

# JOURNAL OF **SWINE** HEALTH & PRODUCTION

Surveillance for PRRSV, PCV2, and IAV  
in Vietnam

*Cuong NV, Carrique-Mas J, Thu HTV, et al*

Potential biosecurity risks associated  
with feed delivery

*Dewey C, Bottoms K, Carter N, et al*

Fine-needle aspiration and cytology  
to evaluate injection-site lesions

*Wiedmeyer CE, Fangman TJ, Schwartz K, et al*

CO<sub>2</sub> system for on-farm euthanasia

*Rice M, Baird C, Stikeleather L, et al*



# Journal of Swine Health and Production

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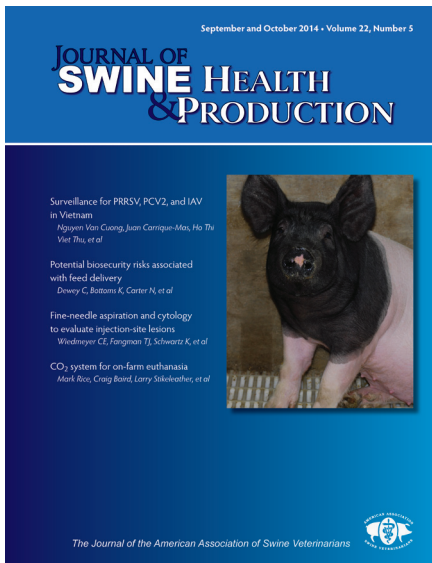
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*quoted from the Executive Editor's message, page 223*

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## PED: Three little letters, infinite implications

By the time this message goes to print, mandatory reporting of clinical cases of porcine epidemic diarrhea (PED) and other swine enteric coronavirus diseases (SECD) under the federal order issued in June will be in full swing and hopefully going smoothly. But as I write this, reporting is in its infancy, and several of the program details are still being defined. The veterinary diagnostic laboratories have just started forwarding information from cases positive for novel swine enteric coronavirus to the United States Department of Agriculture (USDA) through the Laboratory Messaging System (LMS).

It will be interesting to see how this unfolds, though my hope is the process will be streamlined such that it can easily and efficiently become a routine procedure that provides value to the industry. It is essential that data compilation be complete and in a format that can be sorted and evaluated so that we are able to glean as much information as possible from this exercise. As with any database, the information that can be extrapolated is only as robust as the data that

is entered. Veterinary diagnostic laboratory personnel are uploading the positive cases into the LMS, making the burden of reporting much lighter for veterinarians and producers. However, we all have a responsibility to follow up on these cases and do our part to compile the rest of the relevant information, including the clinical picture on the farm. Cooperation across the industry is essential to the significance of the outcome of this program.

*"I look forward to hearing your thoughts on how AASV can and should play a role in the next emerging disease situation."*

While it is unfortunate we did not start collecting the data sooner, this program still has the potential to provide valuable information about the transmission of PED virus (PEDV), if not the likely point source of introduction. This exercise will demonstrate the importance of our national biosecurity program and diagnostic laboratory communication system, as well as highlight the programs and data-management systems that are essential to our success. It will also help to identify shortcomings that must be corrected or advanced prior to the introduction of the next transboundary or foreign animal disease. Furthermore, this program may enlighten us on how to minimize the spread of infectious organisms throughout the national swine herd, a critical task should we ever be faced with a foreign animal disease that must be eradicated promptly.

In addition to highlighting and mitigating risks associated with transmission of swine enteric coronaviruses, this program may also encourage the development and improvement of technologies that will assist in identification, tracking, and monitoring of infectious disease risk and site status. Existing mapping programs are just the tip of the iceberg when it comes to their potential functionality and applications. Fully integrated information systems

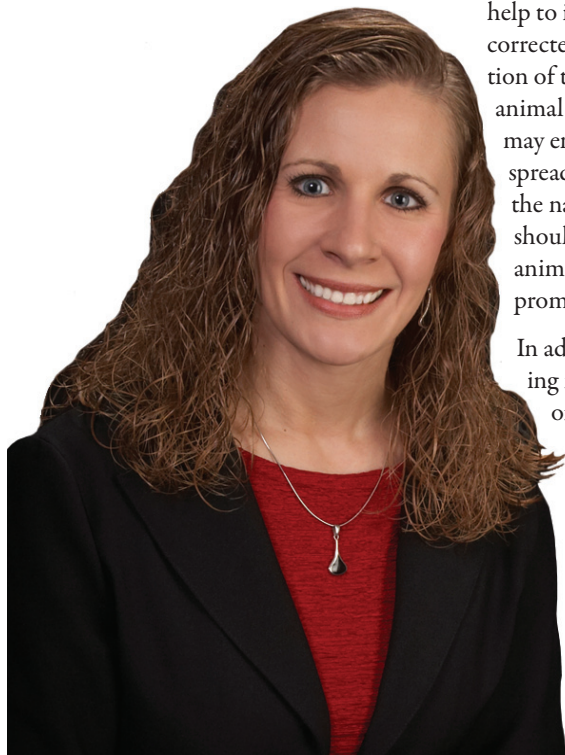
are the way of the future and my hope is this federal order will help fund and facilitate the further development and implementation of this applied information technology so that we may more efficiently and effectively evaluate disease-risk parameters in the future.

While these mapping programs are instrumental in aiding development of local and regional health plans and monitoring protocols, it is important to remember that we live in a global society. We must maintain awareness of international disease threats and be prepared to identify them, both clinically on the farm and definitively at the diagnostic laboratory. It is essential that we be vigilant in monitoring herds for clinical signs of diseases that are considered threats globally; veterinarians truly are the first line of defense on matters involving the health of our nation's livestock herds. It is also imperative that our diagnostic laboratories be equipped with the necessary tests and primers to readily diagnose diseases considered to be global risks.

In addition to the clear devastation caused by this disease and the opportunities PED has given us for improvement, there have also been many benefits that have already been realized. This virus has given us the opportunity (and I would even go so far as to say the obligation) to work together as an industry and as a profession. Staff members and officers of all three key swine associations (National Pork Board, National Pork Producers Council, and the American Association of Swine Veterinarians) have never worked more closely together than they have on issues stemming from this transboundary disease since its introduction in spring 2013.

In fact, the vast amount of time AASV staff has spent on PED-related activities and the breadth of those activities calls into question whether or not the scope of our mission matches that breadth and, if not, whether our mission statement should be revised or labor resources should be redirected. Today,

*President's message continued on page 219*





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*President's message continued from page 217*

it is the mission of the American Association of Swine Veterinarians "to increase the knowledge of swine veterinarians by

- promoting the development and availability of the resources which enhance the effectiveness of professional activities,
- creating opportunities which inspire personal and professional growth,
- advocating science-based approaches to industry issues,
- encouraging personal and professional interaction, and
- mentoring students, encouraging life-long careers as swine veterinarians."

One could easily argue that staff time devoted to PED has been an effective means of "advocating science-based approaches to industry issues." The question becomes whether or not the allocation of time on that particular bullet point is appropriate – be it too little or too much. Regardless of your perception of whether or not the quality and quantity of time spent on PED-related issues is appropriate, it is clear that Tom, Harry, and Sue have had much more added to their collective plate, and yet nothing has gone undone. When you voice your opinions on our mission and how PED and other emerging diseases fit into the context of that mission (and I hope you will), please also take the time to let our AASV staff know how much you appreciate all they do on behalf of swine veterinarians and our association – they certainly deserve the accolades.

In addition to thanking our association's staff, we should also, as an industry, be encouraged by the collective amount of PED research that has been conducted in the past year. There was virtually no clinically relevant information available when herds first became infected with PEDV in the United States, but thanks to industry funds (company, private, and Pork Checkoff) and tireless efforts of industry personnel, we have assimilated a significant amount of information in a relatively brief period of time. With funds allocated in the federal order, we will be able to build on this growing database to provide the necessary information to manage this disease in the most effective way possible.

As with any new disease, we have become acutely aware of our vulnerabilities through our challenges with PED and other emerging SECD. We have also already capitalized on many of the opportunities presented to us through collaboration and research. My hope is we will use the federal order to assimilate more information and improve industry infrastructure. This disease introduction has demonstrated to us the need for improved national and international biosecurity programs and fully integrated data-management systems so we are better prepared to identify, contain, and eliminate the next pathogen that enters our country. I look forward to hearing your thoughts on how AASV can and should play a role in the next emerging disease situation.

Michelle Sprague, DVM  
AASV President

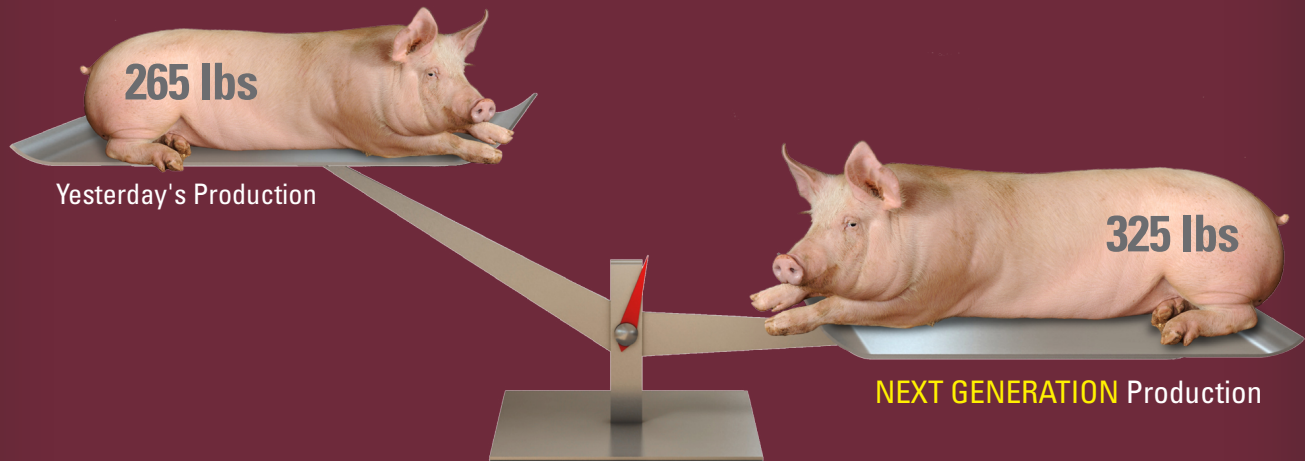






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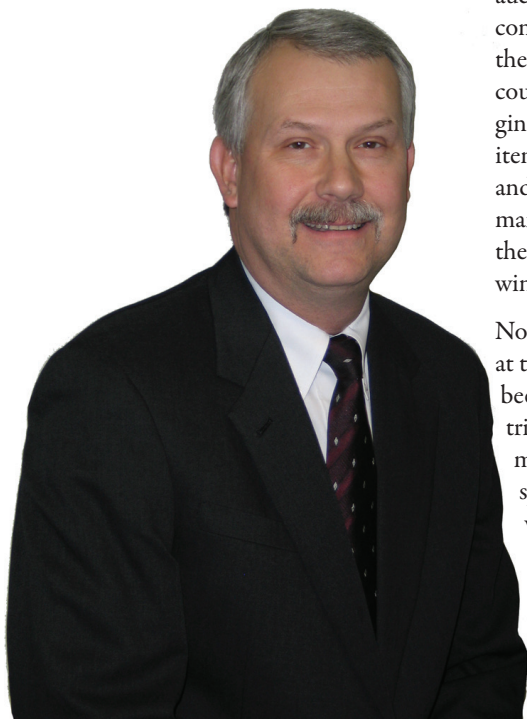


## Integrity, education, success

Over the course of my time with the AASV, I have developed an informal list of my “go to” members. These are members that I tend to lean on when a particular need arises. They always come through with assistance in meeting whatever needs demand attention. One such member has been on my list since my first summer (1994) with the AASP: Dr Rodney Johnson. His passing in late May has left a palpable void, but his life of service to pigs and people left us an example to follow.

The first time I met Rod was the summer of 1994. I had just been hired as the part-time executive liaison for the AASP. We knew many of the same people in the pork industry, but had never really met. We had dinner together at the AVMA annual convention in San Francisco. Right from the start, Rod was encouraging and supportive, offering his assistance however he could help. He never wavered in his offer over the course of the last 20 years. His sage advice was always welcome and valued.

It was evident to me right from the start that Rod valued three things: integrity, education, and helping people to be successful.



He exemplified all three in his service to the pork industry and the veterinary medical profession. Rod was the AASP Practitioner of the Year in 1982. He was president of the AASP in 1985. He delivered the Howard Dunne Memorial Lecture in 1995. He was given the AASV Meritorious Service Award in 2009. Even though Rod transitioned into the role of CEO of the AVMA Professional Liability Trust (PLIT) several years ago, he remained a swine veterinarian at heart and stayed actively involved with the AASV.

*“It was evident to me right from the start that Rod [Johnson] valued three things: integrity, education, and helping people to be successful.”*

In recent years, Rod was focused on the AASV Foundation. He served on the AASVF Board of Directors for many years, leading as the board chair for two terms. It was his leadership on the auction committee that has brought the foundation auction to new levels of donations and bidding. While urging others to donate and bid, Rod also led by example in his generosity to the auction. As the auctioneer, I was always comforted by the sight of Rod right there in the front row for the auction. I could always count on Rod to bid on items that were lagging a bit, even if he really did not want that item. All I needed to do was catch his eye and he would willingly take on the role as a market-maker. If it was an item he wanted, then I was confident that he would be the winning bidder.

Not satisfied with just bidding and buying at the auction, Rod and his wife, Jean, have been donating a complete Minnesota fishing trip for many years. It was always one of the most popular auction items. Word soon spread about the great fishing and the wonderful hospitality experienced on the trip. Rod and Jean were gracious hosts year after year. One of my regrets is that I never bought that fishing trip. Rod also convinced his employer, AVMA PLIT, to donate to the auction every year.

For many years, Rod had also been serving on the AASVF Investment Committee. He was a savvy investor with a keen understanding of wealth-building. He combined that understanding with his passion for helping people, especially veterinarians, to be successful. His vision for the AASVF was based on this premise. Rod was always adamant that the AASV(P) was a major factor in his success as a swine veterinarian. His goal was to make that true for each and every member of the organization today and in the future. He viewed the foundation as a way to ensure the profession of swine veterinary medicine is a viable career for the next generations of veterinarians. He wanted others to enjoy the success that he felt so blessed to have experienced.

I will miss Rod for many reasons. He was a friend and colleague. He was a mentor, cheerleader, and confidante. He was a cool-headed investor when the market was down and a conscientious leader when investing with other people’s money. He was a committed leader who continually demonstrated his integrity through thought, word, and deed. For him, education was life-long and not to be wasted. Success was not something to be taken for granted; rather, it was to be shared.

Rod saw life as a series of opportunities for service and sharing of gifts. We each have similar opportunities in our lives. Let’s use Rod’s example as inspiration so that we can each use those opportunities to be a “go to” person.

Tom Burkgren, DVM  
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In my opinion, the international scope of *JSHAP* articles is very valuable for the growth and improvement of the swine industry worldwide. But with such diversity can come some challenges, and one such challenge is the differences in pharmaceutical (in particular

antimicrobial) usage around the world for swine production. And I am sure I do not need to remind *JSHAP* readers that the use of antimicrobials in food-producing animals has received increased attention worldwide, and the public and media are becoming more engaged in the conversation. While most of us involved in this industry have an understanding and appreciation of the international differences in laws and regulations surrounding medication usage in swine, it is difficult for every author and every reviewer to know every law and every rule with respect to medication usage around the world. For this reason, I respectfully encourage all *JSHAP* readers to take this diversity into consideration when reading a publication in *JSHAP* (or any journal for that matter) involving medication usage or management recommendations.

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*"... the international scope of JSHAP articles is very valuable for the growth and improvement of the swine industry worldwide."*

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Terri O'Sullivan, DVM, PhD  
Executive Editor





# Serological and virological surveillance for porcine reproductive and respiratory syndrome virus, porcine circovirus type 2, and influenza A viruses among smallholder swine farms of the Mekong Delta, Vietnam

Nguyen Van Cuong, MSc; Juan Carrique-Mas, DVM, PhD; Ho Thi Viet Thu, PhD; Nguyen Duc Hien, MSc; Ngo Thi Hoa, PhD; Lam Anh Nguyet, BSc; Pham Hong Anh, MSc; Juliet E Bryant, PhD

## Summary

**Objectives:** To evaluate the feasibility and utility of oral-fluids collection for surveillance of porcine viruses in the Mekong Delta, Vietnam, and to establish baseline serological and virological prevalence estimates for porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), and influenza A virus (IAV) among smallholder farms.

**Materials and methods:** Paired serum and oral-fluids samples from 68 farms (sows, boars, weaners, and growers) were tested during 2011 by reverse transcriptase polymerase chain reaction and enzyme-linked immunosorbent assay for PRRSV, PCV2, and IAV.

**Results:** Low numbers of PRRSV-positive and IAV-positive pigs were detected (1.6% PRRSV viremic, two of 124; 0.8% IAV in oral fluids, one of 124). However, PCV2 detection rates were high in both serum and oral fluids (54.8% and 61.3%, respectively). Overall proportions of pigs seropositive for IAV and PRRSV were 37.9% and 33.9%, respectively. Proportions of pigs seropositive for PRRSV were 48.6% (17 of 35) and 12.1% (four of 33) on vaccinated and unvaccinated farms, respectively. Oral fluids and serum samples yielded comparable prevalence estimates for molecular detection of PCV2, and detected one sample PCR-positive for hemagglutinin of influenza A/H1N1/pdm09.

There was no evidence of PRRSV shedding in oral fluids.

**Implications:** Antibody prevalence estimates based on testing oral fluids may provide an acceptable and useful surrogate for testing serum in future field studies if optimized assays are employed.

**Keywords:** swine, oral fluids, influenza, porcine reproductive and respiratory syndrome virus, Vietnam

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## Resumen - Vigilancia serológica y virológica para el virus del síndrome reproductivo y respiratorio porcino, circovirus porcino tipo 2, y virus de influenza A en granjas porcinas de pequeños agricultores de Mekong Delta, Vietnam

**Objetivos:** Evaluar la viabilidad y utilidad de la recolección de fluidos orales para la vigilancia de virus porcinos en Mekong Delta, Vietnam, y para establecer valores base de la prevalencia serológica y virológica

para el virus del síndrome reproductivo y respiratorio porcino (PRRSV por sus siglas en inglés), circovirus porcino tipo 2 (PCV2 por sus siglas en inglés), y el virus de la influenza A (IAV por sus siglas en inglés) en granjas de pequeños productores.

**Materiales y métodos:** Se analizaron muestras de fluidos orales y sueros pareados de 68 granjas (machos, hembras, lechones de destete, y crecimiento) durante 2011 por medio de la prueba de reacción en cadena

de polimerasa de transcriptasa reversa y la prueba de inmunoabsorción enzimática para PRRSV, PCV2, e IAV.

**Resultados:** Se detectaron bajos números de cerdos positivos al PRRSV y positivos al IAV (1.6% virémicos al PRRSV, dos de 124; 0.8% IAV en fluidos orales, uno de 124). Sin embargo, los índices de detección de PCV2 fueron altos en sueros y fluidos orales (54.8% y 61.3%, respectivamente). En general, las proporciones de cerdos seropositivos al IAV y PRRSV fueron 37.9% y 33.9%, respectivamente. Las proporciones de cerdos seropositivos al PRRSV fueron 48.6% (17 de 35) y 12.1% (cuatro de 33) en granjas vacunadas y no vacunadas, respectivamente. Las muestras de suero y fluidos orales arrojaron valores de prevalencia comparables a la detección molecular de PCV2, y detectaron una muestra positiva al PCR para la hemagglutinina de influenza A/H1N1/pdm09. No hubo evidencia de excreción de PRRSV en fluidos orales.

**Implicaciones:** Los cálculos de prevalencia de anticuerpos basados en pruebas de fluidos orales pueden ofrecer un sustituto aceptable y útil para probar suero en futuros estudios de campo si se emplean pruebas optimizadas.

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## Résumé - Surveillance sérologique et virologique des virus du syndrome reproducteur et respiratoire porcin, du circovirus porcin de type 2, et de l'influenza A dans les fermes porcines de petite taille du Delta du Mékong, Vietnam

**Objectifs:** Évaluer la faisabilité et l'utilité de la collecte de fluides oraux pour la surveillance de virus porcins dans le Delta du Mékong, Vietnam, et établir les estimés des prévalences sérologique et virologique de base pour le virus du syndrome reproducteur et respiratoire porcin (PRRSV), le circovirus porcin de type 2 (PCV2), et le virus de l'influenza A (IAV) dans des fermes de petite taille.

**Matériels et méthodes:** Des échantillons appariés de sérum et des échantillons de fluides oraux provenant de 68 fermes (truies, ver-

rats, porcs sevrés, porc en engraissement) ont été testés durant l'année 2011 par réaction d'amplification en chaîne par la polymérase à l'aide de la transcriptase inverse et par épreuve immunoenzymatique (ELISA) pour PRRSV, PCV2, et IAV.

**Résultats:** Des nombres peu élevés de porcs positifs pour PRRSV et IAV furent détectés (1,6% PRRSV virémiques, 2 sur 124; 0,8% pour IAV dans les fluides oraux, 1 sur 124). Toutefois, les taux de détection de PCV2 étaient élevés autant dans les échantillons de sérum que de fluides oraux (54,8% et 61,3%, respectivement). De manière générale, les taux de porcs séropositifs pour IAV et PRRSV étaient de 37,9% et 33,9%, respectivement. Les taux de porcs séropositifs pour le PRRSV étaient de 48,6% (17 sur 35) et

12,1% (4 sur 33) pour les fermes pratiquant et ne pratiquant pas la vaccination, respectivement. Les échantillons de fluides oraux et de sérum ont donné des résultats d'estimé de prévalence comparables pour la détection moléculaire de PCV2, et ont permis de détecter un échantillon positif par PCR pour l'hémagglutinine du virus influenza A/H1N1/pdm09. Il n'y avait aucune évidence d'excrétion de PRRSV dans les fluides oraux.

**Implications:** Les estimés de prévalence des anticorps basés sur les épreuves effectuées sur les fluides oraux peuvent être des alternatives acceptables et utiles aux tests effectués sur du sérum si des épreuves optimisées sont utilisées.

Pork production is critically important to the national economy and food security of Vietnam, and despite major animal-disease outbreaks, the swine industry of Vietnam has achieved remarkably sustained growth in production and profitability over the last 30 to 40 years. Between 2000 and 2010, the total volume of pork production in Vietnam increased 114%.<sup>1</sup> These increases were due to growth in pig stocks (approximately 3.6% annual growth in swine population between 2000 and 2010, from 23.0 to 49.3 million head), as well as increased efficiencies in production.<sup>2</sup> Animal-health issues facing the industry include fatal epizootics of porcine viruses, endemic circulation of several notifiable diseases (eg, foot-and-mouth disease, classical swine fever), and additional pathogens that reduce efficiency and profitability, some of which may have zoonotic implications for human health (eg, influenza A viruses, *Streptococcus suis*, *Salmonella* serovars, *Trichinella* species, cysticercosis).<sup>2-5</sup>

Major epizootics of porcine high fever disease (PHFD) caused devastating losses to the Vietnamese swine sector in 2007-2010, impacting 53 of 63 provinces and resulting in more than 1,100,000 pigs destroyed in 2010 alone.<sup>6</sup> The principle agent suspected in these outbreaks was porcine reproductive and respiratory syndrome virus (PRRSV). Although PRRSV was clearly a major driver of the explosive outbreaks, experimental studies using a Vietnamese isolate of PRRSV failed to reproduce the severe clinical syndromes seen in the field,<sup>7</sup> suggesting possible co-infections or other co-factors contribut-

ing to the highly pathogenic phenotype. Among the agents suspected of involvement were porcine circovirus type 2 (PCV2), classical swine fever virus, and various bacterial agents (eg, *Pasteurella multocida*, *S suis*, *Mycoplasma hyopneumoniae*, *Haemophilus parasuis*, and *Actinobacillus pleuropneumoniae*).<sup>8</sup> During the PHFD outbreaks of 2007-2010, PRRSV and PCV2 were detected in 80% and 90% of swine cases, respectively, submitted to the National Center for Veterinary Diagnostics, Hanoi, Vietnam.<sup>9</sup> During 2009-2011, approximately 60% of PHFD outbreaks were confirmed positive for PRRSV, while the remaining 40% were negative for PRRSV but positive for PCV2 or other co-infecting agents.

