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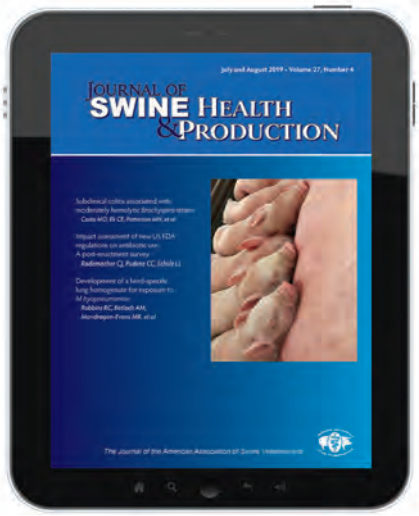

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About the cover...

Napping piglets.

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quoted from the President's message, page 191

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Put more pork on our fork

People like to eat. As incomes increase, people will eat more meat because it is a high-quality source of protein, vitamins, and minerals. Globally, pork is the meat of choice. Pork makes up 36% of the world's meat consumption, followed by poultry (35%) and beef (22%). Currently, net population growth is 78 million people per year and the global middle class is anticipated to rise from 2 billion in 2014 to 4.9 billion by 2030. During 2018, the United States exported 27.4% of the pork produced and is considered the world's least-cost producer of pork. According to the US Department of Agriculture (USDA), the price of pork exported during 2018 averaged only $1.20/lb, or 5.37 billion pounds valued at $6.392 billion. This is despite a difficult export year due to trade disputes, tariffs, and politics. The year going forward promises to be a banner year for all pork-exporting countries due to high demand in China and southeast Asia resulting from the rampant spread of African swine fever. I am confident the National Pork Board (NPB), National Pork Producers Council, and US Meat Export Federation will continue to regain and expand global market share throughout the world. With all this, the outlook for global meat consumption and US pork exports looks very bright!

Domestically, the pork demand story is quite different. Although the United States eats more meat per capita per year than almost any other country at about 264 lbs, pork (50 lbs/person) ranks third behind beef (56.9 lbs/person) and poultry (108.6 lbs/person). Marketing pork in a developed country is not just about being safe, nutritious, and affordable; but may also be about our carbon footprint, animal welfare, and transparency. Our consumers want choice and the free market will dictate the direction of those choices. A few opportunities for swine veterinarians to help advocate and advance our domestic pork sales include pork quality, pork labeling, and promoting facts and being transparent to slow the plant-based "meat" trend.

Pork quality – degree of doneness

Often the difference between a great pork eating experience and a poor pork eating experience is to not overcook pork. Way back in 2011, the USDA changed the recommended end-point cooking temperature for whole muscle pork cuts from 160° F (well-done degree of doneness) to 145° F (medium-rare) with a 3-minute rest, yet this is a relatively unknown fact to the average consumer. According to Jarrod Sutton (oral communication, 2018), NPB's Vice President of Domestic Marketing, an online Pork Checkoff funded survey of 1816 US adults revealed that 69% of consumers are currently over-cooking their pork, targeting well-done or medium-well temperatures. Only 10% of consumers target medium-rare temperatures. Messaging that pork should be cooked to a medium or medium-rare temperature with a moist and slightly rosy center is effective in convincing 54% of consumers to try a lower temperature. The difference in taste, tenderness, and juiciness is dramatic, especially in loins with minimal marbling. Sharing this message at a grass roots level (eg, Operation Main Street) whenever the opportunity presents itself can be one of the most simple and impactful ways to promote pork!

"During 2018, the United States exported 27.4% of the pork produced and is considered the world's least-cost producer of pork."

Pork labels

Another opportunity and pet peeve of mine is misinformation and confusing food labels on pork products in grocery stores. During a recent perusal of my local grocery store's meat counter, a label read "All-Natural Premium Pork, Two Thick Loin Chops, No Antibiotics or Hormones, Humanely and Locally Raised, Vegetarian Fed." What I liked about the label was that a local farmer had found a niche market for his pork chops at $7.89/lb. What I did not appreciate was the insinuation that other pork in the meat case may contain antibiotics (no pork, beef, or poultry does of course) or hormones. If a "no hormones" claim is made, the USDA would require the label to also state "Federal regulations prohibit the use of hormones in pork." Lastly, the cooking instructions on the back label indicated to "heat to 160° F or desired doneness." This prompted me to have a polite but firm discussion on mislabeling with the meat department manager on proper pork cooking temperatures and labeling.

Plant-based diets

There was a 600% (1% to 6%) increase in people identifying as vegan (ie, no animal protein) in the United States from 2014 to 2017 according to a report by Global Data.[1] A larger number of people consider themselves to be vegetarian (eg, my daughter and my barber) or follow a vegetarian-inclined diet. Plant-based eating may not be entirely mainstream yet. But it seems to be more accepted every day, with millennials as the central drivers. Burger King is rolling out the Impossible Whopper across the United States this year as a meatless burger alternative to try to win over vegetarian customers. Beyond Meat, the purveyor of plant-based burgers and sausages, made its stock market debut in April. Vegetarians often cite environmental concerns and animal welfare as reasons for their food choices.[2]

President's message continued on page 191

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President's message continued from page 189

Everyone has a right to their own convictions and food choices. As swine veterinarians, we need to better address and promote scientific facts to the public and continue to reduce our carbon footprint, fund animal welfare research, and be open, vocal, and transparent on the good job we do raising pigs. The facts are that the swine industry has reduced water use by 41% and land use per pig marketed by 78% since 1960. Greenhouse gases from swine production are only 0.4% of total emissions, with 29% coming from transportation and 28% from electricity.[3] Can we do better? Yes, we must. For example, Smithfield, the world's largest hog producer, has committed to a 25% reduction in their carbon footprint by 2025 through "manure-to-energy" projects designed to capture methane from manure to make clean renewable natural gas.[4]

Let's all do our part and continue to be advocates for pork and all of agriculture at every opportunity to help put more pork on our fork.

Nathan Winkelman, DVM
AASV President

Dr Nathan Winkelman and Daurio Passaia, general manager of Fogo de Chão Brazilian Steakhouse restaurant in Washington DC, discussing the recommended cooking temperature for pork.

Photo courtesy of Geni Wren

References

*1. Global Data. *Top trends in prepared foods 2017: Exploring trends in meat, fish and seafood; pasta noodles and rice; prepared meals; savory deli food; soup; and meat substitutes*. New York, NY: Global Trends; 2017.

*2. Vegetarian Times editors. Vegetarianism in America. Vegetariantimes.com. **https://www.vegetariantimes.com/uncategorized/vegetarianism-in-america**. Published May 10, 2017. Accessed May 7, 2019.

*3. US Environmental Protection Agency. Inventory of US greenhouse gas emissions and sinks:1990-2017. **https://www.epa.gov/sites/production/files/2019-04/documents/us-ghg-inventory-2019-main-text.pdf**. EPA430-R-19-001. Published April 11, 2019. Accessed May 7, 2019.

*4. Smithfield's Westerbeek recognized for leadership in renewable energy. National Hog Farmer Web site. **https://www.nationalhogfarmer.com/business/smithfields-westerbeek-recognized-leadership-renewable-energy**. Published May 15, 2019. Accessed May 17, 2019.

* Non-refereed references.

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

[2] Gaddy H et al. A review of recent supplemental iron industry practices and current usage of Uniferon® (iron dextran complex injection, 200 mg/mL) in baby pigs. *AASV*. 2012; 167–171.

[3] Haugegaard J et al. Effect of supplementing fast-growing, late-weaned piglets twice with 200 mg iron dextran intramuscularly. *The Pig Journal*. 2008. 61; 69–73.

[4] Olsen C and Fredericks L. Impact of iron dose and hemoglobin concentration on wean-finish weight gain. *IPVS*. 2018; 910.

PHARMACOSMOS

 PM-048-00

"You know nothing, Jon Snow"

As I write this, the HBO series Game of Thrones (GOT) has just begun its eighth and final season. For those of you that have been living under Casterly Rock since 2011, GOT is based on a series of books recounting a medieval country's seemingly never-ending civil war. The individual fiefdoms are constantly squabbling among themselves over who should rule the kingdom. While the alliances seem to shift around more frequently than air changes in a pig barn, sitting on the Iron Throne becomes a moot point when the White Walkers, an outside invader, threaten to take down the entire kingdom forcing the kids to unify against a common enemy. It falls to a brash young upstart of questionable heritage, Jon Snow, to try to band together the mischievous group of miscreants. At every step, he is met with skepticism and told, "You know nothing, Jon Snow!"

I feel like African swine fever (ASF) is the swine industry's version of the White Walkers. It has existed for a long time and been beaten back over the years until it was contained in a couple of isolated parts of the swine kingdom. The world was aware of it and kept up its guard, but generally speaking, the industry faced bigger issues. Until, one day, the wall that had been protecting the world's swine herds came crashing down allowing the invader to escape and hit at the very heart of the largest pork-producing countries in the world. African swine fever stared us in the face and said, "You know nothing, Jon Snow."

As in GOT, it is not that we really know nothing, it is just that we do not know enough, and we are often quite naïve. We feel threatened because the tools we are used to for diagnosing and fighting endemic diseases do not always work or are not available. In addition, the invader may have also acquired new weapons that we are ill-prepared to deal with - the White Walkers have a dragon with a seriously bad attitude and ASF may be able to cross the oceans in feed ingredients. Fortunately, we are aware that the threat is coming and we have time, although we do not know how much, to shore up our defenses.

Recently, the wardens of North America gathered in Ottawa to discuss how they might come together to harden our defenses and work with the rest of the world to begin to beat back this disease. There were over 150 attendees representing 15 countries. It was a great first step towards international collaboration. Swine industry representatives have been lobbying government leaders to implement surveillance strategies, develop and validate additional diagnostic tools, provide additional border security, conduct educational exercises, fund research to fill knowledge gaps, and secure the necessary resources to prevent and respond to an outbreak. The industry itself has spent producer dollars to fund research into better understanding ASF and to develop tools to enhance biosecurity, data transfer, and producer education. The goal is a smarter Jon Snow.

To their credit, government officials are listening and responding. Regulatory actions have been implemented addressing the importation of potentially contaminated goods. Additional detection tools are being secured and trained to enhance inspection at the borders. Diagnostic tests are being validated and additional sample types are being approved. State and federal animal health officials are exercising their response plans. Intergovernmental collaboration including industry stakeholders is a great starting point and a necessity.

The takeaway message, though, is that government can only do so much. There is a lot more left to do. We need a vaccine, feral swine controls, research to explore risk factors, mechanisms to address depopulation and carcass disposal, enhanced laboratory capacity, international agreements recognizing movement controls, adoption of business continuity strategies, and the list goes on. Protecting our industry falls to each one of us. What can you do? Educate yourself and your clients. Work to enhance on-farm biosecurity. Evaluate the sources of inputs coming onto your farms and select biosecure sources or implement holding times to reduce pathogen threat. Ensure that premises identification numbers are accurate and be willing to share the information animal health officials need to rapidly respond to a disease outbreak. Sign up for the Secure Pork Supply Plan. Attend regional ASF exercises when offered.

"Protecting our industry falls to each one of us. What can you do?"

We've come a long way since this invader broke out of Sardinia and Sub-Saharan Africa in 2007. But the disease continues to wreak havoc throughout parts of Europe, Russia, and Asia and is showing no signs of stopping. As I was watching the latest episode of GOT, it struck me that it was somewhat analogous to the challenge we are facing with ASF. By no means do I mean to minimize in any way the significance of the challenge facing our industry and the people's livelihoods that could be so dramatically affected as a result of an ASF outbreak. However, it is our hope that, by focusing the attention of all swine industry stakeholders on the ASF threat, we will be better able to prevent the introduction of the disease and respond to an outbreak. Hopefully our industry will be able to stand up when threatened and respond, "I may not know everything, but I know enough. And, by the way, I'm the rightful ruler of this kingdom!" Then we can get back to squabbling over the real international question of which is better: bacon, jamon, peameal, serrano, bratwurst, prosciutto, or pancetta?

Harry Snelson, DVM
Executive Director

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Author guideline improvements

I mentioned in my previous message that the journal staff has been working hard to provide updated author guidelines for the journal with the most notable change being the implications section.[1] I wanted to focus on some of the other changes that are now in the author guidelines, which will be reflected in published manuscripts you will see moving forward.

You will now find on the journal website an updated version of the author guidelines. Within the author guidelines, you will see a new table (Table 1) referring to manuscript genres and formatting requirements. The objective of this table is to help authors format their manuscripts correctly and subsequently help with the peer-review process. As I said in my last message, *"a correctly formatted manuscript does facilitate a smoother peer-review process, as a correctly formatted manuscript is easier for reviewers to review."*[1] I often find a quick reference guide, such as Table 1 in the *Journal of Swine Health and Production* (JSHAP) author guidelines, to be quite helpful, so we have provided one for our submitting authors. Historically the JSHAP has not had a word limit on manuscripts. But you will notice in this new table that there are now imposed word limits for our manuscripts in all genre sections. At the risk of seeming repetitive, it is becoming increasingly more challenging to find time to peer-review manuscripts and find peer-reviewers. A long manuscript can be overwhelming and time consuming to review. It can be difficult for reviewers to find time to commit to review a long manuscripts resulting in a delayed peer-review process. Often, manuscripts guilty of being too long really do benefit from being shortened and written with a slightly more succinct message. We feel the word limits that JSHAP has adopted are what are typically found in scientific journals and will not restrict the ability of an author to describe their work appropriately.

What else is new? Another author tool JSHAP has provided is an author checklist. This document reflects all the information that is described in full detail within the author guideline document. But the checklist is just that – a quick list of key style and format criteria to check prior to manuscript submission. For those authors who appreciate a checklist, the journal staff hopes this tool will appeal to you and we encourage all submitting authors to work through the checklist prior to submitting a manuscript.

The journal also now has manuscript templates for authors to use. We will be asking all submitting authors to utilize these genre-specific manuscript templates when preparing their manuscripts for submission. This is just one more tool to help authors with correct formatting.

The full author guidelines are available on the journal section of the AASV website (**www.aasv.org/shap/guidelines**). You will find an abbreviated version published in this issue with information guiding authors to the website for full details and genre templates.

All these changes are very exciting for the journal staff, but it is not just about us! As a reader of the journal, there should be no noticeable change in the final published manuscript except for the change in the implications section. As a submitting author, our hope is that these tools and requirement changes will help you to prepare your manuscript and make efficient use of your time doing so. As a reviewer, our hope is that the peer-review process will be more streamlined for you too.

"You will now find on the journal website an updated version of the author guidelines."

I have just described these changes in 2 very short editorial messages. In my previous message, I wrote about the changes to the implications section[1] and in this message about all the other improvements. The process to develop and implement these tools has taken considerable time and effort. I would like to acknowledge the journal staff and editorial board members for all their hard work and thought that has gone into the process. And, I would like to acknowledge Sherrie Webb specifically for her efforts to get these documents in place. Thank you.

Terri O'Sullivan, DVM, PhD
Executive Editor

Reference

*1. O'Sullivan T. Implications [editorial]. *J Swine Health Prod*. 2019;27(3):115.

* Non-refereed reference.

Peer reviewed

Original research

Subclinical colitis associated with moderately hemolytic *Brachyspira* strains

Matheus O. Costa, DVM, PhD; Courtney E. Ek, BSc; Mo H. Patterson; Roman Nosach, DVM; Susan E. Detmer, DVM, PhD; Champika Fernando, MSc; Gabrielle Paul-McKenzie, DVM; Steve van Ravenstein, DVM; Cole B. Enns, BSc; Matthew E. Loewen, DVM, PhD; Janet E. Hill, PhD; John C. S. Harding, DVM, MSc, DABVP

Summary

Objective: Microbiological and virulence characterization of 2 moderately hemolytic *Brachyspira* strains.

Materials and methods: Clinical isolates were obtained from diarrheic (3603-F2) and healthy (G79) pigs. Phenotypic characterization included assessment of hemolytic activity on blood agar and biochemical profiling. Genotyping was performed by sequencing the nicotinamide adenine dinucleotide oxidase (*nox*) gene, whole genome sequencing, and comparison to relevant *Brachyspira*. Pig inoculation included 4 treatment groups in 2 challenge experiments: negative control (sterile broth media; n = 12), positive control (*Brachyspira hampsonii* genomovar 2 strain 30446; n = 18), and 3603-F2 (n = 12) or G79 (n = 12). Fecal scoring and rectal swabbing for culture were performed daily. Animals were euthanized following onset of mucohemorrhagic diarrhea or between 21 and 28 days post inoculation (dpi). Gross and microscopic pathology were assessed. Terminal colon samples were used to characterize post-infection mucosal ion secretion.

Results: Both strains were moderately hemolytic. Whole genome and *nox* sequencing identified 3603-F2 as *Brachyspira murdochii* and G79 as a novel strain. Both challenge trials revealed intestinal colonization, but no mucohemorrhagic diarrhea. Sporadic watery diarrhea was induced by 3603-F2 associated with a pattern of microscopic lesions similar to pigs with swine dysentery (positive controls). No diarrhea was observed in G79 inoculated pigs, but microscopic lesions were more severe than in controls. Both strains induced greater colonic anion secretory potential than negative controls 21 dpi.

Implications: Allegedly avirulent *Brachyspira* species most closely related to *B murdochii* can be associated with subclinical colitis and may be a concern for grow-finish pigs.

Keywords: swine, swine dysentery, colitis, subclinical diarrhea, spirochetosis

Received: March 8, 2018
Accepted: February 20, 2019

Resumen - Colitis subclínica asociada con cepas de *Brachyspira* moderadamente hemolíticas

Objetivo: Caracterización microbiológica y virulencia de 2 cepas de *Brachyspira* moderadamente hemolíticas.

