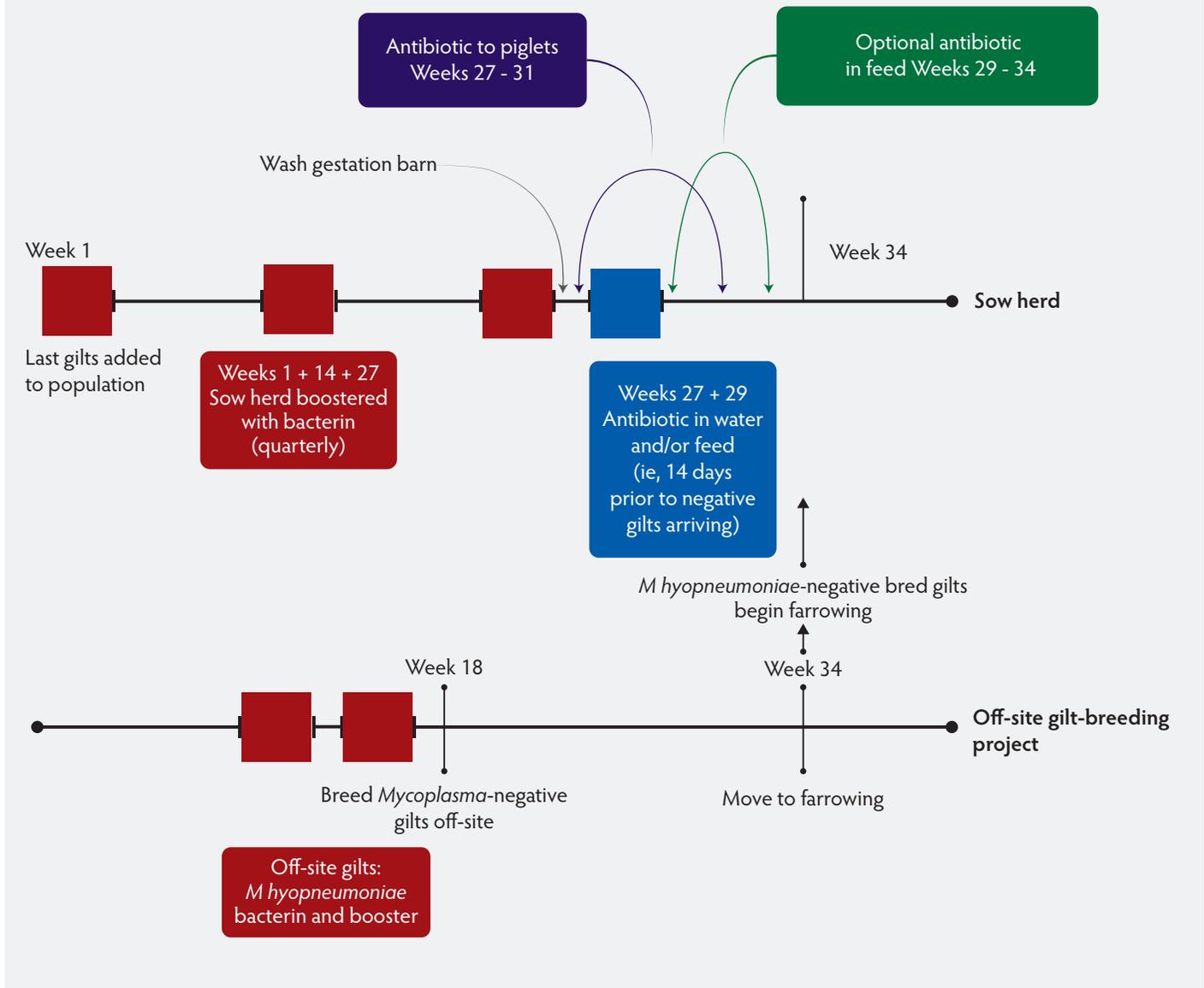
Weeks 37-41

Complete medication of piglets

Weeks 38-42

Begin *M hyopneumoniae* testing to monitor success of elimination program (after completion of piglet medication)

Figure 1: Diagram of a herd closure and medication timeline and activities, including an off-site breeding project, in a review of current *M hyopneumoniae* elimination protocols, with an emphasis on herd closure and medication protocols.



information regardless of publication status. Also, there is no standardized testing protocol to determine the success of *M hyopneumoniae* elimination projects. Previous projects have been evaluated using one or more of the following: presence or absence of *M hyopneumoniae* determined by PCR on nasal or tonsil swabs, interpretation of serological screening results, and clinical signs (coughing). The lack of a standardized testing scheme makes it difficult to compare the outcomes of specific elimination protocols.

While no standard post-elimination testing protocol exists, multiple diagnostic modalities should be utilized to determine elimination success or failure. Previously described ante-mortem sampling methods for *M hyopneumoniae* (nasal swabs, tonsil

swabs, and oral fluids)⁸² have been shown to lack sensitivity, and their utility in low-prevalence situations is less than satisfactory. However, a recently documented ante-mortem sampling method, laryngeal swabs, has demonstrated greater sensitivity⁸² and could be utilized as part of the post-elimination testing protocol. For example, serial collection and submission for *M hyopneumoniae* PCR testing of laryngeal swabs from a subset of *M hyopneumoniae*-negative replacement gilts (sentinels) that enter the farm following elimination is one testing option.

Serological screening of *M hyopneumoniae*-negative replacement gilts serving as sentinels would seem like a practical option for post-elimination testing; however,

vaccination of replacement gilts with an *M hyopneumoniae* bacterin makes differentiating infection-induced antibody response from vaccine-induced antibody response difficult.⁴ Therefore, some farms that have undergone *M hyopneumoniae* elimination have elected to leave replacement gilts unvaccinated. This allows for easy interpretation of serological results; however, the risk for increased severity of *M hyopneumoniae*-related disease is greater if reinfection or novel infection were to occur.