Influenza A viruses (IAVs) circulating in pigs are of particular concern for the Mekong Delta region due to the endemic circulation of highly pathogenic avian influenza (HPAI) within domestic poultry populations,<sup>10</sup> the frequency of mixed rearing of pigs and poultry in backyard farming operations,<sup>11</sup> and the potential role of swine in the emergence of avian-swine-human reassortant viruses.<sup>12</sup> Data from the Mekong Delta suggest that all three major lineages of IAVs in swine (classical swine H1N1, Eurasian avian-like swine H1N1, and North American triple reassortant viruses) co-circulate.<sup>13</sup> Although neither HPAI H5N1 nor low pathogenic avian influenza viruses have been isolated yet from pigs in Vietnam, a novel human-swine reassortant H3N2 was detected in Vietnamese pigs in 2010.<sup>5</sup> Studies of IAV in Vietnamese pigs have shown significant geographic variability in seroprevalence,<sup>14</sup>

from very low levels of circulation (3.1% positive) in semi-commercial farms in a remote northern province<sup>15</sup> to 65% seropositive in intensive farms of the Red River Delta.<sup>16</sup>

Despite the critical imperatives for improved surveillance of swine diseases, the network for animal-disease reporting lacks resources, and veterinary laboratory diagnostics are rarely available, hence few samples are submitted for confirmatory analyses. The lack of baseline prevalence data is due in part to the logistical and technical challenges of sampling animals from small backyard operations; among Vietnamese households raising pigs, approximately 91% have fewer than 10 pigs, and only 6% have more than 30 pigs.<sup>11</sup> Veterinary extension services are limited, and farmers are generally reluctant to restrain animals for collection of blood or nasal swabs. Oral fluids are a diagnostic specimen for detection of many human and veterinary pathogens, and are of increasing interest for routine surveillance activities.<sup>17-19</sup> To the authors' knowledge, oral-fluids-based surveillance has not been evaluated within the context of smallholder farming systems in Vietnam. We hypothesized that oral fluids would present a viable alternative to serum samples for routine surveillance and would assist in overcoming farmer reluctance to sampling, particularly of young piglets. We therefore evaluated the performance of individual and pen-based oral-fluids diagnostics for three of the most important porcine respiratory viruses, PRRSV, PCV2, and IAV, in a province of the Mekong Delta that had previously experienced outbreaks of PHFD.

## Materials and methods

The survey did not require ethical review because the activities comprised part of periodic routine postvaccination monitoring, did not involve animal experimentation, and were implemented by the relevant animal-health authorities of the province.

The survey was implemented by the Sub-department of Animal Health (SDAH) of Can Tho province within the context of periodic routine postvaccination monitoring. The survey was carried out in September 2011 in the Can Tho province of Southern Vietnam, located between latitudes 9°55'08" and 10°19'38" north and longitudes 105°13'38" and 105°50'35" east. With an area of 1409 km<sup>2</sup>, the province is home to approximately 1.2 million people and 5343 pig farms with approximately 126,000 pigs (2011 agricultural census). The province has a total of nine districts and 85 communes. Farms were selected at random (using coin toss and census lists of registered farms) from 21 communes within the eight districts that had a history of confirmed porcine reproductive and respiratory syndrome (PRRS) in 2010 as determined by the SDAH in Can Tho. The number of farms sampled was proportional to the number of farms in the study communes. The study aimed to collect up to six oral-fluids samples and up to 12 blood samples per farm. Farmer consent was obtained with financial compensation, as per standard SDAH practice. Samples were collected from individually confined and group-penned animals. For individually confined animals (sows, boars), one oral-fluids sample was collected per animal. For group-penned pigs (weaners and growers up to 50 weeks old), pen-based oral fluids were collected. Blood samples were collected only from pigs that contributed to oral-fluids collections (ie, were observed to actively chew on ropes).

### Animal sampling method

The protocol for oral-fluids collection was first tested in a pilot study on a local farm. We selected locally produced, 100% cotton, 2-cm diameter woven rope, which was cut into 100-cm sections and unraveled for approximately 10 cm at one end. Ropes were tied to the railings of each pen, and pigs were allowed to chew for 20 minutes under continuous observation. The wet portion of the rope was inserted into a 1-litre re-sealable plastic bag and hand-wrung to extract the fluids; 2 mL was transferred to a cryovial and immediately flash frozen in a liquid nitrogen vapor-cooled

Dewar dry shipper (-140°C) to ensure optimal conditions for subsequent virological testing. After completion of oral-fluids collections, pigs that had actively chewed were restrained by rope, and 6 to 8 mL of whole blood was collected by jugular venipuncture. Serum separation, aliquoting, and transfer to temporary storage at -20°C were performed within approximately 6 hours of collection. All sample collections were transferred to -80°C within 1 week of collection. Sample identification enabled linkage between serum and oral-fluids samples.

### Sample processing

Serum samples were analyzed both individually and pooled for detection of viral pathogens. Pools were prepared by mixing 100 µL of each sample to reflect the same aggregates as the pen-based oral fluids. Nucleic acids (NA) were extracted from sera and oral fluids using 200 µL and the MagNA Pure 96 Viral NA small volume kit (Roche, Basel, Switzerland) and an automated extractor (Roche). Presence of polymerase chain reaction (PCR) inhibitors and NA quality control were assessed by spiking samples with an RNA internal extraction control (equine arterivirus) prior to extraction.<sup>20</sup> The total RNA recovered (60 µL in nuclease-free water) was stored at -80°C until use. Real-time reverse transcriptase PCR (RT-PCR) was performed using primers and probes described for PRRSV<sup>21</sup> and matrix gene of IAV,<sup>22</sup> using SuperScriptIII Platinum One-Step Quantitative kits (Invitrogen, Carlsbad, California) performed in a 25-µL reaction mix on a Chromo4 real-time PCR machine (Biorad, Hercules, California). Molecular screening for influenza was limited to oral-fluids samples, because IAV is not known to cause viremia in swine. Oral fluids positive for IAV by matrix gene PCR were further tested using primer pairs for a swine-specific influenza A nucleoprotein (NP) gene and hemagglutinin subtyping primers for A/H1N1/pdm09, human H3, and avian H5 lineages (current US Centers for Disease Control subtyping primers). Additional testing was subsequently performed using pan-hemagglutinin<sup>23</sup> and pan-neuraminidase<sup>24</sup> primers, a 2× PCR enzyme mix as described for oral-fluids optimization,<sup>25</sup> and products detected by conventional gel electrophoresis. Virus isolation for IAV-positive samples was attempted in embryonated chicken eggs (three eggs per sample) and concurrently for three serial passages in Madin-Darby canine kidney (MDCK)

cells.<sup>22</sup> For PCV2 detection, amplifications were performed using primers and probes<sup>26</sup> that had been used in previous studies in southern Vietnam.<sup>27</sup> The real-time PCV2 PCR was performed in a 25-µL format using TaqMan Universal PCR Master Mix (Applied Biosystems, Carlsbad, California) and Lightcycler480 (Roche).

PRRS virus antibody detection was performed on serum and oral fluids using the HerdChek PRRS X3 (Idexx Laboratories, Westbrook, Maine), designed to detect Chinese, European, and North American lineages of PRRSV. Serum samples were processed according to manufacturer's instructions, whereas oral-fluids processing was modified by decreasing the dilution (1:2 instead of 1:20) and using larger volumes (250 versus 100 µL) and longer incubation (16 hours versus 1 hour).<sup>28</sup> Influenza antibody detection was performed using Influenza A Antibody Test (Idexx Laboratories, Westbrook, Maine), and both serum samples and oral fluids were processed identically following the manufacturer's instructions.

### Statistical analyses

The interquartile range (IQR) of pigs per farm was calculated. Diagnostic yields (numbers of positives) of oral-fluids versus serum samples (representing the same sampled animals) were compared using McNemar's chi-square test; the kappa test was used to measure the level of agreement among tests.<sup>29</sup> The following benchmarks were used for interpretation of kappa test results: 0 to < 0.01 = poor; 0.01 to 0.20 = slight; 0.21 to 0.40 = fair; 0.41 to 0.60 = moderate; 0.61 to 0.80 = substantial; 0.81 to 1 = almost perfect. For PRRSV antibody detection, results from individual serum samples (N = 313), pooled serum samples (N = 84), individual oral-fluids samples (N = 40), and pooled oral-fluids samples (N = 84) were stratified by PRRSV vaccination status and history of disease compatible with PRRS on the farm (abortion in sows and respiratory signs in weaners and growers). All comparisons were made using the chi-square test. Analyses were carried out using R software within the EpiR package (<http://www.r-project.org/>). Comparisons were considered significant at  $P < .05$ .

## Results

### Farm characteristics and sample collection

A total of 68 farms from 21 communes in eight districts were sampled. Of the



68 farms surveyed, 25 (36.8%) were small-scale household farms with three to five sows; 30 (44.1%) were medium size (six to 20 sows); six (8.8%) were larger commercial units (> 20 sows); and seven (10.3%) raised only growers or finishers. The median total number of pigs (all ages) per farm was 24.5 (IQR 16.0 to 75.4), which is representative of the median farm size in the province. Twenty-three farms (33.8%) reported a history consistent with PRRSV infection (abortion in sows or respiratory signs in piglets), as determined by SDAH, and 36 (52.9%) reported vaccination against PRRSV over the past 12 months. Other diseases for which vaccination was carried out included classical swine fever (79.3% of farms); pasteurellosis (69.8% of farms); salmonellosis (67.2% of farms); and foot-and-mouth disease (56.7% of farms).

A total of 124 oral-fluids samples were collected. These corresponded to 40 animals individually penned (gilts, sows, and boars) and 84 animals in pens with ≥ 8 individuals (mostly weaners, growers, and some gilts, range eight to 15) (n = 84 groups). Upon initial exposure to the ropes, most pigs engaged in active chewing. One pen was sampled from 35 farms (51.5%); two pens were sampled from 18 farms (26.5%); three pens were sampled from 10 farms (14.7%); and four to six pens were sampled from five farms (7.4%). Blood was collected from a total of 313 pigs, which were the same animals (individuals or groups) observed chewing the ropes and from which oral fluids were collected (40 from individually penned animals and 273 from 84 pens with eight to 15 animals each).

### Virus detection by PCR in oral fluids and serum

Summary results for the tests performed in matched oral fluids and serum are presented in Table 1. Porcine circovirus type 2 DNA was detected in 54.8% and 61.3% of serum and oral-fluids samples, respectively, indicating that assay sensitivity did not differ significantly by specimen type (Table 2). Results of paired comparisons of oral-fluids and serum samples from individual pigs were more concordant (fair agreement) than those obtained with pooled oral-fluids and serum samples (Table 2). Estimates of overall farm-level, oral-fluids antibody prevalence for IAV and PRRSV did not differ (14.7%, 10 of 68 in each case); however, estimates for pathogen prevalence that were based on

serum antibody were significantly different (IAV 23.5%, 16 of 68) and (PRRSV 8.8%, six of 68) ( $P < .5$ ).

Influenza viral RNA was identified by matrix gene detection in one oral-fluids sample from penned growers (0.8%, one of 124). This sample was confirmed positive using NP primers designed to detect all contemporary swine influenza lineages, and primers for the HA of A/H1N1/pdm09. Virus isolation attempts in eggs and MDCK cells were unsuccessful and depleted the original sample volume. Subsequent attempts to generate amplicons from stored RNA extractions using pan-HA and pan-NA primers did not yield quality sequence reads, and above-threshold cycle threshold (Ct) values for internal RNA controls suggested poor sample quality.

All 124 oral-fluids samples tested negative for PRRSV by RT-PCR, whereas two serum samples tested positive (one pooled sample from a pen of growers with Ct value = 30 and one individually tested sow serum sample with Ct value = 24). The farm with a single pen of PRRSV-positive growers was

a relatively large operation (100 sows, total > 400 pigs) and reported prior use of PRRSV vaccine, although the farmer could not specify the manufacturer. These growers also tested positive for PRRSV antibody in the corresponding pooled oral-fluids sample, but not in the pooled serum sample. The PRRSV PCR-positive sow was from a household with two sows and six piglets, and the farmer reported no PRRSV vaccination. The sow tested negative by ELISA for PRRSV antibody in both oral-fluids and serum samples.

### Antibody detection by ELISA in oral fluids and serum

Antibody detection for IAV and PRRSV in oral-fluids versus serum samples is presented in Table 3. Overall, antibody testing for IAV was more sensitive for serum than for oral-fluids samples, and there was moderate agreement between the sample types. In individually tested pigs, there was a larger differential in antibody prevalence between serum and oral-fluids samples. For pooled samples, sensitivities of the sample types did not differ for IAV antibody detection. This

**Table 1:** Results of virological (PCR) and antibody prevalence testing (ELISA\*) for PCV2, IAV, and PRRSV in oral fluids and serum collected from pigs on 68 farms in Can Tho province, Vietnam, during 2011†

N	Virus testing (PCR)			Antibody testing	
	PCV2 No. pos (%)	IAV No. pos (%)	PRRSV No. pos (%)	IAV No. pos (%)	PRRSV No. pos (%)
<b>Oral-fluids samples</b>					
Individual	40	23 (57.5)	0 (0)	0 (0)	12 (30.0) 9 (22.5)
Pen-based	84	53 (63.1)	1 (1.2)	0 (0)	24 (28.6) 22 (26.2)
Total samples	124	76 (61.3)	1 (0.8)	0 (0)	36 (29.0) 31 (25.0)
<b>Serum samples</b>					
Individual	40	18 (45.0)	ND	1 (2.5)	22 (55.0) 23 (57.5)
Pen (pooled)	84	54 (64.3)	ND	1 (1.2)	25 (29.8) 19 (22.6)
Total samples	124	68 (54.8)	ND	2 (1.6)	47 (37.9) 42 (33.9)

\* HerdChek PRRS X3 ELISA (Idexx Laboratories, Westbrook, Maine). Serum samples were processed according to manufacturer's instructions. For oral fluids, dilution was 1:2 (versus 1:20), volume was 250 µL (versus 100 µL), and incubation time was 16 hours (versus 1 hour).

† For individually confined animals (gilts, sows, and boars), one oral-fluids sample was collected per animal. For group-penned pigs (weaners and growers up to 50 weeks old) pen-based oral fluids were collected (eight to 15 animals/pen). Blood samples were collected only from pigs that contributed to oral-fluids collections, ie, were observed actively chewing ropes.

PCR = polymerase chain reaction; ELISA = enzyme-linked immunosorbent assay; PCV2 = porcine circovirus type 2; IAV = influenza A virus; PRRSV = porcine reproductive and respiratory syndrome virus; pos = positive; ND = not done.

**Table 2:** Detection by PCR of PCV2 viral DNA from 124 oral fluids (OF) and pooled serum (S) samples from pigs surveyed in this study\*

	N	OF(+) PCV2 (%)	S(+) PCV2 (%)	OF(+) S(+)	OF(-) S(-)	OF(+) S(-)	OF(-) S(+)	McNemar		Kappa	
								$\chi^2$	P	Kappa	Level of agreement†
Individual samples	40	23 (57.5)	22 (55.0)	15	10	8	7	0	1	0.24	Fair
Pooled samples	84	53 (63.1)	46 (54.8)	29	14	24	17	0.88	.35	-0.001	Poor
All samples	124	76 (61.3)	68 (54.8)	44	24	32	24	0.87	.35	0.08	Slight

\* Study described in Table 1.

† For kappa test results: 0 to < 0.01 = poor; 0.01 to 0.20 = slight; 0.21 to 0.40 = fair; 0.41 to 0.60 = moderate; 0.61 to 0.80 = substantial; 0.81 to 1 = almost perfect. Comparisons were considered significant at  $P < .05$ .

PCR = polymerase chain reaction; PCV2 = porcine circovirus type 2; (+) = positive; (-) = negative.

**Table 3:** Detection of IAV and PRRSV antibodies in porcine oral fluids (OF) and serum (S) tested by commercial ELISA (HerdChek PRRS X3, Idexx Laboratories, Westbrook, Maine)\*

Antibody test	Sample type	No.	OF(+) IAV antibody (%)	S(+) IAV antibody (%)	OF(+) S(+)	OF(-) S(-)	OF(+) S(-)	OF(-) S(+)	McNemar		Kappa	
									$\chi^2$	P	Kappa	Level of agreement†
IAV	Individual samples	40	30.0	55.0	12	18	0	10	8.10	<.01	0.51	Moderate
	Pooled samples	84	28.6	29.8	16	51	8	9	0	1	0.51	Moderate
	All samples	124	29.0	37.9	28	69	8	19	3.70	.05	0.51	Moderate
PRRSV	Individual samples	40	22.5	57.5	8	16	1	15	10.56	<.001	0.26	Fair
	Pooled samples	84	22.6	27.4	10	52	9	13	0.49	.52	0.30	Fair
	All samples	124	22.6	37.1	18	68	10	28	7.60	.01	0.29	Fair

\* Pigs and sampling described in Table 1.

† For kappa test results: 0 to < 0.01 = poor; 0.01 to 0.20 = slight; 0.21 to 0.40 = fair; 0.41 to 0.60 = moderate; 0.61 to 0.80 = substantial; 0.81 to 1 = almost perfect. Comparisons were considered significant at  $P < .05$ .

IAV = influenza A virus; PRRSV = porcine reproductive and respiratory syndrome virus; ELISA = enzyme-linked immunosorbent assay; (+) = positive; (-) = negative.

pattern was similar for PRRSV antibody detection; prevalence of PRRSV antibody detection was greater in serum samples than in oral-fluids samples, and antibody prevalence was greater when individual samples were tested rather than pools. There was fair to moderate agreement between oral-fluids samples and serum samples in all cases.

### PRRSV ELISA testing results by age, vaccination status, and history of disease on farms

Comprehensive ELISA testing of the 313 individual serum samples yielded an overall

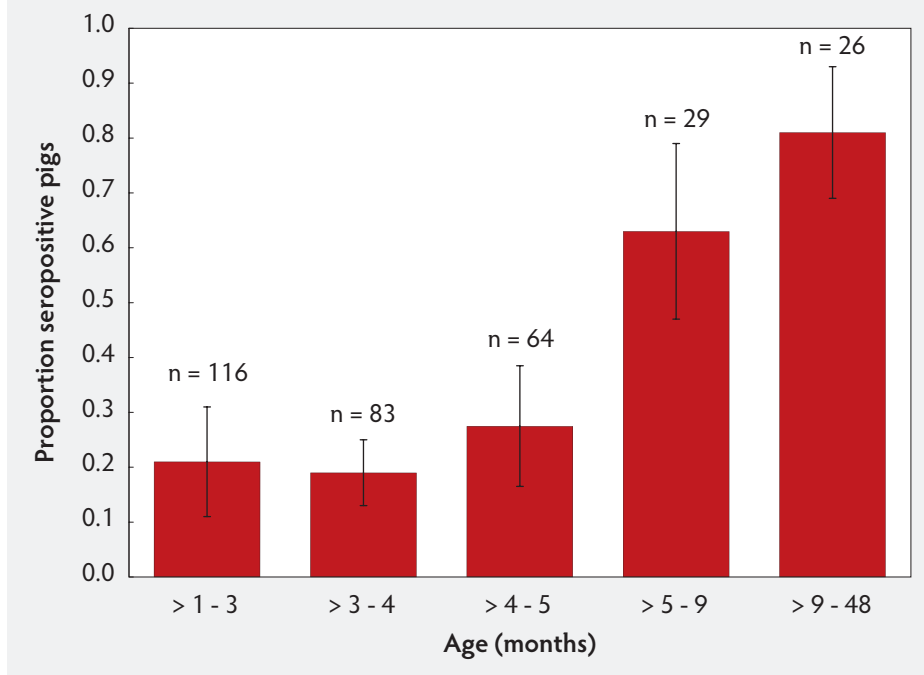
PRRSV seropositivity of 29.1% (24.0% to 34.1%). Older pigs had a greater probability of testing seropositive (Figure 1). Overall PRRSV seropositivity in vaccinated versus unvaccinated farms was 48.6% (17 of 35) and 12.1% (four of 33), respectively. The highest rates of PRRSV seropositivity were found among farms that had vaccinated for PRRSV and had a history of PRRSV disease (67.6%; 95% CI, 68.4%-40.0%), and the lowest for farms with no history of PRRSV or vaccination (6.7%; 95% CI, 1.9%-11.4%;  $P < .001$ ,  $\chi^2$ ). No statistical differences in

rate of seropositivity were observed between samples from unvaccinated farms with and without history of PRRSV disease (8.7% versus 6.7%;  $P = .99$ ,  $\chi^2$ ) (Figure 2). Pooled oral-fluids samples from unvaccinated farms with no history of PRRSV had an unusually high prevalence of seropositivity (26.9%; 95% CI, 9.9%-44.0%).

### Discussion

Our virological and serological analyses confirm endemic co-circulation of PRRSV, PCV2, and IAV within one southern

**Figure 1:** Porcine reproductive and respiratory syndrome virus (PRRSV) antibody detection by ELISA was conducted on individual pig serum samples collected from small farms in Can Tho province, Vietnam, in 2011, as part of routine post-PRRSV vaccination monitoring. Serum samples were stratified by age in months (N = 313). Samples were tested using the HerdChek PRRS 3X ELISA (Idexx Laboratories, Westbrook, Maine). The trend of increasing proportion of PRRSV seropositivity with age was significant ( $P < .05$ : chi-square).



province of Vietnam. We report low levels of PRRSV viremia and IAV shedding in oral fluids, and high levels of both viremia and shedding in oral fluids for PCV2. Antibody detection was more sensitive in serum samples than in oral fluids for both IAV and PRRSV, and there was fair to moderate concordance between the two sample types. Regarding diagnostic efficacy for molecular screening, oral-fluids samples yielded promising results for PCV2 and IAV, but no detections of PRRSV. Detection of PCV2 viral DNA was comparable in oral-fluids and serum samples. In the older pigs, PCV2 was detected significantly more often in oral-fluids samples than in serum samples, indicating prolonged shedding of PCV2 from the respiratory tracts of mature pigs (in contrast to resolution of systemic viremia).

Since our study implementation and sample processing, a number of published investigations have highlighted the need to specifically tailor diagnostic assays for the oral-fluid matrix<sup>30-32</sup> and have thoroughly evaluated the use of oral fluids for monitoring herd health. Panyasing et al<sup>31</sup> document modifications to an influenza blocking NP ELISA similar to those described by Kittawornrat et al<sup>33</sup> for PRRS ELISA, with reportedly

better results; these modifications were not used in the present study. Our failure to find high concordance between assay results for oral fluids and serum for all three pathogens (in particular for PRRSV) are not consistent with the recent reports and may reflect important technical deficiencies in our sample processing. Our results may also reflect inherent variability or bias when evaluating diagnostic protocols using relatively small sample sizes or populations with overall low prevalence of viral shedding.