Materiales y métodos: Se obtuvieron aislados clínicos de cerdos diarreicos (3603-F2) y sanos (G79). La caracterización fenotípica incluyó la evaluación de la actividad hemolítica en agar sangre y el perfil bioquímico. La genotipificación se realizó mediante la secuenciación del gen nicotinamida adenina dinucleótido oxidasa (*nox*), la secuenciación del genoma completo y la comparación con *Brachyspira* relevante. La inoculación de cerdos incluyó 4 grupos de tratamiento en 2 experimentos de desafío: control negativo (medio de caldo estéril; n = 12), control positivo (*Brachyspira hampsonii* genomavariante 2 cepa 30446; n = 18) y 3603-F2 (n = 12) o G79 (n = 12). La puntuación fecal y el hisopado rectal para el cultivo se realizaron diariamente. Los animales fueron eutanasiados después de la aparición de diarrea mucohemorrágica o entre 21 y 28 días después de la inoculación. Se evaluó la patología macroscópica y microscópica. Se utilizaron muestras de colon terminal para caracterizar la secreción de iones de la mucosa después de la infección.

Resultados: Ambas cepas fueron moderadamente hemolíticas. La secuenciación del genoma completo y la secuenciación del *nox* identificaron la 3603-F2 como *Brachyspira murdochii* y la G79 como una cepa nueva. Ambos desafíos revelaron la colonización intestinal, pero no diarrea mucohemorrágica. La diarrea acuosa esporádica fue inducida por la 3603-F2 asociada a las lesiones microscópicas similares a los cerdos con disentería porcina (controles positivos). No se observó diarrea en cerdos inoculados con G79, pero las lesiones microscópicas fueron más severas que en el grupo control. Ambas cepas indujeron un mayor potencial secretor

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

Costa MO, Ek CE, Patterson MH, et al. Subclinical colitis associated with moderately hemolytic *Brachyspira* strains. *J Swine Health Prod.* 2019;27(4):196-209.

de aniones del colon que los controles negativos 21 días después de la inoculación.

Implicaciones: La especie supuestamente avirulenta de *Brachyspira* más estrechamente relacionada con *B murdochii* puede asociarse con colitis subclínica y puede ser importante en los cerdos de crecimiento y finalización.

Resumé - Colite subclinique associée à des souches de *Brachyspira* modérément hémolytiques

Objectif: Caractérisation microbiologique et de virulence de deux souches de *Brachyspira* modérément hémolytiques.

Matériels et méthodes: Des isolats cliniques furent obtenus de porcs diarrhéiques (3603-F2) et en santé (G79). La caractérisation phénotypique incluait l'évaluation de l'activité hémolytique sur gélose au sang ainsi qu'un profil biochimique. Le génotypage fut effectué par séquençage du gène de la nicotinamide adénine dinucléotide oxydase (*nox*), le séquençage du génome entier, et par comparaison à des *Brachyspira* appropriés. L'inoculation de porcs comprenait quatre groupes de traitement dans deux infections expérimentales: témoins négatifs (milieu de culture stérile; n = 12), témoins positifs (*Brachyspira hampsonii* genomovar 2 souche 30446; n = 18), et 3603-F2 (n = 12) ou G79 (n = 12). Le pointage fécal et un écouvillonnage rectal pour culture ont été effectués quotidiennement. Les animaux furent euthanasiés à la suite du début d'une diarrhée muco-hémorragique ou entre 21 et 28 jours post-inoculation. Les lésions macroscopiques et microscopiques ont été évaluées. Des échantillons de colon terminal furent utilisés afin de caractériser la sécrétion post-infection d'ions provenant de la muqueuse.

Résultats: Les deux souches étaient modérément hémolytiques. Le séquençage du génome entier et du gène *nox* permit d'identifier la souche 3603-F2 comme étant *Brachyspira murcochii* et G79 comme une nouvelle souche. Les deux infections expérimentales ont permis d'observer de la colonisation intestinale, mais pas de diarrhée muco-hémorragique. Une diarrhée aqueuse sporadique fut induite par la souche 3603-F2 associée avec un patron de lésions microscopiques similaires à celles de porcs avec de la dysenterie (témoins positifs). Aucune diarrhée ne fut observée chez les porcs inoculés avec la souche G79, mais les lésions microscopiques étaient plus sévères que chez les animaux témoins. Les deux souches ont induit un plus grand potentiel sécrétoire d'anions que les témoins négatifs au jour 21 post-inoculation.

Implications: Des espèces de *Brachyspira* supposées avirulentes et apparentées de près à *B murdochii* peuvent être associées à de la colite subclinique et représentées un souci chez les porcs en période de croissance-finition.

Before current molecular techniques were employed for bacterial characterization, strong β-hemolysis on culture plates was suggested as an indicator of virulence for *Brachyspira* species.[1-3] Further characterization of *Brachyspira* species using biochemical profiling was also subject to discussion, culminating with the identification of atypical isolates based on molecular methods.[4,5] These atypical isolates are often recovered from pigs with diarrhea, including allegedly non-pathogenic species which display weak to strong β-hemolysis activity or strains of pathogenic *Brachyspira* species that do not induce strong β-hemolysis.[6-10] This knowledge gap on *Brachyspira* virulence determinants led to the characterization of *Brachyspira murdochii* strains from diarrheic and healthy pigs across the globe.[4,6-8]

In western Canada, diagnostic surveillance during the past 8 years has revealed an increasing proportion of *B murdochii* cases associated with clinical diarrhea (Figure 1). A recent investigation led to the identification of two unique *Brachyspira* isolates: 3603-F2 was recovered from pig feces submitted to the Molecular Microbiology Research Laboratory at the University of Saskatchewan following a diagnostic investigation of watery diarrhea in a grow-finish farm and G79 recovered from a rectal swab of a healthy grower pig in a grow-finish commercial operation with a history of severe *Brachyspira hampsonii*-associated colitis. Both farms were in central Saskatchewan, Canada[6] but had no known epidemiological link. Both *Brachyspira* strains were initially identified as closely related to *B murdochii* based on partial sequencing of the nicotinamide adenine dinucleotide oxidase (*nox*) gene, a commonly utilized gene target for *Brachyspira* identification.[11] Based on the unusual presentation of the previously mentioned isolates, the objectives of this study were to conduct a detailed phenotypic and genotypic characterization of the isolates and to evaluate their pathogenicity in susceptible pigs using an experimental challenge model. Pathogenicity evaluation included the use of electrophysiology to investigate changes in the colonic function. This is a more objective approach than histopathology, as a measurement of absorptive and secretory capacities are generated.[12] Here we hypothesize that these 2 *Brachyspira* isolates affect colonic secretory and absorptive function, despite minimal observed histological changes.

Materials and methods

This work was designed and conducted in accordance with the Canadian Council for Animal Care and approved by the University Animal Care Committee at the University of Saskatchewan (Protocol No. 20130034).

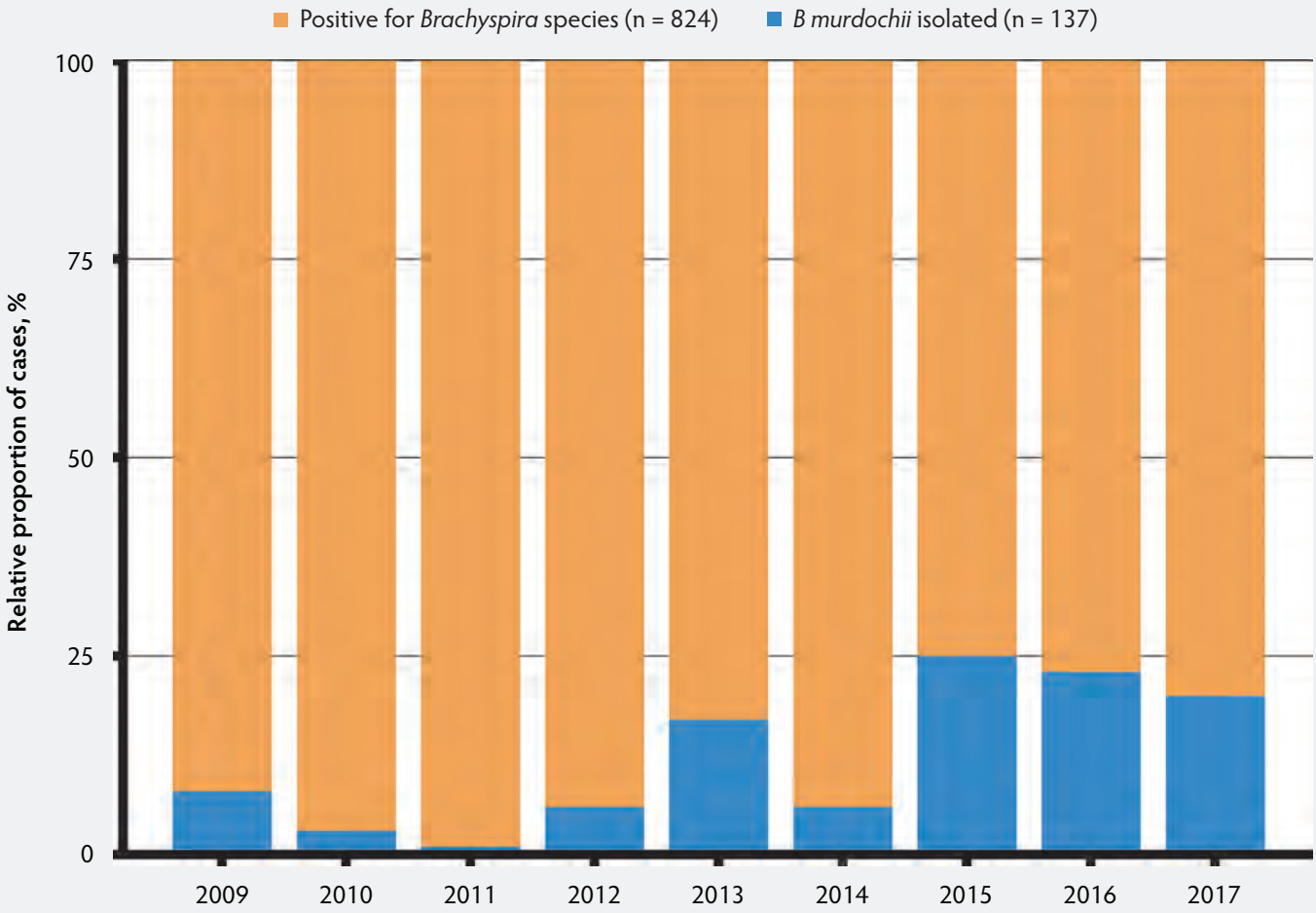
Bacterial strains and cultivation

Bacterial isolation and culture were performed as previously described.[6] Briefly, fecal samples were plated on selective blood agar (BJ), and incubated anaerobically at 42° C for 96 hours.[13] *Brachyspira* 3603-F2 (hereafter 3603-F2) was isolated from an 11-week-old pig presenting with watery diarrhea (no mucus or blood) at the time of collection. *Brachyspira* G79 (hereafter G79) was isolated from a rectal swab from a healthy 23-week-old pig.[6]

Phenotypic characterization of isolates

Isolates were grown in JBS broth (brain heart infusion with 5% [vol/vol] fetal calf serum, 5% [vol/vol] sheep's blood, and 1% [wt/vol] glucose) for biochemical profiling. An aliquot of 3 mL of 48-hour broth culture from each isolate was pelleted and washed twice with API zym suspension medium before being diluted to an optical density of 5 (MacFarland standard) supplied with the API zym kit (bioMerieux Inc, Durham, North Carolina). Isolates were then tested for enzymatic activity using API zym kit strips as suggested by the manufacturer. A second aliquot from each isolate culture (1 mL) was pelleted, washed twice and re-suspended in 0.85% saline for spot indole and hippurate broth assays, which were performed as previously described.[14] Isolate β-hemolysis activity was evaluated while grown on Columbia agar containing 5% sheep blood. Strong hemolysis was characterized by the formation of depigmented translucent areas on agar, whereas weak hemolysis meant

Figure 1: Relative proportion of cases in western Canada associated with *Brachyspira murdochii* based on all samples positive for any *Brachyspira* species in western Canada from January 1, 2009 to September 30, 2017.



pigmented, opaque areas. Hemolysis falling between these extremes (strong, weak) were considered moderate β-hemolysis. Type strains *Brachyspira hyodysenteriae* ATCC 27164^T, *B murdochii* ATCC 51284^T, and *Brachyspira pilosicoli* ATCC 51139^T were included as controls and for further comparisons in all tests.

nox amplification and sequencing

Partial *nox* sequences were amplified using *Brachyspira* genus-specific primers as previously described.[11] Amplicons were purified using a commercial kit (EZ-10 spin column PCR product purification kit, Bio Basic Canada Inc, Markham, Ontario, Canada) and sequenced using the amplification primers. Raw sequence data was assembled and edited using Pregap4 and Gap4, sequence alignments were performed with CLUSTALw, and phylogenetic trees were calculated in PHYLIP using the F84 distance matrix and neighbor-joining methods.[15,16]

Whole genome sequencing

For each isolate, genomic DNA was extracted and purified from 3 mL of JBS broth culture using a modified salting out procedure.[17] Whole genome sequencing was conducted using a shotgun sequencing approach with established manufacturer's protocols for the Roche GS Junior instrument (454 Life Sciences, Branford, Connecticut). Pyrosequencing data were processed using the default on-rig procedures from 454/Roche and assembled using gsAssembler (454 Life Sciences, Branford, Connecticut). The 3603-F2 and G79 genome sequence similarity to other known *Brachyspira* species was calculated using Average Nucleotide Identity (ANIm) by MUMmer within the JSpecies software package.[18] Virulence gene sequences were aligned to sequences from reference strains using BLASTn.[19]

Challenge experiment 1 – strain 3603-F2

Pigs. The methods used herein were modified from Costa et al.[20] Healthy, 5-week-old barrow pigs (n = 24) of average body weight compared to their cohorts were purchased from a single porcine reproductive and respiratory syndrome negative commercial farm in Saskatchewan, Canada with no clinical history of swine dysentery or previous laboratory diagnosis of *Brachyspira*. Upon arrival at the isolation facility, pigs were randomly allocated to treatment groups using a random number generator. Animals were acclimated for 7 days prior to inoculation. Rectal swabs and feces collected at -5, -2, and 0 days post inoculation (dpi) were cultured on BJ media as previously described to detect any *Brachyspira* species infection acquired before inoculation on 0 dpi. Pigs were fed a non-medicated, mash starter diet containing wheat, barley, soybean meal, canola

meal, vitamins, and minerals *ad libitum* for the duration of the trial, with the exception of 12 hour fasting periods prior to each of 3 inoculations. Three treatment groups were used: a negative control (sterile broth, n = 6), a positive control (*B hampsonii* genomovar 2 strain 30446, n = 6) and the experimental group (3603-F2, n = 12). One 3603-F2 challenged pig was removed from the trial at 10 dpi due to an unrelated injury, and all associated data from this pig was excluded from the analyses. Each treatment group was housed in separate animal biosafety level 2 (BSL-2) rooms in a series of 1.2 × 1.8 m^2 pens with solid concrete floors. Positive control and treatment pigs were housed 2 pigs/pen, whereas negative controls were housed 3 pigs/pen. Pens were scraped daily but not washed in order to promote fecal-oral transmission of *Brachyspira*.

Inoculation. On three consecutive days beginning 0 dpi, pigs were sedated using azaperone (6 mg/kg intramuscular; Stresnil, Elanco, Guelph, Canada) and intragastrically inoculated using an 18 French feeding tube. Feed was removed 12 hours prior to each inoculation and was replaced 2 to 3 hours post inoculation to avoid feed aspiration. Each pig from the positive control group or the experimental group received 10 mL of inoculum JBS broth containing 10^8 genomic equivalents/mL of their respective *Brachyspira* strain on each day. The negative control group received sterile JBS broth.

Clinical assessment. Observation of clinical signs was performed twice per day to evaluate responsiveness, skin color, body condition, respiratory effort, and fecal consistency of all pigs. Feces were scored from 0 to 4 based on physical appearance and consistency: 0 = normal, formed; 1 = soft, wet cement consistency; 2 = runny or watery diarrhea; 3 = mucoid diarrhea; 4 = bloody diarrhea (with or without mucus). Because clinical observers (n = 3) were not blinded to treatment group, they rotated daily among rooms to minimize observer bias as much as possible. Observers were trained through several previous *Brachyspira* inoculation trials using the same scoring system. Beginning 5 dpi, fecal samples were collected daily using rectal swabs for culture followed by species identification by polymerase chain reaction (PCR).

Pathology. Pigs were humanely euthanized by cranial captive bolt and exsanguination within 48 hours of displaying mucohemorrhagic diarrhea (fecal score 4) or between 21 and 28 dpi if no diarrhea was observed. Necropsy examination focused on the gastrointestinal tract. Cecum and spiral colon were linearized and longitudinally opened, and the colon was divided into thirds (proximal, apex, and distal). Gross lesion severity was scored based on the presence of characteristic lesions of swine dysentery including hyperemia, congestion, edema, necrosis, fibrin, and mucus by a single pathologist blinded to treatment group. Colonic tissue from the apex spiral colon was collected for *Brachyspira* culture, PCR, and sequencing. Colon samples were fixed in 10% buffered formalin for 24 to 48 hours prior to processing for paraffin embedding. Hematoxylin and Eosin (HE) and Warthin-Faulkner (WF) staining was conducted on 4 µm sections of embedded tissue. Microscopic lesions in the spiral colon and cecum were scored based on the severity of the inflammation and necrosis: 0 = no lesions; 1 = minimal to mild necrosis of superficial enterocytes with minimal inflammatory infiltrates; 2 = moderate necrosis and attenuation of enterocytes with mild to moderate inflammatory infiltrates; 3 = severe necrosis (erosion or ulceration present) with moderate inflammatory infiltrates predominantly consisting of neutrophils. The presence of *Brachyspira*-like organisms was scored from 0 to 3 in WF stained sections: 0 = no spirochetes observed; 0.5 = a single gland contained a few spirochetes; 1 = small numbers of spirochetes in multiple glands; 2 = many spirochetes within several glands; 3 = many spirochetes forming thick mats in numerous glands. After scoring each section, an overall pathology impression score was assigned to each sample. Samples of colon for detection of *Salmonella* on brilliant green agar following enrichment in selenite broth and ileum for detection of *Lawsonia intracellularis* by PCR[21] were submitted from each animal to Prairie Diagnostic Services Inc for differential diagnostics.