Evaluation of clinical signs (coughing)⁴ in the downstream pig flow and replacement gilts that enter the farm following elimination should also be included in the post-elimination testing regime. Additionally, lung tissue collection and submission for *M hyopneumoniae* PCR testing from dead pigs in

these populations rounds out a multipronged approach for post-elimination testing.

The authors' goal for this commentary was not to recommend a specific *M hyopneumoniae* elimination protocol, but rather to review the basic principles, describe specific protocols utilized by practitioners, and discuss the merits of implementing an *M hyopneumoniae* elimination project. The elimination protocol best suited for a particular farm or system will hinge on the facilities, pig flow, gilt availability, location, production type, and other unique aspects specific to the farm or system. Due to the advantages of *M hyopneumoniae*-free production previously discussed, implementation of an *M hyopneumoniae* elimination project should be considered if it is feasible for a farm or system to adhere to the specific guidelines set forth by the elimination protocol. Interest in utilizing proven disease-elimination techniques has never been higher. Continued interest and focus on the development of innovative methods and strategies for disease elimination will be necessary to help combat health challenges faced by the swine industry, now and in the future.

Implications

- *M hyopneumoniae* is a significant cause of economic loss to swine producers, and successful elimination from a production system can result in improved animal welfare, increased production, decreased production costs, and reduced antimicrobial usage.
- The multitude of elimination protocols that have been described and successfully executed can be tailored to fit the unique aspects associated with a particular farm or system and their goals.
- Increased focus and effort on the development of novel disease elimination techniques and strategies will be vital to combat health challenges in the future.

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Pyburn to lead Checkoff's science and technology team

The National Pork Board announced that Dave Pyburn, DVM, has been named the new senior vice president of science and technology. Pyburn joined the Pork Checkoff staff in 2013 and was serving as assistant vice president in the science and technology department. As senior vice president of science and technology, Pyburn will report to Chief Executive Officer Chris Hodges and lead the science programs and research priorities of the National Pork Board. He will also participate in the six volunteer pork producer committees that assist in prioritizing scientific focus and will manage the on-staff team of experts.

Dr Pyburn is a respected and demonstrated leader in the swine science industry and has proven himself as both a qualified academic and a leader of his team," said Hodges. "Dave's professional history encompasses on-the-farm practical work and government experience, and he was also previously the director of veterinary science at the National Pork Producers Council. Dave has many progressive ideas for this team and our industry, and we are looking forward to him starting his new role immediately."

Pyburn joined the National Pork Board in 2013 and previously served 13 years as the senior veterinary medical officer at the United States Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) Veterinary Services. In that role, he was responsible for setting the department's priorities, budget, and implementation of swine health programs. Pyburn was with the National Pork Producers Council from 1997 to 2000 and, prior to that, a practicing veterinarian in Iowa. He is also a member of several professional organizations including American Veterinary Medical Association, Iowa Veterinary Medical Association, United States Animal Health Association, National Institute for Animal Agriculture, and American Association of Swine Veterinarians (AASV). Through AASV, he currently serves on the Foreign Animal Disease Committee, the Pork Safety Committee, and the Influenza Committee.

For more information, contact Dave Pyburn at DPyburn@pork.org or 515-223-2634.



Dave Pyburn, DVM, NPB's new senior vice president of science and technology

New educational series: How to beat the long-term effects of seasonal pig stress

Pork producers know that high summer temperatures can lead to heat stress and poor pig performance, but they may not know how long those effects can last and how much they cost if not addressed correctly. These topics were the focus of the Pork Checkoff's newest educational opportunity, "Assessing and understanding the impact of seasonal loss of productivity," a free, four-part Webinar series that was presented in August.

"The Checkoff's Animal Science Committee was pleased to again bring this type of research-based information to all producers this year," said Chris Hostetler, director of animal science at the National Pork Board. "The subject of the series affects all producers regardless of farm size or location, yet producers have few tools to combat the effects of summer heat. However, being aware of its long-term impact is the first step."

To learn more, go to www.pork.org/animalscience. For more information, contact Chris Hostetler at CHostetler@pork.org or 515-223-2606.

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Consumer outreach: Pork Education Center now open

Last June, the new Pork Education Center in Fair Oaks, Indiana, opened its doors to help connect consumers with how pork is produced. Funded in part by a \$2 million Pork Checkoff investment, the youth-focused center aims to provide visitors a transparent look at a working farrow-to-weaning farm. Through interactive displays and a challenging rope-climbing course, the new 7000 ft²

Pork Education Center, which is part of the overall Fair Oaks' Pig Adventure, focuses on all-things pork – from nutrition and recipes to facts about responsible, sustainable pork production and pigs' contribution to human medicine.

For more information, contact Angela Anderson at AAnderson@pork.org or 515-223-2623.



The new Pork Education Center in Fair Oaks, Indiana



AASV NEWS

Nominate exceptional colleagues for AASV awards

Do you know an AASV member whose dedication to the association and the swine industry is worthy of recognition? The AASV Awards Committee requests nominations for the following five awards to be presented at the upcoming AASV annual meeting in New Orleans.

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

Meritorious Service Award – Given annually to an individual who has consistently given time and effort to the

association in the area of service to the AASV members, AASV officers, and the AASV staff.

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

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

as well as given tirelessly in service to the AASV and the swine industry.

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

Nominations are due December 15. The nomination letter should specify the award and cite the qualifications of the candidate for the award. Submit to AASV, 830 26th Street, Perry, IA 50220-2328; Fax: 515-465-3832; E-mail: aasv@aasv.org.

Is your practice on “The List”?