The oral-fluids screening for IAV yielded one positive (one of 124; 0.8%), and subtyping by PCR confirmed that the sample was positive for hemagglutinin of A/H1N1/pdm09. Because we were unable to confirm the partial HA or NA amplicons by sequencing and did not sequence internal gene fragments, it remains unclear whether the detected virus was similar to pH1N1 currently circulating in people, or was an independent lineage or mixed virus. We anticipate further reports from government swine-surveillance activities that will clarify the complex situation of co-circulating reassortant subtypes in the region. The fact that IAV virus isolation from oral fluids was not successful suggests the presence of virus-inactivating factors within saliva (such as IAV antibodies), dilution effects in

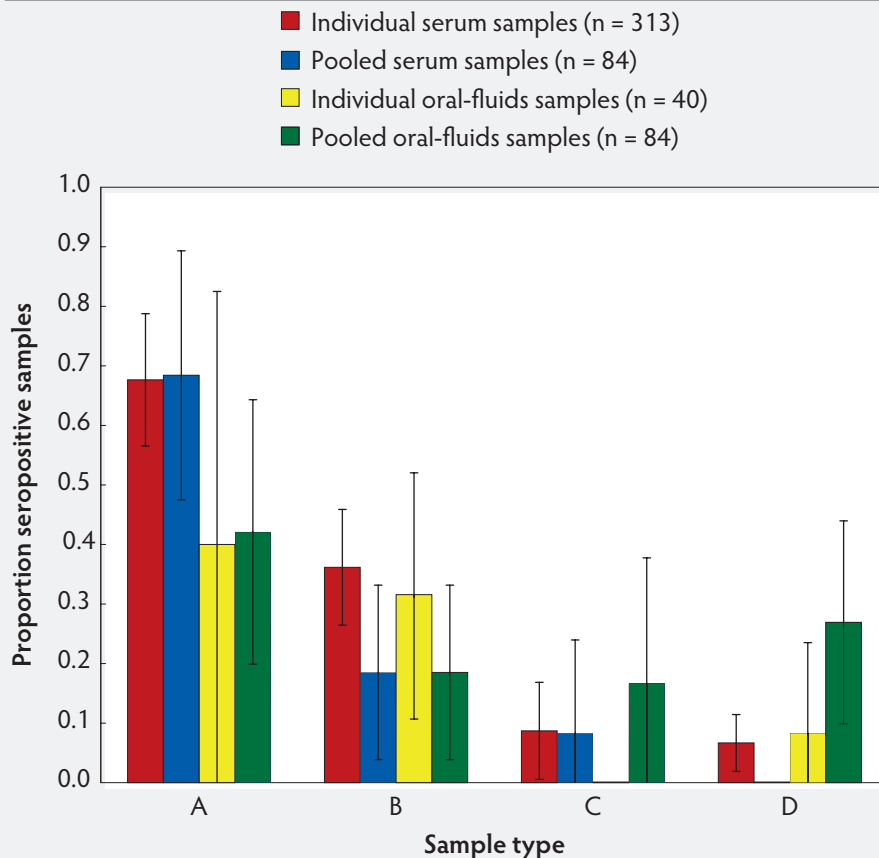
saliva, or sample degradation that impaired infectivity but did not entirely degrade RNA. Current swine surveillance programs continue to focus exclusively on use of nasal-swab specimens, and it remains to be seen whether optimization protocols for oral-fluids virus isolations will be accepted in the Vietnam context.

Conventional individual testing of serum samples by PRRSV ELISA revealed the expected age-dependent increase in PRRSV seropositivity, as well as a significant relationship between seropositivity, PRRS vaccination status, and history of PRRSV disease on farms. The observed PRRSV seropositivity in approximately 12% of unvaccinated farms that did not report PRRSV disease might reflect asymptomatic seroconversion to wild-type field virus, inaccuracy in reporting PRRSV vaccination status, secondary transmission of live attenuated vaccine virus, or all three. The JAX-1 vaccine (based on an attenuated virus of the highly pathogenic Chinese lineage) has been licensed for use in Vietnam since 2008, but was not used in Can Tho province during the time of the survey collections in 2011. The vaccines used on the survey farms at that time were commercial vaccines from Singapore, Germany, and Spain that were based on North American or European lineages of PRRSV, and would not have been detected by the RT-PCR used for screening. Thus, the two RT-PCR-positive detections from one sow and one pen of growers indicate asymptomatic infections with circulating wild-type virus.

Although the infrastructure and laboratory capacity for swine-disease surveillance in Vietnam is limited, government authorities regularly engage in vaccination campaigns for high-priority diseases, and postvaccination monitoring activities afford an opportunity to conduct cross-sectional surveys of viral prevalence. In general, field-based investigations face challenges in obtaining farmer consent for blood collection from pigs, particularly from piglets. Because oral-fluids collections are perceived as posing little or no risk to livestock health, large numbers of diagnostic samples can be easily collected at low cost by staff with limited animal-handling experience. It might be particularly productive to implement oral-fluids collections for case clusters of swine with clinical respiratory disease. We conclude that oral-fluids collection shows promise for future field research on respiratory porcine viruses in Vietnam. However, widespread



**Figure 2:** Prevalence of detection of antibody against porcine reproductive and respiratory syndrome virus (PRRSV) in individual animal serum samples (n = 313), pooled serum samples (n = 84), individual oral-fluids samples (n = 40), and pooled oral-fluids samples (n = 84) from pigs in small farms in Can Tho province, Vietnam, as described in Table 1. A = samples from farms with both history of PRRSV and PRRSV vaccination; B = samples from farms with PRRSV vaccination and no history of PRRSV; C = samples from farms with history of PRRSV and no PRRSV vaccination; D = samples from farms with neither history of PRRSV nor PRRSV vaccination. Samples were tested using the HerdChek PRRS 3X ELISA (Idexx Laboratories, Westbrook, Maine). Differences between A and D were significant ( $P < .001$ ; chi-square).



implementation will require standardization of field sampling techniques and careful adoption of optimized and validated diagnostic assays.

## Implications

- PRRSV, IAV, and PCV2 are endemic in swine farms of the Mekong Delta, with moderate levels of PRRSV and IAV transmission and nearly ubiquitous PCV2 circulation.
- Oral fluids provide comparable sensitivity to serum for molecular detection of PCV2.
- Oral-fluids screening can provide an acceptable surrogate for serum samples to estimate overall exposure to porcine respiratory viruses and may prove particularly useful in the context of developing countries.

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## Conflict of interest

None reported.

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# A qualitative study to identify potential biosecurity risks associated with feed delivery

C. Dewey, DVM, MSc, PhD; K. Bottoms, MSc; N. Carter, BA; K. Richardson

## Summary

**Objectives:** To identify management and operational functions, recommended by feed-company personnel and swine producers, that have the potential to decrease the risk of pathogens being transmitted among swine farms through movement of feed trucks.

**Materials and methods:** Focus groups and key-informant interviews were conducted with feed company representatives (21), including managers, dispatchers, and truck drivers, and also with swine producers (15). Questions explored biosecurity measures that would reduce risk of pathogen transmission at the farm, feed-company, and feed-truck

levels. Participants were asked to rate these biosecurity management changes by economic and logistic feasibility and likelihood of reducing pathogen transmission.

**Results:** The results provide an understanding of the roles of the farm, feed truck, and feed company in biosecurity management surrounding delivery of feed to swine farms and the need for education about how pathogens move among farms. Examples include pest control and truck washing, dispatching trucks according to farm disease status, drivers not entering the barn, reducing exposure of trucks to deadstock and manure, and educating all industry personnel.

**Implications:** All swine industry personnel must think about their roles in pathogen transmission associated with feed delivery and consider implementing changes and developing an industry standard that could reduce this risk. Veterinarians may take the responsibility of educating others in the industry about risks identified in the scientific literature that are associated with pathogen transmission. Biosecurity is everyone's concern: everyone has a role to play in reducing the potential risk.

**Keywords:** swine, biosecurity, feed delivery, qualitative research, focus groups

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## Resumen - Un estudio cualitativo para identificar los riesgos de bioseguridad potenciales asociados con la entrega de alimento

**Objetivos:** Identificar las funciones de manejo y operacionales identificadas por el personal de compañías de alimento y productores porcinos, que tienen el potencial de reducir el riesgo de transmisión de patógenos entre granjas porcinas a través del movimiento de camiones de alimento.

**Materiales y métodos:** Se realizaron entrevistas a informantes clave y grupos de enfoque con representantes de las compañías de alimento (21), incluyendo gerentes, despachadores, y conductores de camión, y también productores porcinos (15). Las preguntas exploraron medidas de bioseguridad que redujeran riesgos de transmisión de patógenos en la granja, la compañía de

alimento, y a nivel de camión de alimento. A los participantes se les pidió que calificaran estos cambios de manejo de bioseguridad por la viabilidad económica y logística y la posibilidad de reducir la transmisión de patógenos.

**Resultados:** Los resultados proveen un entendimiento del papel que juegan la granja, el camión de alimento, y la compañía de alimento en el manejo de la bioseguridad alrededor de la entrega de alimento a granjas porcinas y la necesidad de educación sobre la manera como los patógenos se mueven entre las granjas. Algunos ejemplos incluyen el control de pestes y lavado de camión, despacho de camiones de acuerdo al estatus de enfermedad de la granja, conductores que no entran al granero, reducción de exposición de camiones a animales muertos y excretas, y la educación de todo el personal de la industria.

**Implicaciones:** Todo el personal de la industria porcina deben pensar en su papel en la transmisión de patógenos asociados con la entrega de piensos y considerar la implementación de los cambios y el desarrollo de un estándar del sector que podrían reducir este riesgo. Los veterinarios pueden tomar la responsabilidad de educar a otros en la industria sobre los riesgos identificados en la literatura científica que están relacionados con la transmisión de patógenos. La bioseguridad es asunto de todos: todos tienen un papel que jugar en la reducción del riesgo potencial.

## Résumé - Étude qualitative pour identifier les risques potentiels de biosécurité associés à la livraison d'aliments

**Objectifs:** Identifier les activités opérationnelles et de gestion mentionnées par le personnel de compagnies d'alimentation et les producteurs de porcs qui ont le potentiel de diminuer le risque de transmission d'agents pathogènes entre les fermes porcines via les déplacements des camions de moulée.

**Matériels et méthodes:** Des groupes d'intérêt et des entrevues des intervenants clés ont été menés auprès de représentants de compagnie d'aliments (21), incluant des gérants, des répartiteurs, et des conducteurs

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de camion, de même que des producteurs de porcs (15). Les questions portaient sur les mesures de biosécurité qui réduiraient le risque de transmission d'agents pathogènes à la ferme, à la meunerie, et par les camions. On demandait aux participants de classer ces changements aux mesures de biosécurité en fonction de leur faisabilité logistique et financière et leur probabilité à réduire la transmission d'agents pathogènes.

**Résultats:** Les résultats fournissent une compréhension des rôles de la ferme, du camion de moulée, et de la meunerie dans la gestion de la biosécurité entourant la livraison de nourriture aux fermes porcines et le besoin d'éducation sur les modes de transmission des agents pathogènes entre les fermes. Citons par exemple, le contrôle de la vermine et le lavage des camions, la répartition des camions en fonction du statut sanitaire de la ferme, le conducteur de camion n'entrant pas dans les bâtiments, diminuer l'exposition des camions aux animaux morts et au fumier, et éduquer tout le personnel de la compagnie.

**Implications:** Tout le personnel de l'industrie porcine doit réfléchir à son rôle dans la transmission des agents pathogènes associée à la livraison de nourriture et considérer mettre en place des changements et développer des standards qui pourraient réduire ce risque. Les vétérinaires pourraient prendre la responsabilité d'éduquer les autres membres de l'industrie sur les risques identifiés dans la littérature scientifique qui sont associés avec la transmission des agents pathogènes. La biosécurité concerne tous les intervenants et tous ont un rôle à jouer dans la réduction des risques potentiels.

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**B**iosecurity protocols are important in reducing the introduction and transmission of pathogens among swine farms.<sup>1</sup> In the North American swine industry, biosecurity protocols are essential to ensuring market stability, maintaining export opportunities, and minimizing public health concerns related to foodborne illness.<sup>2</sup> Some pathogens affecting swine can be transmitted by contaminated clothing, shoes, equipment, and vehicles, and in contaminated feed.<sup>1,2</sup> Previous research has shown that delivery of feed has the potential to be involved in the transmission of disease among swine farms.<sup>3</sup> Although we are unaware of literature directly linking an outbreak to feed delivery, research in other

areas has shown there is a risk related to contaminated feed itself, as well as to contaminated trucks, tires, boots, clothing, and other fomites.

Salmonellosis, a common cause of foodborne illness in Canada, is the second most common cause of bacterial foodborne illness in the United States.<sup>4-6</sup> Infection causes gastrointestinal illness in humans, and severe illness and even death can occur in vulnerable individuals.<sup>7</sup> Swine can act as asymptomatic carriers.<sup>8-13</sup> Salmonellae have been isolated from pigs,<sup>14,15</sup> boots,<sup>15</sup> flies,<sup>14,15</sup> rodents,<sup>14-17</sup> bird feces,<sup>15</sup> feed,<sup>3,17</sup> and feed-ingredient samples<sup>3,17</sup> on swine farms. In one study, salmonellae were isolated from 2.8% of on-farm feed and feed-ingredient samples, and from 46.7% of swine farms.<sup>17</sup> In another study, salmonellae were present on 22.7% of feed trucks sampled, either in the grain box or in the feed itself.<sup>3</sup> The authors concluded that feed trucks could serve as a source of *Salmonella* organisms, and recommended that trucks be washed and disinfected between loads. Pigs fed a diet contaminated with *Salmonella* can become infected but remain clinically healthy.<sup>9,18</sup>

Porcine reproductive and respiratory syndrome (PRRS) is one of the most widespread and economically important diseases in the North American swine industry.<sup>19,20</sup> Infection causes reproductive failure in sows, morbidity and mortality at multiple production stages, and large production and economic losses.<sup>20</sup> Replacement animals and semen are the most common sources of PRRS virus (PRRSV) transmission, although vehicles, fomites, aerosols, and insects also play a role.<sup>20</sup> People act as mechanical vectors for PRRSV; viral RNA has been detected on coveralls, boots, and other fomites after contact with infected pigs.<sup>21</sup> Furthermore, workers who contact infected pigs can transmit the virus when they enter a population of susceptible pigs without changing boots and coveralls or washing their hands.<sup>21,22</sup> Basic sanitation protocols limit the transmission of PRRSV.<sup>22</sup> Additional research showed that PRRSV could be introduced to a swine facility after an inoculated carrier (snow and water or soil samples) was affixed to the vehicle's wheel well, and the virus was subsequently introduced at the barn's entrance.<sup>23,24</sup> Additionally, a PRRSV-positive herd status has been significantly associated with the feed truck visiting another herd without being washed prior to arrival.<sup>25</sup>

A variety of other pathogens can be moved from one farm to another on fomites such as boots, clothing, hands, and vehicles. These include *Brachyspira hyodysenteriae*,<sup>26</sup> transmissible gastroenteritis virus (TGEV),<sup>27,28</sup> *Lawsonia intracellularis*,<sup>29</sup> and *Escherichia coli*.<sup>30,31</sup> This information highlights the potential risk associated with feed delivery via contaminated fomites such as hands, boots, and coveralls, as well as the feed and feed trucks themselves.

In order to investigate these issues in more detail, a qualitative approach was used. Qualitative methods provide an added dimension to research because they allow investigators to identify and explore the issues important to the study population.<sup>32</sup> Qualitative research uses methods such as focus groups and key-informant interviews to gather participants' knowledge, lived experiences, and perspectives.<sup>32-34</sup> Qualitative methods also aid researchers in understanding the issues and context surrounding a subject.<sup>32</sup> Such methods produce relevant results applicable to the study population.<sup>33</sup> This study used focus groups and key-informant interviews to gather information about biosecurity best practices in the feed industry and to explore concerns surrounding delivery of feed to swine farms. In order to understand a variety of perspectives, discussions included feed-company managers, dispatchers, and truck drivers, and swine producers. The discussions were used to identify the biosecurity protocols currently in place regarding delivery of feed and to determine the changes participants thought could be implemented to further reduce the risk of disease transmission associated with feed delivery. The first objective of this study was to summarize the key management and operational functions identified by feed-company personnel and swine producers as having the potential to affect the risk of disease transmission among swine farms. The second objective was to have participants identify economically and logistically feasible operational approaches that are expected to reduce the potential risk of disease transmission.

## Materials and methods

This study received approval from the University of Guelph Research Ethics Board.

## Study participants

The study consisted of three focus groups and 18 key-informant interviews, and participants

included feed-company personnel and swine producers. Discussions were held during October through December, 2012.

Participants were recruited with assistance from the Ontario Agri Business Association (OABA) and Ontario Pork. Twelve feed companies were contacted through OABA, and managers from seven of these companies agreed to participate. Additionally, a representative from another feed company was recruited when a member of the research team made a presentation at a swine industry meeting. Managers from four of the eight feed companies were asked to participate further by allowing the researchers to contact some of their employees (drivers, dispatchers, sales personnel) and ask them to take part in key-informant interviews. Three of these four feed companies agreed and facilitated participation of their employees. Twenty-two swine producers were contacted through Ontario Pork and the University of Guelph: one did not respond, two declined to participate, three were not available during the proposed time frame, and one agreed to participate but did not attend their scheduled focus group.

The focus groups and key-informant interviews included a total of 21 feed-company personnel and 15 swine producers. The feed-company personnel represented eight Ontario feed companies and included eight managers, six feed-truck drivers, four dispatchers, one sales person, one production supervisor, and one customer-service representative. Each participant was provided with a letter that included background information about the importance of reducing pathogen transmission among swine farms, the reasons the research was focused on the feed industry, and the purpose and format of the study. Participants were informed that the discussions would be audio-taped (H2next Handy Recorder; Zoom, Japan) and professionally transcribed, and they agreed to keep the discussion confidential, signed a consent form, and received a \$50 gift card in compensation for their participation. Additionally, each producer who took part in the study was asked to provide information about their farm – what type of operation it was, how many sites it included, how many pigs it had, and how often bulk or bagged feed or both were received.

### Structure of focus groups and key-informant interviews

The focus groups and most of the key-informant interviews were facilitated by one of the

authors and observed by a second author. The observer led five of the 18 key-informant interviews. All of the focus-group and face-to-face key-informant interviews were held within 2 hours of Guelph. Some key-informant interviews were by telephone. Each focus group met once. The facilitator welcomed the participants and described the purpose of the project and the consent form. Then a series of standardized, open-ended questions were asked that were then followed by questions that encouraged participants to clarify and elaborate on their comments. Specifically, these questions asked about diseases considered to be among the three most important in the swine industry, participant knowledge about how diseases are transmitted from farm-to-farm, current biosecurity protocols at feed-company and farm levels, and changes that could be implemented to further reduce the potential risk of pathogens being transmitted during the delivery of feed.

### Rating of management ideas

The observer recorded management ideas that emerged during the discussion. After completion of all focus groups and key-informant interviews, the researchers collated the recommendations that emerged. This information was sent to all participants for whom e-mail addresses were available, including 18 feed-company personnel and 14 producers. Participants were asked to rate all recommendations on the basis of three criteria: their effectiveness for disease control, ease of implementation, and economic feasibility. On a scale of 1 to 5, a rating of 5 meant the idea was rated in a positive way (good for disease control, easy to implement, economically feasible) and a rating of 1 meant the idea was rated in a negative way (not good for disease control, hard to implement, not economically feasible). Responses were received from 25 of the possible 32 participants who were reached by e-mail. Not all 25 respondents ranked all of the management ideas, but 17 to 23 ratings were received for each idea.

### Transcript analysis

Transcripts from the focus groups and key-informant interviews were examined in order to identify the ideas, themes, and opinions expressed by participants. The researchers identified the swine diseases that the participants considered most important and summarized participant understanding of how diseases are transmitted from farm to

farm and whether diseases can be transmitted in the feed itself. Information was summarized to highlight current procedures at the feed-company, dispatcher, truck-driver, and farm or producer levels and the changes or improvements participants felt could be made. The researchers also noted the similarities and differences in the opinions and comments from feed-company personnel and producers.

## Results

### Producer information

The producers who participated in this study represented a variety of farm types and sizes. Eleven farms were farrow-to-finish and four were finisher only. Four producers had one-site operations, three had two-site operations, and seven had three-site operations. One producer did not indicate the number of sites. Eleven producers had sows, with a mean of 1355 (standard deviation [SD] = 1261), a minimum of 120, and a maximum of 4500 sows. Ten producers had nursery pigs, with a mean of 12,120 (SD = 15,310), a minimum of 300, and a maximum of 45,000 pigs. Fourteen producers had finisher pigs, with a mean of 16,282 (SD = 21,146), a minimum of 400, and a maximum of 75,000 pigs. All producers received bulk feed on a regular basis, and 10 received bagged feed on a regular basis.

### Important diseases and knowledge about how diseases are transmitted

Table 1 shows the pathogens that cause the diseases identified by participants as being among the three most important in swine production. Some participants listed only one or two diseases. Four participants (two managers, one driver, and one producer) included pneumonia; four (one manager, two drivers, and one producer) included scours; and two drivers were not familiar with any specific pathogens or disease problems in pigs. Table 2 presents participant responses when asked how diseases spread from farm to farm. Mechanisms by which participants thought pathogens could be transmitted in the feed itself included birds, rodents, fomites, trucks, raw ingredients, bulk pipe hoses, and people. Personnel of all types indicated that salmonellae could be transmitted in the feed. Other pathogens mentioned included transmissible gastroenteritis virus, PRRSV, *E. coli*, influenza A virus, and *B. hyodysenteriae*. This study was conducted before porcine epidemic diarrhea was a clinical problem in US and Canadian swine herds.

**Table 1:** Pathogens that cause diseases identified during focus-group discussions and key-informant interviews as being among the three most important in swine production\*

Pathogens that cause the diseases	Managers	Dispatchers, sales personnel, other employees	Drivers	Producers
<i>Actinobacillus pleuropneumoniae</i>	Yes	Yes	No	Yes
<i>Brachyspira hyodysenteriae</i>	Yes	No	No	Yes
Foot-and-mouth disease virus	Yes	No	Yes	No
<i>Haemophilus parasuis</i>	No	No	Yes	Yes
Influenza A virus	Yes	Yes	Yes	Yes
<i>Lawsonia intracellularis</i>	Yes	No	No	Yes
<i>Mycoplasma hyopneumoniae</i>	Yes	No	No	Yes
Porcine circovirus type 2	Yes	Yes	Yes	Yes
Porcine reproductive and respiratory syndrome virus	Yes	Yes	Yes	Yes
<i>Salmonella enterica</i> serovars	Yes	Yes	Yes	Yes
<i>Streptococcus suis</i>	Yes	No	No	Yes
Transmissible gastroenteritis virus	Yes	Yes	Yes	Yes

\* Focus-group discussions and key-informant interviews with 15 swine producers and 21 feed-company employees (including managers, dispatchers, and truck drivers) were conducted to examine the potential risks of disease transmission associated with feed delivery. Participants were asked about the important diseases in swine production. "Yes" response indicates that at least one person in that category mentioned the disease. "No" response indicates that no one in the category mentioned the disease. However, "no" cannot be interpreted as participants being unaware of the disease.

### Currently implemented protocols and related issues

The following information summarizes by topic the key points of discussion regarding biosecurity protocols.

**Feed mill.** All mills represented were certified under the Hazard Analysis and Critical Control Points system and follow a program that includes, among other things, regular testing of raw ingredients for *Salmonella*, collection of drag swabs from locations around the mill and testing for *Salmonella*, keeping the mill and the equipment clean, and pest control. However, rodent and bird control were identified as challenges for some mills.