***Brachyspira* culture of feces**. Rectal swabs were plated on BJ and incubated anaerobically using a commercial gas pack (Oxoid Limited, Basingstoke, United Kingdom) at 42° C for 96 hours. Zones of β-hemolysis were semi-quantified after 48 and 96 hours by the number of streaks observed (1+ to 4+) as well as the strength of hemolysis (strong, moderate, or weak). The absence of hemolysis was recorded as culture negative. After 96 hours of incubation, all the plates which were positive for hemolysis were tested through *nox* sequencing to confirm the species of *Brachyspira* detected. For analytical purposes, a pig was considered colonized if *Brachyspira* was isolated from rectal swabs between 5 and 21 dpi.

Challenge experiment 2 – strain G79

Methods for this experiment were predominantly the same as challenge experiment 1, with main differences related to the number and assignment of pigs across the treatment groups. The animals used were from the same source as challenge experiment 1. However, upon arrival to the BSL-2 facility, pigs were blocked by weight and then assigned to different treatment groups. This experiment had 3 treatment groups: negative control (sterile broth, n = 6), positive control (*B hampsonii* genomovar 2 strain 30446, n = 12) and the experimental group (G79, n = 12). In addition, the clinical observers were blinded to identity of the G79 and positive control groups. Daily fecal samples were collected from 5 to 21 dpi with euthanasia occurring within 48 hours of pigs displaying mucohemorrhagic diarrhea or between 21 and 26 dpi if no mucohemorrhagic diarrhea was observed. All other procedures were performed as described for challenge experiment 1.

Colonic mucosa ion secretory capacity

Positive control pigs from both trials were excluded from the analyses because they were euthanized when they presented with mucohemorrhagic diarrhea (at peak clinical signs). By contrast, pigs in the negative control, 3603-F2 and G79 groups were euthanized at the end of the experiments (after 21 dpi) because they did not develop mucohemorrhagic diarrhea. Therefore, electrogenic secretory analyses included the 3603-F2 inoculated pigs (n = 12), negative controls for 3603-F2 (n = 6), G79-inoculated pigs (n = 12), and negative controls for G79 (n = 6). Briefly, segments of spiral colon (apex region, midpoint between the cecocolic junction and the transversal colon) were harvested immediately after euthanasia. Segments were washed with Krebs buffer (pH 7.4, containing 113 mM NaCl, 5 mM KCl, 1.6 mM Na_2HPO_4, 0.3 mM $NaH_2PO_4 \bullet H_2O$, 25 mM $NaHCO_3$, 1.1 mM $MgCl_2 \bullet 6H_2O$, 2.2 mM $CaCl_2 \bullet 2H_2O$, and 10 mM glucose) chilled to 4° C. Samples were immediately transported to the lab in Krebs buffer gassed with 95% O_2 and 5% CO_2 where the serosa (visceral peritoneum) and longitudinal and circular muscle

layers of the colonic wall were removed. Pieces of stripped mucosa (2-12 tissue replicates of each segment per pig) were then placed on 1 cm^2 Ussing chamber inserts and placed into the Ussing chamber (Physiologic Instruments, San Diego, California). Each reservoir was independently gassed with 95% O_2 and 5% CO_2. A heated circulating water bath maintained the bathing buffer in the Ussing chamber constantly at 37° C. Transepithelial potential differences were short-circuited to 0 mV with a voltage clamp using Ag-AgCl electrodes and 3 M KCl agar bridges (Physiologic Instruments, San Diego, California) on apical and basolateral sides.

Samples were allowed to equilibrate for 20 minutes before the addition of any drugs. A 1mV pulse every 30 seconds was used to determine the resistance and tissue viability from the resulting current. Changes in short-circuit current (I_{sc}) were measured following tissue exposure to different drugs. After a steady state was reached, 10 μM of the adrenergic agonist isoproterenol (I6504; Sigma Aldrich) was added to both the apical and basolateral sides of the chamber to increase cyclic adenosine monophosphate (cAMP) and stimulate cAMP activated channels, such as the cystic fibrosis transmembrane conductance regulator gene (CFTR). After a steady state was reached, 0.1 mM of carbachol (C4382; Sigma Aldrich) was added to both the apical and basolateral sides of the chamber. This cholinergic agonist increases intracellular Ca^{2+} activating calcium dependent ion transport channels. After a steady state was reached, 10 μM of forskolin (F6886; Sigma Aldrich) and 1 mM of 1 M 3-isobutyl-1-methylxanthine (IBMX; I5879; Sigma Aldrich) were added to the apical and basolateral sides of the Ussing chamber causing a massive irreversible and sustained elevation in cAMP to fully induce cAMP-activated secretion. Finally, after a steady state was reached, 0.1 mM bumentanide (B3023; Sigma Aldrich) was added to the basolateral side of the Ussing chamber to inhibit the basolateral Na^+-K^+-$2Cl^-$ co-transporter 1 (NKCC1).

Statistical analysis

Except for the third cluster analyses, the statistical analysis was performed independently for each animal experiment using Prism 7.0c (GraphPad Software, La Jolla, California) and Stata 14 (StataCorp, College Station, Texas). Potential group differences in the frequency of dichotomized clinical, microbiological and pathological outcome measures (diarrhea, colonized, terminal culture results) were assessed using a Fisher's exact test. Potential differences in continuous and ordinal outcome measures (days to first fecal culture positive, % fecal samples culture positive) between the *Brachyspira* inoculated groups were compared using Kruskal-Wallis or Mann-Whitney U test as appropriate.

A multivariate approach was used to assess potential group differences in histological lesion severity and mucosal secretory capacity. This enabled multiple histological or physiological outcome measures to be compared among the group simultaneously. Three cluster analyses (Ward's linkage; Euclidean dissimilarity measure) were performed. Histological lesion severity (scaled-ranks of colonic and cecal necrosis and inflammation, WF staining scores) was analyzed separately for each challenge experiment as previously described.[22] A third cluster analysis was used to assess potential differences in colonic mucosa ion secretory capacity in the spiral colon using electrical I_{sc} generated in Ussing chambers. For this, negative control, 3603-F2, and G79 pigs were included and the I_{sc} results of multiple tissue replicates were averaged into a composite score for each pig. Data from both challenge experiments were analyzed together to increase the delineating power of the cluster analysis.

For all cluster analyses, the appropriate number of clusters was determined using the post-hoc Duda-Hart Je(2)/Je(1) and Calinski stopping rules. Kruskal-Wallis and post hoc Dunn tests with Sidak multiple comparison adjustments were used to assess how the clusters differed in terms of the underlying histological or ion secretory capacity variables. For all analyses, $P < .05$ was considered statistically significant *a priori*.

Results

Microbiological characterization

Phenotypic characterization. A summary of the phenotypic characterization results is found in Table 1. β-Hemolytic activity for 3603-F2 and G79 was moderate in both cases, whereas the *B murdochii* type strain displayed only weak β-hemolysis on blood agar plates (Figure 2). Both test strains and the *B murdochii* type strain were positive for β-glucosidase activity. The only strain which was positive for the indole spot test and α-glucosidase activity was *B hyodysenteriae*.

Phylogenetic analysis. Figure 3 is a visual representation of genetic similarities of different *Brachyspira* species based on the alignment of partial *nox* gene sequences (801 bp). Strain 3603-F2 clusters with the type strain of *B murdochii*, as expected since their partial *nox* sequences were 99% identical to each other. Strain G79 is separated from both *Brachyspira innocens* and *B murdochii* with good bootstrap support, as their *nox* sequence was 97% and 96% similar, respectively. Neither G79 nor 3603-F2 strains clustered near the pathogenic *B hyodysenteriae*, *B hampsonii,* or *B pilosicoli*.

Whole genome sequencing. Shotgun sequencing resulted in 154,057 high quality reads (average read length of 478 bp) with 28.04% G/C content for strain 3603-F2, and 182,906 high-quality reads (average read length of 404 bp) with 28.23% G/C content for strain G79. Assembled and annotated genome sequences have been deposited to the National Center for Biotechnology Information Genbank database with accession numbers JQIU00000000 (G79) and JJMJ00000000 (3603-F2). Table 2 shows the ANIm of strains 3603-F2, G79 in comparison to the complete genome sequences of *B hyodysenteriae* WA1 (GenBank accession NC_012225), *B pilosicoli* 95/1000 (NC_014330), *Brachyspira intermedia* PWS/A (NC_017243), *B hampsonii* genomovar II (strain 30446, ALNZ00000000), *B hampsonii* genomovar I (strain 30599, GCA_000334935), *Brachyspira suanatina* AN4859/03 (GCA_001049755), and *B murdochii* DSM 12563 (synonym of ATCC 51284T, NC_014150). Whole genome sequences ANIm values of < 95% correspond to different bacterial species as defined by DNA-DNA hybridization.[18] The ANIm values ranged from 85.89% to 98.25% between all species. Strain 3603-F2 was found to be 98.25% identical to the *B murdochii* type strain. Strain G79 was < 95% similar to any *Brachyspira* species included in the analysis. Its closest relatives were *B innocens* (93.4%) and *B murdochii* (93.2%).

To identify putative hemolysin genes in the genome of strains 3603-F2 and G79, gene sequences of *tly*A, *tly*B, *tly*C, and *hly*A from *B hyodysenteriae* WA1 (NC_012225), a pathogenic strain, were used as a reference in comparison to the studied strains. Predicted open reading frames with significant similarity to all 4 putative hemolysin genes were identified in the genomes of 3603-F2

Table 1: Phenotypic profiles of strains 3603-F2 and G79 and other *Brachyspira* species type strains.

Isolate	β-hemolysis	Indole	Hippurate	α-Galactosidase activity	α-Glucosidase activity	β-Glucosidase activity
*B murdochii**	Weak	-	-	-	-	+
*B hyodysenteriae**	Strong	+	-	-	+	+
*B pilosicoli**	Weak	-	+	+†	-	-
3603-F2	Moderate	-	-	-	-	+
G79	Moderate	-	-	-	-	+

* type strain.
† weak positive.

and G79: *tly*A (83% nucleotide identity to both strains), *tly*B (87% identity to strain 3603-F2 and 85% to strain G79), *tly*C (86% identity to strain 3603-F2 and 84% to strain G79), and *hly*A (95% identity to strain 3603-F2 and 86% to strain G79). In parallel, *B murdochii* (NC_014150) *tly*A, *tly*B, *tly*C, and *hly*A sequence similarities to the same *B hyodysenteriae* were 83%, 88%, 84%, and 95%, respectively. When investigating the G79 and 3603-F2 hemolysin genes' homology to the non-pathogenic *B innocens* ATCC 29796 (GCA_000384655), the only gene present in the *B innocens* genome was *tly*A, which had an 85% nucleotide identity to G79 and 84% to 3603-F2.

Strain virulence evaluation

Challenge experiment 1 – strain 3603-F2. A summary of clinical and microbiological findings from this trial are presented in Table 3. Median fecal scores were significantly greater in the 3603-F2 group compared to negative control but none of the pigs in either group developed mucohemorrhagic diarrhea during the study period. However, 5 of 11 (45%) of the 3603-F2-inoculated animals had scattered episodes of watery diarrhea that ceased within 12 hours, while 10 of 11 (91%) of the 3603-F2 pigs were colonized with β-hemolytic spirochetes subsequently identified as strain 3603-F2 by partial *nox* sequencing. *Brachyspira* characterized by moderate β -hemolysis was cultured from the colonic mucosa collected at termination from all except one pig (pig 53) from the 3603-F2 group. Interestingly, one pig (pig 61) was culture positive with 2^+ β-hemolysis on 6 dpi and developed watery diarrhea on 7 dpi despite having no β-hemolysis observed on blood agar plates from samples collected on that same day.

The majority of the positive control *B hampsonii* inoculated animals (4 of 6, 67%) developed mucohemorrhagic diarrhea as expected, and 5 of 6 pigs in this group became colonized. Partial *nox* gene sequencing confirmed *B hampsonii* strain 30446 in terminal colon samples from 4 of 6 (67%) pigs. All samples tested negative for *L intracellularis* by PCR and *Salmonella* by isolation.

Necropsy examinations revealed mildly inflamed and hyperemic cecal mucosa in 3 of 11 (27%) 3603-F2-inoculated pigs, but the entire length of the spiral colon had no visible lesions. No other gross lesions were observed in the remaining 3603-F2 inoculated pigs or in the controls. The 2 *B hampsonii* inoculated pigs that remained healthy also had no gross lesions, but the other 4 pigs presented with mild to severe typhylocolitis with mucohemorrhagic and necrotic exudate.

The 3603-F2-inoculated pigs had histologic evidence of mild to moderate inflammation, luminal mucous accumulation, and limited epithelial necrosis throughout the colon and cecum (all scores ≤ 2; Figure 4). Sections from the large intestine of the *B hampsonii* pigs typically had more severe lesions featuring large mats of mucous and bacteria associated with focal epithelial necrosis. The negative control group had no or mild lesion scores (all ≤ 1). Spirochetes were observed in 11 of 12 colonic sections from 3603-F2-inoculated pigs, whereas the clinically affected *B hampsonii*-inoculated group had both moderate and large numbers of spirochetes visible. No spirochetes were observed in colonic sections of all negative control pigs, except one with very low numbers.

Using cluster analysis, pigs were assigned to 1 of 4 'severity clusters' based on the severity of histological lesions in the colon and cecum and their colonic spirochete score (Figure 5). Two of the 4 *B hampsonii* inoculated pigs with mucohemorrhagic diarrhea at termination comprised the highest severity cluster (cluster 4). Six 3603-F2 inoculated pigs were grouped in cluster 3 (moderate severity) along with the other 2 *B hampsonii* pigs that had mucohemorrhagic diarrhea at termination. Cluster 3 mainly featured pigs with moderate colonic inflammation and necrosis. The remaining five 3603-F2 inoculated pigs were grouped within cluster 2 (mild severity) along with 2 negative-control and 2 non-diarrheic *B hampsonii* pigs. Severity cluster, however, was not associated with fecal shedding.

Challenge experiment 2 – strain G79. A summary of clinical and microbiological findings for this trial is presented in Table 3. Positive control (*B hampsonii* strain 30446) pigs developed mucohemorrhagic diarrhea (5 of 12, 42%). None of the negative control animals developed any clinical diarrhea (mucohemorrhagic or otherwise) during the experimental period. Five of 12 G79 inoculated pigs, and 2 of 4 control pigs developed one or more days of intermittent mild diarrhea of wet cement consistency. Despite observing mucohemorrhagic diarrhea in *B hampsonii*-inoculated pigs, median fecal scores did not differ across group ($P = .08$), largely because the pigs developing mucohemorrhagic diarrhea did so very acutely then were terminated. Ten of 12 (83%) G79-inoculated pigs, and 7 of 12 (58%) *B hampsonii* strain 30446 pigs became colonized by their respective inocula strain. At termination, colonic contents from 6 of 12 (50%) G79 and 6 of 12 (50%) *B hampsonii* group animals were culture-positive for their respective inoculum species, confirmed by partial *nox* gene sequencing. None of the negative

control animals shed β-hemolytic bacteria at any point during this study. All samples were negative for *L intracellularis* by PCR.

Necropsy examination of G79-inoculated and negative control pigs revealed no visible gross abnormalities in ceca and large intestines, whereas 6 of 12 (50%) *B hampsonii* pigs had moderate to severe, multifocal to diffuse colitis associated with mucohemorrhagic and fibrinonecrotic lesions consistent with swine dysentery. Histological lesions were severe in the *B hampsonii*-inoculated pigs with 6 of 12 (50%) showing moderate to severe inflammation and necrosis of their colonic and cecal mucosa. By contrast, histopathology was milder in the G79-inoculated and negative control pigs, consistent with the lack of gastrointestinal clinical signs. Animals were grouped into 3 clusters based on their histological lesion severity and spirochetal scores (Figure 6). Five of 12 *B hampsonii* inoculated pigs, including 4 with mucohemorrhagic diarrhea at termination and 1 non-diarrheic *B hampsonii* pig (pig 173) grouped in the highest severity cluster (Cluster 3). The moderate severity cluster (Cluster 2) comprised the remaining *B hampsonii* pigs, the majority (8 of 12) of G79 pigs, and 1 negative control animal. The low severity cluster (Cluster 1) comprised the majority (5 of 6) of control pigs, as well as 4 of 12 G79 and 2 of 12 *B hampsonii* inoculated pigs, all of which were clinically normal at termination except 1 *B hampsonii* inoculated pig (pig 181) that had mucohemorrhagic diarrhea of 1-day duration at the time of necropsy (an acute case).

Colonic mucosa ion secretory capacity

The electrogenic anionic secretory response of spiral colon (apex) mucosa was investigated in pigs inoculated with both tested strains (3603-F2 and G79) and negative controls in Ussing chambers by characterizing changes in I_{sc} following the movement of ions between the apical (luminal) and basolateral (stromal or circulation) aspects of the colonic epithelium. Each of the 4 drugs used stimulated or inhibited a different ion channel pathway, and collective responses were used to assess an increase or decrease in intestinal secretion in ex vivo tissues. Although no pigs had diarrhea when the colonic mucosal tissues were collected for Ussing chamber analyses, their secretory responses grouped into 3 distinct clusters (normal, moderate, and markedly enhanced; Figure 7). There were clear differences between the *Brachyspira*-inoculated and negative control pigs but the 3603-F2 and G79 inoculated pigs had similar responses. The majority of 3603-F2 (8 of 10) and G79 (9 of 12) inoculated pigs had moderately or markedly enhanced secretory responses (positioned in clusters 2 or 3) compared to the majority of negative control pigs (10 of 12) which grouped into cluster 1, considered normal. Specifically, pigs in clusters 2 and 3 had greater responses to isoproterenol (an adrenergic stimulator of CFTR chloride secretion), carbachol (stimulator of calcium-activated chloride channels), forskolin/IBMX (direct stimulation of CFTR-based chloride secretion), and bumentanide (inhibitor of basolateral to apical chloride movement through Na^+-K^+-$2Cl^-$ co-transporters).