Does your veterinary practice provide opportunities for veterinary students to observe and practice the skills needed to become a swine veterinarian? If the answer is “yes,” please make sure you’re on “The List.” Veterinary students rely on practitioners in the field to provide practical, hands-on experience, and one way they find out about opportunities in swine practice is by checking the AASV’s list of internships and externships at <https://www.aasv.org/internships/index.php>.

Thanks to recent efforts by AASV Alternate Student Delegate Emily Mahan-Riggs and Webmaster David Brown, the internship-externship list has been updated and expanded. With enhanced searching capabilities and the inclusion of more detailed

information in the listings, students are better able to search and identify opportunities matching their goals, thus reducing the number of queries to busy practitioners.

The opportunities on “The List” are divided into two categories: 1) internships, loosely defined as experiences lasting 8-14 weeks, often in the summer, and often paid; and 2) externships, which are generally 1-2 weeks in length, often unpaid, and typically involving fourth-year veterinary students. The availability of lodging, transportation, or both, and the opportunity to work on a research project, are additional factors that are noted within the listings.

If your practice or company is already on the internship-externship list, thank you! Please

contact AASV if changes to your listing are indicated. If you are not on the list, please contact AASV (aasv@aasv.org) to provide your information. Veterinary students who are interested in swine practice will appreciate it!

Don’t forget, the AASV Foundation provides externship grants of up to \$500 per student for participating in a 2-week or longer swine externship with an AASV-member practitioner. See <https://www.aasv.org/students/externgrant.htm> for details and encourage eligible veterinary students to take advantage of this assistance.



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AASV Annual Meeting Program

"Standing on the shoulders of giants: Collaboration and teamwork"

SATURDAY, FEBRUARY 27

7:30 AM - 12:30 PM

Web-based PRRS risk assessment training for the breeding herd

8:00 AM

Entrance examination: American Board of Veterinary Practitioners, Swine Health Management

Pre-conference seminars

1:00 PM – 5:30 PM

- Seminar #1: AASV's got talent (practice tips)
Jeff Harker, chair
- Seminar #2: Pharmaceutical hot topics
Sam Holst, chair
- Seminar #3: Current issues in swine reproduction
Glen Almond, chair
- Seminar #4: Swine welfare
Sherrie Webb, chair
Jamee Amundson, moderator
- Seminar #5: Operation Main Street training
Al Eidson, chair

SUNDAY, FEBRUARY 28

Canadian Swine Veterinarians

8:00 AM – 12:00 noon

Pre-conference seminars

8:00 AM – 12:00 noon

- Seminar #6: Blisters and quarantines, no exports – oh, my!
Patrick Webb, chair
David Pyburn, moderator
- Seminar #7: Zoonoses, workplace safety, and mental health:
The confluence of pigs and people
Jennifer Koeman, chair
- Seminar #8: Diagnostics
Kent Schwartz, chair

Seminar #9: Tools to improve feed efficiency and reduce feed cost
Mike Tokach and John Patience, co-chairs

Seminar #10: Swine medicine for students
Angela Supple and Jeremy Pittman, co-chairs

Research topics

8:00 AM – 12:00 noon

Session chair: Chris Rademacher

- 8:00 AM: Comparative analysis of vaccine efficacy of live-attenuated virus, whole-virus inactivated and alphavirus vectored subunit vaccines against antigenically distinct swine influenza A viruses
Eugenio Abente
- 8:15 AM: Novel reassortant human-like H3N2 and H3N1 influenza A viruses detected in pigs are virulent and antigenically distinct from endemic viruses
Daniela Rajao
- 8:30 AM: Effect of sow vaccination and maternally derived antibodies on IAV infection
Fabian Chamba Pardo
- 8:45 AM: Airborne transmission of highly pathogenic avian influenza virus. Is the swine industry ready?
Carmen Alonso
- 9:00 AM: Particle size association with PRRS and PED viruses in aerosols from acutely infected pigs under field conditions
Carmen Alonso
- 9:15 AM: PCV as delivery virus vector to express PRRSV epitopes
Pablo Pineyro
- 9:30 AM: Developing sampling guidelines for oral fluid-based PRRSV surveillance
Marisa Rotolo
- 9:45 AM: BREAK
- 10:15 AM: Evaluation of the pathogenesis differences of the US PEDV prototype and S-INDEL-variant

Current program information is online at <https://www.aasv.org/annmtg>

strains and examination of the cross-protective immunity of two strains in weaned pigs

Qi Chen

- 10:30 AM An evaluation of porcine epidemic diarrhea virus survival in individual feed ingredients in the presence or absence of a liquid antimicrobial
Scott Dee
- 10:45 AM Effect of thermal mitigation of porcine epidemic diarrhea virus (PEDV)
Roger Cochrane
- 11:00 AM PEDV antibody responses in fecal and oral fluid specimens
Luis Gimenez-Lirola
- 11:15 AM Stochastic modeling to determine number of laryngeal swab sample pools and sample collections needed for low *Mycoplasma hyopneumoniae* group prevalences
Cassandra Fitzgerald
- 11:30 AM Detection of *Actinobacillus pleuropneumoniae* ApxIV toxin antibody in serum and oral fluid specimens from pigs inoculated under experimental conditions
Wendy Gonzalez
- 11:45 AM Discovery of a novel pestivirus in piglets with congenital tremors and reproduction of disease following experimental challenge
Bailey Arruda
- 12:00 noon Session concludes

Poster session: Veterinary Students, Research Topics, and Industrial Partners

12:00 noon – 5:00 PM

Poster authors present from 12:00 noon to 1:00 PM
Poster session continues on Monday, 9:00 AM to 5:00 PM

Concurrent sessions

1:00 PM – 5:15 PM

- Session #1 **Student Seminar**
Alex Ramirez and Maria Pieters, co-chairs
- Session #2 **Industrial Partners**
- Session #3 **Industrial Partners**
- Session #4 **Industrial Partners**