Trucks delivering ingredients to the mill are inspected for cleanliness and asked to

declare their previous load. Suppliers are asked what programs they have in place to ensure the quality of their product; incoming product is rejected if mill personnel feel it has been compromised.

Traffic control at the mill is a concern – the mill's own feed trucks, supplier trucks, and customer trucks all enter the mill yard. There is little control over traffic and little knowledge about where incoming vehicles have been; feed-mill personnel expressed concern that such vehicles could be bringing pathogens on-site.

At most mills, personnel ask visitors and contractors where they have been and take note of what visitors are wearing. Some people are allowed in the mill for tours, although producers and drivers are generally kept out of production areas. However, some

producers felt there should be more control over where visitors are allowed to go. Some mills are quite strict regarding visitor traffic, whereas others are more relaxed and have few restrictions.

Employees are trained on basic hygiene and biosecurity. Drivers are encouraged to wash their hands when they come into the mill and to generally stay clean, although compliance is variable.

In the past, empty feed bags were returned to the mill and refilled; however, bags are no longer reused due to biosecurity concerns. Some mills do not accept returned feed at all, and some specifically do not accept returns from swine farms. Some companies have incorporated micro-bin systems to reduce handling of feed and feed ingredients.

**Feed.** Generally, incoming ingredients and some batches of finished feed are sampled and tested for *Salmonella* and mycotoxins. High temperature and steam during the pelleting process are thought to reduce pathogen loads in the finished product. However, mash feed is not heat treated and therefore presents a greater risk than pelleted feed. Some feed mills installed netting to try to keep birds out of the loading area, but that intervention has not worked well.

**Dispatcher.** It is difficult to manage scheduling when feed is ordered without sufficient notice. The dispatcher's best tool is advance orders, as last-minute deliveries are difficult to incorporate into an existing schedule. Thus, delivery of the feed, rather than biosecurity, may be the dispatcher's first priority. Feed mills designate certain farms and production systems as "high-biosecurity," but the criteria for defining farms as such are not entirely clear. The dispatcher tries to accommodate producers with a pyramid in mind. Sow breeders and multiplier herds are at the top of the pyramid and will receive feed at the beginning of the week. Next will be nursery barns, and finishers generally receive feed at the end of the week. Dispatchers also try to schedule deliveries to high-biosecurity, high-health farms first, and low-biosecurity, low-health farms last. Breeding sites generally have priority over commercial farms because of the way an outbreak would affect the industry as a whole. This pyramid structure occurs at most, but not all, feed companies. Delivery sequence for some mills is based primarily on location and convenience. Such routes are generally



**Table 2:** Participant knowledge of ways that diseases can be transmitted among farms\*

Means of disease transmission	Managers	Dispatchers, sales personnel, other employees	Drivers	Producers
Aerosolization	Yes	Yes	Yes	Yes
Birds	Yes	Yes	Yes	Yes
Deadstock trucks	Yes	No	No	Yes
Delivery of bagged feed	Yes	No	Yes	Yes
Direct pig-to-pig contact	Yes	Yes	Yes	Yes
Equipment	Yes	No	No	Yes
Feed	Yes	Yes	Yes	Yes
Feed sales personnel	No	No	No	Yes
Feed trucks	Yes	Yes	Yes	Yes
Fomites	Yes	Yes	Yes	Yes
Hoses for delivery of bulk feed	No	No	Yes	Yes
Improper deadstock management	Yes	No	No	No
Livestock trucks	Yes	Yes	Yes	Yes
Manure and spreading equipment	Yes	No	Yes	Yes
People	Yes	Yes	Yes	Yes
Rodents	Yes	Yes	Yes	Yes
Service people (electricians)	No	Yes	No	Yes
Supplies (veterinary, semen)	Yes	No	No	Yes
Traffic routes	Yes	No	Yes	Yes
Veterinarians and the clinic	No	Yes	Yes	Yes

\* Study described in Table 1. Questions were asked about the transmission of disease and whether it can be transmitted via the listed methods. "Yes" response indicates that at least one person in that category mentioned this route of disease transmission. "No" response indicates that no one in the category mentioned this route of disease transmission. However, "no" cannot be interpreted as participants being unaware that disease could be transmitted this way.

planned according to the most economical way for the feed to be delivered. The dispatcher's information about disease status sometimes comes from the producer, but also from sales personnel, the veterinarian, a neighbour, or from driver observations. One feed-company representative stated that producers tend to be open to sharing information if their farm is "clean," but less so if their farm has problems with disease. Feed-company personnel stated that such information is shared only when it is something really critical. The feed company may realize that there has been an outbreak on a particular farm only if the producer orders medicated feed or if the truck driver notices there is more deadstock than usual. In case of a known outbreak on a farm, the dispatcher schedules delivery to that farm for the end of the day, and the truck is washed immediately afterwards. The driver is advised to spray the truck tires with disinfectant on the way into the farm and on the way out. If feed is being delivered to a neighbour of a farm with a known outbreak, the route is changed to avoid having the truck pass the infected farm. Producers often request delivery of their feed first thing on Monday morning with a clean truck; however, mills have limited resources and it is not possible to provide this service for everyone.

**Feed trucks.** There is a general move towards using tanker trucks instead of box trucks, with augers being preferred over blowers. All participants agreed that box trucks present the highest biosecurity risk, followed by tanker trucks that blow feed into the bins, then by tanker trucks that auger the feed. Box trucks are considered the highest risk because the driver has to get in and out of the feed compartment in order to move dividers and sweep out feed. In this way, the inside of the compartment and even the feed itself could become contaminated. Tanker trucks allow the feed to be loaded and unloaded without being touched. Auger trucks in particular minimize the contact required between the feed bin and the truck. The whole fleet is generally washed every weekend using high-pressure hoses with hot water and soap. For some companies, the soap includes a disinfectant. Participants were aware that if a truck does not dry completely after being washed, moisture can promote the growth of some pathogens. Some companies have their own washing facilities, while others use a commercial truck wash. Trucks are often washed more

than once per week, for example, if a customer requests a clean truck, if a sow herd or other high-biosecurity herd needs feed mid-week, or if the driver visits a farm that is considered dirty and high-risk or that is positive for a specific pathogen. Generally, producers felt that drivers take pride in keeping their trucks clean, but that trucks should be washed more often. Feed-company personnel and producers acknowledged that it is not practical to wash the trucks between farms or even every day. Some trucks have onboard disinfectant sprayers. For the others, the driver has a hand-held disinfectant canister. It was once common practice for the driver to spray truck tires prior to entering every swine site. Generally, this is now done only at producer request, but some companies still spray regularly, especially at sow farms. Disinfecting tires was discussed at length. Most participants realized that the contact time is probably insufficient to kill pathogens, and that the disinfectant will not be effective if there is organic material on the tires. The practice of disinfecting tires is generally viewed as being cosmetic by both feed-company personnel and producers. Producers expressed concern that although trucks might be cleaned prior to being loaded, they then enter a high-traffic loading bay that is rarely washed or disinfected.

**Driver.** There is an important link between the driver and the dispatcher – the driver talks to producers, makes on-farm observations, and can relay this information to the dispatcher. Drivers usually receive biosecurity training. They are instructed on cleanliness of their hands and footwear and the inside of the truck's cab. Drivers try their best to keep a tidy truck and to keep themselves clean. At some feed mills, they are provided with disinfectant spray for the floor mats, pedals, and steering wheel, multiple sets of gloves, and disposable plastic boots. They are often instructed to stay away from the barn, to avoid going inside the barn, and to use a shovel if the feed needs to be moved, instead of using their hands or feet. Disposable plastic boots are worn by the drivers if the producer makes that request, or if the farm is considered high-risk. All participants seemed to understand the importance of such protection, but there was concern about the risk to the driver, expressed by both the feed-company personnel and producers, because these plastic boots are slippery, too big, and easily ripped. The boots are especially dangerous in the winter and have resulted in several workplace accidents. The general feeling was that

disposable plastic boots don't work well and something different needs to be investigated. Drivers are provided with several pairs of rubber or leather gloves or both. They try to keep them clean and dry, but they are used at multiple farms. The producers felt that wearing the same pair of gloves at multiple farms is a biosecurity concern, and that the drivers should be provided with disposable gloves. This presents a challenge in the colder months, when drivers need warm gloves. Customer requests are listed electronically on the bill of lading. Generally, the driver complies with the producer's requests, even if that means going inside the barn.

**Bulk feed.** The biggest concern with delivery of bulk feed relates to the use of blow pipes, which are moved from farm to farm. Truck drivers are generally careful about placement of the pipe and attempt to avoid dragging it through mud or manure, but it can be challenging to keep clean.

**Bagged feed.** Bagged feed is considered a higher biosecurity risk than bulk because there is more personal contact with bagged feed. A bag could get stepped on or dropped on the ground by accident. Often, the driver is asked to enter a farm building in order to deliver bags. Producers do realize that bagged feed is a risk and try to limit the amount they order. Feed-company personnel expressed concern that many producers ask that bags be delivered right into the feed room. The producers we spoke with knew that having bags delivered to the feed room is a risk and were surprised to hear that some producers still asked drivers to deliver the bags into the barn. Alternatively, some producers ask that the bags be left on the loading chute from which pigs are shipped. This is a concern for the driver with respect to manure contamination. The ideal situation, according to feed-company personnel and producers, would be for bagged feed to be delivered to a separate building (a shed or garage). Then the producer would be responsible for taking the bags to the barn at a later time. Alternatively, bags could be unloaded truck-to-truck at the end of the laneway.

**Producer.** Biosecurity protocols vary extensively between farms. Producers often ask to be the first feed delivery of the day, not necessarily asking for a clean truck, but making the assumption that because it is first thing in the morning, the truck is clean. Producers need to communicate to the mill exactly what they want. Deadstock management was a real concern. Recently,

marked improvements have been made in the management of deadstock, and producers tend to locate the bins on the edge of the property. In some cases, however, the deadstock bin is located right next to the feed bin or the laneway. In this case, run-off from the bin has been observed draining across areas where the driver of the truck has to drive or walk. There is also concern about how deadstock are moved from the farm to the bin; deadstock may be dragged across high-traffic areas. Some of the drivers we spoke with complained of deadstock being left in inappropriate areas (such as near the feed bin or on the laneway). Producers are aware of these issues and feel that the ideal situation is for deadstock to be composted or incinerated on-site. The cleanliness of the feed bin area is important. Some producers have their feed bins fenced in, with the pipes extending outside the fence. This prevents the driver from getting close to the bin and crossing paths with the producer. Feed-company personnel expressed concern that last-minute feed orders disrupt the dispatcher's plan for the day and make sequencing, with respect to biosecurity, more challenging. Ideally, producers should plan their orders to decrease the frequency with which the feed company must deliver to the farm: less interaction means less risk for the producer. Bigger bins would mean less frequent deliveries. Occasionally, producers order more feed than there is room for in the bin and the driver needs to decide what to do with the extra feed. Producers were aware of these issues, but stated that unforeseen circumstances make inventory management challenging. Producers need to maintain clean, dry yards – this concern was expressed by both feed-company personnel and producers. The lane needs to be well drained so that trucks are not driving through mud, manure, or puddles. Some producers we spoke with were aware of this issue and make an effort to maintain a clean, dry yard without deadstock, straw, manure, or other debris. If producers are able to maintain a clean yard, people will respect it more. A cluttered or dirty yard may give the impression that the producer does not think biosecurity is important. Producers could try to schedule delivery of feed so that other traffic, such as livestock trucks, are not at the farm at the same time and that equipment used to spread manure is not being used at the same time feed is being delivered. In particular, drivers did not want to drive over manure that was spilled in the laneway or the yard.

## New management ideas

Management ideas discussed in the focus groups and key-informant interviews are summarized in tables 3 through 6. They are categorized according to the level of implementation: feed company (Table 3), dispatcher (Table 4), driver (Table 5), or producer (Table 6). Ideas are organized in descending order of average overall rating, and the average rankings for disease control, ease of implementation, and economic feasibility are provided.

## Discussion

The focus groups and key-informant interviews revealed that swine producers and feed-company personnel recognize the importance of biosecurity in ensuring a sustainable swine industry. Not only is biosecurity fundamental to economic sustainability, it is also important in maintaining freedom from disease that is key to swine productivity and to maintaining both local and export markets. Participants in this study discussed the many protocols they already have in place to reduce the potential biosecurity risk associated with delivery of feed. They stressed that biosecurity is a responsibility shared across all levels, and that everyone has a role to play in ensuring these protocols are carried out effectively.

Participants were first asked how diseases are transmitted from farm to farm. Managers and producers seemed to have the most knowledge about the different ways diseases can be transmitted. Participants from all groups were aware that contact between an infected animal and one that is susceptible to a pathogen is the most important route of transmission, but a variety of other means were mentioned (Table 2).<sup>1,35,36</sup> Participants were also asked to list specific diseases that could be transmitted in the feed, if they thought that was a possibility. A previous study showed that *Salmonella* can be transmitted in the feed,<sup>3</sup> and at least one person from management, drivers, and producers identified this as a possibility. Some participants also thought that PRRSV and TGEV could be transmitted in the feed itself; however, this is not supported by scientific evidence. This opinion highlights the importance of increased education for people in the industry so that feed-company personnel and swine producers understand which pathogens can be found in feed and which are not expected to be found in feed.

Participants in this study generated a large number of recommendations for protocols that could further reduce the risk of disease

transmission associated with delivery of feed. Some ideas that were highly rated in terms of disease control and economic and logistic feasibility are discussed here. One recurring theme was that visitor access should be restricted, both at the feed mill and at the farm. Studies have shown that boots can become contaminated with *Salmonella*,<sup>15</sup> and that boots, coveralls, and hands can become contaminated with PRRSV.<sup>21</sup> When they do not change their clothing or footwear after contacting infected pigs, people can act as mechanical vectors for a variety of pathogens, including *B. hyodysenteriae*,<sup>26</sup> *E. coli*,<sup>30</sup> TGEV,<sup>27</sup> and PRRSV.<sup>21</sup> These studies highlight the importance of restricting visitor access whenever possible. Specific recommendations from previous studies include the following: do not allow visitors to enter the bagged-feed storage area at the feed mill; ensure sales personnel visiting farms follow good biosecurity protocols (including showering in and changing coveralls); have producers provide a container so the driver can leave the bill of lading at the feed bin; and never ask or allow feed-company personnel to enter the barn.

Ensuring adequate pest control at the feed company was also rated highly by participants, and several studies have shown there is

**Table 3:** Average ratings for feed-company-level management changes to enhance biosecurity on a scale of 1 to 5\*

Recommendation	Rating category		
	Disease control	Ease of implementation	Economic feasibility
Pest control (rodents and birds)	4.44	4.67	4.44
Truck-washing facilities dedicated to feed trucks (not shared with livestock trucks)	4.67	3.89	3.76
Exclude visitors from the area where bagged feed is stored	4.00	4.09	4.22
Visitor sign-in book recording recent contact with livestock	3.71	3.94	4.41
Do not return skids or pallets to the mill	4.00	4.09	3.57
Maintain a central database for disease status on farms	4.26	3.48	3.83
Wash feed trucks more often (more than once per week)	4.35	3.61	3.39
Scoring system for farms based on production type and biosecurity measures to plan the sequencing of deliveries	4.06	3.29	4.00
Do not allow bulk or bagged product to be returned to the mill	4.22	3.57	3.39
Returned skids or pallets are washed, disinfected, and dried at the mill	4.22	3.04	3.13
Use preferred truck types: auger > blower > box	3.67	3.06	2.39
Purchase tankers with a side compartment dedicated to bagged feed	3.00	2.94	2.82
Wash and dry feed trucks daily in a heated bay located at the feed mill	4.32	2.09	1.82
Have one feed truck dedicated to high-health herds	4.04	2.17	1.74

\* Study described in Table 1. For each recommendation, each column represents the average rating of one of the three categories on a scale of 1 to 5, with 5 the most positive rating and 1 the least positive rating.



**Table 4:** Average rating for dispatcher-level management recommendations to enhance biosecurity on a scale of 1 to 5\*

Recommendation	Rating category		
	Disease control	Ease of implementation	Economic feasibility
Plan delivery route to visit high-health, high-biosecurity herds first and low-health, low-biosecurity herds last	4.65	3.74	4.00
Plan sequence of delivery for bagged feed, with high-health, high-biosecurity herds visited first	4.06	3.67	3.39
Sequence deliveries in the absence of disease, eg, sow herds first and finisher herds last; all-in, all-out first and continuous flow last	4.28	3.50	3.17
Give producer 45 minutes advance warning before the truck is scheduled to arrive; producer can then arrange to meet the driver on arrival	3.26	3.16	3.74
Plan deliveries within production systems so that one system can have a feed truck for the day	3.95	2.50	2.45

\* Study described in Table 1. For each recommendation, each column represents the average rating of one of the three categories on a scale of 1 to 5, with 5 the most positive rating and 1 the least positive rating.

a risk of rodents and birds transmitting disease. *Salmonella* serovars have been isolated from bird feces on swine farms.<sup>15</sup> Rodents have tested positive for a variety of pathogens that infect swine, including *Salmonella*,<sup>14,15</sup> *Bordetella bronchiseptica*,<sup>16</sup> *Pasteurella* species,<sup>16</sup> *E coli*,<sup>16</sup> *Campylobacter jejuni*,<sup>16</sup> *B hyodysenteriae*,<sup>16,37</sup> and rotavirus.<sup>16</sup> Rodents are not carriers of PRRSV.<sup>38,39</sup>

Throughout discussions with feed-company personnel and swine producers, the subject of deadstock management came up frequently, and the recommendation that producers keep deadstock properly contained was highly rated. Deadstock and run-off from carcasses may act as reservoirs for pathogens.<sup>40</sup> The use of truck-washing facilities dedicated to feed trucks and not shared with livestock trucks was rated highly among participants. Since livestock trucks have direct contact with animals, they are considered to be a bigger risk than feed trucks.

Participants also stressed the importance of planning the route for feed delivery so that high-health, high-biosecurity herds are visited first, and low-health, low-biosecurity herds are visited last. This aligns with recommendations made by the Food and Agriculture Organization (FAO) and the Canadian Swine Health Board, who advise that feed deliveries be made in the order of health status, with high-health farms being visited early in the week, and contaminated facilities being visited later in the week.<sup>1,41</sup> Additionally, the FAO recommends that nucleus herds receive deliveries after the truck has been properly decontaminated and has had 2 days of down time.<sup>1</sup>

Some ideas were rated highly in terms of disease control, but were generally considered difficult to implement because of their poor economic and logistic feasibility. These include washing and drying feed trucks daily, washing and disinfecting the blow pipe between farms, and having bagged feed delivered to a separate room so that it can be fumigated before entering the barn. Although these ideas may have a measurable impact on disease control, participants considered them too costly, too challenging, or both to implement in the current system.

Several management ideas were related to infrastructure challenges or more global-industry ideas that cannot be addressed on existing farms or in a short time-frame. Farm layout in particular was identified as an issue: farms that have not been designed to enhance biosecurity would require infrastructure changes. Several identified issues are important considerations when designing new farms. Firstly, a variety of traffic uses the same lane – manure equipment, livestock trucks, deadstock trucks, feed trucks, and service vehicles. Both feed-company personnel and producers expressed concern that the feed-truck driver does not know when other types of traffic were last on-farm or exactly where they drove. Ideally, there would be separate lanes for different types of traffic, but in many cases there is only one lane at each farm. This highlights the importance of scheduling pigs and manure movement separately from feed delivery. Secondly, the location of the feed bin is a concern. There were reports of feed bins located next to the

deadstock bin or compost pile, the load-out chute, the manure pit, or directly underneath exhaust fans. Producers realize that bin placement can be a biosecurity issue, but it would be challenging to relocate existing bins. Ideally, the bin would be located on the perimeter of the property and away from high-traffic areas.

Some broader themes identified include the need for increased communication, collaboration, education, and research. There is a need for increased communication between feed companies and producers, especially in terms of disease status. The feed company needs this information in order to make the best decisions regarding the sequence of deliveries. The producers we spoke with are aware of the importance of informing the feed company of an outbreak so that feed deliveries can be sequenced properly. Throughout our discussions, there was concern that government, academic, and industry organizations are approaching these issues independently. The industry would like to see more collaboration among the different sectors. Feed-company personnel also felt there should be more collaboration among commodity groups (swine, poultry, and cattle), since feed companies do not necessarily make that distinction in the delivery of feed. Both feed-company personnel and swine producers expressed interest in development of a set of minimum standards that everyone adheres to, with additional precautions to be taken in case of a disease outbreak. Finally, there is a need for science-based recommendations. Some participants felt

**Table 5:** Average rating for driver- or sales-personnel-level management recommendations to enhance biosecurity on a scale of 1 to 5\*

Recommendation	Rating category		
	Disease control	Ease of implementation	Economic feasibility
Ensure sales personnel follow good biosecurity protocols when calibrating the mill	4.48	4.83	4.87
Feed company personnel do not enter the barn	4.52	4.70	4.83
Increase driver education to understand why certain protocols must be followed	4.26	4.37	4.47
On tanker trucks, keep bagged feed compartment clean	4.11	4.47	4.26
Wash and disinfect floor mats regularly	4.09	4.23	4.27
Drivers report biosecurity incidents or observations to the feed company	3.71	4.00	4.29
Drivers wear disposable boots or clean reusable over-boots when leaving the cab of the vehicle	3.86	3.96	4.17
Multiple pairs of reusable boots available for drivers; clean, disinfect, and dry boots after on-farm use	3.86	2.83	3.09
Checklist of farm-specific biosecurity protocols for the driver, who signs off on all protocols	3.53	3.63	4.05
Bulk delivery trucks completely cleaned out before leaving a farm	3.56	3.79	3.72
Drivers wear a new pair of disposable gloves at each farm	3.45	3.48	3.67
Disinfect bag carts, trolleys, and loading ramps between loads	3.83	3.13	3.30
Ensure the blow pipe doesn't touch the ground or mud	3.61	2.78	3.48
Wash and disinfect the blow pipe between farms	3.64	2.13	2.65
For tanker and box trucks: install a coarse mesh so that the driver cannot enter the feed compartment	3.18	2.17	2.50
Wash sales-personnel vehicles between farms	3.45	2.09	2.00
Removable slatted plastic floor inserts for box trucks that can be cleaned and disinfected	3.17	2.33	1.94

\* Study described in Table 1. For each recommendation, each column represents the average rating of one of the three categories on a scale of 1 to 5, with 5 the most positive rating and 1 the least positive rating.

that certain recommendations are based on marketing and are not necessarily backed by scientific evidence. The people we spoke with are generally happy to implement biosecurity protocols as necessary, but they need to know that scientific evidence supports these decisions. Additionally, education is important in ensuring that feed-truck drivers and producers understand the science behind biosecurity recommendations. If they understand the reasoning behind specific recommendations, they may be more likely to comply. Some of the drivers we spoke with expressed interest in having fact sheets outlining the diseases that are important in swine production, how they affect pigs, and how they are transmitted.

This work has provided valuable insight into participant knowledge and application of biosecurity protocols related to delivery of feed. It has increased awareness of this issue

among feed-industry personnel and swine producers. The qualitative, participatory approach utilized here was well received by participants. They appreciated that we wanted to know their thoughts and ideas about the issues and to obtain their input about what is important and what improvements might be feasible. The researchers have gained a much better understanding of the issues and the complexity involved with delivery of feed. Additionally, the focus-group approach facilitated sharing ideas and knowledge among participants and allowed them to learn from others in their field. An added benefit of the approach was that some producers had not thought about what they can do to prevent diseases from being picked up on their farm and moved elsewhere by a feed truck. Generally, their focus is to prevent pathogens from coming into their own farms. However, this expanded thinking is very important to the swine industry as a whole.