Figure 2: Degree of hemolysis on selective blood agar plates induced by A) strain G79, moderate; B) *Brachyspira murdochii* type strain, weak; C) strain 3603-F2, moderate; and D) *Brachyspira hampsonii* genomovar II strain 30446, strong.

Figure 3: Phylogenetic tree displaying the relatedness of moderately hemolytic *Brachyspira* isolates 3603-F2 and G79 to the *Brachyspira* species type strains. This tree is based on an 801 bp alignment of partial *nox* gene sequences. Bootstrap values are indicated on branches.

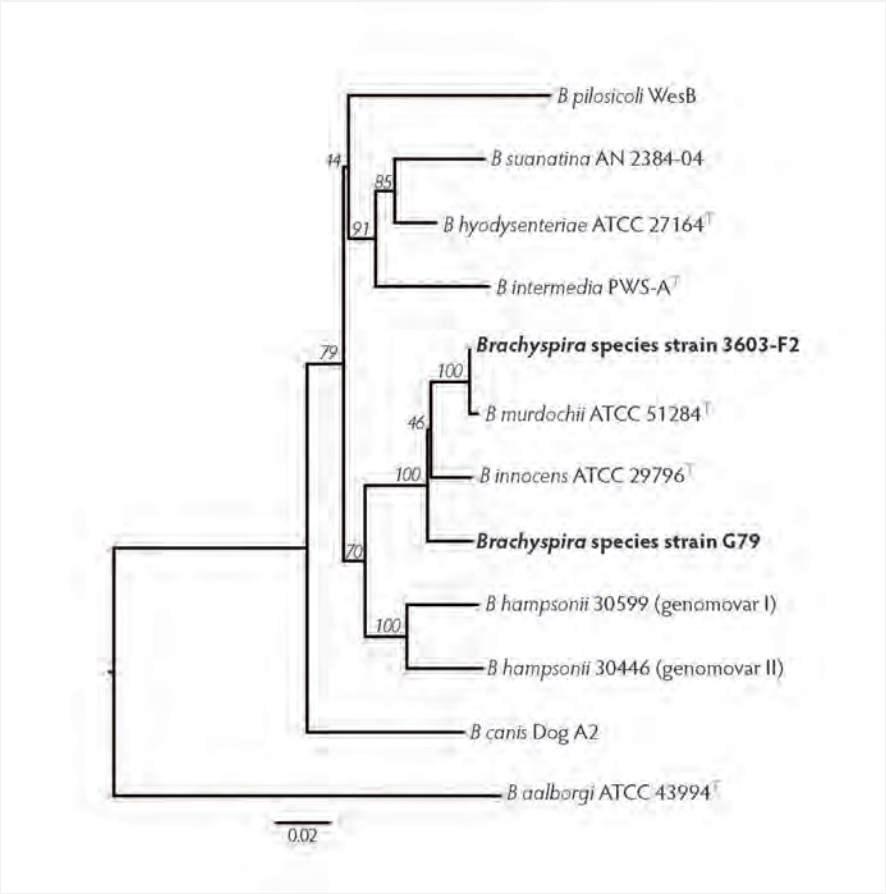


Table 2: Whole genome sequence ANIm results based on MUMmer*

	B innocens	*B intermedia*	*B pilosicoli*	Strain G79	Strain 3603-F2	*B hyodysenteriae*	*B hampsonii 30599*	*B hampsonii 30446*	*B murdochii*
B suanatina	86.09	93.28	86.32	86.01	86.09	92.74	89.31	88.81	86.02
B murdochii	93.43	86.06	85.56	93.2	98.43	86.01	88.04	87.62	
B hampsonii 30446	87.65	89.01	85.6	87.63	87.67	88.78	93.63		
B hampsonii 30599	87.86	89.44	85.62	87.89	87.88	89.27			
B hyodysenteriae	86.06	91.94	86.37	86.13	86.04				
Strain 3603-F2	93.51	86.33	85.5	93.03					
Strain G79	93.42	86.05	85.68						
B pilosicoli	85.68	86.63							
B intermedia	86.45								

* ANI values > 95% have been demonstrated to correspond to different bacterial species as defined by DNA-DNA hybridization. The highest scores for each experimental strain are underlined.
ANIm = average nucleotide identity.

Discussion

Spirochetes of the *Brachyspira* genus are of interest to the pork industry due to their ability to cause mucoid or hemorrhagic diarrhea. Three species, *B hyodysenteriae*, *B pilosicoli* and *B hampsonii*, have targeted diagnostic tests available in most diagnostic laboratories due to their clinical and economic significance. Since 2009, western Canada submissions by veterinarians revealed a growing number of samples positive for *B murdochii* in feces of colonic tissue samples. The characterization of 2 such *Brachyspira* isolates, which by many would be considered non-pathogenic and non-production limiting, is described here. Both isolates were identified as closely related to *B murdochii* by sequencing of the *nox* gene, and speciation by whole genome sequencing revealed similar results. Strain G79 was recovered from a healthy grower pig on a farm with a clinical history of severe mucohemorrhagic diarrhea associated with *B hampsonii*. Strain 3603-F2 was isolated from a diagnostic sample collected from a pig with diarrhea. In the current study, neither strain caused mucohemorrhagic diarrhea, but they were also not avirulent, which is a relevant finding for the western Canadian swine industry. Strain 3603-F2 induced microscopic lesions similar to, albeit less severe than, *B hampsonii* along with sporadic episodes of watery diarrhea in roughly half of the inoculated pigs. The G79 strain induced a histopathologic lesion pattern more severe than control pigs, but only sporadic loose stool (consistency of wet cement) in a small number of pigs and overall fecal scores no more severe than controls. While pigs inoculated with either strain had intestinal secretory responses that were greater than controls, it must be emphasized that these responses were measured 3 to 4 weeks after inoculation and may be different if measured during periods of loose or watery feces. However, most of the 3603-F2 or G79 inoculated pigs had secretory responses that differed from negative control pigs. Whether or not these pathological and physiological changes would result in diarrhea in commercial farms is not fully understood, but it is noteworthy that 3603-F2 was originally isolated from a pig with diarrhea submitted to our diagnostic laboratory. Moreover, pigs on many grow-finish farms have been observed with sporadic mild diarrhea or loose feces, but diagnostics including specialized culture for *Brachyspira* are rarely performed. Therefore, novel *Brachyspira* strains, such as 3603-F2 and G79, are unlikely to be identified even though they may be associated with subclinical colitis and mild diarrhea.

Brachyspira murdochii has been previously reported to account for 9.4% to 25.3% of all *Brachyspira* isolated from diagnostic surveillance cases.[5,23] In contrast, we have observed an increasing proportion of cases positive for *B murdochii* in the last 6 years (range, 1.3%-25.1%), in comparison to all submitted samples suspicious of *Brachyspira* infection. This disparity may be due to different diagnostic approaches used. Commercial diagnostic laboratories offer bacterial species-specific PCRs targeting only the proven pathogenic *Brachyspira* species, whereas the data shown in Figure 1 are based on an untargeted approach including culture on selective agar followed by PCR and sequencing of the *nox* gene. This combination of methods is more sensitive than a species-specific PCR-only approach,[11,20] and allows for the detection of any *Brachyspira* species and mixed infections. Other authors have reported a high incidence of *B murdochii* in pigs with chronic wasting disease, as well as catarrhal colitis associated with extensive epithelial colonization by the spirochetes.[24,25] These observations provide evidence for the potential role for *B murdochii* and other moderately hemolytic *Brachyspira* species in colitis, poor performance, and unthriftiness in the grow-finish barn.

Culture characteristics are useful to detect the presence of *Brachyspira* within a sample but are insufficient to speciate isolates as observed by others.[14,26] Here we described isolates with β-hemolytic capabilities in between strong and weak, thus classified as moderate (Figure 2). Although not a direct

Table 3: Clinical results from *Brachyspira* inoculation experiments.

Challenge experiment 1	Negative control	Strain 3603-F2	Positive control (*B hampsonii*)
No. pigs inoculated	6	11‡	6
Median fecal score (IQR)	0.04 (0.13)[a]	0.17 (0.07)[b]	1.05 (0.81)[c]
Frequency of watery diarrhea	0/6	5/11 (45%)	0/6
Frequency of mucoid or bloody diarrhea (%)	0 (0)[a]	0 (0)[a]	4 (67)[b]
Frequency of colonization/*Brachyspira* shedding (%)*	NA	10 (91)	5 (83)
Median days to 1st positive fecal culture (IQR)	NA	5 (5)	5 (5)
Median % daily fecal samples culture positive (IQR)*	NA	35.3 (41)	56.3 (55)
Frequency of positive *Brachyspira* culture at termination (%)‡	NA	10 (91)	4 (67)

Challenge experiment 2	Negative control	Strain G79	Positive control (*B hampsonii*)
No. pigs inoculated	6	12	12
Median fecal score (IQR)	0 (0)	0 (0.08)	0.05 (0.69)
Frequency of watery diarrhea (%)	0 (0)	1 (8.3)	0 (0)
Frequency of mucoid or bloody diarrhea (%)	0 (0)[a]	0 (0)[a]	5 (42)[b]
Frequency of colonization/*Brachyspira* shedding (%)*	NA	10 (83)	7 (58)
Median days to 1st positive fecal culture (IQR)	NA	6 (3)	9 (5)
Median % daily fecal samples culture positive (IQR)*	NA	50 (37)	11.7 (42)
Frequency of positive *Brachyspira* culture at termination (%)‡	NA	6 (50)	6 (50)

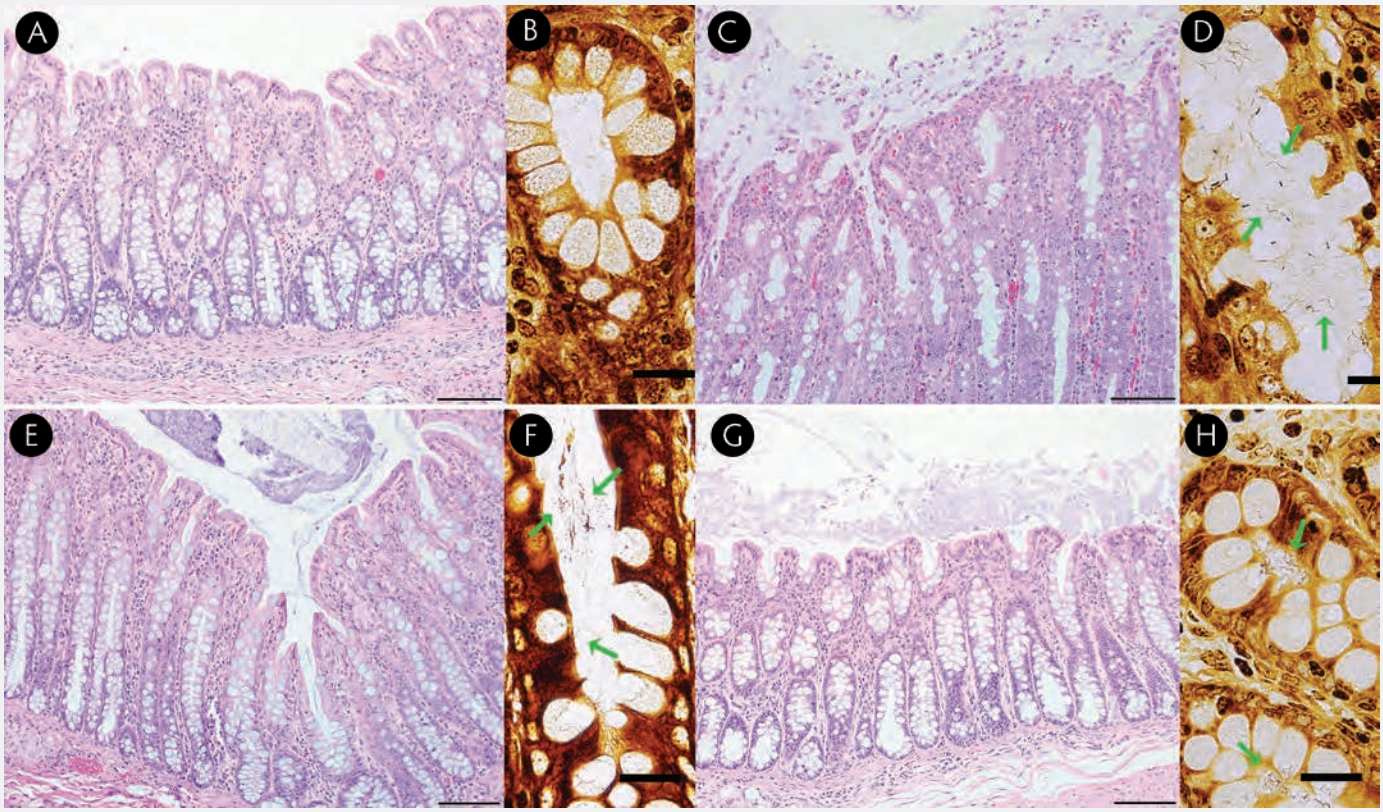
* Colonization is defined as positive fecal culture between 5 and 21 dpi.
† One pig in 3603-F2 group was removed from the experiment at 10 dpi due to an unrelated injury.
‡ Positive *Brachyspira* culture on selective media from swab of colonic contents.
[a,b,c] Superscripts within row differ statistically ($P < .05$). Exact test was used for frequency variables. Kruskal-Wallis with post hoc Mann-Whitney was used for continuous variables.
IQR = interquartile range; NA = not applicable; dpi = days post inoculation.

indicator of virulence, this phenotypic characteristic maybe helpful to practitioners exploring alternative causes of mild diarrhea and sub-optimal grow-finish performance. The identity of strain 3603-F2 was confirmed to be *B murdochii* after whole genome sequencing and partial *nox* sequence analysis. Pigs inoculated with this strain presented with intermittent episodes of watery diarrhea and microscopic lesions. Interestingly, the pattern of microscopic lesions observed in a portion of 3603-F2-inoculated pigs clustered together with pigs that developed mucohemorrhagic diarrhea following inoculation with *B hampsonii* (Figure 5). Previous reports of pigs with loose stools and colitis associated with the isolation of *B murdochii* corroborate the pathogenicity of various *B murdochii* isolates.[24,27] Although a clear difference in severity of disease caused by the two strains was described herein, subclinical intestinal disease and sporadic episodes of diarrhea have been shown to affect gut health and pig performance.[28]

However, as discussed previously, a high load of non-pathogenic spirochetes may be responsible for cases of mild colitis and diarrhea.[24,27,29] It is worth noting that in challenge experiment 2, only 5 of 12 (42%) *B hampsonii*-inoculated pigs developed mucohemorrhagic diarrhea, and one of these had only mild necrotic lesions in the colon. Fecal shedding (reported as frequency of colonization or *Brachyspira* shedding from positive fecal sample cultures between 5 and 21 dpi) mostly reflects an isolate's ability to colonize the colon. The lack of clustering between shedding and lesions reported mainly speaks to the ability of a given isolate to cause histopathologic lesions, which we have demonstrated to be mild. However, secretory capacity was still affected (as shown in Figure 7). Furthermore, it is important to remember that we and others observed < 100% morbidity when performing experimental challenges,[20,30,31] so this challenge experiment was less effective than normal, despite being performed under similar experimental conditions and season as challenge experiment 1. Furthermore, even though the pathologist was blinded to pig identity for both trials, the distribution of 3603-F2 and *B hampsonii* pigs, but not control pigs, across histology clusters (Figure 5) had a distinct pen bias indicating that housing and location factors contributed to lesion severity. Thus, it is always important to exercise caution when extrapolating results of challenge experiments, because if repeated, different results may occur.

Comparison of the whole genome sequence of G79 (approximately 3 million bp) to other recognized *Brachyspira* species corroborated the phylogenetic analysis based on partial *nox* gene (801 bp) sequences and indicate its distinction from both *B innocens* and *B murdochii*. We also investigated the

Figure 4: Hematoxylin and eosin (HE; bar = 200 μm) and Warthin-Faulkner (WF; bar = 20 μm) stained porcine colon from the challenge experiments. A) Negative control pig with normal colon, HE stain. B) Negative control pig with no spirochetes, WF stain. C) Positive control (30446) pig with moderate to severe muconecrotic colitis, HE stain. D) Positive control (30446) pig with many spirochetes in glands (arrows), WF stain. E) Strain 3046-F2 inoculated pig with moderately increased mucus, minimal necrosis and mild colitis, HE stain. F) Strain 3046-F2 inoculated pig with small numbers of spirochetes in glands (arrows), WF stain. G) Strain G79 inoculated pig with a mild mucus increase and minimal colitis, HE stain. H) Strain G79 inoculated pig with occasional glands containing many spirochetes (arrows), WF stain.