MONDAY, FEBRUARY 29

General session: Standing on the shoulders of giants: Collaboration and teamwork

8:00 AM – 12:30 PM

Program chair: **George Charbonneau**

- 8:00 AM **Howard Dunne Memorial Lecture**
Emergence of *Brachyspira hampsonii* in western Canada: A collaborative success
John Harding
- 9:00 AM **Alex Hogg Memorial Lecture**
Whose shoulders are we standing on?
Peggy Anne Hawkins
- 10:00 AM BREAK
- 10:30 AM Neonatal immunology and vaccinology: Timing is everything
Dick Hesse
- 11:00 AM Fair Oaks Pig Adventure: Public perception of what we do
Megan Inskeep
- 11:30 AM On-farm welfare audits: Preparing your clients
Chris Rademacher
- 12:00 noon It's about us, not me! Communication, cooperation, coordination and collaboration
Dale Polson
- 12:30 PM LUNCHEON

Concurrent session #1: Swine enteric coronaviruses

2:00 PM – 5:30 PM

Session chair: **Chris Rademacher**

- 2:00 PM Review of PEDV disinfectant research
Derald Holtkamp
- 2:25 PM PEDV in feed
Steve Dritz
- 2:50 PM PEDV prevalence
Bob Morrison
- 3:15 PM BREAK
- 3:45 PM PEDV outbreak management
Sara Hough
- 4:10 PM PEDV gilt acclimatization
Pete Thomas
- 4:35 PM PEDV vaccination
Trevor Schwartz
- 5:00 PM Panel discussion
Paul Yeske, Pete Thomas, Sara Hough, and Joe Connor
- 5:30 PM Session concludes

Current program information is online at <https://www.aasv.org/annmtg>

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Concurrent session #2: PRRS, *Mycoplasma*, and influenza

2:00 PM – 5:30 PM

Session chair: Mark Engle

- 2:00 PM PRRS epidemiology: Data from the swine health monitoring project
Bob Morrison
- 2:25 PM PRRS: emerging isolates
Chad Smith
- 2:50 PM *Mycoplasma hyopneumoniae* elimination
Paul Yeske
- 3:15 PM Gilt acclimation options for *Mycoplasma hyopneumoniae* infected breeding herds
Matthew Turner
- 3:40 PM BREAK
- 4:10 PM *Mycoplasma hyosynoviae*: Sample collection and findings from diagnostic case review
Paisley Canning
- 4:35 PM Interspecies movement of influenza A viruses
Marie Culhane
- 5:00 PM Considerations for vaccines against influenza A virus
Amy Vincent
- 5:30 PM Session concludes

Concurrent session #3: Antibiotic use – 2017 and beyond

2:00 PM – 5:30 PM

Session chair: Sam Holst

- 2:00 PM Current status of GFI 213 and VFD rule
Jennifer Koeman
- 2:20 PM Implementing the VFD rule: Feed company perspective
Ronny Moser
- 2:40 PM Implementing the VFD rule: Practitioner perspective
Paul Ruen
- 3:00 PM Pork production without antibiotics
Jessica Risser
- 3:20 PM BREAK

- 3:50 PM Antibiotic use in practice
Laura Bruner
- 4:10 PM Treatment decision trees
Locke Karriker
- 4:30 PM Considerations for preserving antibiotic use
Mike Apley
- 4:50 PM Roundtable Q&A
All speakers
- 5:30 PM Session concludes

TUESDAY, MARCH 1

General session: Emerging disease response

8:00 AM – 12:00 noon

Session chair: George Charbonneau

- 8:00 AM Emerging disease response: Global roadmap but local drivers
Corrie Brown
- 8:30 AM Modeling the transboundary risk of PEDV: Introduction of the virus to the US from China via contaminated feed ingredients in the presence or absence of treatment
Scott Dee
- 9:00 AM The next one! A Canadian vision for emerging disease preparedness
Doug MacDougald
- 9:30 AM Swine Health Information Center (SHIC): Enhancing our preparedness
Paul Sundberg
- 10:00 AM BREAK
- 10:30 AM Lessons learned from high-path avian influenza (HPAI)
Craig Rowles
- 11:00 AM Keep your feet on the ground but stick your head in the “cloud”
Rodger Main
- 11:30 AM Roundtable Q&A
All speakers
- 12:00 noon Session concludes



Current program information is online at <https://www.aasv.org/annmtg>

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FOUNDAATION NEWS

AASV Foundation issues call for research proposals: \$60,000 available

As part of its mission to fund research with direct application to the profession, the American Association of Swine Veterinarians Foundation seeks research proposals for funding in 2016. Proposals are **due January 29, 2016** and may request a maximum of \$30,000 (US\$) per project. A maximum of \$60,000 will be awarded across two or more projects. The announcement of projects selected for funding will take place at the AASV Foundation Luncheon in New Orleans, Louisiana, on Sunday, February 28, 2016 (awardees may be notified in advance).

Proposed research should fit one of the five action areas stated in the AASV Foundation mission statement (see sidebar).

The instructions for submitting proposals are available on the AASV Foundation Web site at <https://www.aasv.org/foundation/2016/research.php>. Proposals may be submitted by mail or e-mail (preferred).

A panel of AASV members will evaluate and select proposals for funding, on the basis of the following scoring system:

- Potential benefit to swine veterinarians/swine industry (40 points)
- Probability of success within timeline (35 points)
- Scientific/investigative quality (15 points)
- Budget justification (5 points)
- Originality (5 points)

For more information, or to submit a proposal:

AASV Foundation, 830 26th Street, Perry, IA 50220-2328; Tel: 515-465-5255; Fax: 515-465-3832; e-mail: aasv@aasv.org.