This study has some limitations, the biggest of which is selection bias – participants were recruited through a convenience sample selected by OABA and Ontario Pork. Feed-company personnel and producers who chose to participate may have done so because they already understood the importance of biosecurity. As a result, our sample may represent those who are already doing well in this area and may not include feed companies or producers who have fewer protocols in place.

This study has identified many important factors related to biosecurity and the surrounding issues. The next step is to determine the frequency with which certain practices are being implemented.

## Implications

- Biosecurity is a responsibility shared among all members of the industry, and

**Table 6:** Average rating for producer-level (farm-level) management recommendations to enhance biosecurity on a scale of 1 to 5\*

Recommendation	Rating category		
	Disease control	Ease of implementation	Economic feasibility
Provide a container where the driver can leave the mill order without going near the barn	4.32	4.95	5.00
Don't allow feed-company personnel to enter the barn for any reason	4.48	4.74	4.91
Contain deadstock in proper bins with lids	4.70	4.68	4.55
The producer shares the disease status of the farm, informing the feed company when the herd has a new outbreak	4.70	4.30	4.83
The driver never enters the barn to deliver bagged feed	4.52	4.30	4.83
1. Driver leaves the bags in a shed	4.26	4.26	4.21
2. Driver leaves the bags on a cart that staff pull inside the barn or feed room	3.41	3.94	4.22
3. Feed loaded into the barn from the outside via a chute	4.28	3.33	3.11
4. Bags off-loaded truck-to-truck at the end of the laneway	3.94	3.06	3.28
Area around the bottom of the feed bin is kept clean and tidy	4.00	4.57	4.83
Producer orders an appropriate amount of feed; no leftovers go back to the mill	3.95	4.41	4.77
Storage area for bagged feed is kept clean and tidy	3.84	4.47	4.74
Rodent control	4.25	4.15	4.30
Producers report biosecurity breeches to the mill; driver can be reminded of protocols	3.89	4.26	4.53
Signs indicate controlled access and restricted access zones (where to park, where not to go) and ensure compliance	4.00	4.21	4.42
Keep farm lane clean, dry, well drained; driver need not drive or walk through manure, mud, or run-off from the deadstock bin	4.65	3.91	4.00
Garbage (eg, gloves, disposable plastic boots) disposed of on-farm	4.13	4.13	4.17
Producer always washes hands prior to handling feed	3.47	4.26	4.42
Bagged feed stored off the floor	3.32	4.32	4.26
Producer plans timing of feed delivery; manure not being spread when feed truck arrives	4.22	3.35	4.27
Checklist of farm-specific biosecurity protocols for driver to sign to confirm they followed all protocols	3.26	3.79	4.42
Chain and a sign at the end of the laneway to remind driver about biosecurity	3.20	4.05	4.00
Pipes for delivery of feed are producer-owned and stay at each farm	3.68	4.11	3.37
Producer requests specific biosecurity protocols from feed company	3.78	3.43	3.70
Producers order bulk feed instead of bagged feed	3.67	3.61	3.44
Producer provides farm boots for the driver	3.27	3.36	4.00
Appropriate feed-bin placement (not near exhaust fans, deadstock, loading chute, manure pump-out, main barn entrance)	4.25	2.95	2.95
Use blow pipe extensions so driver need not get close to the feed bin or barn	3.17	3.33	3.28
Bagged feed delivered to a separate room or building so that it can be fumigated before being carried into the feed room	4.05	2.89	2.79
Locate feed bins at the edge of the property	3.94	1.72	1.61
Retrofit bins so that when feed is being delivered via an auger truck, the driver can open the bin remotely without leaving the cab	3.28	1.33	1.39

\* Study described in Table 1. For each recommendation, each column represents the average rating on a scale of 1 to 5, with 5 the most positive rating and 1 the least positive rating.



individuals of each sector need to work together to enhance biosecurity for the industry as a whole.

- There is diversity of opinion regarding the issues that are most important and the interventions that could be implemented in order to further decrease the risk of pathogen transmission associated with delivery of feed.
- The swine industry is willing to implement changes, but wants to know there is scientific evidence to support these changes.
- There is great interest in development of an industry standard for best practices related to the delivery of feed.
- There is a need for education concerning biosecurity issues, and veterinarians can play a role in this.

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## Conflict of interest

None reported

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\*Non-refereed references.



# CONVERSION TABLES

## Weights and measures conversions

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

## Temperature equivalents (approx)

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

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

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

## Conversion chart, kg to lb (approx)

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

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

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

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

# Fine-needle aspiration and cytology as an antemortem method for evaluating injection-site lesions

Charles E. Wiedmeyer, DVM, PhD, Diplomate ACVP; Thomas J. Fangman, DVM, MS, Diplomate ABVP; Kent Schwartz, DVM, MS; Brian Payne, DVM

## Summary

**Objectives:** To apply a fine-needle aspirate (FNA) technique to evaluate grossly visible injection-site reactions by cytologic examination and determine agreement with gross and histopathological findings.

**Materials and methods:** Two trials were conducted. In both, pigs were vaccinated with porcine circovirus type 2 vaccine at weaning and 17 days later. Seven days after the second vaccination, pigs with grossly visible injection-site lesions were selected (Trial 1, n = 40; Trial 2, n = 12). In Trial 1, pigs were manually restrained for the FNA procedure. In Trial 2, pigs were sedated and the FNA procedure was conducted using

two different-sized hypodermic needles (18-gauge and 22-gauge). After the FNA procedure, pigs were euthanized and the injection-site lesions and lymph nodes dissected and submitted for histopathologic interpretation. All cytologic preparations were examined by a board-certified veterinary clinical pathologist.

**Results:** In Trial 1, the cytologic interpretation of the samples was mild lymphocytic to mixed inflammation. Lesions were suggested to be the result of an immunologic response to the vaccine, not hemorrhage or abscess. In Trial 2, no differences were detected between preparations made with an 18-gauge or 22-gauge needle. Cytologic and

histological findings agreed, reporting low to moderate numbers of lymphocytes and macrophages, with low numbers of neutrophils, foreign material, and bacteria.

**Implications:** The FNA procedure described is a potential technique practitioners can utilize to characterize tissue-reaction lesions without the need for euthanasia or surgical biopsy.

**Keywords:** swine, antemortem, injection sites, fine-needle aspirate, cytology

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**Accepted:** February 11, 2014

## Resumen - La aspiración de aguja fina y la histología como un método antemortem para evaluar las lesiones en el sitio de la inyección

**Objetivos:** Utilizar la técnica de aspiración de aguja fina (FNA por sus siglas en inglés) para evaluar lesiones visibles por reacciones en el sitio de inyección por medio de la evaluación histológica y determinar la concordancia entre los hallazgos macro e histopatológicos.

**Materiales y métodos:** Se realizaron dos pruebas. En ambas, los cerdos fueron vacunados con una vacuna de coronavirus porcino tipo 2 al destete y 17 días después. Siete días después de la segunda vacunación, se seleccionaron los cerdos con lesiones visibles en el sitio de inyección (Prueba 1, n = 40; Prueba 2, n = 12). En la Prueba 1,

se contuvo manualmente a los cerdos para el procedimiento FNA. En la Prueba 2, se sedó a los cerdos y se realizó el procedimiento FNA utilizando dos agujas hipodérmicas de diferentes tamaños (calibre 18 y calibre 22). Después del procedimiento FNA, los cerdos fueron sacrificados y las lesiones del sitio de la inyección y los nódulos linfáticos fueron disecados y enviados para la interpretación histopatológica. Todas las preparaciones histológicas fueron examinadas por un patólogo clínico veterinario certificado.

**Resultados:** En la Prueba 1, la interpretación histológica de las muestras fue desde inflamación linfocítica ligera hasta mixta. Se sugirió que las lesiones eran resultado de una respuesta inmunológica a la vacuna, no había hemorragia ni absceso. En la Prueba 2, no se detectaron diferencias entre las prepara-

ciones hechas con aguja calibre 18 o calibre 22. Los hallazgos histológicos y citológicos concordaron, reportando bajos números a moderados de linfocitos y macrófagos, con números bajos de neutrófilos, material extraño, y bacterias.

**Implicaciones:** El procedimiento FNA descrito es una potencial técnica que los médicos pueden utilizar para caracterizar las lesiones de reacción del tejido sin la necesidad de hacer una eutanasia o biopsia quirúrgica.

## Résumé - Aspiration à l'aiguille fine et cytologie comme méthode ante-mortem pour évaluer les lésions aux sites d'injection

**Objectifs:** Utiliser une technique d'aspiration à l'aiguille fine (FNA) pour évaluer par examen cytologique les réactions aux sites d'injection visibles à l'œil nu et déterminer l'accord avec les trouvailles des examens macroscopiques et histopathologiques.

**Matériels et méthodes:** Deux essais ont été réalisés. Dans les deux, des porcs furent vaccinés avec le vaccin contre le coronavirus de type 2 au sevrage et 17 jours plus tard. Sept jours après la deuxième administration, les porcs avec des lésions visibles au site

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d'injection ont été sélectionnés (Essai 1, n = 40; Essai 2, n = 12). Dans l'Essai 1, les porcs étaient contentionnés manuellement pour la procédure de FNA. Dans l'Essai 2, les porcs étaient mis sous sédation et la procédure de FNA effectuée en utilisant des aiguilles hypodermiques de deux tailles différentes (18-gauge et 22-gauge). Suite à la procédure de FNA, les porcs étaient euthanasiés et les lésions aux sites d'injection ainsi que les nœuds lymphatiques furent disséqués et soumis pour examen histopathologique. Toutes les préparations cytologiques furent examinées par un pathologiste clinique vétérinaire certifié.

**Résultats:** Dans l'Essai 1, l'interprétation cytologique des échantillons était une légère inflammation lymphocytaire à mixte. Les lésions semblaient être le résultat d'une réponse immunologique au vaccin, et non une hémorragie ou un abcès. Dans l'Essai 2, aucune différence ne fut détectée entre la préparation faite avec l'aiguille 18-gauge ou l'aiguille 22-gauge. Les trouvailles cytologiques et histologiques concordaient, rapportant des quantités faibles ou modérées de lymphocytes et de macrophages, avec de faibles quantités de neutrophiles, de matériel étranger, et de bactéries.

**Implications:** La procédure de FNA décrite est une technique potentielle que les praticiens peuvent utiliser pour caractériser des lésions associées à des réactions tissulaires sans avoir à euthanasier l'animal ou prélever chirurgicalement une biopsie.

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**P**igs in commercial production units are routinely vaccinated to aid in prevention of a variety of diseases. Most vaccines used today are safe and efficacious and result in a very low incidence of complications. Occasionally, vaccination can cause adverse systemic reactions that may result in poor production or death. Additionally, local reactions can cause permanent tissue damage, resulting in undesirable carcass quality and economic losses.<sup>1</sup> Local tissue reactions that can occur are granulomatous or lymphocytic inflammation, hemorrhage (ie, hematoma), fibrosis, abscessation (sterile or septic), or a combination of these.<sup>1</sup> Each type of reaction has a characteristic cell and tissue architecture that may be discernible by cytologic examination. Defining the type of reaction is useful in order to institute prevention or control. Currently, there are no routinely utilized, simple techniques to characterize the type of local

tissue reaction present without pig sacrifice. This study describes the use of fine-needle aspirate and cytologic examination as an antemortem technique to characterize local vaccine reactions. The purpose of this study was to apply a fine-needle aspirate (FNA) technique to evaluate grossly visible injection-site reactions by cytologic examination and determine its agreement with gross and histopathologic findings. Additionally, the optimal needle size used to perform the aspirate was evaluated. By using cytology to characterize lesions, decisions regarding future vaccination management or protocols can be developed.

## Materials and methods

The studies were approved by the institutional animal care and use committees of Boehringer Ingelheim Vetmedica, Inc (Trial 1) and the Iowa State University (Trial 2).

Two trials were conducted. The first trial utilized 139 pigs housed in a commercial production unit. Pigs had received an intramuscular (IM) porcine circovirus type 2 vaccine administered at weaning (21 days of age) and a second dose 17 days later (38 days of age). Both injections were administered in the same anatomical location on the pig, the left cervical region. Seven days after the second dose, 69 pigs with grossly visible swelling at the injection site were identified. Of these, 40 pigs were conveniently selected and manually restrained for the FNA procedure. The lesion was manually isolated and punctured by inserting the needle into the lesion several times at different angles in order to obtain tissue within the needle and its hub ("woodpecker method") using a 1.5-inch, 22-gauge hypodermic needle (no syringe was attached). The cellular contents in the needle and hub were expelled onto a clean glass microscope slide by pushing 5 mL of air retained in a syringe through the hypodermic needle. To spread the cellular material onto the slide, another clean glass slide was placed on top of the material and the slides slowly slid apart as for a standard cytologic preparation. Both cytologic preparations were air dried and sent for examination by a board-certified veterinary clinical pathologist (CEW). After FNA acquisition, the pigs were returned to their pens.

Trial 2 was conducted to compare the cytologic findings obtained by FNA with gross and histopathologic findings. Additionally, the effect of needle size on cytologic findings was evaluated. In Trial 2, a population of 1495 pigs within a different commercial

production unit was utilized. Pigs had been subjected to the identical vaccination protocol using the same vaccine as in Trial 1. Forty percent of the pigs receiving this injection protocol demonstrated injection-site swellings. Twelve pigs with grossly visible lesions at the site of the vaccine (Figure 1) were selected by reaching into an affected pen and removing one pig from each of 12 pens. The selected pigs were then sedated with xylazine (2.0 mg per kg IM) and ketamine (20.0 mg per kg IM). Two separate cytologic samples were obtained per pig following the same FNA technique used in the first trial, but using two different-sized hypodermic needles (18-gauge and 22-gauge). The only difference between the FNA techniques utilized during Trial 2 versus Trial 1 was the addition of a sanitization step. The site chosen for FNA and approximately 1cm around the site was wiped with an alcohol-soaked gauze several times to remove surface fecal matter. The cytologic preparations were air-dried and sent for interpretation to a board-certified clinical pathologist. Following the FNA procedure, pigs were humanely euthanized. The injection-site lesion and regional lymph node were dissected from the neck area and submitted to Iowa State University Veterinary Diagnostic Laboratory for histopathologic interpretation by a diagnostic pathologist.

All cytology slides from both groups of animals were stained with a Wright-Giemsa stain and characterized for cellularity, cell populations present and their relative percentages, and the presence of organisms or foreign material. A final interpretation was determined, based on the following cytologic criteria. Granulomatous inflammation was characterized by a predominance of macrophages, lymphocytic inflammation by a predominance of lymphocytes. Hemorrhagic inflammation or hematoma was determined by the presence of macrophages containing hemosiderin or displaying erythrophagocytosis, and abscesses were defined by an infiltrate of degenerate or non-degenerate neutrophils with or without bacteria.<sup>2</sup> Fibrosis cannot be reliably identified by cytology, but a cytologic preparation with low cellularity may suggest fibrosis. Mixed inflammation is best characterized by an overlap of cell populations from each cytologic category. The sensitivity and specificity of cytologic compared to histopathologic findings was determined using a 2 × 2 table.

**Figure 1:** Pigs received an intramuscular porcine circovirus type 2 vaccine administered at weaning (21 days of age) and a second dose 17 days later (38 days of age). Both injections were administered in the left cervical region. Seven days after the second dose, pigs with a grossly visible swelling at the injection site were identified, as illustrated (Trial 2).



## Results

In Trial 1, at least one of the two cytologic samples taken per pig contained adequate material for interpretation. Nearly all of the samples were of low cellularity and displayed mostly small mature lymphocytes with lesser numbers of nondegenerate neutrophils and red blood cells. In the background, a mixed population of bacteria was noted. The cytologic interpretation of the samples with these findings was mild lymphocytic to mixed inflammation. The bacteria present were believed to be environmental contaminants, most likely from fecal material on the skin of the pig. On the basis of the cytologic findings, it was suggested that the vaccine reactions were a result of an immunologic response to the vaccine and not hemorrhage or abscess. Due to the low cellularity, fibrosis could not be completely ruled out. From this portion of the study, it was concluded that the FNA technique was adequate for cytologic characterization of the lesions (Figure 2). However, it was recommended that the site be lightly cleaned prior to the FNA procedure to avoid fecal contamination of the sample.

In Trial 2, no differences in cytologic quality were noted between the preparations made with an 18-gauge or 22-gauge needle. Cytologic findings from this group varied from granulomatous to mixed inflammatory response characterized by macrophages, small lymphocytes, and very low numbers of nondegenerate neutrophils. Histopathologic examination revealed subacute to severe mononuclear cell infiltrations, primarily macrophages with lesser numbers of lymphocytes, within muscle and connective tissue. Histopathologic examination also revealed that neutrophils, foreign material, or bacteria were not a prominent feature in any of the lesions examined. On the basis of these results, it was determined that the cytologic and histopathologic methods agreed. The 2 × 2 table analysis revealed a sensitivity of 100%; specificity could not be determined due to the lack of negative findings (ie, no cells observed on cytologic preparations or tissue for histopathologic examination) on both cytology and histopathology, respectively.

## Discussion

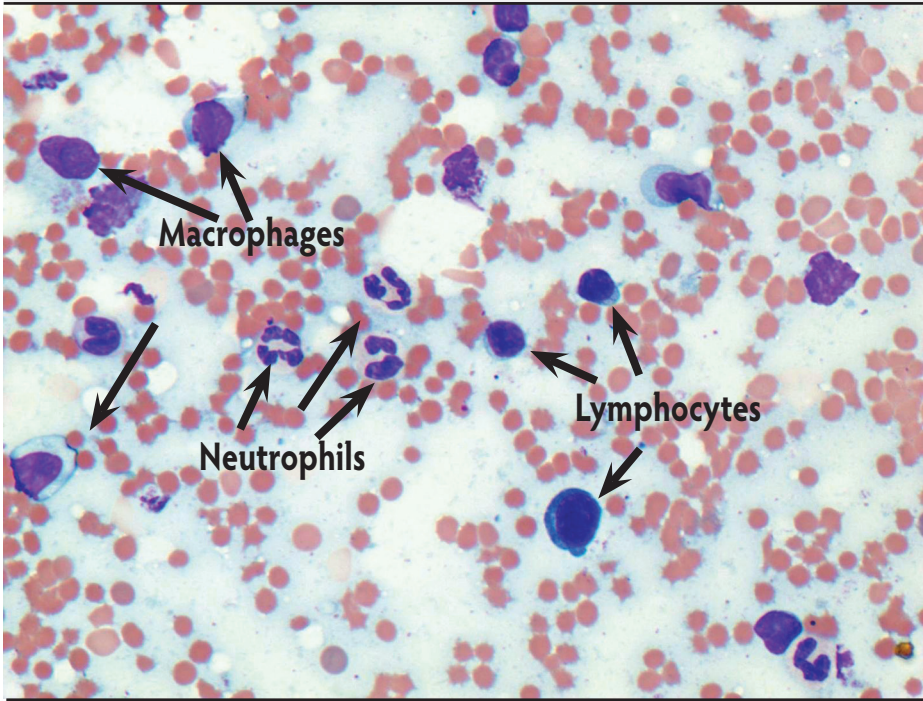
This study demonstrates that the FNA technique described in this paper, and cytologic examination of the samples, can be utilized to obtain cellular components from a raised injection-site lesion. This technique can provide useful information regarding the cytologic characteristics of a vaccine-reaction lesion. While cytologic characterization can provide information about the type of tissue reaction, the exact cause of the reaction cannot be discerned by this technique. Additionally, cytologic examination cannot provide an entirely comprehensive architectural characterization of the lesion as can histopathologic examination, but it is able to provide relative proportions of the cells present. We believe this technique will be most useful for discriminating between rather benign vaccine reactions (ie, granulomatous or lymphocytic) and abscesses. A finding of mostly neutrophils, with or without bacteria, indicates the presence of an abscess, which may be the result of an unsanitary vaccination. If FNA and cytologic examination reveal the presence of an abscess, management control measures can be employed for future prevention. If FNA and cytologic examination routinely reveal other cellular processes, management protocols may be directed at the type of vaccine used rather than the vaccine procedure.

## Implications

- These studies provide a description of how a practitioner can collect needle-aspirate samples and evaluate the cause of injection-site lesions.
- To ensure adequate cytologic preparation for examination, clean the site to be sampled of debris and feces, spread the sample on clean glass slides, and either ship air dried or stain the cytologic preparations in house with hematology-cytology stains.
- Either a 22-gauge or 18-gauge needle with proper technique can provide adequate cytologic preparations for interpretation.
- This technique can characterize tissue-reaction lesions without the need for pig euthanasia or surgical biopsy.
- Use of the cytologic examination of samples collected by fine-needle aspiration allows practitioners to evaluate the causes of injection-site lesions and alter vaccination protocols if necessary.



**Figure 2:** Fine-needle aspirate cytological preparation of an injection-site lesion (500× magnification). This cytological slide reveals adequate cellularity with a mixture of non-degenerate neutrophils, small lymphocytes, and macrophages set within a background of red blood cells. The interpretation is mild mixed inflammation. Vaccination and sampling methods described in Figure 1.



## Acknowledgments

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## Conflict of interest

None reported.

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# Carbon dioxide system for on-farm euthanasia of pigs in small groups

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## Summary

Certain swine-farm operations require the regular euthanizing of multiple pigs on almost a daily basis. These animals may be too large for the small-scale methods of euthanasia used for nursing pigs and therefore may require the use of individual mechanical methods approved by the American Veterinary Medical Association (AVMA), such as gunshot and captive bolt. These methods may be unpleasant for workers and pose additional handling and

carcass-disposal challenges. Considerable research has been done using the AVMA-recommended carbon dioxide (CO<sub>2</sub>) method for mass depopulation of swine in the case of an exigent situation. This paper details a method for adapting that CO<sub>2</sub> methodology for euthanizing small groups of pigs. The system does not require direct worker contact with individual animals or manual handling of carcasses. The concept involves use of a standard high-pressure CO<sub>2</sub> cylinder and a small euthanasia chamber,

which can be a small dump-type trailer to allow easy transport to a disposal site. A detailed description of the CO<sub>2</sub> application system and method is provided so that producers can construct a suitable system from readily available low-cost components.

**Keywords:** swine, euthanasia, carbon dioxide, AVMA, on-farm

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## Resumen - Sistema de dióxido de carbono para la eutanasia en la granja de grupos pequeños de cerdos

Ciertos sistemas porcinos requieren la eutanasia regular de múltiples cerdos casi a diario. Estos animales pueden ser muy grandes para ser sacrificados mediante los métodos de eutanasia a pequeña escala utilizados para cerdos en destete y por lo tanto pueden requerir el uso de métodos mecánicos individuales aprobados por la Asociación Americana Médica Veterinaria (AVMA por sus siglas en inglés), tales como un disparo y la bala cautiva. Estos métodos pueden ser desagradables para los trabajadores y pueden crear problemas adicionales de manejo y eliminación de la canal. Se ha realizado una investigación exhaustiva utilizando el método de dióxido de carbono (CO<sub>2</sub>) recomendado por el AVMA para la despoblación masiva en el caso de una situación apremiante. Este

documento detalla un método para adaptar esa metodología de CO<sub>2</sub> para la eutanasia de pequeños grupos de cerdos. El sistema no requiere el contacto directo del trabajador con los animales de manera individual o el manejo manual de las canales. El concepto incluye el uso de un cilindro estándar de CO<sub>2</sub> de alta presión y una pequeña cámara de eutanasia, que puede ser un pequeño furgón de descarga para permitir el fácil transporte a un sitio de desecho. Se provee una descripción detallada del método y sistema de aplicación del CO<sub>2</sub>, para que los productores puedan construir un sistema adecuado con componentes de bajo costo ya disponibles.