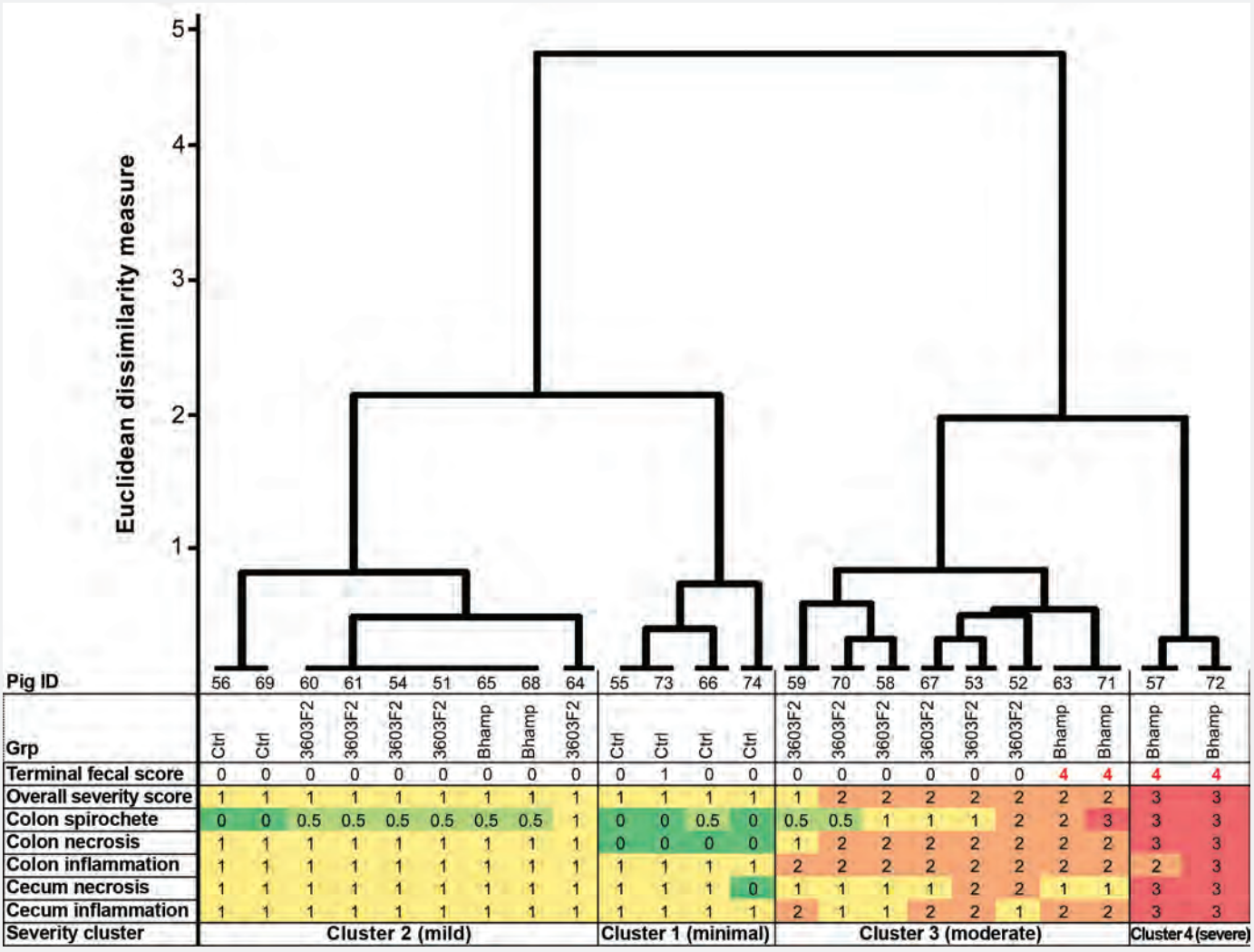


presence of hemolysin genes (*tly*A, *tly*B, *tly*C, and *hly*A) in the genomes of 3603-F2 and G79. Orthologues of these putative virulence genes were identified in the genomes of both strains. It is important to stress that the role of these hemolysins in the pathophysiology of *Brachyspira* species is still under debate.[9,10] At this point, a cautious diagnostic interpretation is warranted in grow-finish diarrhea cases where any weakly or moderately hemolytic *Brachyspira* is detected since the virulence attributes and pathogenesis of these strains and species are poorly understood. Even with known limitations, the authors strongly believe that animal challenge experiments are the most definitive diagnostic test available but are rarely performed.

In this study, we provided evidence that 2 *Brachyspira* strains (3603-F2 and G79) induced significant changes in ion transport capacity across the colonic mucosa when compared to negative controls (Figure 7). These changes were characterized by an increased luminal anion secretory potential. Electrolyte secretion by epithelia is coupled with ion, nutrient, and water absorption.[32] Augmented luminal anion secretion capacity was identified by stimulation of CFTR (apical anion channel), through indirect (isoproterenol) and direct (forskolin) increased production of cAMP, and inhibition of NKCC1 (basolateral anion channel) by bumentanide. Other pathogens that employ CFTR overstimulation to induce secretory diarrhea include *Vibrio cholerae* and enterotoxigenic *Escherichia coli*.[33,34] Although diarrhea was mild (3603-F2) to non-existent (G79), the change in anion secretory capacity 21 days after infection suggests a persistent, subclinical disease by allegedly non-pathogenic *Brachyspira*. A previous study reproduced subclinical ileitis under controlled conditions after *L intracellularis* infection of naïve pigs, resulting in reduced average daily gain (37%-42%) compared to controls.[35] This impact in pig performance was estimated to represent a loss of approximately $3.40 per pig in commercial farm settings.[36] Together with the histopathology and electrophysiology data presented, it is important to consider that the subclinical colitis caused by *Brachyspira* has the potential to lead to adverse effects on animal performance (including average daily gain) as seen in subclinical ileitis. The work described here did not aim to investigate the impact of these atypical *Brachyspira* isolates on weight gain, which would encompass a much different study design (including trials being performed outside a BSL-2 facility, and stocking densities should be similar to those in commercial operations). The authors strongly encourage further investigation on this issue.

For this study, we chose to use a multivariate statistical approach (cluster analyses) rather than more traditional univariate statistical analyses (parametric or non-parametric

Figure 5: Dendrogram displaying 3603-F2 challenge trial cluster analysis of the severity of histological lesions in large intestine of inoculated pigs. Twenty-four pigs from 3 inoculation groups (negative control [Ctrl], positive control *B hampsonii* genomovar II strain 30446 [Bhamp], *Brachyspira* strain 3603-F2 [3603-F2]) are arranged into 4 lesion severity clusters based on the severity of colonic and cecal inflammation and necrosis (scored 0-3 for each pig) and a semi-quantitative assessment of spirochetes in proximity of colonic epithelium based on examination of Warthin-Faulkner stained slides (scored 0-3). The cluster analysis uses Ward's linkage and Euclidean dissimilarity measure and was performed on scaled-ranks of severity scores as described by Kaufman and Rousseeuw.[22] Terminal fecal scores (0 = formed to 4 = mucohemorrhagic diarrhea) are included to show consistency of feces on the morning before necropsy.



approaches such as *t*-tests, ANOVA or Mann-Whitney, Kruskal-Wallis, etc), due to the small groups sizes and multiple outcomes reflecting overall health of the pigs. Unlike univariate statistical approaches where each outcome is assessed independently, multivariate statistical approaches assess multiple outcome variables simultaneously. For instance, multiple histopathologic scores representing inflammation, infiltration, and necrosis in several intestinal organs were assessed collectively because overall gut health is reflective of the sum of these outcomes. Many multivariate statistical techniques use visualization to assess group differences rather than statistical inference (generation of *P* values). Univariate and multivariate approaches each have advantages and disadvantages, but both are legitimate if used appropriately. The use of multivariate approaches overcome issues pertaining to multiple comparisons and adjustments (generating *P* values for numerous outcomes measured on the same pigs and finding some significant by chance), a fundamental problem of applying repeated univariate tests. In multivariate analyses including the cluster analyses used herein, the Euclidean dissimilarity measure is commonly used due to its simplicity. Similar to the standard genomic dendrogram, the Euclidean dissimilarity measure compares the relative length of the vertical lines separating individual animals and clusters, with greater relative length reflecting greater dissimilarity. However, the absolute values of the Euclidean dissimilarity measure (eg, the 0-5 scale along the y-axis of Figure 5) is not directly interpretable in terms of animal physiology or performance. But when combined with a heat map or cluster mat of raw data, the relative distances between animals and clusters allows for meaningful and relevant visual interpretation of the data and potential group differences or trends.

Although not as severe as swine dysentery, the results presented herein provide evidence that *Brachyspira* strains 3603-F2 and G79 induce microscopic lesions and sporadic clinical disease in susceptible pigs, along with altered mucosal ion transport capacity that may contribute to diarrhea. As the pork industry moves towards reducing the use of antibiotics in production, enteric organisms capable of inducing mild disease will become more relevant. Improved understanding of the production impact and the development of methods to mitigate loses due to subclinical and mild intestinal disease is warranted.

Implications

- Neither *B murdochii* strain 3603-F2 nor *Brachyspira* strain G79 caused swine dysentery-like colonic lesions or muco-hemorrhagic diarrhea in trial pigs.
- *Brachyspira* resembling non-pathogenic species induced microscopic lesions in a similar pattern to, but milder than, *B hampsonii* and *B hyodysenteriae*.
- Changes to the mucosal ion transport capacity following inoculation with allegedly non-pathogenic *Brachyspira* suggest a subclinical form of colitis.

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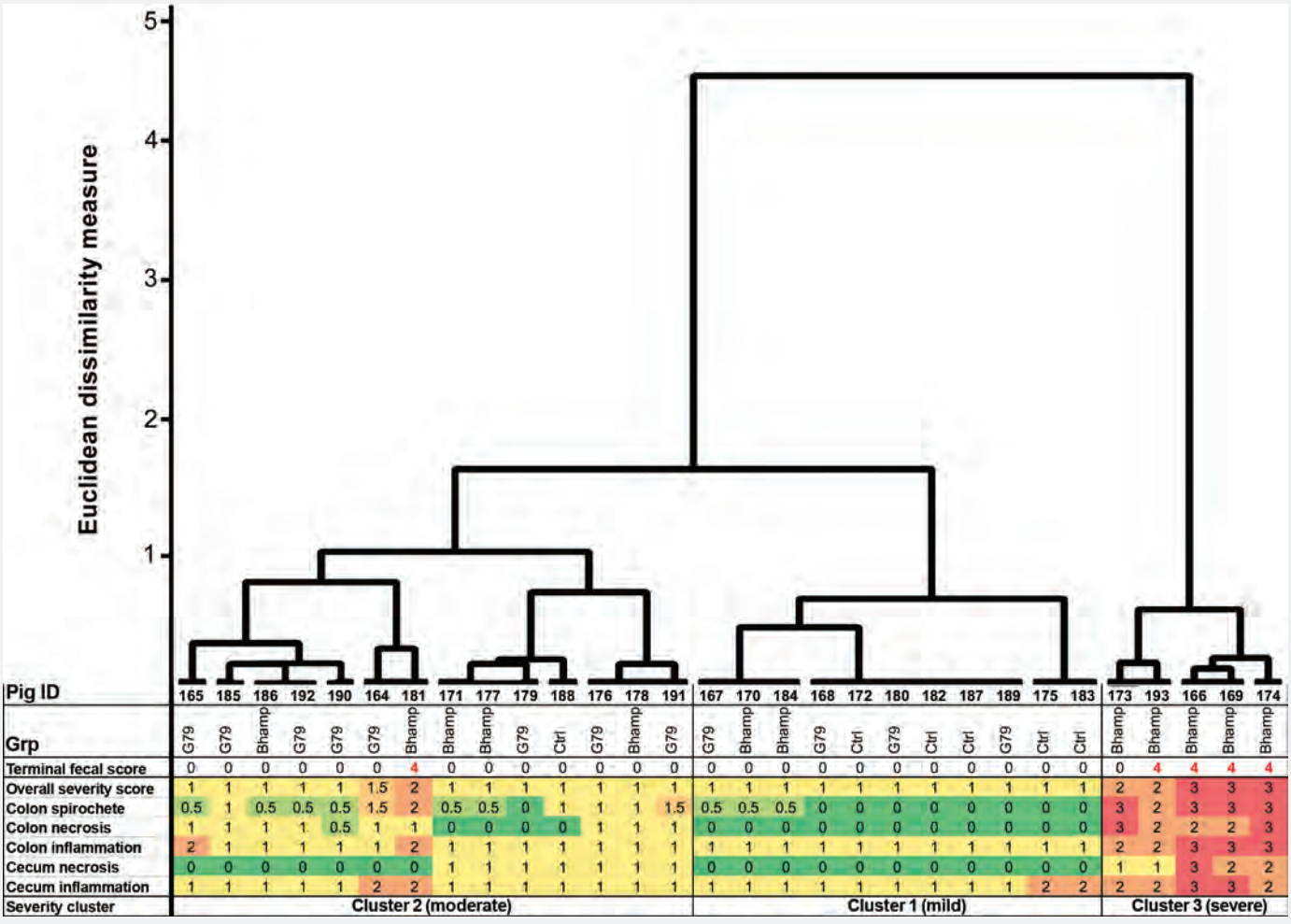
Conflict of interest

None reported.

Disclaimer

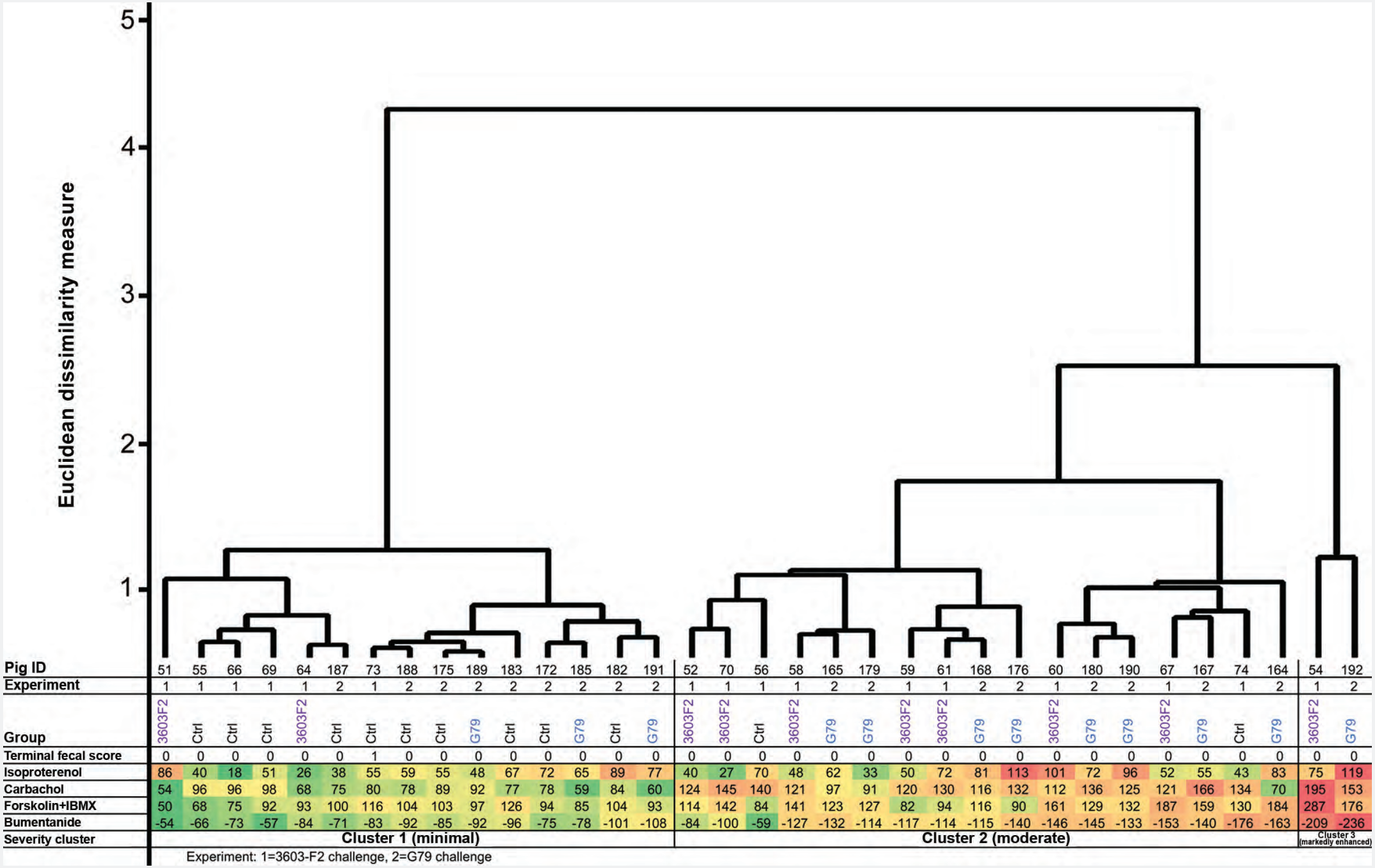
Scientific manuscripts published in the *Journal of Swine Health and Production* are peer reviewed. However, information on medications, feed, and management techniques may be specific to the research or commercial situation presented in the

Figure 6: Dendrogram displaying G79 cluster analysis of the severity of histologic lesions in large intestine of inoculated pigs. Thirty pigs from 3 inoculation groups (negative control [Ctrl], positive control *B hampsonii* genomovar II strain 30446 [Bhamp], *Brachyspira* strain G79 [G79]) are arranged into 3 lesion severity clusters based on the severity of colonic and cecal inflammation and necrosis (scored 0-3 for each pig) and a semi-quantitative assessment of spirochetes in proximity of colonic epithelium of Warthin-Faulkner stained slides (scored 0-3). The cluster analysis uses Ward's linkage and Euclidean dissimilarity measure and was performed on scaled-ranks of severity scores as described by Kaufman and Rousseeuw.[22] Terminal fecal scores (0 = formed to 4 = mucohemorrhagic diarrhea) are included to show consistency of feces on the morning before necropsy.



Pig ID	165	185	186	192	190	164	181	171	177	179	188	176	178	191	167	170	184	168	172	180	182	187	189	175	183	173	193	166	169	174
Grp	G79	G79	Bhamp	G79	G79	G79	Bhamp	Bhamp	Bhamp	G79	Ctrl	G79	Bhamp	G79	G79	Bhamp	Bhamp	G79	Ctrl	G79	Ctrl	Ctrl	G79	Ctrl	Ctrl	Bhamp	Bhamp	Bhamp	Bhamp	Bhamp
Terminal fecal score	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4
Overall severity score	1	1	1	1	1	1.5	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	3	3	3
Colon spirochete	0.5	1	0.5	0.5	0.5	1.5	2	0.5	0.5	0	1	1	1	1.5	0.5	0.5	0.5	0	0	0	0	0	0	0	0	3	2	3	3	3
Colon necrosis	1	1	1	1	0.5	1	1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	3	2	2	2	3
Colon inflammation	2	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	3	3	3
Cecum necrosis	0	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	3	2	2
Cecum inflammation	1	1	1	1	1	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	3	3	2
Severity cluster	Cluster 2 (moderate)														Cluster 1 (mild)											Cluster 3 (severe)				

Figure 7: Cluster dendogram displaying analysis of the colonic secretory capacity in *Brachyspira* inoculated pigs. Thirty-four pigs from 3 inoculation groups (negative control [Ctrl], *Brachyspira* strain G79 [G79], and *Brachyspira* strain 3603-F2 [3603-F2]) are arranged into 3 clusters based on their secretory responses. Specifically, the intensity of ion movement through the mucosa for each pig is represented numerically, measured by the change in a short-circuit current (I_{SC}). Experiment 1 reflects challenge trial 1 (strain 3603-F2) and experiment 2 reflects challenge trial 2 (strain G79). The cluster analysis uses Ward's linkage and Euclidean dissimilarity measure. Terminal fecal scores (0 = formed to 4 = mucohemorrhagic diarrhea) are included to show consistency of feces on the morning before necropsy which was performed 3 to 4 weeks post inoculation.