AASV Foundation Mission Statement

The mission of the American Association of Swine Veterinarians Foundation is to empower swine veterinarians to achieve a higher level of personal and professional effectiveness by

- Enhancing the image of the swine veterinary profession,
- Supporting the development and scholarship of students and veterinarians interested in the swine industry,
- Addressing long-range issues of the profession,
- Supporting faculty and promoting excellence in the teaching of swine health and production, and
- Funding research with direct application to the profession.

Fine weather, keen competition highlight AASV Foundation Golf Outing

Landsmeer Golf Club greeted 14 teams of AASV Foundation golfers on August 20 with the weather every golfer dreams of, but rarely encounters on an Iowa summer afternoon: sunny yet cool with a light, refreshing breeze. Truly a perfect day! The 56 golfers responded with strong golfing and lively, good-natured competition. There appeared to be a bullseye on the back of Dave Bomgaars' team, which has consistently placed high in past outings and also enjoyed a bit of home course advantage at this year's Orange City location. At the end of the day, only a single point separated the top three teams, with Bomgaars' foursome (himself, Dave Iverson, Tom Marsteller, and Doug Sullivan) finishing in third place after the tiebreaker with Boehringer Ingelheim's team of Jeff Blythe, Doug Nold, Cory Puetz,



The team from Pipestone Veterinary Services took top honors at this year's AASV Foundation Golf Outing. Left to right: Michael Menke, Adam Schelkopf, Luke Minion, Steve Menke.

Photo courtesy of Andrew Kleis, Insight Wealth Group

AASV Foundation news continued on page 343

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It took a tie-breaker to determine the second and third-place teams. The Boehringer Ingelheim-hosted team won the tie-breaker to take second-place. Left to right: Doug Nold, Tom Wetzell, Cory Puetz, and Jeff Blythe.

Photo courtesy of Andrew Kleis, Insight Wealth Group



Elanco Animal Health foursome of (left to right) Dave Iverson, Dave Bomgaars, Doug Sullivan, and Tom Marsteller finished only one stroke behind the leader but lost the tie-breaker to receive third place.

Photo courtesy of Andrew Kleis, Insight Wealth Group

and Tom Wetzell. Both teams recorded a score of 59. The outing's top honors went to the Pipestone Veterinary Services team of Michael Menke, Steve Menke, Luke Minion, and Adam Schelkopf, achieving a final team score of 58. In addition to the team competition, several contests scattered across the course tested and rewarded the skills of the individual golfers as well.

The generous support of sponsors enhanced the event for all participants and helped ensure its financial success. Harrisvaccines sponsored the beverage cart to keep golfers refreshed on the course. Golf hole sponsors, including Chr Hansen, Elanco Animal Health, Insight Wealth Group, Merck Animal Health, NPPC, Phibro Animal Health,

and Zoetis, hosted on-course activities and giveaways. At the conclusion of the afternoon of golf, Boehringer Ingelheim Vetmedica, Inc, sponsored the awards dinner, where Dr Josh Ellingson, this year's outing coordinator, announced the following team and individual contest winners.

Championship flight

First place team hosted by Pipestone Veterinary Services (score of 58): Michael Menke, Steve Menke, Luke Minion, Adam Schelkopf

Second place team hosted by Boehringer Ingelheim Vetmedica (score of 59): Jeff Blythe, Doug Nold, Cory Puetz, Tom Wetzell

Third place team hosted by Elanco Animal Health (score of 59): Dave Bomgaars, Dave Iverson, Tom Marsteller, Doug Sullivan

First flight

First place team hosted by Zoetis (score of 66): Tom Buelt, Josh Ellingson, Tim Stuart, Paul Thomas

Second place team hosted by Zoetis (score of 66): Kent Schwartz, Steve Sornsen, Ron White, Jeff Zimmerman

Third place team hosted by Iowa State University (score of 66): Eric Burrough, Drew Magstadt, Tiffany Magstadt, Chris Rademacher

Second flight

First place team hosted by Merck Animal Health (score of 69): Jack Creel, Steve Schmitz, Michelle Sprague, Steve Sprague

Second place team hosted by NPPC (score of 69): Pete Houska, Greg Thornton, Chris Van Beck, Marv Van Den Top

Third place team hosted by Boehringer Ingelheim Vetmedica (score of 75): Kyle Clymer, Justin Rustvold, Paul Schmid, Hillary Snyder

Individual contests

Longest drive, women (hole #6):

Tiffany Magstadt

Longest drive, men (hole #8):

Jared Terpstra

Longest drive (hole #12): Jeff Kindwall

Longest putt (hole #9): Darrell Neuberger

Closest to 150-yard marker (hole #5):

Pete Houska

Closest to the pin (hole #4): Dave Iverson

Closest to the pin (hole #7):

Jared Terpstra

Closest to the pin (hole #11):

Tom Marsteller

The annual golf fundraiser provides support for AASV Foundation programs, including veterinary-student scholarships, travel stipends to assist students attending the AASV Annual Meeting, research grants, swine externship grants, and tuition support for students taking courses at the Swine Medicine Education Center.

Jazz It UP – Give Generously in The Big Easy!

What better place than New Orleans to support the AASV Foundation? The city of New Orleans, with all of its charm, has been known as the birthplace of jazz for nearly a century. The nickname “The Big Easy” comes from a renowned dance hall of the early nineteen hundreds, but not until 1987 did the name become universally adopted after the movie based on the James Conway crime novel of the same name indelibly etched it into American culture. The Big Easy maintains the image of relaxation and slow, easy-going ways to unwind and live on the fun side of life. Likewise, the AASV Foundation Auction Committee invites you relax, be generous, and enjoy the music while you invest in the future of the AASV. Our success depends on you, the membership, so help us put together another fun-filled auction night at our annual meeting. We are confident of our endeavor and the commitment of the AASV members.