## Résumé - Système au dioxyde de carbone pour l'euthanasie à la ferme de porcs en petits groupes

Il est nécessaire sur certaines fermes porcines de procéder à l'euthanasie régulière

de plusieurs porcs presque sur une base quotidienne. Ces animaux peuvent être trop gros pour les méthodes d'euthanasie à petite échelle utilisées pour les porcs en pouponnière et ainsi peuvent nécessiter l'utilisation de méthodes mécaniques approuvées par l'American Veterinary Medical Association (AVMA), telles que l'arme à feu et le percuteur. Ces méthodes peuvent être déplaisantes pour les travailleurs en plus de poser des défis supplémentaires en ce qui concerne des manipulations supplémentaires et la disposition des carcasses. De nombreuses recherches ont été effectuées sur la méthode recommandée par l'AVMA d'utilisation du dioxyde de carbone (CO<sub>2</sub>) pour une dépopulation massive de porcs dans un cas où la situation l'exige. Cet article décrit une méthode pour adapter la méthode au CO<sub>2</sub> afin d'euthanasier des petits groupes de porcs. Le système ne nécessite pas de contact direct de l'employé avec des animaux individuellement ou la manutention de carcasses. Le concept implique l'utilisation d'un cylindre standard de CO<sub>2</sub> sous haute pression et une petite chambre à euthanasie, qui peut être du type petite remorque à bascule pour permettre le transport facile à un site de disposition des carcasses. Une description détaillée du système à CO<sub>2</sub> et de la méthode à utiliser sont fournies afin de permettre aux producteurs de construire un système approprié à l'aide de composantes disponibles et peu coûteuses.

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Carbon dioxide (CO<sub>2</sub>) provides an attractive means for euthanasia of swine when applied in accordance with the American Veterinary Medical Association (AVMA) recommendations.<sup>1</sup> The AVMA panel recommends in their 2013 guidelines that CO<sub>2</sub> be introduced at a flow rate between 10% and 30% of chamber volume per minute, which gives a wash-in time constant of 5 minutes (wash-in is the inflow of CO<sub>2</sub> that purges the air).<sup>1</sup> As detailed in a pilot study by Meyer and Morrow,<sup>2</sup> this CO<sub>2</sub> injection rate results in an average CO<sub>2</sub> volume fraction of 63.5% in the chamber after 5 minutes of wash-in. This study demonstrated the feasibility of CO<sub>2</sub> for on-farm depopulation of adult pigs.

### Safety advantages of CO<sub>2</sub>

Safe, humane, and practical methods for euthanizing small groups of swine are important to the pork industry and to farm personnel. Carbon dioxide offers the potential for meeting overall humane and safety requirements. For example, use of CO<sub>2</sub> does not require restraint of individual pigs or application of mechanical means that can be hazardous and stressful for personnel.<sup>3</sup> Application of CO<sub>2</sub> allows for pigs to be treated in groups rather than individually. Minimizing the number of workers required simplifies personnel training. It is well established that CO<sub>2</sub> results in rapid depressant, analgesic, and anesthetic effects. Concentrations of CO<sub>2</sub> < 30 volume percent are seemingly not aversive to pigs.<sup>4,5</sup> Carbon dioxide produces quicker loss of consciousness than inert gas hypoxia when administered by gradual displacement methods,<sup>6</sup> and gradual displacement administration of CO<sub>2</sub> is less likely to cause pain due to ocular and nasal nociceptor activation prior to loss of consciousness.<sup>1</sup> Further, when CO<sub>2</sub> is gradually administered to young pigs at a constant displacement rate of either 10% or 20% of the container volume per minute, unconsciousness occurs within 80 to 124 seconds at approximately 22 volume percent CO<sub>2</sub> concentration, and the increase in plasma concentrations of cortisol, norepinephrine, and lactate after exposure to CO<sub>2</sub> do not differ from those observed following the physical methods of captive bolt and electrocution.<sup>6</sup> Unlike nitrogen and argon, which must be held within a very tight range of concentration to reduce oxygen (O<sub>2</sub>) levels below 2% of the total volume for effective killing, CO<sub>2</sub> can render pigs unconscious and kill over a wide

range of concentrations, even when O<sub>2</sub> is greater than 2% of the total volume.<sup>7</sup> Meyer and Morrow<sup>2</sup> point out that other advantages of CO<sub>2</sub> as a euthanasia agent include its ready availability and relatively low cost, its nonflammable and non-explosive properties, and the rapid reversal of its toxic effects after accidental exposure of personnel by prompt removal from the area (unlike other gases such as carbon monoxide). Carbon dioxide poses minimal hazard when used with properly designed equipment.<sup>2</sup>

### CO<sub>2</sub> system requirements

The main components of a CO<sub>2</sub> system for on-farm euthanasia of small batches of pigs can be listed as follows: a small euthanasia chamber with no air leaks in floor or sides; a translucent cover for the chamber, such as a clear polyethylene film secured but not sealed to the top edges of the chamber walls; a hose or pipe for delivery of the CO<sub>2</sub> to the floor area of the chamber; a means for delivering CO<sub>2</sub> to the euthanasia chamber so that a volume of CO<sub>2</sub> equal to the volume of the chamber can be delivered in a total of 5 minutes; and a method of supplying and metering this amount of CO<sub>2</sub>. This paper will recommend a method which allows use

of a high-pressure CO<sub>2</sub> cylinder of standard size (22.2 kg, or other size as desired) and a low-cost means for storing and metering the required amount of CO<sub>2</sub> gas for each euthanasia treatment.

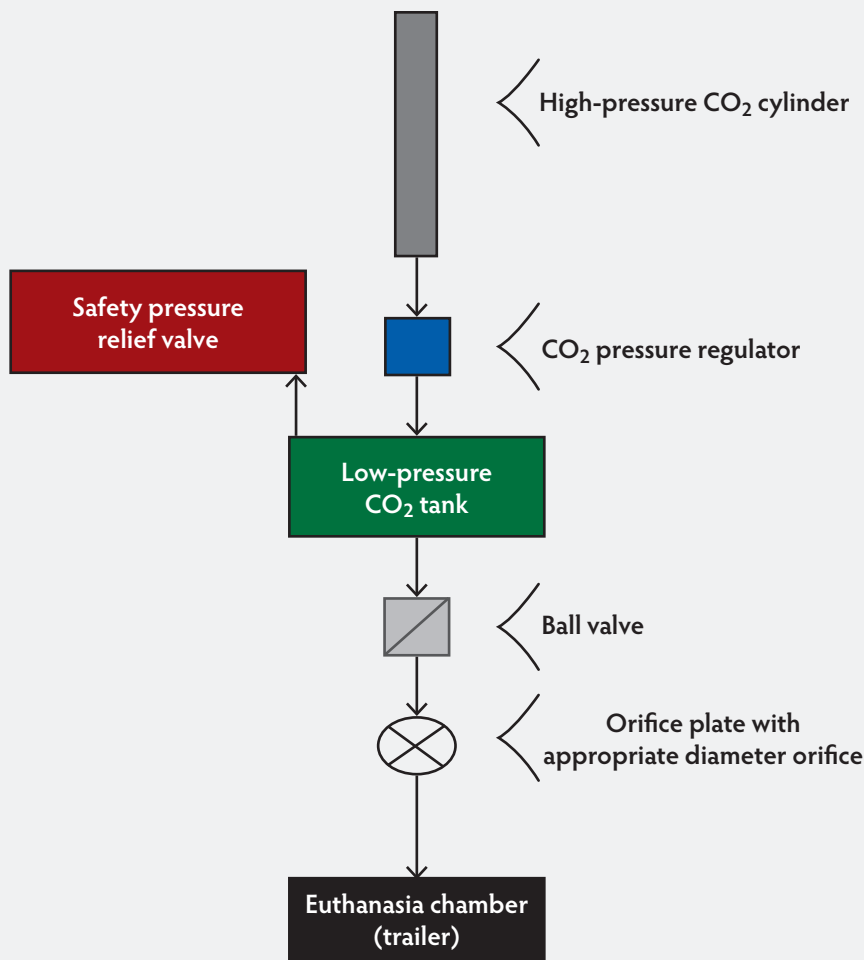
### The euthanasia chamber (dump-type trailer)

The euthanasia chamber should be sized to hold pigs of the anticipated size and number. Transport density recommendations can be used to estimate the required chamber size.<sup>8</sup> The height of the chamber walls should allow adequate head room for the pigs. Excessive height should be avoided, since that space will require additional CO<sub>2</sub> volume. The floor and walls of the chamber must be airtight. If the euthanasia chamber is to be mobile and used as a dump-trailer body, the tailgate should be hinged to allow for convenient dumping. A smaller sliding or hinged doorway will facilitate the movement of pigs from the loading chute into the trailer. A sliding door such as that shown in Figure 1 allows the door to be opened without interfering with the loading chute or other obstructions.

**Figure 1:** A standard dump trailer or truck modified for use as a swine euthanasia chamber. A sliding tailgate section and top hinges added to this truck bed facilitates loading of live pigs and then dumping the carcasses once the pigs have been euthanized. The sides and tailgate are sealed with expanding foam to form a gas-tight seal. A tarpaulin or plastic sheeting is placed over the truck bed to reduce air mixing and carbon dioxide washout.



**Figure 2:** Block diagram of a CO<sub>2</sub> application system for releasing high-pressure CO<sub>2</sub> into a low-pressure tank for application as a swine euthanasia chamber as described in Figure 1. The CO<sub>2</sub> is released slowly into the low-pressure tank through a pressure regulator to avoid tank and regulator freezing. When the low-pressure tank has reached the predetermined pressure set by the regulator, the valve to the euthanasia chamber is opened, allowing the orifice to control CO<sub>2</sub> flow into the chamber. This flow rate must meet the American Veterinary Medical Association guidelines for use of CO<sub>2</sub> for euthanizing pigs. A pressure-relief valve with a pressure rating slightly above the required pressure, and well below the maximum tank pressure rating, should be used to avoid over-pressurizing the low-pressure tank. The pressure regulator maintains the preset pressure in the low-pressure tank between application events.



### The euthanasia chamber cover

The top of the chamber can be covered with a clear or translucent polyethylene sheet secured with tape. The clear or translucent material is recommended to avoid having a dark space that pigs do not want to enter. The interface between the cover and the top edges of the side walls need not be airtight: in fact, there needs to be some air leakage at the top to allow for air to purge out of the space during CO<sub>2</sub> wash-in. This cover can be left on the chamber at all times, but prior to each use, it should be checked to confirm that it is well secured and in good condition.

If damaged, it must be replaced prior to the next CO<sub>2</sub> treatment.

### Delivery of CO<sub>2</sub> into the euthanasia chamber

Carbon dioxide should be delivered to the chamber via a hose or flexible pipe, preferably 2.5 to 7.6 cm in diameter, inserted through the top cover and extending down to within 5.1 to 7.6 cm of the floor. The larger the hose diameter, the lower the CO<sub>2</sub> gas exit velocity and the lower the noise, turbulence, and mixing of CO<sub>2</sub> and air. The hose may be placed near one of the side

walls, preferably near the mid-span of the wall, although this is not critical. The CO<sub>2</sub>, which is heavier than air, will diffuse and fill the chamber from the floor to the top. Studies have shown that distribution manifolds are not needed to get uniform CO<sub>2</sub> distribution in the chamber.<sup>9</sup>

### The CO<sub>2</sub> storage and metering system

Figure 2 shows a schematic of the component arrangement. The model euthanasia chamber, shown in Figure 3, was a dump trailer with a volume of approximately 1.7 m<sup>3</sup> (1.8 m wide × 1.2 m long × 0.8 m high). The CO<sub>2</sub> delivery system must be capable of supplying 20% of the chamber volume per minute during the 5-minute application period. In 5 minutes, the system delivers a volume of CO<sub>2</sub> equal to the chamber volume, meeting the AVMA guideline.<sup>1</sup> For large euthanasia-chamber volumes, the standard high-pressure CO<sub>2</sub> cylinder may not supply the required flow rate directly to the chamber, because the high CO<sub>2</sub> gas outflow could cause freeze-up in the CO<sub>2</sub> cylinder regulator and distribution line. Multiple cylinders could be connected in parallel using a manifold, but this adds an undesirable degree of complexity. The illustrated design uses one standard high-pressure CO<sub>2</sub> cylinder with a standard pressure regulator that can be set and locked to the required pressure, allowing the low-pressure tank to fill at a rate slow enough to avoid line freeze-up. The suggested scenario is that the storage tank refills immediately after each euthanasia treatment, allowing enough time for the CO<sub>2</sub> gas to approach ambient temperature prior to the next treatment. The required pressure depends on the volume of the low-pressure CO<sub>2</sub> tank and the euthanasia-chamber volume being serviced. As shown in Figure 2, the pressure regulator is used to pre-charge the low pressure tank to ensure there is adequate CO<sub>2</sub> to perform the euthanasia. The low-pressure tank is then allowed to empty into the euthanasia chamber through a quarter-turn valve and orifice sized to allow the tank to bleed down to atmospheric pressure in 5 minutes. Our low-pressure CO<sub>2</sub> tank was a recycled 1000-L liquid petroleum (LP) gas tank with the original LP fittings removed. The LP tank was chosen because of its adequate pressure rating and availability and because it was configured with the necessary ports needed for the CO<sub>2</sub> gas connections. If some other type of tank is used, it must be rated for the pressures that will be required to store the CO<sub>2</sub>.



**Figure 3:** A prototype CO<sub>2</sub> application system assembled and used to verify the performance of the swine euthanasia system illustrated and described in figures 1 and 2. A pressure-relief valve has been added to a used propane tank to serve as the low-pressure CO<sub>2</sub> tank. An orifice plate (Figure 4) was included in the discharge hose to control the CO<sub>2</sub> gas flow into the dump trailer used as a model euthanasia chamber.



### Initial purging of the low-pressure CO<sub>2</sub> tank

The low-pressure tank is purged of air and residual LP gas fumes by filling it several times with CO<sub>2</sub> while allowing the gases in the tank to bleed out of the top ports. This initial purging can be enhanced by fabricating a CO<sub>2</sub> inlet tube to carry the incoming CO<sub>2</sub> to the bottom of the tank, allowing the CO<sub>2</sub> to displace the air and any other gases with a minimum of mixing. An alternate approach to initial purging is to place in the tank approximately 2 kg of dry ice pellets or chips per cubic meter of tank volume so that the CO<sub>2</sub> gas from sublimation will purge the tank of air and other gases.

### Calculating the pressure required for the low-pressure CO<sub>2</sub> tank

Initially, the low-pressure tank must be filled with enough CO<sub>2</sub> gas so that when it flows to the euthanasia chamber and equilibrates (after 5 minutes) to atmospheric pressure, the volume of CO<sub>2</sub> released to the chamber will be equal to the volume of the chamber. In order to calculate the initial pressure required in the low-pressure tank we apply the ideal gas law which says

$$P_1 V_1 = P_2 V_2 \text{ (Equation 1)}$$

$$P_1 = P_2 V_2 \div V_1 \text{ (Equation 2)}$$

where P<sub>1</sub> and P<sub>2</sub> are absolute pressures.<sup>10</sup> In the described swine euthanasia system, P<sub>1</sub> is the pressure required in the low-pressure tank; P<sub>2</sub> is atmospheric pressure, 10<sup>5</sup> Pascal (Pa); V<sub>1</sub> is the volume of the low-pressure tank, in this case approximately 1.0 m<sup>3</sup>; and V<sub>2</sub> is the total volume occupied by the CO<sub>2</sub> after it expands. Then V<sub>2</sub> is the volume of the storage tank plus the volume of the chamber. The size of the model euthanasia chamber was 1.7 m<sup>3</sup>, which gives a total V<sub>2</sub> volume of 1.0 + 1.7 = 2.7 m<sup>3</sup>.

Equations 1 and 2 allow us to compute the initial pressure required in the low-pressure CO<sub>2</sub> tank.  $P_1 = (10^5 \times 2.7) \div 1.0 = 270$  kPa absolute pressure or approximately 170 kPa gauge pressure.

Equations 1 and 2 assume a constant temperature expansion that is not really the case. There will be a temperature decrease due to the expansion of the CO<sub>2</sub>. However, our calculations, applying the ideal gas law,<sup>10</sup> indicate that at these low pressures, the temperature change is < 0.2°C. Increasing this

computed pressure by approximately 10%, which in this case would bring the gauge pressure P1 to about 187 kPa, provides a safety factor to compensate for a pressure difference due to changes in temperature and other system variables. This should account for the loss of pressure that may occur and ensures that there will be enough CO<sub>2</sub> to meet AVMA guidelines. This was verified during our testing by measuring the change in weight of the high-pressure CO<sub>2</sub> cylinder before and after pressurizing the low-pressure tank (when the tank initially contained CO<sub>2</sub> at atmospheric pressure). Since CO<sub>2</sub> gas at 20°C and one atmosphere occupies approximately 0.55 m<sup>3</sup> per kg, approximately 3.1 kg of CO<sub>2</sub> was required for a 1.7-m<sup>3</sup> chamber.

If a low-pressure storage tank of a different size is chosen, the pressure appropriate for that tank must be computed. For example, for a 0.6-m<sup>3</sup> low-pressure tank used with a 1.7-m<sup>3</sup> euthanasia chamber (the same size as the model), this would work out to P1 = 383 kPa absolute pressure or approximately 283 kPa gauge pressure. Adding the 10% safety factor as recommended, the required gauge pressure is approximately 311 kPa for a 0.6-m<sup>3</sup> low-pressure tank to supply a euthanasia chamber with a volume of 1.7 m<sup>3</sup>.

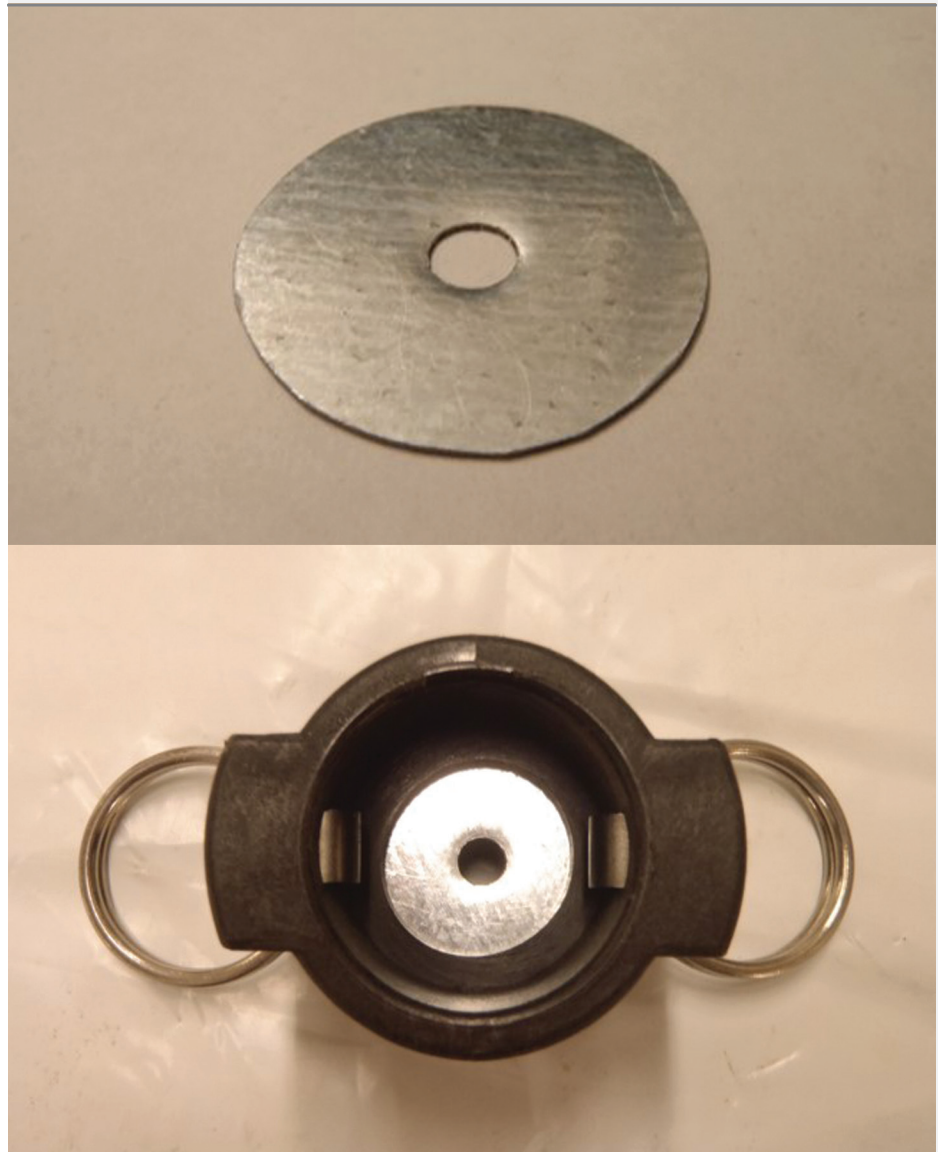
### Orifice for setting the flow rate

In the hose or pipe connecting the low-pressure tank to the euthanasia chamber, there must be a metering orifice sized to allow the CO<sub>2</sub> gas in the low-pressure tank to expand into the euthanasia chamber at the rate of approximately 20% of the chamber volume per minute. This means the orifice should allow the low-pressure tank to reach atmospheric pressure in 5 minutes once the valve to the euthanasia chamber is opened, allowing gas flow to begin. In this model, a fixed orifice was fabricated by drilling a hole in a disk of 0.95 mm thick stainless steel sheet metal (Figure 4). For the 1000-L storage tank and 1.7-m<sup>3</sup> chamber, an orifice hole of 0.67-cm diameter was needed to provide a 5-minute bleed-down time. Figure 5 shows typical test data for gauge pressure, % CO<sub>2</sub>, and % O<sub>2</sub> versus time in seconds.

### Practical application

The CO<sub>2</sub> system described can be easily constructed for use on-farm. Table 1 contains a list of materials used for the prototype system. The high-pressure CO<sub>2</sub> cylinder

**Figure 4:** An orifice plate inserted into the tank fitting to control the flow of CO<sub>2</sub> from the low-pressure tank to the chamber in the swine euthanasia system illustrated and described in Figure 2. The top figure is the orifice plate made of 0.95 mm thick stainless steel sheet metal. The bottom figure shows the orifice plate mounted in the quick-connect coupling used to connect the low-pressure tank to the chamber shown in Figure 3.