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References

1. Harris DL, Glock RD, Christensen CR, Kinyon JM. Inoculation of pigs with *Treponema hyodysenteriae* (new species) and reproduction of the disease. *Vet Med Small Anim Clinic*. 1972;67:61-64.

2. Kinyon JM, Harris DL, Glock RD. Enteropathogenicity of various isolates of *Treponema hyodysenteriae*. *Infect Immun*. 1977;15:638-646.

3. ter Huurne AA, Muir S, van Houten M, van der Zeijst BA, Gaastra W, Kusters JG. Characterization of three putative *Serpulina hyodysenteriae* hemolysins. *Microb Pathog*. 1994;16:269-282.

4. Thomson JR, Smith WJ, Murray BP, Murray D, Dick JE, Sumption KJ. Porcine enteric spirochete infections in the UK: surveillance data and preliminary investigation of atypical isolates. *Anim Health Res Rev*. 2001;2:31-36.

5. Clothier KA, Kinyon JM, Frana TS, Naberhaus N, Bower L, Strait EL, Schwartz K. Species characterization and minimum inhibitory concentration patterns of *Brachyspira* species isolates from swine with clinical disease. *J Vet Diagn Invest*. 2011;23:1140-1145.

6. Patterson AH, Rubin JE, Fernando C, Costa MO, Harding JC, Hill JE. Fecal shedding of *Brachyspira* spp. on a farrow-to-finish swine farm with a clinical history of "*Brachyspira hampsonii*"-associated colitis. *BMC Vet Res*. 2013;9:137. doi:10.1186/1746-6148-9-137

7. Jacobson M, Gerth Löfstedt M, Holmgren N, Lundeheim N, Fellstrom C. The prevalences of *Brachyspira* spp. and *Lawsonia intracellularis* in Swedish piglet producing herds and wild boar population. *J Vet Med B Infect Dis Vet Public Health*. 2005;52:386-391.

8. Osorio J, Carvajal A, Naharro G, Rubio P, La T, Phillips ND, Hampson DJ. Identification of weakly haemolytic *Brachyspira* isolates recovered from pigs with diarrhoea in Spain and Portugal and comparison with results from other countries. *Res Vet Sci*. 2013;95:861-869.

9. Mahu M, De Pauw N, Vande Maele L, Verlinden M, Boyen F, Ducatelle R, Haesebrouck F, Martel A, Pasmans F. Variation in hemolytic activity of *Brachyspira hyodysenteriae* strains from pigs. *Vet Res*. 2016;47:66. doi:10.1186/s13567-016-0353-x

10. Mahu M, Boyen F, Canessa S, Zavala Marchan J, Haesebrouck F, Martel A, Pasmans F. An avirulent *Brachyspira hyodysenteriae* strain elicits intestinal IgA and slows down spread of swine dysentery. *Vet Res*. 2017;48:59. doi:10.1186/s13567-017-0465-y

11. Rohde J, Rothkamp A, Gerlach GF. Differentiation of porcine *Brachyspira* species by a novel *nox* PCR-based restriction fragment length polymorphism analysis. *J Clin Microbiol*. 2002;40:2598-2600.

12. Clarke LL. A guide to Ussing chamber studies of mouse intestine. *Am J Physiol Gastrointest Liver Physiol*. 2009;296:G1151-1166.

13. Jenkinson SR, Wingar CR. Selective medium for the isolation of *Treponema hyodysenteriae*. *Vet Rec*. 1981;109:384-385.

14. Fellström C, Gunnarsson A. Phenotypical characterisation of intestinal spirochaetes isolated from pigs. *Res Vet Sci*. 1995;59:1-4.

15. Staden R, Beal KF, Bonfield JK. The Staden package, 1998. *Methods Mol Biol*. 2000;132:115-130.

16. Felsenstein J. PHYLIP-phylogeny inference package (Version 3.2). *Cladistics*. 1989;5:164-166.

17. Martin-Platero AM, Valdivia E, Maqueda M, Martinez-Bueno M. Fast, convenient, and economical method for isolating genomic DNA from lactic acid bacteria using a modification of the protein "salting-out" procedure. *Anal Biochem*. 2007;366:102-104.

18. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci USA*. 2009;106:19126-19131.

19. Chen Y, Ye W, Zhang Y, Xu Y. High speed BLASTN: an accelerated MegaBLAST search tool. *Nucleic Acids Res*. 2015;43:7762-7768.

20. Costa MO, Hill JE, Fernando C, Lemieux HD, Detmer SE, Rubin JE, Harding JC. Confirmation that "*Brachyspira hampsonii*" clade I (Canadian strain 30599) causes mucohemorrhagic diarrhea and colitis in experimentally infected pigs. *BMC Vet Res*. 2014;10:129. doi:10.1186/1746-6148-10-129

21. Jones GF, Ward GE, Murtaugh MP, Lin G, Gebhart CJ. Enhanced detection of intracellular organism of swine proliferative enteritis, ileal symbiont intracellularis, in feces by polymerase chain reaction. *J Clin Microbiol*. 1993;31:2611-2615.

22. Kaufman L, Rousseeuw PJ. *Finding groups in data: an introduction to cluster analysis*. 2nd ed. Hoboken, New Jersey: John Wiley & Sons; 2009.

23. Verspohl J, Feltrup C, Thiede S, Amtsberg G. Diagnosis of swine dysentery and spirochaetal diarrhea. III. Results of cultural and biochemical differentiation of intestinal *Brachyspira* species by routine culture from 1997 to 1999. *Dtsch Tierarztl Wochenschr*. 2001;108:67-69.

24. Komarek V, Maderner A, Spergser J, Weissenböck H. Infections with weakly haemolytic *Brachyspira* species in pigs with miscellaneous chronic diseases. *Vet Microbiol*. 2009;134:311-317.

25. Jensen TK, Christensen AS, Boye M. *Brachyspira murdochii* colitis in pigs. *Vet Pathol*. 2010;47:334-338.

26. Ohya T, Araki H, Sueyoshi M. Identification of weakly beta-hemolytic porcine spirochetes by biochemical reactions, PCR-based restriction fragment length polymorphism analysis and species-specific PCR. *J Vet Med Sci*. 2008;70:837-840.

27. Weissenböck H, Maderner A, Herzog AM, Lussy H, Nowotny N. Amplification and sequencing of *Brachyspira* spp. specific portions of *nox* using paraffin-embedded tissue samples from clinical colitis in Austrian pigs shows frequent solitary presence of *Brachyspira murdochii*. *Vet Microbiol*. 2005;111:67-75.

*28. Paradis MA, McKay RI, Wilson JB, Vessie GH, Winkelman NL, Gebhart CJ, Dick CP. Subclinical ileitis produced by sequential dilutions of *Lawsonia intracellularis* in a mucosal homogenate challenge model. *Proc AASV*. Toronto, Canada. 2005:189-191.

29. Neef NA, McOrist S, Lysons RJ, Bland AP, Miller BG. Development of large intestinal attaching and effacing lesions in pigs in association with the feeding of a particular diet. *Infect Immun*. 1994;62:4325-4332.

30. Rubin JE, Costa MO, Hill JE, Kittrell HE, Fernando C, Huang Y, O'Connor B, Harding JC. Reproduction of mucohaemorrhagic diarrhea and colitis indistinguishable from swine dysentery following experimental inoculation with "*Brachyspira hampsonii*" strain 30446. *PLoS ONE*. 2013;8:e57146. doi:10.1371/journal.pone.0057146

31. Burrough ER, Strait EL, Kinyon JM, Bower LP, Madson DM, Wilberts BL, Schwartz KJ, Frana TS, Songer JG. Comparative virulence of clinical *Brachyspira* spp. isolates in inoculated pigs. *J Vet Diagn Invest*. 2012;24:1025-1034.

32. Gawenis LR, Hut H, Bot AG, Shull GE, de Jonge HR, Stien X, Miller ML, Clarke LL. Electroneutral sodium absorption and electrogenic anion secretion across murine small intestine are regulated in parallel. *Am J Physiol Gastrointest Liver Physiol*. 2004;287:G1140-1149.

33. Golin-Bisello F, Bradbury N, Ameen N. STa and cGMP stimulate CFTR translocation to the surface of villus enterocytes in rat jejunum and is regulated by protein kinase G. *Am J Physiol Cell Physiol*. 2005;289:C708-716.

34. Kimberg DV, Field M, Johnson J, Henderson A, Gershon E. Stimulation of intestinal mucosal adenyl cyclase by cholera enterotoxin and prostaglandins. *J Clin Invest*. 1971;50:1218-1230.

35. Paradis M-A, Gebhart CJ, Toole D, Vessie G, Winkelman NL, Bauer SA, Wilson JB, McClure CA. Subclinical ileitis: Diagnostic and performance parameters in a multi-dose mucosal homogenate challenge model. *J Swine Health Prod*. 2012;20:137-141.

36. Pommier P, Keita A, Pagot E, Duran O, Cloet PR. Comparison of Tylvalosin with Tylosin for the control of subclinical ileitis in swine. *Rev Med Vet*. 2008;159:579-582.

* Non-refereed reference.

Peer reviewed

Brief Communication

Impact assessment of new US Food and Drug Administration regulations on antibiotic use: A post-enactment survey of swine practitioners

Christopher J. Rademacher, DVM; Christopher C. Pudenz, BA; Lee L. Schulz, PhD

Summary

Following a 2016 pre-enactment survey, 42 swine veterinary practitioners were surveyed in 2017 to assess post-enactment impacts of the revised Veterinary Feed Directive (VFD). The survey evaluated veterinarian-client-patient relationships, client recruitment, VFD fees and creation, record keeping, education and training, business costs, and changes in antibiotic usage and on-farm management.

Keywords: swine, veterinary feed directive, economics, antibiotics

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Resumen - Evaluación del impacto de las nuevas regulaciones de la Administración de Alimentos y Medicamentos de los Estados Unidos sobre el uso de antibióticos: Una encuesta post-promulgación a los veterinarios especialistas en cerdos

Después de una encuesta previa a la promulgación en 2016, en 2017 se encuestó a 42 veterinarios especialistas en cerdos para evaluar los impactos post-promulgación de la Directiva Veterinaria de los Alimentos revisada (VFD). La encuesta evaluó las relaciones veterinario-cliente-paciente, el reclutamiento de clientes, las tarifas y la creación de la VFD, el registro de datos, la educación y la capacitación, los costos comerciales y los cambios en el uso de antibióticos y en el manejo en la granja.

Résumé – Évaluation de l'impact des nouvelles règlementations du US Food and Drug Administration sur l'utilisation des antibiotiques: Un sondage post-promulgation des praticiens porcins

À la suite du sondage pré-promulgation réalisé en 2016, 42 vétérinaires praticiens porcins ont été sondés de nouveau en 2017 pour évaluer les impacts post-promulgation des directives vétérinaires sur les aliments (Veterinary Feed Directive, VFD). Le sondage a évalué les relations vétérinaire-client-patient, le recrutement de clients, la création et les frais de VFD, la tenue de dossier, l'éducation et la formation, les coûts d'affaire, et les changements dans l'utilisation d'antibiotiques et la gestion à la ferme.

On April 13, 2012, the US Food and Drug Administration (FDA) issued Guidance for Industry (GFI) 209 "to inform the public of FDA's current thinking on the use of medically important antimicrobial drugs in animal agriculture."[1,2] On December 12, 2013, FDA issued GFI 213 to provide "information to sponsors of certain antimicrobial new animal drug products who are interested in revising conditions of use for those products consistent with FDA's Guidance for Industry (GFI) #209" and to "set timelines for stakeholders wishing to comply voluntarily with this guidance."[3,4] On June 3, 2015, FDA issued the revised Veterinary Feed Directive (VFD) which "established requirements relating to the distribution and use of VFD drugs and animal feeds containing such drugs,"[5] and became effective on October 1, 2015. Full implementation of FDA's GFIs and VFD final rule was set for December 2016 with enforcement commencing on January 1, 2017.

The GFIs and VFD final rule direct the use of medically important antibiotics (defined as antibiotics that are important for therapeutic use in human medicine) in livestock for therapeutic purposes only. Therapeutic purposes are defined as either treatment, control, or prevention of disease.[2] These policies are focused on use of medically important antibiotics given in mass medication formats, either through the feed or the water. Use of medically important antibiotics in feed requires a VFD order from the veterinarian to the producer and feed manufacturer. Medically important antibiotics used in water requires a veterinary prescription. Another aim of these policies was to require that if producers wanted to use medically important antibiotics, they could only do so under the guidance of a veterinarian with a valid veterinarian-client-patient relationship (VCPR). This endows the veterinarian with the responsibility for making medical decisions for the farm, with the producer bearing responsibility in following the medical directions of the veterinarian. Another aim of these policies was to eliminate the use of medically important antibiotics for growth promotion use. Collectively, these new regulations have changed the ways that antibiotics are used in livestock production.

From a 2016 survey of practicing swine veterinarians on VFD preparation, Schulz and Rademacher[6] reported that extensive preparation and education was being done by veterinarians and their producers to help ensure a smooth transition to the new antibiotic-use guidelines. The results also

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Rademacher CJ, Pudenz CC, Schulz LL. Impact assessment of new US Food and Drug Administration regulations on antibiotic use: A post-enactment survey of swine practitioners. *J Swine Health Prod.* 2019;27(4):210–220.

suggested that there were varying views on the definition of a VCPR, plans for meeting the additional record keeping requirement and delivery of VFDs, fees associated with providing VFDs, costs to swine veterinary business operations, and reduction in the use of antibiotics in feed as a result of the VFD. A follow-up survey was conducted in 2017 to assess post-enactment impacts of the revised VFD.

Materials and methods

The survey protocol was approved by the Iowa State University Institutional Review Board (IRB ID 16-489) prior to distribution of the survey. Data collection procedures for this study were similar to those used for the 2016 survey as described by Schulz and Rademacher.[6] Questions from the 2016 survey were revised to reflect post-January 1, 2017 status of the new antibiotic-use guidelines. In addition, new questions were added to the survey to elicit information on the fee structure for writing VFDs and prescriptions, the level of production (ie, group or lot, site, flow, or system), and average number of pigs for which a VFD was written. The data for this study are from a convenience sample of practicing swine veterinarians. Swine veterinary practitioners attending the 2017 Iowa State University (ISU) James D. McKean Swine Disease Conference held in Ames, Iowa, on November 2-3, 2017 were surveyed. The conference attracts veterinarians, students, academic faculty and staff, and allied industry personnel. Of the 305 conference attendees, 125 practicing swine veterinarians were identified at conference check-in and given a paper survey.

To increase survey response and expand distribution, input was sought from ISU faculty and staff who were familiar with swine production systems and swine-focused veterinary clinics to identify additional practicing swine veterinarians to be surveyed. A convenience sample of 35 practicing swine veterinarians from the upper Midwest region of the United States who did not attend the conference were surveyed electronically using Qualtrics (Qualtrics, Provo, Utah). The Qualtrics survey questionnaire sent to practicing swine veterinarians was identical to the one distributed at the conference. A customized email invitation for the Qualtrics survey was sent on November 16, 2017 with reminders sent to non-respondents on 2 occasions 1 week apart. The Qualtrics survey was closed on December 18, 2017. Data retrieved from returned surveys were compiled and summarized using descriptive statistics.

After several introductory questions gathering practitioner demographic (ie, location, experience) and clientele base (ie, independent, contractor, or contract grower; phase of production; annual pig sales) information, the practitioners surveyed were asked a series of questions about how the VFD requirements impacted their business operations as well as swine production in general. Specifically, questions targeted veterinarian-client-patient relationships, client recruitment, VFD fees and creation, record keeping, education and training, business costs, and changes in antibiotic usage and on-farm management.

Results

Response rate and respondent profile

The response rate for the survey distributed at the ISU James D. McKean Swine Disease Conference was 23.2%, 29 of 125 practitioners who received a paper survey returned a completed survey. Thirteen of 35 practitioners who received a Qualtrics survey returned a completed survey, a 37.1% response rate. Therefore, there were 42 practicing swine veterinarians in the final sample. However, a few participants only partially completed the survey. The number of respondents for each question of interest are presented in Tables 1 through 12.

Comprehensive questions about veterinarian demographic details (eg, private vs corporate practice or employed by an integrator) were not included in the survey and, therefore, it cannot be confirmed if the study sample is representative of the entire practicing swine veterinarian population. Respondents' primary geographic location were in states with the largest number of swine operations and inventories: 24 veterinarians practiced in Iowa and 9 in Minnesota. Other states represented include Illinois (3 respondents), Indiana (2 respondents), Kansas (1 respondent), Missouri (1 respondent), and Nebraska (1 respondent). According to the 2012 US Census of Agriculture, these states represent 30% of US swine operations and 67% of the US pig inventory.[7]

The average number of hogs marketed annually by the responding veterinarians' clientele were 0 (1.5% of clients), 1 to 4999 (13.1% of clients), 5000 to 19,999 (23.0% of clients), 20,000 to 49,999 (23.2% of clients), and 50,000 or more (39.2% of clients). For the 7 states represented, 74% of all operations have annual sales of 1 to 4999 hogs, while 26% of operations have annual sales of 5000 or more hogs according to the 2012 US Census of Agriculture.[7] Thus, the clients served by the veterinarians within our sample had larger operations than the census averages.