Donate your auction item(s) by December 1

The committee is currently working on putting together donations, so make your commitments as soon as possible. If you have questions or just want to discuss possibilities, please contact any of the committee members. Download the donation form at <https://www.aasv.org/foundation> and submit a description and image of your

item(s) by December 1. Your contribution will be recognized in the printed auction catalog as well as on the auction Web site, and your name will appear in the JSHAP full-page spread recognizing all of our auction item donors. If that’s not enough, there’s a good chance Dr Harry Snelson will say something witty about your donation in the AASV e-Letter, too!

The AASV Foundation is committed to ensuring the future of the swine veterinary profession. Proceeds from the auction enable funding for AASV Foundation programs, including

- Administering endowments for the Howard Dunne and Alex Hogg Memorial Lectures
- Administering the Hogg Scholarship for a swine veterinarian pursuing an MS or PhD
- Administering funding for Veterinary Student Scholarships
- Co-sponsoring travel stipends for veterinary students attending the AASV Annual Meeting
- Providing swine externship grants to veterinary students
- Funding swine research with direct application to the profession

AASV Foundation Auction Committee

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- Administering funding for the National Pork Industry Foundation Internship Stipends
- Providing support for Heritage Videos
- Tuition support for out-of-state veterinary students to attend the Swine Medicine Education Center.



The US Veterinary Feed Directive (VFD) has changed

The new VFD regulation became effective October 1, 2015

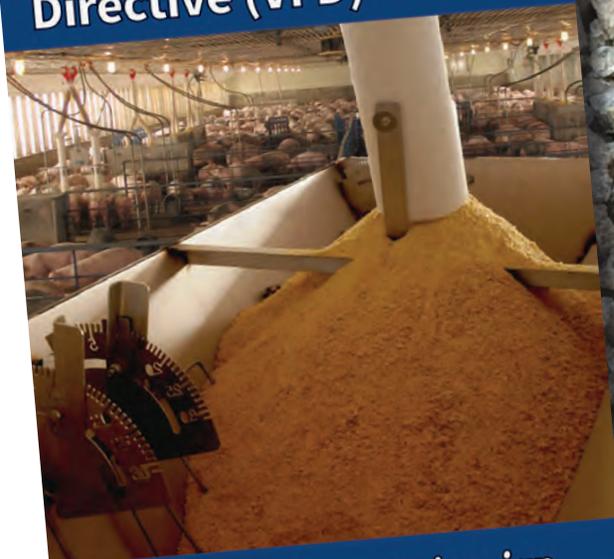
The use of any feed-grade antimicrobial with a VFD label is now subject to the new regulation. This includes tilmicosin, florfenicol, and avilamycin, which are already VFD drugs labeled for use in swine.

Pharmaceutical manufacturers will transition other medically important, feed-grade antimicrobials to VFD labels by December 2016. Essentially all swine antibiotics will be affected, except bacitracin, carbadox, bambarmycin, ionophores, and tiamulin. These antibiotics will remain available for growth promotion or over-the-counter (OTC) distribution, or both.

The AASV has prepared and mailed a brochure to all US members that highlights the responsibilities of the veterinarian issuing a VFD, the information required on a VFD, the need for a veterinary-client-patient relationship, and additional items of interest. The brochure is available online at www.aasv.org/aasv/publications.htm.

The AASV urges swine veterinarians to become familiar with the regulation, which is available – along with additional information and updates – on the FDA's Veterinary Feed Directive Web page: <http://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/ucm071807.htm>.

Changes in the Veterinary Feed Directive (VFD)



What the swine veterinarian needs to know



Extra-label use of feed-grade antimicrobials remains **ILLEGAL**.

Questions about VFDs?

Contact:

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Pounds of paperwork

The pork industry is data driven. It's one of the things that actually drew me to swine veterinary medicine. Pork producers and veterinarians want to collect and analyze data about all aspects of the industry to better understand the implications of what we do. I started my career at a large swine and turkey production company in North Carolina in 1990. The company collected a lot of data, mostly focused on understanding the economics of our business. Every month we met as a management team and spent hours poring over reams of computer printouts, evaluating costs to the fourth decimal point. The running joke was that we should just do away with measuring our performance by analyzing production parameters such as farrowing rates and average daily gains and instead just look at pounds of paperwork per pound of pork.

As the years passed and the company grew, we expanded our data-collection efforts to evaluate additional aspects of the business, such as the impacts of animal health on performance. It also became evident that we could benefit from understanding how our performance compared with that of other pork producers, so we started sharing our data – confidentially, of course. This enabled

us to benchmark our performance economically, but it also provided an opportunity to enhance decisions based on animal health. By sharing disease information, we could better locate new facilities, prepare for disease exposure, and monitor herd-health status to benefit disease control and eradication efforts.

"During a large-scale disease outbreak, sharing data by spreadsheet is extremely inefficient."

Recent disease-control challenges such as pseudorabies eradication, porcine reproductive and respiratory syndrome area control, influenza surveillance, and porcine epidemic diarrhea virus monitoring have illustrated the need for the ability to share disease data across the industry. The implementation of premises identifiers and the capability to trace animal movements have made disease epidemiology more meaningful and useful. There are some significant challenges yet to overcome, however, regarding the ability to efficiently utilize all the available data.

First, producers have to be willing to share their premises data and disease status information at some level that allows for effective decision making. Obviously, it is necessary to maintain the confidentiality of the data, but there are mechanisms to ensure that security. We really have very little understanding of the prevalence, distribution, and severity of disease challenges facing the US swine industry. We are currently seeing encouraging steps to address this information gap with such programs as the Swine Health Monitoring Project. Expansion of the project is necessary if it is to become truly representative of US pork production. The National Animal Health Monitoring System conducted by the US Department of Agriculture's Center for Epidemiology and Animal Health is another example of data collection and analysis at the national level.