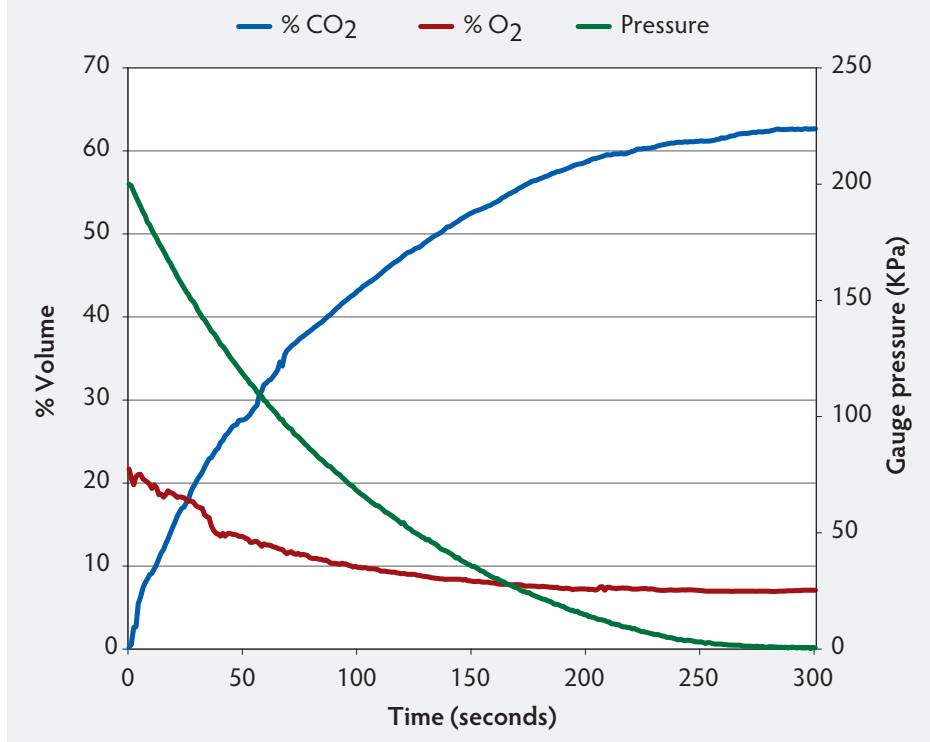


and pressure gauges should be stored inside a building for security and protection from weather. There should be easy access for convenient cylinder exchange, which may occur frequently depending on usage. For example, since the 1.0-m<sup>3</sup> chamber requires approximately 3.1 kg of liquid CO<sub>2</sub> per treatment cycle, a standard 22.2-kg, high-pressure CO<sub>2</sub> cylinder should provide about seven treatments before the pressure becomes too low to recharge the low-pressure tank. The overall concept allows the use of CO<sub>2</sub> without sophisticated controls or skilled personnel. Steps for use are essentially as follows.

First the high-pressure CO<sub>2</sub> regulator is set to the required pressure. In the prototype system, that pressure is 187 kPa. This allows a low flow rate of CO<sub>2</sub> from the high-pressure cylinder, continuing until the low-pressure tank reaches 187 kPa. This can be the normal state, which means that any time a chamber or trailer load of pigs is to be euthanized, the CO<sub>2</sub> is ready for application. Once the euthanasia chamber is located near the low-pressure tank and the delivery hose is inserted into chamber with the end of the hose approximately 5.1 to 7.6 cm above the floor of the chamber, the ball valve on the



**Figure 5:** Typical data showing the changes in the low-pressure storage tank pressure, CO<sub>2</sub> concentration, and O<sub>2</sub> concentration in a model swine euthanasia chamber over time. The pressure in the tank decreases as the CO<sub>2</sub> flows into the chamber through an orifice plate. As the CO<sub>2</sub> flows into the chamber, CO<sub>2</sub> concentration increases and the air is displaced, reducing O<sub>2</sub> concentration.



outlet of the low-pressure CO<sub>2</sub> tank can be fully opened and the orifice will control the flow to fill the chamber in approximately 5 minutes. Flow rate is not constant with this arrangement, but the average is approximately 20% of the chamber volume per minute, which is what AVMA requires. Once the low-pressure CO<sub>2</sub> tank bleeds down to zero gauge pressure, the ball valve is closed, allowing the tank to refill over a period of time so that it will be ready for the next application. At this point, the CO<sub>2</sub> hose can be pulled out of the chamber and the cover re-secured in that spot. As a final step, the euthanasia chamber or trailer with pigs and CO<sub>2</sub> gas should remain sealed for at least 15 to 20 additional minutes prior to transport and disposal. This provides enough time to ensure that all pigs are dead, with no risk of revival when exposed to air. Pigs that may have survived CO<sub>2</sub> exposure must be humanely killed using an alternate AVMA-approved method, such as captive bolt or gunshot to the head.

The CO<sub>2</sub> system should be used only in well-ventilated areas, and all normal safety precautions for handling high-pressure cylinders and CO<sub>2</sub> should be followed. If a used LP tank is to be utilized, it should be

thoroughly purged of LP liquid and vapor. Air in the tank must also be purged and replaced with CO<sub>2</sub> before the initial euthanasia application. As a safeguard against over-pressurizing any tank used for this application, a pressure relief valve should be included in the system.

## Implications

- If designed and operated properly, a CO<sub>2</sub> system can be a safe and effective option for on-farm euthanasia of groups of pigs.
- The number of pigs that can be euthanized in one batch is limited only by the size of the chamber and provision of the appropriate flow rate of CO<sub>2</sub> for that chamber size.

## Acknowledgement

Supported by a USDA APHIS cooperative agreement and a Department of Homeland Security Science and Technology Division Interagency Agreement (System to Administer Inhaled Gases for Mass Depopulation of Swine in a National Emergency).

## Conflict of interest

None reported.

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**Table 1:** List of materials required to construct a carbon dioxide gas swine euthanasia system

Item description	Vendor	Part no.
22.2 kg high-pressure CO <sub>2</sub> cylinder*	Air Gas, Radnor, PA	CD 50
Compressed-gas pressure regulator†	Air Gas, Radnor, PA	HCL3000540
Pressure regulator adapter, CGA320 to CGA580‡	Air Gas, Radnor, PA	RAD64003956
Safety pressure-relief valve§	McMaster-Carr, Atlanta, GA	48435K81
Pressure gauge¶	McMaster-Carr, Atlanta, GA	4000K721
Used LP gas tank used for low-pressure CO <sub>2</sub> storage**	NA	NA
Pneumatic hose, gas regulator to storage tank††	Lowe's, Wilkesboro, NC	Various lengths
Pneumatic coupler set, quick connect, two sets needed‡‡	Lowe's, Wilkesboro, NC	235470
Orifice to control low-pressure CO <sub>2</sub> flow	Fabricate	NA
Ball valve, 1-1/4-inch NPT, full port§§	Lowe's, Wilkesboro, NC	369200
Hose leading to chamber¶¶	Agri Supply, NC	18415
Plastic film, clear, 6 mil***	Agri Supply, NC	11424

\* Tank size and vendor optional.

† CO<sub>2</sub>-N<sub>2</sub> compatible regulator.

‡ CO<sub>2</sub> gas cylinder to compatible gas regulator.

§ Pressure set above desired tank pressure but well below tank-rated pressure.

¶ 0-60 psi range adequate.

\*\* 250-gallon (950 L).

†† Pressure rated, ¼-inch NPT ends, use shortest available length. NPT = National Pipe Thread.

‡‡ Make connections more convenient.

§§ Should not restrict flow when wide open.

¶¶ Hose should be 2.45-5.08 cm ID to allow low-velocity CO<sub>2</sub> flow.

\*\*\* Polyethylene sheeting for euthanasia chamber, various sizes.

CO<sub>2</sub> = carbon dioxide gas; N<sub>2</sub> = nitrogen gas; LP = liquid petroleum; NA = not applicable; NPT = National Pipe thread.



## New common industry audit platform announced

The National Pork Board (NPB) shared plans for a new common industry audit platform for pork producers, packers, and processors. The audit platform will use the existing Pork Quality Assurance Plus (PQA Plus) program as its foundation to serve as a common audit approach for the industry. The main goal of the common audit process is to assure consumers of the care taken by farmers and processors regarding animal well-being and food safety. The concept of a common audit was first introduced at the 2013 National Pork Industry Forum. The NPB then convened packers and pork producers to explore a credible and affordable solution for assuring animal well-being.

In 2011, the Pork Checkoff's board of directors met with European counterparts who complained that audit programs in

their countries were duplicative, costly, and inefficient. The new common platform announced at World Pork Expo seeks to create and standardize a common process that will

- meet individual company and customer needs,
- focus on outcome-based criteria that measure animal welfare,
- provide clarity to producers with regard to audit standards and expectations,
- minimize duplication and prevent over-sampling, and
- ensure greater integrity of the audit process through consistent application.

The new framework has several components. These include a new audit tool, requirements for auditor training and biosecurity, and a platform that will allow the results to be

shared to prevent duplicative audits. This tool is currently being tested on farms across the country and will be reviewed before finalizing the audit.

The Industry Audit Task Force includes producers and veterinarians representing the American Association of Swine Veterinarians, as well as packer representatives from Cargill, Farmland/Smithfield, Hatfield, Hormel, JBS, Seaboard, Triumph, and Tyson. The NPB cannot deploy the standards of the program without the direct involvement of packers and processors. Many packers have agreed to support the new common industry audit by promising to utilize the standard when conducting third-party audits.

For more information, contact Sherrie Webb at [SWebb@pork.org](mailto:SWebb@pork.org) or 515-223-3533.

## National Pork Board elects new officers

Dale Norton, a pork producer from Bronson, Michigan, was elected president of the National Pork Board at the organization's June board meeting in Des Moines, Iowa. The National Pork Board comprises 15 farmer-directors representing America's pig farmers.

"As a producer-director of the Pork Checkoff, I see so much opportunity in the year ahead," Norton said. "There is great consumer interest in farming and understanding how food is raised and marketed. The Pork Checkoff is up for the challenges facing our industry, chief among them managing diseases like porcine epidemic diarrhea. Sharing our stories of success in research, education, and promotion will be a priority for me as we introduce our new 5-year strategic plan."

Norton is a partner in Kendale Farm, Bronson, Michigan, which is primarily a 1450-sow farrow-to-wean operation that also finishes about a third of the pigs. He is involved with a cow-calf operation and raises corn, soybeans, hay, green beans, peppers for processing, and seed corn on more than 3500 acres. Nationally, he is serving his second 3-year term on the National Pork Board. He had served as the Pork Checkoff's representative on the US Farmers and Ranchers Alliance and serves on the Swine Health Committee.

Serving with Norton as vice president is Brad Greenway, a pork producer from Mitchell, South Dakota. Derrick Sleezer, a pork producer from Cherokee, Iowa, will

continue as treasurer. The three executive officers will serve 1-year terms in their positions beginning immediately.

"As we look forward to the next 5 years, our industry is excited to engage with food-service and retail leaders, as well as consumers, underscoring the versatile, nutritious product that we offer to shoppers in the United States and worldwide," Norton said. "It's important that producers continue to build trust and share our commitment with our customers."

For more information, contact John Johnson at [JJohnson@pork.org](mailto:JJohnson@pork.org) or 515-223-2765.

## National Pork Board members approved

United States Secretary of Agriculture Tom Vilsack announced three new appointments and two reappointments to the National Pork Board. Each member will serve a 3-year term. The new members were nominated by the National Pork Producers Delegate Body dur-

ing Forum in Kansas City last March. The new members representing pork producers are Brett Kaysen, Nunn, Colorado; Steven Rommereim, Alcester, South Dakota; and Craig Rowles, Carroll, Iowa. Members reappointed to the board include Henry Moore,

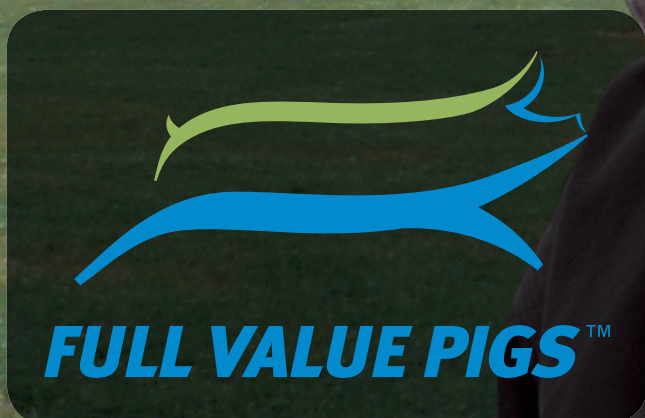
Clinton, North Carolina; and Glen Walters, Forsyth, Georgia.

For more information, contact John Johnson at [JJohnson@pork.org](mailto:JJohnson@pork.org) or 515-223-2765.

*NPB news continued on page 257*



**It's about you.  
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It's about working together to find you more profit.

*Full Value Pigs* is more than a metric or a tool. It's a set of beliefs that together, we can make your business better. It's about taking a holistic approach to disease management and herd health. It's about feed optimization and getting the most out of your biggest input. It's about marketing your pigs at the right weight and at the right time, giving you a precision harvest. It's about access and the assurance that you'll be able to sell your products to your preferred buyer. It's about feeding the world. But most of all, *Full Value Pigs* is about growing your business.



## USDA orders mandatory PEDV reporting for US pork producers

On June 5, Secretary of Agriculture Tom Vilsack issued a federal order requiring pork producers, veterinarians, and diagnostic laboratories to report positive occurrences of porcine epidemic diarrhea virus (PEDV), porcine deltacoronavirus, (PDCoV), or other novel swine enteric coronaviruses that meet the case definition. If a sample is submitted to a National Animal Health Laboratory Network laboratory for testing and is found to be positive, duplicate reporting is not required. Reporting by producers or veterinarians must be directed to the state

animal-health official or the United States Department of Agriculture's (USDA's) Animal and Plant Health Inspection Service Assistant District Director located in the state in which the herd resides.

The USDA requires the following specific reporting information to be submitted:

- Premises identification number (PIN) or an alternative premises location identifier (if you do not have a PIN, go to [www.pork.org/pintag](http://www.pork.org/pintag));

- Date of sample collection;
- Type of unit being sampled (eg, sow, nursery, finisher);
- Test methods used to make the diagnosis; and
- Diagnostic test results.

Additional details on compliance can be found on the USDA Web site or at [www.pork.org](http://www.pork.org).

## Pork Academy sessions online

In case you missed World Pork Expo last June, you can still view the 2014 Pork Academy sessions online. These include sessions on safe pig handling, industry productivity

analysis, sow bridge-pork bridge, reproductive decision tree, world market economics and the importance of trade, PEDV research update, PEDV-lessons learned and next

steps, sustainability in pork production, and pain management. To view the sessions online, go to [www.pork.org](http://www.pork.org) and click Resources and then Conferences.

## New Safe Pig Handling Tool introduced

The Checkoff's new Safe Pig Handling Tool helps keep animal caretakers safe, including new workers who may not have a basic understanding of pig behavior and animal handling knowledge. Working with producers to identify key areas where guidance is needed, educational materials, including a video, photos, and handouts, were designed

to help provide training in barns. Content from the Pork Quality Assurance Plus and Transport Quality Assurance programs were incorporated to create cohesive worker messages. The Safe Pig Handling materials reinforce safety concepts while depicting real-life, specific situations encountered daily in pork production. The materials can be

used in one-on-one meetings, small groups, and self-study training sessions. For more information, go to [www.pork.org](http://www.pork.org).

For more information, contact Sherrie Webb at [SWebb@pork.org](mailto:SWebb@pork.org) or 515-223-3533.

## Porkcares.org updated to engage visitors

The redesigned [porkcares.org](http://porkcares.org) provides a comprehensive online resource on responsibly raising pigs. New features are designed to inform, engage, and inspire visitors while conveying the positive story of modern

pork production. The Web site serves as a credible, authoritative resource for facts and information about responsible pig farming practices to food-chain customers and engaged consumers.

For more information, contact Angela Anderson at [AAnderson@pork.org](mailto:AAnderson@pork.org) or 515-223-2623.



# swine influenza

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## FDA secures full industry engagement on antimicrobial resistance strategy

The US Food and Drug Administration (FDA) has announced the first of its progress reports on its strategy to promote the judicious use of antimicrobials in food-producing animals. All 26 drug manufacturers affected by Guidance for Industry (GFI) #213 have now agreed to fully engage in the strategy by phasing out the use of medically important antimicrobials in food-producing animals for food-production purposes and phasing in the oversight of a veterinarian for the remaining therapeutic uses of such drugs. While GFI #213 specified a 3-year timeframe (until December 2016) for drug sponsors to complete the recommended changes to their antimicrobial products, some sponsors have already begun to implement them.

The FDA is committed to updating the public on the progress that drug sponsors have made in aligning their products with GFI #213 and intends to do so on a 6-month basis. The FDA's progress reports will summarize current and pending actions taken by sponsors to align with the guidance, including the type of action (eg, withdrawal, change in marketing status) and, when possible, without revealing confidential business information (CBI), the type of animal for which the drug is approved for use, and the type of application (pioneer, generic, combination).

As of June 30, 2014, FDA reports the following progress in the animal-health industry's engagement in GFI #213:

The last sponsor, Pharmaq AS, has agreed in writing to engage in the judicious use strategy and has consented to allow FDA to publicly acknowledge its participation. With this addition, all 26 sponsors of 283 affected applications have now confirmed in writing their intent to engage with FDA as defined in GFI #213 and have given FDA consent to identify them as participants. Please see FDA's March 26, 2014 update for a list of companies that had previously committed

to the strategy (<http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/JudiciousUseofAntimicrobials/ucm390738.htm>).

There have been two published label changes, one to withdraw a production claim and one to change a product's marketing status from over-the-counter to available by prescription only. These changes are documented in the online chart of Applications Affected by GFI #213 (<http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/JudiciousUseofAntimicrobials/ucm390429.htm>), and FDA will continue to update this chart in real time when label changes are approved.

One additional drug label change is currently pending. The change is from over-the-counter marketing status to prescription status. More details about the product and the change in labeling will be available after the paperwork is complete. The level of summary detail provided in this update for pending supplemental applications is limited by the need to

protect CBI. To avoid revealing CBI, either directly or indirectly, the level of summary detail provided for future updates regarding GFI #213-related pending actions may change as this voluntary initiative progresses and the pool of affected applications gets smaller. Given the small number of pending and completed changes at this time, FDA cannot provide more information about the type of drug being affected (eg, application type, species, indication) in this update without revealing protected information.

Thirty-one approvals for affected products have been withdrawn to date, and there are no drug approval withdrawals currently pending. After an approval is voluntarily withdrawn, that product can no longer be marketed or sold in the United States.

The FDA will continue to work with the animal pharmaceutical industry, animal producers, and the veterinary community to address antimicrobial resistance and preserve the effectiveness of antimicrobials of human health importance.

## Mandatory reporting of novel coronaviruses in swine

Secretary of Agriculture Tom Vilsack issued a federal order on June 5 requiring producers, veterinarians, and diagnostic laboratories to report presumptive or confirmed positive occurrences of porcine epidemic diarrhea virus (PEDV), porcine deltacoronavirus (PDCoV), or other novel swine enteric coronaviruses that meet the case definition ([http://www.aphis.usda.gov/animal\\_health/animal\\_dis\\_spec/swine/downloads/secd\\_case\\_definition.pdf](http://www.aphis.usda.gov/animal_health/animal_dis_spec/swine/downloads/secd_case_definition.pdf)). An occurrence may be the initial detection of disease or a reoccurrence of previously detected disease. If a sample is submitted

to a National Animal Health Laboratory Network (NAHLN) laboratory for testing and is found positive, duplicate reporting by the herd owner, producers, veterinarians, and others with knowledge of the disease is not required. Reporting must be directed to the State Animal Health Official or the Animal and Plant Health Inspection Services (APHIS) Assistant District Director (previously referred to as the Area Veterinarian in Charge) located in the state in which the herd resides.

*AASV news continued on page 261*





**FLORVIO™**  
(florfenicol)



# A GREAT NEW WAY TO SEE YOUR PIGS GET BETTER FASTER.

**Introducing FLORVIO™ (Florfenicol) 2.3% Concentrate Solution.** It's the fast, no-nonsense treatment option for swine respiratory disease including *Streptococcus suis*. As the only labeled water treatment for *S. suis*, FLORVIO gets the job done without the stress of an injectible antibiotic. See your pigs get better faster.

For more details about FLORVIO call your Novartis Animal Health representative or visit [www.florvio.com](http://www.florvio.com)

Warning: Swine intended for human consumption must not be slaughtered within 16 days of the last treatment.  
NOT FOR HUMAN USE. KEEP OUT OF REACH OF CHILDREN. Avoid direct contact with skin, eyes, and clothes.  
Caution: Do not use in swine intended for breeding. See product label for directions for use and additional information.

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 **NOVARTIS**  
ANIMAL HEALTH



**FLORVIO™ (florfenicol) an Antimicrobial  
2.3% Concentrate Solution**

**For Oral Use in Swine Drinking Water Only.**

**Caution:** Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian.

ANADA #200-519, Approved by FDA.

**DESCRIPTION:** Florfenicol (Oral Concentrate) is a synthetic broad-spectrum antibiotic. Each milliliter (mL) of Florvio™ 2.3% Concentrate Solution contains 23 mg florfenicol.

**INDICATIONS:** Florvio™ 2.3% Concentrate Solution is indicated for the treatment of swine respiratory disease associated with *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Salmonella choleraesuis* and *Streptococcus suis* in swine.

**DOSAGE AND ADMINISTRATION:** For proportioner: To produce drinking water with a final concentration of 400 mg/gallon (100 ppm): Fill the bottle of Florvio™ 2.3% Concentrate Solution with water to the fill line (The Fill line is: 4 liters volume for the 2.17 liter product bottle). Add the contents of the bottle to the mixing tank. Mix thoroughly. Confirm that the proportioner is set to deliver 1:128 (0.8%). Turn on the proportioner. Verify that the drinkers are operational.

**For Bulk Tank:** To produce drinking water with a final concentration of 400 mg/gallon (100 ppm): Add the Florvio™ 2.3% Concentrate Solution to the drinking water in the bulk tank. Use one 4L bottle (undiluted) of Florvio™ 2.3% Concentrate Solution for every 125 gallons of water. The medicated water should be administered as the only source of drinking water for five (5) consecutive days. Medication should be initiated promptly when swine respiratory disease is diagnosed.

**PRECAUTIONS:** Do not use this product at any other proportioner setting. This will result in precipitation of product. This product is not recommended for use in automatic water proportioners if water hardness is greater than 275 ppm. Water proportioners should be tested for accuracy before use. Do not use or store this product in galvanized metal watering systems or containers. Do not operate chlorinators while administering medication.

**RESIDUE WARNINGS:** Swine intended for human consumption must not be slaughtered within 16 days of the last treatment. Use of this product in a manner other than indicated with dosages in excess of those included on this label may result in illegal drug residues in edible tissues.

**WARNINGS: NOT FOR HUMAN USE. KEEP OUT OF REACH OF CHILDREN. This product contains material that can be irritating to skin and eyes. Avoid direct contact with skin, eyes, and clothes. In case of accidental eye exposure, flush with water for 15 minutes. In case of accidental skin exposure, wash with soap and water. Remove contaminated clothing. Consult a physician if irritation persists. The Material Safety Data Sheet (MSDS) contains more detailed occupational safety information.**

For customer service and/or a copy of the MSDS, call 1-800-843-3386. For adverse effects reporting, call 1-800-843-3386.

**PRECAUTION:** The effects of florfenicol on swine reproductive performance, pregnancy and lactation have not been determined. Do not use in swine intended for breeding.

**ADVERSE REACTIONS:** Perianal inflammation may occur transiently following treatment.

Made in United Kingdom.

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NAH/FLO/23mg/4L-BS/2

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Greensboro, NC 27408

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Rev. date: 04/14

NVSFL07148966LR



The following specific reporting information must be submitted:

- Premises identification number (PIN) or an alternative premises location identifier;
- Date of sample collection;
- Type of unit being sampled (eg, sow, nursery, finisher);
- Test methods used to make the diagnosis; and
- Diagnostic test results.

In addition, the producer must develop and implement, in collaboration with the accredited herd veterinarian, state veterinarian, or APHIS veterinarian, a herd management plan that addresses the following:

- Biosecurity of visitors and vehicles entering or exiting the premises;
- Monitoring employee biosecurity;
- Periodic herd-health observation;
- Animal movement (both into and out of the herd);
- Cleaning and disinfection of facilities;

- Diagnostic testing to monitor the status of the herd infection and assess efficacy of control strategies; and
- Maintenance of records on pig movement that are accessible to state or federal animal-health officials upon request.