The largest percentage of swine clients served by veterinarians had farrow-to-finish production (39.2%), followed by wean-to-finish (21.3%), breeding-farrowing (18.8%), finishing (12.2%), nursery (5.6%), gilt developer unit (2.7%), boar stud (0.2%), and other (0.1%). The largest segment of swine clients were independent producers (64.5%), followed by contractors or integrators (21.7%) and contract growers (13.8%).

These general demographic characteristics of the survey participants suggests a reasonable degree of representation of clients served by veterinarians was achieved despite use of convenience recruitment techniques. However, due to not asking certain questions in the survey and the small sample size, we were not able to make comparisons across several factors that characterize the entire population of swine veterinary practitioners. Therefore, the study results may not be generalizable to all practicing swine veterinarians and may not represent the entire US swine industry. Nonetheless, this work reports one of the first attempts to track progress toward adjusting to the new antibiotic-use guidelines.

Veterinarian-client-patient relationship

All respondents were aware of their respective state's VCPR definition. Twenty-one of the 41 responding veterinarians (51.2%) believed that only 1 visit per year was needed to maintain a VCPR, whereas 11 veterinarians (26.8%) thought 2 visits and 7 veterinarians (17.1%) thought 4 visits were needed to maintain a VCPR (Table 1). Two veterinarians replied that they did not know how many visits were required to have a VCPR. One common concern often voiced from practicing veterinarians was an obligation to make site visits solely for the purpose of writing VFDs. More than half of 41 survey respondents (24 veterinarians; 58.5%) felt as though they were conducting more site

visits per operation with the new VFD regulations. Veterinarians were asked how many sites within an operation they felt were necessary to visit to fulfill the VCPR requirements. The majority of respondents (26 of 41; 63.4%) felt it necessary to visit all sites, while 14 respondents (34.1%) felt that they had to visit more than 1 site, but not all sites. Only 1 veterinarian replied that visiting 1 site was sufficient to satisfy the VCPR requirement. These results differ somewhat from the 2016 survey responses where a larger proportion of veterinarians (56.0%) envisioned visiting 2 or more sites, but not all sites, compared to the proportion (40.0%) that anticipated visiting all sites.

Client recruitment

Regarding client recruitment, 17 veterinarians (41.5%) reported being approached by new clients for the purpose of writing VFDs (Table 2). Fourteen veterinarians (34.1%) accepted new clients that approached them specifically to provide VFDs, but only 6 of them (14.6%) admitted to actively recruiting new clients to meet minimum requirements

Table 1: Survey questions regarding the veterinarian-client-patient relationship*

	No. reporting	% reporting
How many visits in a year do you think is required for a swine producer and veterinarian to have a VCPR? (n = 41)		
1 visit	21	51.2
2 visits	11	26.8
3 visits	0	0.0
4 visits	7	17.1
5 visits	0	0.0
6 or more visits	0	0.0
I don't know	2	4.9
Have you made more visits per operation to write VFDs? (n = 41)		
Yes	24	58.5
No	17	41.5
In order to fulfill the VCPR requirement for a producer how many sites do you visit? (n = 41)		
1 site	1	2.44
2 or more sites (but not all sites)	14	34.15
All Sites	26	63.41

* A convenience sample of practicing swine veterinarians attending the 2017 ISU James D. McKean Swine Disease Conference or who practice in the upper Midwest region of the United States were surveyed regarding their opinions of the impact of the new antibiotic-use guidelines on pork production and the practice of swine veterinary medicine during the first year of enforcement. Forty-two completed or partially completed surveys were returned.

VCPR = veterinarian-client-patient relationship; VFD = Veterinary Feed Directive; ISU = Iowa State University.

Table 2: Survey questions regarding client recruitment*

	No. reporting	% reporting
Have you been approached by new potential clients due to the VFD? (n = 41)		
Yes	17	41.5
No	24	58.5
Have you accepted new clients that approached you specifically to provide VFDs? (n = 41)		
Yes	14	34.1
No	27	65.9
Have you recruited new clients specifically to meet minimum requirements to provide VFDs? (n = 41)		
Yes	6	14.6
No	35	85.4

* Study details are described in Table 1.

VFD = Veterinary Feed Directive.

to provide VFDs. These results would only apply to veterinarians working for veterinary clinics. Prior to the new regulations, there were anecdotal reports of some producers who would purchase their medically important antibiotics over-the-counter from local feed suppliers rather than routinely use veterinarians. Once VFDs or prescriptions were required for antibiotic administration of a medically important antibiotic to a population of pigs, it is reasonable to hypothesize that the client pool for veterinarians increased.

VFD fees

The mean fee per VFD written for existing clients was $23.75 and for new clients was $24.19 (Table 3). Only 36 and 31 veterinarians, respectively, responded to this question which is most likely due to some respondents being employed by production companies and therefore do not charge for VFDs they write. Based on client operation size, a median difference of approximately $2.50 to $5.00 per VFD was found for operations who marketed between 1 and 49,999 pigs. Similarly, a median difference of $2.50 to $7.50 per prescription was observed across client operation sizes (Table 3). In general, it appears that veterinarians charged clients with larger operations more for VFDs and prescriptions. Thirty-five of the 40 responding veterinarians (87.5%) that are charging for a VFD include this expense as a separate line item in their invoice. When compared to veterinarians who also write prescriptions, only 21 of the 37 responding veterinarians (56.8%) include prescriptions as a separate line item, rather than writing prescriptions as part of a consultation or service fee.

Table 3: Survey responses regarding VFD and prescription fees for new and existing clients*†

	Type of client	No. reporting	Cost, mean (SD), $	Cost, median, $
1 to 4999 marketings/year				
VFD	New	0	NR	NR
	Existing	1	20.00 (NA)	20.00
Prescription	New	0	NR	NR
	Existing	1	20.00 (NA)	20.00
5000 to 19,999 marketings/year				
VFD	New	6	20.42 (4.01)	20.00
	Existing	8	22.19 (4.90)	22.50
Prescription	New	4	13.75 (4.79)	12.50
	Existing	4	16.25 (4.79)	17.50
20,000 to 49,999 marketings/year				
VFD	New	23	24.89 (6.05)	25.00
	Existing	24	23.65 (6.47)	25.00
Prescription	New	11	21.36 (7.45)	20.00
	Existing	10	20.50 (7.62)	20.00
≥ 50,000 marketings/year				
VFD	New	0	NR	NR
	Existing	1	35.00 (NA)	35.00
Prescription	New	0	NR	NR
	Existing	0	NR	NR
All respondents				
VFD	New	31‡	24.19 (5.82)	25.00
	Existing	36‡	23.75 (6.17)	25.00
Prescription	New	15	19.33 (7.53)	20.00
	Existing	15	19.33 (6.78)	20.00

* Study details are described in Table 1.
† The survey instrument collected swine-client marketings per year using categorical variables, ie, the percentage that would fall into each size category: 1 to 4999; 5000 to 19,999; 20,000 to 49,999; or 50,000 or more. For this analysis, the midpoint of each category (and endpoint of the upper and lower bound category) was used to calculate the weighted average marketings per year.
‡ Two survey respondents did not report swine client marketings per year but did report VFD charges for new and existing clients; these responses are included in "all respondents."
VFD = Veterinary Feed Directive; NR = none reported; NA = not applicable.

VFD creation

Veterinary Feed Directives can be written for various levels of production. When the 42 respondents were asked for which level of production they most frequently wrote VFDs, 18 (42.9%) responded the pig flow level, while 12 (28.6%) responded the site level and 9 (21.4%) responded the group or lot level (Table 4). The mean number of pigs covered by a written VFD was 5916 pigs with a median of 2600 and a standard deviation of 9070 (Table 5). Over half of the respondents (22 of 39) wrote VFDs for 2400 to 9999 pigs, while 11 (28.2%) respondents wrote VFDs for fewer than 2400 animals and 6 respondents (15.4%) wrote VFDs for more than 9999 animals. To generate VFDs, veterinarians predominately used an electronic VFD service (34 of 41; 82.9%) but had also made their own VFDs (7 of 41; 17.1%) or used a VFD form from a drug sponsor (4 of 41; 9.8%) (Table 6). For drug prescriptions, most veterinarians responded that they used a form they had created (28 of 41; 68.3%), while others used an electronic prescription service (13 of 41; 31.7%) or a prescription form provided by a drug sponsor (4 of 41; 9.8%).

VFD record keeping

Veterinary Feed Directives must be retained for 2 years by the producer, feed distributor, and the veterinarian. Almost two-thirds of the 41 responding veterinarians (63.4%) had used a third-party service (eg, Global VetLink [GVL]) in order to meet this requirement, while 11 (26.8%) used existing staff (Table 7). Independent of how the VFD was generated, veterinarians reported to have delivered VFDs to producers via a third-party electronic service (28 of 41; 68.3%), email (22 of 41; 53.7%), hard copies (18 of 41; 43.9%), fax (12 of 41; 29.3%), and method of producer (8 of 41; 19.5%) or feed supplier (6 of 41; 14.6%) preference.

Education and training

Since the implementation of the new guidelines, veterinarians and staff had attended meetings (including webinars) (34 of 41; 82.9%), read literature (32 of 41; 78.0%), and created information bulletins to distribute to staff (13 of 41; 31.7%) to learn about the VFD requirements (Table 8). To help educate their clients, veterinarians sponsored in-clinic meetings (including webinars) (16 of 40; 40.0%), met with clients in person (35 of 40; 87.5%), sent a notice of requirements in a regularly published newsletter (20 of 40; 50.0%), and created

Table 4: Survey responses regarding the level of production for which a VFD was most frequently written*

	Level of production, No. (%)			
Marketings/year†	**Group or lot**	**Site**	**Flow**	**System**
1 to 4,999	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)
5000 to 19,999	0 (0.0)	3 (37.5)	5 (62.5)	0 (0.0)
20,000 to 49,999	7 (26.9)	7 (26.9)	10 (38.5)	2 (7.7)
≥ 50,000	1 (20.0)	0 (0.0)	3 (60.0)	1 (20.0)
All respondents	9 (21.4)‡	12 (28.6)‡	18 (42.9)	3 (7.1)

* Study details are described in Table 1.
† The survey instrument collected swine-client marketings per year using categorical variables, ie, the percentage that would fall into each size category: 1 to 4999; 5000 to 19,999; 20,000 to 49,999; or 50,000 or more. For this analysis, the midpoint of each category (and endpoint of the upper and lower bound category) was used to calculate the weighted average marketings per year.
‡ Two survey respondents did not report swine client marketings per year but did report the level of production they most often write a VFD for; this response is included in "all respondents."
VFD = Veterinary Feed Directive.

Table 5: Survey responses regarding the number of pigs per VFD*

	No. reporting	**Mean (SD)**	**Median**
	39	5916 (9070)	2600
Average No. of pigs		**No. reporting (%)**	
< 1200		5 (12.8)	
1200 to 2399		6 (15.4)	
2400 to 4999		15 (38.5)	
5000 to 9999		7 (17.9)	
10,000 to 19,999		3 (7.7)	
≥ 20,000		3 (7.7)	

* Study details are described in Table 1.
VFD = Veterinary Feed Directive.

Table 6: Survey questions regarding types of VFD and prescription forms used*

	No. reporting	% reporting†
Have you used a pre-made VFD form or created your own? (n = 41)		
Used electronic VFD service (eg, GVL)	34	82.9
Used VFD form provided by a drug sponsor	4	9.8
Created VFD form for your clinic	7	17.1
Other	0	0.0
Have you used a pre-made prescription form or created your own? (n = 41)		
Used electronic prescription service (eg, GVL)	13	31.7
Used prescription form provided by a drug sponsor	4	9.8
Created prescription form for your clinic	28	68.3
Other	0	0.0

* Study details are described in Table 1.
† Percentages may reflect multiple answers from individual survey respondents.
VFD = Veterinary Feed Directive; GVL = Global VetLink.

Table 7: Survey questions regarding record keeping and VFD delivery to producers*

	No. reporting	% reporting†
FDA requires a record of every VFD be kept for a period of 2 years. What have you done to meet the additional record keeping requirement? (n = 41)		
No changes	4	9.8
Used existing staff	11	26.8
Hired new staff	1	2.4
Used a third-party service (eg, GVL)	26	63.4
Other	0	0.0
How do you provide VFDs to producers? (n = 41)		
Whatever the producer prefers	8	19.5
Whatever the feed supplier prefers	6	14.6
Third party electronic service (eg, GVL)	28	68.3
Fax	12	29.3
Email	22	53.7
Hard copies	18	43.9
Other	0	0.0

* Study details are described in Table 1.
† Percentages may reflect multiple answers from individual survey respondents.
VFD = Veterinary Feed Directive; FDA = Food and Drug Administration; GVL = Global VetLink.

an information bulletin (15 of 40; 37.5%). The frequency of updated training on VFD requirements varied, but the largest percentage of respondents believed updated training should occur annually for both staff (26 of 40; 65.0%) and clients (26 of 39; 66.7%).

Cost of VFD regulation implementation

When evaluating the business costs associated with VFD regulation implementation, there were more non-responders (n = 19) than for most of the other survey questions. This is most likely due to responding veterinarians either being employed by production companies or being young, associate veterinarians who are not involved in the financial dealings of the clinic. Six survey respondents (14.6%) had 1 to 5 years of experience in swine veterinary practice and an additional 6 respondents (14.6%) had 6 to 10 years of experience.

Descriptive statistics and distribution of annual cost estimates regarding writing and delivery of VFDs, maintaining records for

Table 8: Survey questions regarding education and training on VFD requirements*

	No. reporting	% reporting†
Since January 1, 2017, what have you done to educate yourself and staff on VFD requirements? (n = 41)		
I (we) have not done any education in 2017	4	9.8
Attended meetings (including webinars) to learn more about the VFD	34	82.9
Read literature on the VFD	32	78.0
Created an information bulletin on the VFD to distribute to staff	13	31.7
Other	0	0.0
Since January 1, 2017, what have you done to educate your swine clients on VFD requirements? (n= 40)		
I (we) have not done any education in 2017	4	10.0
Sponsored in-clinic meetings (including webinars) to present information and discuss requirements	16	40.0
Met in-person with clients to discuss requirements	35	87.5
Sent a notice of requirements to clients in a regular newsletter	20	50.0
Created an information bulletin to distribute to clients	15	37.5
Other	0	0.0

* Study details are described in Table 1.
† Percentages may reflect multiple answers from individual survey respondent.
VFD = Veterinary Feed Directive.

VFDs, educating clients and others (eg, nutritionists and feed suppliers), training staff on VFD requirements, and other components are presented in Table 9. Writing and delivering VFDs was the largest annual cost across all respondents with a mean value of $4051 and a median value of $3000. The annual cost for maintaining VFD records was similar in expense with a mean value of $3561 and a median value of $1000. The lowest annual cost to business operations was training staff on VFD requirements (mean of $787; median of $500). Costs recorded in the "other" category by 2 respondents were listed as the cost of the GVL software and additional staff required to write and store the VFDs. Generally, the costs slightly increased as client operation size increased, most likely due to the increase in the number of VFDs that would be written annually.

Table 10 shows the perceived amount of burden the respondents felt that VFD requirement compliance has had on veterinarians, feed suppliers, producers, and consulting nutritionists. Overall, burden to comply with the VFD is considered moderate. The highest amount of burden is believed to be on feed suppliers followed closely by producers and veterinarians.

Impact of antibiotic-use regulations

The reality of FDA's antibiotic-use guidelines is that producers and veterinarians have had more conversations about judicious antibiotic use of medically important antibiotics in feed or water. Overall, a perceived reduction in the amount of antibiotics used was reported, however, the magnitude of the reduction varied. The largest percentage of responding veterinarians (9 of 20; 22.5%) indicated a 21% to 30% perceived reduction in the use of antibiotics in feed by their clients as a result of the new antibiotic regulations (Table 11). Thirteen (32.5%) respondents perceived a 51% to 100% reduction of antibiotic use in feed among their clients. Swine veterinarians also reported a perceived increase in the amount of injectable (19 of 40 respondents; 47.5%) and water-soluble antibiotics used (30 of 41 respondents; 73.2%) since the VFD regulations were implemented.

Management changes due to regulations

One of the most important changes in the new regulations was the removal of medically important antibiotics for growth promotion. In response, it appears that most clients dealt with this change by eliminating all uses of antibiotics for growth promotion (58.8%), while another 17.0% of clients reduced use of antibiotics for growth promotion (Table 12). About 24% of clients changed to the use of non-medically important antibiotics for growth promotion. These results were different than those reported in the 2016 survey where veterinarians were predominately recommending replacing the medically important antibiotics with non-medically important antibiotics for growth promotion (52.9%).[6]

Responding veterinarians (n = 37) reported that increased vaccinations (30; 81.1%) were the primary management change made due to the new antibiotic regulations. Increasing non-antibiotic feed additives (21; 56.8%), modifying biosecurity (18; 48.6%), and modifying nutrition (14; 37.8%) were other common responses. One of the concerns veterinarians had during the previous survey regarding the new regulations was having enough documentable evidence to justify their recommendations to use medically important antibiotics. Thirty-nine of the 41 responding veterinarians in the present survey (95.1%) felt they had collected the needed health diagnostic information to defend or justify their antibiotic-use recommendations.