Willingness to share data is just part of the equation, however. There also needs to be an effective and efficient method to transfer the data to someone, group or agency, who can coordinate and analyze the data. This step can be a significant technical challenge. An example of this is the need to transfer diagnostic laboratory information between laboratories, clients, researchers, and regulatory officials. All of these groups have their own databases and spreadsheets. Standardizing the data and connecting the disparate databases to facilitate electronic transfer of the data is no easy feat. Particularly during times of animal health emergency, it is critical that the exchange and analysis of data promotes business continuity by not unnecessarily delaying the movement of animals and animal products. During a large-scale disease outbreak, sharing data by spreadsheet is extremely inefficient.

While many of the key swine veterinary diagnostic laboratories have made great strides in standardizing their data, much work remains to enable the seamless transfer of data across all pertinent stakeholders and for all pathogens. On the positive side, the technology exists to bridge these databases and facilitate the transfer of data. However, obtaining the necessary funding and committing the technical resources remain the key stumbling blocks to implementation of this technology. Veterinarians and pork producers need to make this a priority issue and not wait on the government to make it happen.

Harry Snelson, DVM
Director of Communications



CUMULATIVE INDEX

The *Journal of Swine Health and Production* cumulative index is updated online throughout the year as issues go to press. Articles can be accessed via the “Search” function and from the Abstracts page, <http://www.aasv.org/shap/abstracts/>.

Index by title 2015

- Case report describing the clinical course of porcine epidemic diarrhea in a commercial boar stud and return of the stud to service after whole-herd inoculation with porcine epidemic diarrhea virus. McCarty M, Petznick T, Kuster C, et al. *J Swine Health Prod.* 2015;23(5):264–271.
- Characterization of histopathologic lesions among pigs with overgrown claws. Newman SJ, Rohrbach BW, Wilson ME, et al. *J Swine Health Prod.* 2015;23(2):91–96.
- Collection of oral fluid from individually housed sows. Pepin B, Liu F, Main R, et al. *J Swine Health Prod.* 2015;23(1):35–37.
- Correlation of *Lawsonia intracellularis* semi-quantitative fecal polymerase chain reaction assay results with the presence of histologic lesions of proliferative enteropathy and positive immunohistochemical staining. Burrough ER, Rotolo ML, Gauger PC, et al. *J Swine Health Prod.* 2015;23(4):204–207.
- Depletion of bromadiolone in tissues of hogs following oral exposure. Enouri S, Dekroon K, Friendship R, et al. *J Swine Health Prod.* 2015;23(6):298–305.
- Early indicators of iron deficiency in large piglets at weaning. Bhattarai S, Nielsen JP. *J Swine Health Prod.* 2015;23(1):10–17.
- Effect of fenbendazole on shedding and embryonation of *Ascaris suum* eggs from naturally infected sows. Pittman JS, Myers GH, Stalder KJ, et al. *J Swine Health Prod.* 2015;23(5):252–263.
- Effect of short (10- or 12-day) or standard (14- or 18-day) periods of estrus suppression with allyl trenbolone on estrus synchronization and fertility in pubertal gilts. De Rensis F, Mazzoni C, Saleri R, et al. *J Swine Health Prod.* 2015;23(2):97–99.
- Elimination of *Mycoplasma hyopneumoniae* from breed-to-wean farms: A review of current protocols with emphasis on herd closure and medication. Holst S, Yeske P, Pieters M. *J Swine Health Prod.* 2015;23(6):321–330.
- Elimination of porcine respiratory coronavirus by early weaning and segregation. Burlatschenko S, Arsenault C. *J Swine Health Prod.* 2015;23(4):208–213.
- Evaluation of disinfection protocols to reduce virus transmission via livestock transport vehicles using model trailers and experimental conditions. Schneider PT, Zhang J, Ramirez A, et al. *J Swine Health Prod.* 2015;23(6):306–316.
- Evaluation of time and temperature sufficient to inactivate porcine epidemic diarrhea virus in swine feces on metal surfaces. Thomas PR, Karriker LA, Ramirez A, et al. *J Swine Health Prod.* 2015;23(2):84–90.
- Experimental inoculation of neonatal piglets with feed naturally contaminated with porcine epidemic diarrhea virus. Pillatzki AE, Gauger PC, Madson DM, et al. *J Swine Health Prod.* 2015;23(6):317–320.
- Hoof lesions and lameness in sows in three Greek swine herds. Lisgara M, Skampardonis V, Kouroupides S, et al. *J Swine Health Prod.* 2015;23(5):244–251.
- Immunological, virological, and pathological evaluation of a single dose versus two doses of a one-dose porcine circovirus type 2 subunit vaccine under experimental conditions. Seo HW, Duy DT, Park C, et al. *J Swine Health Prod.* 2015;23(4):214–219.
- Influence of age at weaning and feeding regimes on the postnatal morphology of the porcine small intestine. Al Masri S, Hünigen H, Al Aiyani A, et al. *J Swine Health Prod.* 2015;23(4):186–203.
- Monitoring influenza A virus infection in pigs by using a competitive enzyme-linked immunosorbent assay to detect virus antibodies in pen-based oral-fluid specimens. Strutzberg-Minder K, Boehmer J, Fischer S, et al. *J Swine Health Prod.* 2015;23(3):126–131.
- Persistence of porcine reproductive and respiratory syndrome virus and porcine circovirus type 2 in bacterial biofilms. Jacques M, Grenier D, Labrie J, et al. *J Swine Health Prod.* 2015;23(3):132–136.
- Postpartum injection of human chorionic gonadotrophin: Effects on sow ovarian follicles. Zemitis J, Bouwman EG, Langendijk P, et al. *J Swine Health Prod.* 2015;23(3):137–139.
- Survey of serum vitamin D status across stages of swine production and evaluation of supplemental bulk vitamin D premixes used in swine diets. Arnold J, Madson DM, Ensley SM, et al. *J Swine Health Prod.* 2015;23(1):28–34.
- Susceptibility of 45 recent French field isolates of *Streptococcus suis* to florfenicol. Lequeux G, Le Drean E. *J Swine Health Prod.* 2015;23(3):140–142.
- The course of rectal and vaginal temperature in early postpartum sows. Stiehler T, Heuwieser W, Pfützner A, et al. *J Swine Health Prod.* 2015;23(2):72–83.
- Thiamine-responsive neurological disorder of swine. Hough SD, Jennings SH, Almond GW. *J Swine Health Prod.* 2015;23(3):143–151.
- Variation in porcine reproductive and respiratory syndrome virus open reading frame 5 diagnostic sequencing. Stricker AM, Polson DD, Murtaugh MP, et al. *J Swine Health Prod.* 2015;23(1):18–27.