Herd owners or veterinarians failing to promptly report a presumptive or confirmed positive case or to follow a herd management plan may be subject to civil penalties or revocation of veterinary accreditation, and additional requirements (hold order, quarantine, permitting, or other restrictions on movement of pigs) may be placed on their premises by state or federal animal-health officials.

The actual federal order and additional supporting documents can be found on the United States Department of Agriculture Web site (<http://www.aphis.usda.gov/animal-health/secd>). Additional information can be viewed on the AASV PEDV web page (<http://www.aasv.org/aasv%20website/Resources/Diseases/PorcineEpidemicDiarrhea.php>).

## Call for papers – AASV 2015 Student Seminar

### Veterinary Student Scholarships

The American Association of Swine Veterinarians announces an opportunity for veterinary students to make a scientific presentation during the Student Seminar at the AASV Annual Meeting in Orlando, Florida, on Sunday, March 1, 2015. Interested students are invited to submit a one-page abstract of a research paper, clinical case study, or literature review for consideration. The submitting student must be a current (2014-2015) student member of the AASV at the time of submission, and must not have graduated from veterinary school prior to March 1, 2015. Submissions are limited to one (1) abstract per student.

Abstracts and supplementary materials must be **received** by Dr Alex Ramirez ([alex@aaasv.org](mailto:alex@aaasv.org)) by **11:59 PM Central Daylight Time on Monday, September 22, 2014** (firm deadline). All material must be submitted electronically. Late abstracts will not be considered. You should receive an e-mail confirming the receipt of your submission. If you do

not receive this confirmation e-mail, you must contact Dr Alex Ramirez ([alex@aaasv.org](mailto:alex@aaasv.org)) by Wednesday, September 24, 2014, with supporting evidence that the submission was made in time; otherwise, your submission will not be considered for judging. The abstracts will be reviewed by an unbiased, professional panel consisting of a private practitioner, an academician, and an industry veterinarian. Fifteen abstracts will be selected for oral presentation in the Student Seminar at the AASV Annual Meeting. Students whose papers are selected will be notified by October 15, 2014, and will be expected to provide the complete paper or abstract, reformatted for publication, by November 17, 2014.

To help defray the costs of attending the AASV meeting, Zoetis provides a \$750 honorarium to the student presenter of each paper selected for oral presentation during the Student Seminar.

Each veterinary student whose paper is selected for oral presentation also competes for one of several veterinary student scholarships awarded through the AASV



Foundation. The oral presentations will be judged to determine the amount of the scholarship awarded. Zoetis funds a \$5000 scholarship for the student whose paper, oral presentation, and supporting information are judged best overall. Elanco Animal Health provides \$20,000 in additional funding, enabling the AASV Foundation to award \$2500 each for 2<sup>nd</sup> through 5<sup>th</sup> place, \$1500 each for 6<sup>th</sup> through 10<sup>th</sup> place, and \$500 each for 11<sup>th</sup> through 15<sup>th</sup> place.

Abstracts that are not selected for oral presentation in the Student Seminar will be considered for participation in a poster session at the annual meeting. Zoetis and the AASV fund a stipend of \$250 for each student who is selected and participates in the poster presentation. In addition, the presenters of the top 15 poster abstracts compete for awards ranging from \$200 to \$500 in the Veterinary Student Poster Competition.

Complete information for preparing and submitting abstracts is available on the AASV Web site at [www.aasv.org/annmtg/2015/studentseminar.htm](http://www.aasv.org/annmtg/2015/studentseminar.htm). Please note: the rules for submission should be followed carefully. For more information, contact the AASV office (Tel: 515-465-5255; Fax: 515-465-3832; E-mail: [aasv@aasv.org](mailto:aasv@aasv.org)).

## 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 Orlando.

**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 26<sup>th</sup> Street, Perry, IA 50220-2328; Fax: 515-465-3832; E-mail: [aasv@aasv.org](mailto:aasv@aasv.org).

## Call for submissions – Industrial Partners

The American Association of Swine Veterinarians invites submissions for the Industrial Partners portion of the 46<sup>th</sup> AASV Annual Meeting, to be held February 28-March 3, 2015, in Orlando, Florida. This is an opportunity for commercial companies to make brief presentations of a technical, educational nature to members of the AASV.

As in the past, the oral sessions will consist of a series of 15-minute presentations scheduled from 1:00 to 5:00 PM on Sunday, March 1. A poster session will take place on the same day. Poster authors will be required to be stationed with their posters from 12:00 noon until 1:00 PM, and the posters will remain on display throughout the afternoon and the following day for viewing by meeting attendees.

Restricted program space necessitates a limit on the number of presentations per company. Companies that are members of the *Journal of Swine Health and Production* Industry Support Council (listed on the back cover of each issue of the journal) may submit two topics for oral presentation. Sponsors of the AASV e-Letter may submit an additional topic for oral presentation. All other companies may

submit one topic for oral presentation. Each company may also submit one topic for poster presentation (poster topics may not duplicate oral presentations). All topics must represent information not previously presented at the AASV Annual Meeting or published in the meeting proceedings.

Topic titles, a brief description of the presentation content, and presenter information (name, address, telephone and fax numbers, e-mail address) must be received in the AASV office by October 1, 2014. Please identify whether the submission is intended for oral or poster presentation. Send submissions via mail, fax, or e-mail to

Commercial Sessions, AASV  
830 26<sup>th</sup> Street, Perry, IA 50220-2328  
Fax: 515-465-3832  
E-mail: [aasv@aasv.org](mailto:aasv@aasv.org).

Authors will be notified of their acceptance by October 15, 2014, and must submit the paper for publication in the meeting proceedings by November 17, 2014. All presentations – oral and poster – will be published in the proceedings of the meeting. Papers for poster presentations are limited to one page of text plus one table or figure. Papers for oral presentations may be up to five pages in length (including tables and figures), when formatted according to the guidelines provided to authors upon acceptance of their presentations. Companies failing to submit papers in a timely manner will not be eligible for future participation in these sessions.







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3. Enter a PIN into the box beside Premises ID and click "Lookup."  
**PINs issued after May 9, 2006, do not contain the letters O or I.**
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**If the addresses don't match, double-check to make sure you entered the correct PIN. If there is still a problem, contact the Pork Checkoff Service Center at the number below.**
5. To print your barcode click on the "Barcode Labels" link which will open up a PDF you can print.  
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# Controlling PRRS Goes Beyond The Bottle

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package to help you control PRRS.



## Swine vets go to Washington

The AASV's executive committee held their eighth annual meeting with regulators, legislators, and researchers in Washington, DC. Drs Michelle Sprague, Ron Brodersen, George Charbonneau, Matt Anderson, Tom Burkgren, and Harry Snelson, along with the leadership from the American Association of Bovine Practitioners (AABP), were hosted May 5 and 6 by the Governmental Relations Division of the American Veterinary Medical Association (AVMA). This annual meeting is an excellent opportunity to offer continued stakeholder input on issues of importance to swine veterinarians.

Participants met with representatives from the following USDA agencies: Food Safety and Inspection Service (FSIS), Animal and Plant Health Inspection Service (by phone), Food and Drug Administration (FDA), and the Department of Homeland Security (DHS). In addition, we spent some time with research leaders from the Agricultural Research Service (ARS) and the National Institute for Food and Agriculture (NIFA). We also had a chance to hear what issues were driving the allied producer groups, including the National Pork Producers Council (NPPC) and the National Milk Producers Federation. The NPPC also scheduled time for our group to visit with

the offices of their congressional representatives on Capitol Hill. Following are some highlights of those discussions.

Dr Gary Sherman from NIFA described the Veterinary Medicine Loan Repayment Program now in its fifth year. The inaugural group just completed their 3-year program. They are eligible to reapply if they have at least \$20,000 in debt remaining. Annually, there have been approximately 180 to 200 under-served or shortage areas identified by the state animal-health officials. The program is able to fill approximately 45 of those shortages annually. Over the 5 years of the program, approximately 250 veterinarians have been accepted into the program.

Dr Peter Johnson, also from NIFA, described a new initiative for research funding involving public-private partnerships targeting innovation institutes. Each of the institutes is eligible for up to \$25 million to fund initiatives. This may be an additional funding opportunity for food-animal researchers interested in exploring such topics as antimicrobial resistance.

The main topic of interest with representatives from FSIS and FDA was the issue of violative drug residues. The majority of residues continue to be associated with cull dairy cows and bob veal calves. Market swine continue to have an exceptionally low rate of residues. However, the incidence of penicillin G residues in cull sows remains somewhat elevated since 2012, when FSIS validated a testing protocol allowing for the identification of penicillin G residues. In order to gain a better understanding of the significance of these findings, we asked FSIS to provide us a quarterly report outlining penicillin violations in sows. Additional discussions with FDA and FSIS are ongoing to determine any possible resolution to this issue.

In addition to the residue issue, we also discussed with FDA the issues of proposed changes to the Veterinary Feed Directive (VFD), compounding for food animals, and pain mitigation.

Drs Bernadette Dunham, Bill Flynn, and Craig Lewis reported that the agency was reviewing comments received regarding the VFD proposed rule. The goal was to publish a final rule, most likely in early 2015. The agency is aware of the challenges associated with the lack of approved products for use in pain mitigation for food-producing animals. They did reaffirm that the extra-label use of approved drugs for pain mitigation would be appropriate as long as all extra-label drug-use criteria could be met. They also reported that basically all manufacturers of feed-grade antimicrobials had indicated their intention to comply with directives to remove growth promotion claims from their products within the timeframe outlined in the FDA's guidance documents.

Dr John Clifford, Chief Veterinary Officer for the United States, joined the group by phone to discuss the proposed mandatory reporting plan and porcine epidemic diarrhea virus (PEDV) in general. He assured the group that it was not the intent of USDA to impose movement restrictions on producers who complied with a herd management plan developed in collaboration with either their herd veterinarian or a state or federal area veterinarian.

Lastly, we met with Dr Julie Brewer from the DHS. She updated the group on the status of the proposed National Bio and Agro Defense Facility. The 580,200-square-foot facility is to be built in Manhattan, Kansas, and will replace the aging facility on Plum Island. The cost estimate for the facility is now estimated at \$1.2 billion (\$900 million federal funds and \$300 million from Kansas). They propose that their 2015 budget request will complete the necessary congressional funding, and the project is slated for completion by 2022. Upon its completion, the USDA will be responsible for research, training, and diagnostic activities at the facility, while DHS will work to advance vaccine development and maintain the facility.

*Advocacy continued on page 267*







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Reference: 1. Buhr BL, Hurley T, Tonsor G, Zering K, DiPietre D. Comprehensive economic analysis of Improvest adoption by the US pork industry. *Am Assoc Swine Vet.* 2014;201-206.

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## Brief Summary

**CAUTION:** Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian.

**DESCRIPTION:** IMPROVEST is a sterile solution containing Gonadotropin Releasing Factor Analog-Diphtheria Toxoid Conjugate. Each mL contains 0.2 mg Gonadotropin Releasing Factor Analog-Diphtheria Toxoid Conjugate, 150 mg of diethylaminoethyl-dextran hydrochloride, 1 mg chlorocresol, sodium hydroxide as needed to adjust pH and water for injection.

**INDICATIONS FOR USE:** IMPROVEST is indicated for the temporary immunological castration (suppression of testicular function) and reduction of boar taint in intact male pigs intended for slaughter.

**DOSAGE AND ADMINISTRATION:** IMPROVEST should be administered via subcutaneous injection into the post auricular region of the neck. A safety injector should be used, preferably one which has a dual safety system providing both a needle guard and a mechanism to prevent accidental operation of the trigger. Each intact male pig should receive two 2-mL doses of IMPROVEST. The first dose should be administered no earlier than 9 weeks of age. The second dose should be administered at least 4 weeks after the first dose. Pigs should be slaughtered no earlier than 3 weeks and no later than 10 weeks after the second dose. In case of misdosing, the animal should be re-dosed immediately.

**CONTRAINDICATIONS:** Do not use IMPROVEST in intact male pigs intended for breeding because of the disruption of reproductive function. Not approved for use in female pigs and barrows.

## WARNINGS:

### WITHDRAWAL PERIODS:

No withdrawal period is required when used according to labeling.

**Not for Human Use. Keep Out of Reach of Children.**

## USER SAFETY WARNINGS:

**Warning for person administering IMPROVEST:** Accidental self injection could affect reproductive physiology of both men and women and may adversely affect pregnancy.

**Pregnant women should not administer this product. Women of childbearing age should exercise extreme caution when handling this product.** Special care should be taken to avoid accidental self injection and needle stick injury when administering the product. Protective clothing including, but not limited to, safety glasses and gloves should be worn. Use a safety injector, preferably one which has a dual safety system providing both a needle guard and a mechanism to prevent accidental operation of the trigger. In case of eye contact, rinse immediately with copious amounts of water. In case of skin contact, wash immediately with soap and water. The product should be stored safely out of the reach of children. As a reminder, it is the prescribing veterinarian's responsibility to inform drug administrators of the user safety warnings associated with IMPROVEST.

### Advice to the user in the event of accidental self injection:

In the event of accidental self injection, wash the injury thoroughly with clean running water. Seek prompt medical attention and take the package leaflet with you. Do not administer the product, and/or any other product with a similar action, in the future.

**Advice to the physician:** Accidental self injection could affect reproductive physiology of both men and women and may adversely affect pregnancy. If self injection with IMPROVEST is suspected, reproductive physiology should be monitored by assay of testosterone or estrogen levels (as appropriate). The risk of a physiological effect is greater after a second or subsequent accidental injection than after a first injection. The patient should be advised not to administer IMPROVEST, and/or any other product with a similar action, in the future.

For customer service, to report suspected adverse reactions or to obtain a copy of the Material Safety Data Sheet (MSDS) call 1-888-963-8471.

**PRECAUTIONS:** Subcutaneous injection in intact male pigs can cause a transient local injection site reaction that may result in trim loss at slaughter.

**ADVERSE REACTIONS:** The field study observations from field effectiveness studies were consistent with the observations made during the target animal safety studies of transient inflammation at the injection sites. IMPROVEST did not cause unusual clinical signs or an unexpected frequency or severity of injection site reactions. Adverse events, as reported, were not uniquely attributable to IMPROVEST.

**STORAGE INFORMATION:** Store under refrigeration at 2°-8°C (36°-46°F). Once broached, product may be stored under refrigeration for 28 days. Store bottles in carton until used. Protect from light. Protect from freezing.

**HOW SUPPLIED:** IMPROVEST is available in the following package sizes:  
20 mL bottle, 100 mL bottle, 250 mL bottle, 500 mL bottle.

Revised: January 2013

NADA # 141-322, Approved by FDA

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In addition, the group also met with swine researchers from the ARS and NIFA to discuss the priorities and direction for swine health, production, and welfare research in the face of continued declines in research funding. Dr Joan Lunney provided an update on the on-going research into viral and host genetics associated with porcine reproductive and respiratory syndrome.

Finally, the AASV leadership also had an opportunity to meet with their individual legislators on Capitol Hill to discuss legislation and funding concerns associated with swine health and production. The key issues discussed included funding for the National Animal Health Laboratory Network and

discussions regarding the impact PEDV has had on producers and practitioners.

This annual meeting affords our leadership an opportunity to interact with the leadership from AABP and the AVMA's Government Relations Division on a broad range of topics that potentially have a significant impact on the practice of food-animal veterinary medicine. Although it requires a time commitment away from practice responsibilities, I think all would agree that it is time well spent, and the association benefits from their participation.

Harry Snelson, DVM  
Director of Communications





# The evolution of the *Journal of Swine Health and Production*

## Informing and speaking for swine veterinarians

### Striving for excellence through the years

## AASPN Newsletter

Volume 1 • Number 1 July-August 1989



### Control Measures for Pseudorabies

• Timothy J. Loula

The question whether pseudorabies is a major, economically important swine disease has been hotly debated for years. This debate also has involved the issue of eradication. The availability of safe, effective, and economical vaccines has led to a national program to eradicate pseudorabies. As a practicing swine health consultant, it is my job to promote profitable swine production and to reduce the risk of economic loss. In many areas of the United States, including southern Minnesota where I practice, pseudorabies is endemic and control measures must be practiced routinely. The national program for pseudorabies eradication that began in January 1989 does not rely on the control of pseudorabies but that practitioners be aware of methods to eradicate the disease from the client's premises eventually.

**Prevention**  
The most important source of new infections is carrier swine brought into the susceptible susceptible herd. Most pigs brought onto farms are not

care of the pigs for three to four weeks. If off-farm facilities are used, the area should be checked for the possible presence of the pseudorabies virus (PRV). After 3 weeks, the new animals should be tested for PRV and any other diseases to be monitored. This reduces the risk of introducing infected animals or animals that may have picked up PRV in transit. When we are dealing with breeding stock farms or commercial operators who buy breeding stock at hog shows, we try to get the pig blood tested twice, 2 weeks apart, before entry. Only a small percentage of farms actually practice this preventative measure. Annual spread of PRV has been halted when other sources of infection cannot be confirmed. This is often the case in heavily infected areas. (continues on page 4)

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**1989**

This newsletter is the effort of several members, including our Executive Secretary, Dr. Tomas Nisula, and continues on page 12

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## AMERICAN ASSOCIATION OF SWINE PRACTITIONERS

### NEWSLETTER

September-October, 1991 Vol. 3, No. 5

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Volume 1, Number 1 January, 1993

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Volume 2, Number 1 January and February, 1994

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**1995**

What's your interpretation?  
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**2000**

The Official Journal of the American Association of Swine Practitioners

Nursery close out

Feed cost / lb gain

FE

scamples!

## JOURNAL OF Swine Health and Production

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**2014**

The Journal of the American Association of Swine Veterinarians



# Reporting a suspected foreign animal disease

## What to look for:

Since clinical signs for many foreign animal diseases resemble those of common endemic diseases, a complete history is essential to identify factors that raise suspicion of foreign animal disease. Pay attention to the following:

- Disease onset
- Foreign visitors or recent travel by employees
- Garbage feeding
- Which species are infected
- Recent animal introductions
- Consumption of foreign foodstuffs by employees
- Presence of vesicles/blisters
- Lack of response to treatment

## What to do:

1. Stay on the premises! Do not leave the farm unless absolutely necessary – and then only after thorough disinfection.
2. Contact the federal Assistant District Director (ADD) for your state or the State Veterinarian's office.
  - a. A list of the federal ADDs' contact numbers by state can be found at [www.aphis.usda.gov/wps/portal/banner/contactus/sa\\_animal\\_health/](http://www.aphis.usda.gov/wps/portal/banner/contactus/sa_animal_health/).
  - b. A list of State Veterinarians' contact numbers can be found at [www.usaha.org/Portals/6/StateAnimalHealthOfficials.pdf](http://www.usaha.org/Portals/6/StateAnimalHealthOfficials.pdf).

## What happens next:

1. The ADD will dispatch a Foreign Animal Disease Diagnostician (FADD) as quickly as possible to initiate an investigation. The ADD or State Animal Health Official will provide direction regarding your movements and information for you to convey to the staff and owners at the premises of concern. Your client will benefit from your interpretation and reassurance during this phase.
2. The FADD may set up an appointment to visit the premises, assess the disease situation, collect and submit laboratory samples, execute disease control actions if necessary, and file a report with the ADD.
3. The ADD will assign a priority level to the laboratory submissions which will govern the response of the federal lab(s).
4. Further actions may be taken at the discretion of the ADD in consultation with the FADD, State Veterinarian, and USDA Emergency Programs staff.
5. Laboratory results will be reported to the ADD who, in turn, will notify the State Veterinarian and the FADD. The FADD will then notify the practitioner and the owner.

It is important that you contact the ADD or State Veterinarian immediately if you suspect an FAD. To avoid laboratory contamination and possible disease spread, do not send samples to the diagnostic lab yourself.



**USDA Emergency Contact: 800-940-6524**



# Beyond our oath: Integrity, intensity, professionalism

**46<sup>th</sup> AASV Annual Meeting**

February 28 - March 3, 2015

Orlando, Florida

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**For more information:**

**<https://www.aasv.org/annmtg>**



# UPCOMING MEETINGS

## 24<sup>th</sup> Annual Swine Health and Production Conference

September 9, 2014 (Tue)  
Western Illinois University Union, Macomb, Illinois

Hosted by Carthage Veterinary Service, Ltd

For more information:

Karen Jacquot, Training and Education Coordinator  
PO Box 220, Carthage, IL 62321

Tel: 217-357-2811; Fax: 217-357-6665

E-mail: [kjacquot@hogvet.com](mailto:kjacquot@hogvet.com)

Web: <http://www.hogvet.com/conf-overview.htm>

## Allen D. Leman Swine Conference

September 13-16, 2014 (Sat-Tue)

St Paul RiverCentre, St Paul, Minnesota

For more information:

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1365 Gortner Ave, 462 Veterinary Medical Center  
St Paul, MN 55108

Tel: 800-380-8636 or 612-624-3434; Fax: 612-625-5755

E-mail: [vmedce@umn.edu](mailto:vmedce@umn.edu)

Web: <http://www.LemanSwineConference.org>

## 2014 USAHA and AAVLD Joint Annual Meeting

October 16-22, 2014 (Thu-Wed)

Sheraton Kansas City at Crown Center, Kansas City, Missouri

Hosted by United States Animal Health Association (USAHA)  
and American Association of Veterinary Laboratory Diagnosticians  
(AAVLD)

For more information:

Web: <http://www.usaha.org/Home.aspx>

## 2014 Leman China Swine Conference

October 20-22, 2014 (Mon-Wed)

Qujiang International Conference Center, Xi'an, China

Organized by the University of Minnesota

For more information (China):

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Room 919, Qinghe Qiangyou Building

Haidian District, Beijing, China 100085

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E-mail: [cisile@126.com](mailto:cisile@126.com)

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

For more information (United States):

Dr Bob Morrison

Tel: 612-625-9276

E-mail: [bobm@umn.edu](mailto:bobm@umn.edu)

Web: <http://www.cvm.umn.edu/lemanchina/>

## Swine Disease Conference for Swine Practitioners

November 13-14, 2014 (Thu-Fri)  
Ames, Iowa

Hosted by Iowa State University

For more information:

Conference Planning and Management

Iowa State University

1601 Golden Aspen Drive #110, Ames, IA 50010

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

E-mail: [registrations@iastate.edu](mailto:registrations@iastate.edu)

## 2014 North American PRRS Symposium and PED Update

December 5-6, 2014 (Fri-Sat)

Intercontinental Chicago Magnificent Mile  
505 N Michigan Ave, Chicago, Illinois

For more information:

Megan Kilgore

Kansas State University

Tel: 785-532-4528

E-mail: [vmce@vet.k-state.edu](mailto:vmce@vet.k-state.edu)

## American Association of Swine Veterinarians 46<sup>th</sup> Annual Meeting

February 28-March 3, 2015 (Sat-Tue)

Buena Vista Palace Hotel and Spa, Orlando, Florida

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](mailto:aasv@aasv.org)

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

## 7<sup>th</sup> International Symposium on Emerging and Re-emerging Pig Diseases

June 21-24, 2015 (Sun-Wed)

Kyoto International Conference Center, Kyoto, Japan

For more information:

E-mail: [iserpd2015@ics-inc.co.jp](mailto:iserpd2015@ics-inc.co.jp)

Web: <http://emerging2015.com>

## 24<sup>th</sup> International Pig Veterinary Society Congress

June 6-10, 2016 (Mon-Fri)

Dublin, Ireland

For more information:

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



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





**American Association of Swine Veterinarians**  
830 26<sup>th</sup> Street  
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## Photo Corner



A modern farrow-to-finish farm in central China

*Photo courtesy of Dr John Waddell*

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