Index by author 2014

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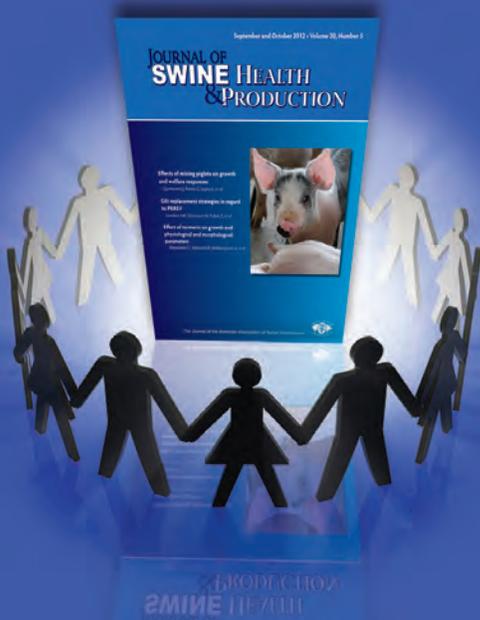
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Thank you, reviewers

Working together and creating
a journal to be proud of!

The editorial staff of the Journal of Swine Health and Production would like to acknowledge the invaluable assistance of the following individuals for their service as referees for the manuscripts that were reviewed between September 23, 2014 and September 22, 2015.

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Hans Coetzee	Lee Johnston	Andres Perez	Scott Whisnant
Joe Connor	Cassandra Jones	Mike Pierdon	Nate Winkelman
Russ Daly	Hannes Kauffold	Maria Pieters	Paul Yeske
Peter Davies	Andrea Ladinig	Zvonimir Poljak	Beth Young
Scott Dee	Karl Heinz Lahrman	Megan Potter	Jeff Zimmerman
Josepha deLay			

We apologize if we have inadvertently left a reviewer's name off the list.

UPCOMING MEETINGS

Passion for Pigs “Learn to Earn” Tour

November 3, 2015 (Tue): Dayton, Ohio
November 18, 2015 (Wed): Orange City, Iowa
December 8, 2015 (Tue): Columbia, Missouri

For more information:

Julie A. Lolli, Executive Coordinator

Tel: 660-657-0570

E-mail: julie.nevets@nevetsrv.com

Web: <http://www.passionforpigs.com>

Antibiotic Stewardship: From Metrics to Management

November 3-5, 2015 (Tue-Thu)
Crowne Plaza Atlanta Midtown, Atlanta, Georgia
Hosted by: National Institute for Animal Agriculture

For more information:

National Institute for Animal Agriculture

13570 Meadowgrass Drive, Suite 201

Colorado Springs, CO 80921

Tel: 719-538-8843; Fax: 719-538-8847

E-mail: niaa@animalagriculture.org

Web: <http://www.animalagriculture.org/2015-Antibiotics-Symposium>

2015 ISU James D. McKean Swine Disease Conference

November 5-6, 2015 (Thu-Fri)
Ames, Iowa

Hosted by Iowa State University

For more information:

Tel: 515-294-6222; Fax: 515-294-6223

E-mail: registrations@iastate.edu

Web: <http://www.extension.iastate.edu/registration/events/conferences/swine/>

2015 North American PRRS Symposium

December 5-6, 2015 (Sat-Sun)
Intercontinental Hotel
505 N Michigan Avenue, Chicago, Illinois

For more information:

Web: <http://ksvma.site-ym.com/?NAPRRS>

2016 Pig-Group Ski Seminar

February 3-5, 2016 (Wed-Fri)
Copper Mountain, Colorado

For more information:

Lori Yeske

Pig Group

39109 375th Ave, St Peter, MN 56082

Tel: 507-381-1647

E-mail: pyeske@swinevetcenter.com

Web: <http://www.pigski.net>

American Association of Swine Veterinarians 47th Annual Meeting

February 27-March 1, 2016 (Sat-Tue)
Hyatt Regency New Orleans, New Orleans, Louisiana

For more information:

American Association of Swine Veterinarians

830 26th Street, Perry, IA 50220-2328

Tel: 515-465-5255; Fax: 515-465-3832

E-mail: aasv@aasv.org

Web: <http://www.aasv.org/annmtg>

24th International Pig Veterinary Society Congress

June 6-10, 2016 (Mon-Fri)
Dublin, Ireland

For more information:

Web: <http://www.ipvs2016.com>

World Pork Expo

June 8-10, 2016 (Wed-Fri)
Iowa State Fairgrounds, Des Moines, Iowa
Hosted by the National Pork Producers Council

For more information:

Alicia Newman

National Pork Producers Council

10676 Justin Drive, Urbandale, IA 50322

Tel: 515-278-8012; Fax: 515-278-8014

E-mail: newmana@nppc.org

Web: <http://worldpork.org>



For additional information on upcoming meetings: <https://www.aasv.org/meetings/>



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830 26th Street
Perry, IA 50220-2328

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A moment shared at University of Missouri

Photo courtesy of Tina Smith

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