Discussion

On January 1, 2017, GFIs 209 and 213 and the revised VFD took effect. With a 3-year

Table 9: Survey responses regarding per year costs to veterinary business operations*†

	No. reporting	Cost, mean (SD), $	Cost, median, $
5000 to 19,999 marketings/year			
Writing and delivering VFDs	5	2860 (2796)	2400
Maintaining records for VFDs	2	1000 (0)	1000
Educating clients and others on the VFD requirements	3	433 (493)	200
Training staff on VFD requirements	2	300 (283)	300
Per year for other	0	NR	NR
20,000 to 49,999 marketings/year			
Writing and delivering VFDs	12	4143 (3700)	2800
Maintaining records for VFDs	7	4659 (9133)	1000
Educating clients and others on the VFD requirements	7	822 (862)	300
Training staff on VFD requirements	9	895 (877)	500
Per year for other‡	1	3600 (NA)	3600
≥ 50,000 marketings/year			
Writing and delivering VFDs	3	5667 (3786)	4000
Maintaining records for VFDs	1	1000 (NA)	1000
Educating clients and others on the VFD requirements	2	3500 (3536)	3500
Training staff on VFD requirements	0	NR	NR
Per year for other	0	NR	NR
All respondents			
Writing and delivering VFDs	20	4051 (3446)	3000
Maintaining records for VFDs	10	3561 (7663)	1000
Educating clients and others on the VFD requirements	12	1171 (1673)	650
Training staff on VFD requirements	11	787 (826)	500
Per year for other‡	2§	11,800 (11,597)	11,800

* Study details are described in Table 1.
† The survey instrument collected swine-client marketings per year using categorical variables, ie, what percentage would fall into each size category: 1 to 4999; 5000 to 19,999; 20,000 to 49,999; or 50,000 or more. For this analysis, the midpoint of each category (and endpoint of the upper and lower bound category) was used to calculate the weighted average marketings per year. One respondent had swine clients with 1 to 4999 marketings per year but did not report costs to veterinary business operations.
‡ Costs listed in this category were GVL software cost and hired employee to spend ½ time writing VFDs.
§ One survey respondent did not report swine client marketings per year but did report per year other costs to veterinary business operations; this response is included in "all respondents."
VFD = Veterinary Feed Directive; NR = none reported; NA = not applicable; GVL = GlobalVetLink.

implementation timeline from the time the GFIs were published, these regulations had already begun to influence antibiotic-use practices. According to the FDA 2016 Summary Report on Antimicrobials Sold or Distributed for Use in Food-Producing Animals, sales of medically important antibiotics decreased by 14%.[8] Sales to US swine producers made up 37% of the medically important antibiotics that were sold to livestock in 2016, so it is reasonable to assume that a portion of this overall decrease was in preparation for the new regulations to take effect. In 2016, there were many conversations among veterinarians and regulatory officials about how many visits to each site would fulfill the VCPR definition of timely visits. Some states have defined what constitutes timely, whereas others have not. Our survey results were varied with 51.2% of veterinarians thinking an annual visit would suffice, but the rest of the respondents felt it would take 2 or 4 visits per year to be considered timely. Many swine owners today have pigs that are raised on many different sites. Most of the veterinarians surveyed felt that they needed to visit all sites to have a valid VCPR, but there was a significant number of veterinarians that felt they needed to visit more than one site, but not all sites, within the operation to be in compliance.

Table 10: Survey responses regarding the perceived burden to comply with VFD requirements*

	Perceived burden, No. reporting (%)			
Population	**No burden**	**Little burden**	**Moderate burden**	**Very burdensome**
Veterinarians	0 (0.0)	14 (34.1)	24 (58.5)	3 (7.3)
Feed suppliers	0 (0.0)	6 (14.6)	24 (58.5)	11 (26.8)
Producers	2 (4.9)	16 (39.0)	21 (51.2)	2 (4.9)
Consulting nutritionists	5 (12.8)	20 (51.3)	14 (35.9)	0 (0.0)

* Study details are described in Table 1.
VFD = Veterinary Feed Directive

Table 11: Survey questions on perceived reduction in antibiotic usage due to new regulations*

	No. reporting (%)
What percentage have your swine producers reduced the use of antibiotics in feed as a result of the VFD? (n = 40)	
0%	0 (0.0)
1% to 10%	4 (10.0)
11% to 20%	7 (17.5)
21% to 30%	9 (22.5)
31% to 40%	1 (2.5)
41% to 50%	6 (15.0)
51% to 60%	1 (2.5)
61% to 70%	4 (10.0)
71% to 80%	4 (10.0)
81% to 90%	0 (0.0)
91% to 100%	4 (10.0)
In your opinion, how has the VFD changed the use of antimicrobials in water and injectable in US swine production?	
Water (n = 41)	
Increased	30 (73.2)
Decreased	2 (4.9)
Not changed	8 (19.5)
I do not know	1 (2.4)
Injectable (n = 40)	
Increased	19 (47.5)
Decreased	1 (2.5)
Not changed	18 (45.0)
I do not know	2 (5.0)

* Study details are described in Table 1.
VFD = Veterinary Feed Directive.

For those veterinarians who charge for writing VFDs, the mean fee for both new and existing clients was approximately $24 per VFD (median fee of $25), in contrast to an anticipated fee of $27 to $30 per VFD based on the 2016 survey.[6] The standard deviation was also cut in half compared to the 2016 survey, indicating that charges for VFDs are much more consistent across the industry. Prescription prices were less than the price of VFDs (median value of $20). While most of the VFDs are listed as a separate line item on a veterinary invoice, it is more common for prescriptions to be included as part of a consultation fee.

There is a fair amount of variation regarding what level of production a VFD is written for. Flow (generally defined as pigs that originated from the same breeding herd but raised in several different locations after weaning) was the most common production level, but there were many veterinarians who wrote VFDs specifically for the site and some even down to the individual lot level. Most veterinarians surveyed used an electronic service to both issue and store written VFDs. However, many veterinarians surveyed still used computer-generated forms rather than utilizing an electronic prescription service.

These survey results provide evidence that the new regulations have resulted in a perceived decreased usage of antibiotics in feed. The most common response was a 21% to 30% perceived decrease in antibiotic usage, but nearly a third of respondents believe that the reduction is anywhere from 50% to 100%. One of the biggest changes in antibiotic usage was their overall removal for growth promotion. In the 2016 survey, the majority of respondents predicted that their clients would shift from medically important to non-medically important antibiotics for growth promotion as there are several products now

available and new products being evaluated. It appears though, that most of their clients eliminated either all or part of their antibiotics used for growth promotion, thus most likely contributing to the overall decrease in antibiotic usage.

Due to this survey using a convenience sample, there are some limitations to this data. There is certainly potential for biases based on the sampling technique and the geographic region from which the sample was derived. The readers should take this into consideration and not extrapolate the results of this survey to the entire US swine industry. The low response rate also necessitates caution when interpreting results. It is unknown whether collecting survey responses by type of veterinary practice, eg, private vs corporate practice or employed by a large integrator, would have affected the results. However, the swine veterinary practice demographics collected did demonstrate variability and responses were from US states with the highest concentration of swine production. Informed by these results, future surveys should employ a randomized questionnaire distribution method and include questions to provide a more complete picture of how the antibiotic-use guidelines continue to impact pork production and the practice of swine veterinary medicine in the United States.

Implications

- Improved veterinarian oversight of antibiotics used in US swine production was a key response from survey participants.
- Survey respondents reported the occurrence of more discussions between swine veterinarians and producers about the use of antibiotics and antibiotic alternatives.
- Survey respondents perceived a reduction of antibiotic use in feed as a result of the VFD regulations.

Acknowledgments

Conflict of interest

None reported.

Disclaimer

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References

*1. Guidance for Industry on the judicious use of medically important antimicrobial drugs in food-producing animals; Availability. *Fed Regist*. 2012;77(72):22328-22329.

*2. US Department of Health and Human Services, Food and Drug Administration, Center for Veterinary Medicine. Guidance for Industry #209, The judicious use of medically important antimicrobial drugs in food producing animals. April 13, 2012. http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM216936.pdf. Accessed June 6, 2017.

*3. Guidance for Industry on new animal drugs and new animal drug combination products administered in or on medicated feed or drinking water of food-producing animals: Recommendations for drug sponsors for voluntarily aligning product use conditions with Guidance for Industry #209; Availability. *Fed Regist*. 2013;78(239):75570-75571.

*4. US Department of Health and Human Services, Food and Drug Administration, Center for Veterinary Medicine. Guidance for Industry #213, New animal drugs and new animal drug combination products administered in or on medicated feed or drinking water of food-producing animals: Recommendations for drug sponsors for voluntarily aligning product use conditions with GFI #209. December 2013. http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM299624.pdf. Accessed June 6, 2017.

*5. Veterinary Feed Directive; Final Rule. *Fed Regist*. 2015;80(106):31708-31735. To be codified at 28 CFR §514 and §558.

Table 12: Survey questions regarding management changes made to address antibiotic regulations and growth promotion use*

	No. reporting	Mean (SD), %	Median, %
Percentage of swine clients who:			
Eliminated all uses of antibiotics for growth promotion	38	58.8 (42.3)	66.9
Reduced use of antibiotics for growth promotion	38	17.0 (29.2)	0.0
Moved to non-medically important growth promotants	38	24.2 (33.4)	7.5
Other	38	0.0 (0.0)	0.0
	No. reporting		**% reporting†**
Changes producers have made (n = 37)			
Modified biosecurity	18		48.6
Increased vaccinations	30		81.1
Increased non-antibiotic feed additives	21		56.8
Modified nutrition	14		37.8
Modified housing	6		16.2
Modified animal purchase strategies	5		13.5
Modified population density	3		8.1
Other	0		0.0

* Study details are described in Table 1.
† Percentages may reflect multiple answers from individual survey respondents.

6. Schulz LL, Rademacher CJ. Food and Drug Administration Guidance 209 and 213 and Veterinary Feed Directive regulations regarding antibiotic use in livestock: A survey of preparation and anticipated impacts in the swine industry. *J Swine Health Prod*. 2017;25(5):247-255.

*7. US Department of Agriculture, National Agricultural Statistics Service. 2012 census of agriculture, United States summary and state data. **http://www.agcensus.usda.gov/Publications/2012/Full_Report/Volume_1,_Chapter_1_US/usv1.pdf**. Published May 2014. Accessed June 6, 2017.

* 8. US Food and Drug Administration. 2016 summary report on antimicrobials sold or distributed for use in food-producing animals. **https://www.fda.gov/downloads/forindustry/userfees/animaldruguserfeeactadufa/ucm588085.pdf**. Published December 2017. Accessed July 9, 2018.

* Non-refereed references.

Conversion tables

Weights and measures conversions

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

Temperature equivalents (approx)

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

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

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

Conversion chart, kg to lb (approx)

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

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

Development of a herd-specific lung homogenate for exposure to *Mycoplasma hyopneumoniae* under field conditions

Rebecca C. Robbins, DVM, PhD; Alyssa M. Betlach, DVM; Maria R. Mondragon-Evans, MVZ; Maria Pieters, DVM, PhD

Summary

The swine industry is known for holding high standards of disease control and elimination. However, partial disease control for *Mycoplasma hyopneumoniae* at the farm level has been evident and has driven initiatives for unconventional health management strategies. Several approaches focused on gilt exposure for *M hyopneumoniae* using a herd-specific lung homogenate have been performed in the field. Nevertheless, variations in efficacy are apparent and a publicly available protocol for producing *M hyopneumoniae* lung homogenate under field conditions is not available. In this practice tip, a protocol is described for developing a herd-specific lung homogenate for *M hyopneumoniae* exposure intended for use in veterinary-supervised elimination or control programs. A herd-specific lung homogenate inoculum, free of secondary respiratory pathogens for the herd of intended use and with an adequate *M hyopneumoniae* concentration, was obtained through extensive diagnostic testing and evaluation of *M hyopneumoniae* localization within the lung. Molecular methods were applied to characterize the *M hyopneumoniae* present in the lung and to evaluate the genomic stability of the bacterium during the exposure process. In doing so, a herd-specific *M hyopneumoniae* lung homogenate for gilt acclimation was obtained under field conditions.

Keywords: swine, *Mycoplasma hyopneumoniae,* gilt acclimation, lung homogenate, disease control and elimination

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Resumen - Desarrollo de un homogeneizado de pulmón hato-específico para la exposición a *Mycoplasma hyopneumoniae* en condiciones de campo

La industria porcina es conocida por mantener altos estándares de control y eliminación de enfermedades. Sin embargo, el control parcial de la enfermedad causada por *Mycoplasma hyopneumoniae* a nivel de granja ha sido evidente y ha impulsado iniciativas para desarrollar estrategias de control de salud no convencionales. En el campo, se han desarrollado varios enfoques centrados en la exposición a la hembra primeriza contra *M hyopneumoniae* con un homogeneizado de pulmón hato-específico. Sin embargo, la variación en la eficacia es evidente y no se dispone de un protocolo publicado para producir el homogeneizado pulmonar con *M hyopneumoniae* en condiciones de campo. En este consejo práctico, se describe un protocolo para la preparación de un homogeneizado de pulmón hato-específico para la exposición de *M hyopneumoniae* destinado a ser utilizado en programas de eliminación o control supervisados por veterinarios. A través de extensas pruebas diagnósticas y la evaluación de la localización de *M hyopneumoniae* dentro del pulmón, se obtuvo un inóculo hato-específico de un homogeneizado de pulmón, libre de patógenos respiratorios secundarios para ser utilizado en el hato previsto y con una concentración adecuada de *M hyopneumoniae*. Se utilizaron métodos moleculares para caracterizar al *M hyopneumoniae* presente en el pulmón y para evaluar la estabilidad genómica de la bacteria durante el proceso de exposición. Al hacerlo, se obtuvo un homogeneizado de pulmón de *M hyopneumoniae* específico para la aclimatación de hembras primerizas en condiciones de campo.

Résumé – Développement d'un homogénat de poumon spécifique de troupeau pour exposition à *Mycoplasma hyopneumoniae* dans des conditions de terrain

L'industrie porcine est reconnue pour le maintien de standards élevés en ce qui a trait à la maitrise et à l'élimination des maladies. Toutefois, à la ferme la maitrise partielle de l'infection par *Mycoplasma hyopneumoniae* est évidente et a entrainé des initiatives pour des stratégies non-conventionnelles de gestion de la santé. Plusieurs approches ont misé sur l'exposition de cochettes à *M hyopneumoniae* en utilisant un homogénat de poumon spécifique au troupeau ont été réalisées sur le terrain. Cependant, des variations dans l'efficacité sont apparentes et un protocole disponible à tous pour produire en condition de terrain un homogénat pulmonaire contenant *M hyopneumoniae* n'est pas disponible. Dans la présente astuce de pratique, un protocole est décrit pour développer et utiliser, sous supervision vétérinaire, un homogénat pulmonaire spécifique de troupeau contenant *M hyopneumoniae* dans le cadre de programmes de maitrise ou d'élimination. Un inoculum d'homogénat de poumon spécifique de troupeau, exempt d'agents pathogènes respiratoires secondaires pour le troupeau sélectionné et avec une concentration adéquate de *M hyopneumoniae*, fut

RCR, MRM-E: Seaboard Foods, Guymon, Oklahoma.

AMB, MP: College of Veterinary Medicine, University of Minnesota, St Paul, Minnesota.

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obtenu à la suite d'épreuves diagnostiques nombreuses et à l'évaluation de la localisation de *M hyopneumoniae* dans le tissu pulmonaire. Des méthodes moléculaires furent utilisées afin de caractériser les *M hyopneumoniae* présents dans le poumon et pour évaluer la stabilité génomique de la bactérie durant le processus d'exposition. Ainsi, un homogénat de poumon spécifique de troupeau contenant *M hyopneumoniae* pour l'acclimatation des cochettes fut obtenu dans des condition de terrain.

Veterinarians are responsible for applying their knowledge to improve animal health and welfare. The swine industry aims for high herd health to rear healthy pigs and safe pork. To do so, veterinarians, producers, industry professionals, and scientists attempt to implement practical and science-driven solutions that can be applied in the field. The herd veterinarian is tasked with recommending solutions based on professional judgement, scientific literature, experience, field research, and consultation with colleagues and experts. Historically, herd management practices have evolved in response to issues faced in the field and are adopted as ethically and scientifically substantiated solutions. In the case of disease control, the swine industry has been keen to develop and apply strategies towards disease management and elimination, including the use of biosecurity and the modification of production practices to decrease the detrimental effect of disease transmission (eg, early weaning[1] and all-in/all-out production[2]). In cases where ideal disease control cannot be achieved with the available tools, novel solutions are generated.

The administration of a herd-specific infectious product for disease control has been used in veterinary medicine to confer complete and strain-specific protection when other measures have proven inadequate to contain the disease process. In some instances, administration of a herd-specific tissue homogenate is the best option for a controlled exposure to indigenous pathogens when the exposure is intended to protect the larger population. Use of herd-specific tissue homogenate for controlled exposure requires veterinary oversight and must adhere to any applicable regulations ensuring that it does not adversely affect the health and performance of the individual animal exposed. For example, the control of viruses (ie, porcine parvovirus and porcine enterovirus) known to cause stillbirths, mummification, embryonic deaths, and infertility has been achieved by exposing dams to infectious feedback material composed of feces or tissues from contaminated litters.[3,4] This exposure serves to homogenize herd immunity and acclimatize incoming gilts to prevent herd disequilibrium. Immunity to porcine epidemic diarrhea virus (PEDV) and porcine rotavirus has been accomplished by using pre-farrow oral controlled exposure of dams with infectious feedback material[5,6] resulting in protection of piglets through the development of humoral and cell-mediated immunity.

Mycoplasma hyopneumoniae causes a chronic respiratory condition in pigs known as enzootic pneumonia (EP), which affects herds worldwide.[7,8] Control measures for EP include the use of immunization, antimicrobial medication, increased biosecurity practices, parity segregation, all-in/all-out movement, and elimination strategies.[9] However, in certain situations such as gilt acclimation, partial control can be obtained with the use of these measures, even if they are employed in combination. Thus, veterinary professionals have proposed the use of alternative measures to control *M hyopneumoniae* infections in the field, which are tailored to be herd-specific and include pathogen exposure using lung homogenate.

Statement of the problem

Replacement gilts play an important role in the dynamics of a sow farm, as approximately half of the herd is replaced with young females every year for genetic improvement and maintenance of parity structure.[10] However, every new batch of replacement females needs to be evaluated for their potential to cause disturbance of the sow farm dynamics, especially as it pertains to infectious agents. Incoming gilts may introduce new pathogens not currently prevalent in the herd or be naïve to existing pathogens on the recipient sow farm. Gilt health status is closely surveilled before and after transportation and during introduction to the recipient herd. Assurance from suppliers regarding freedom from economically important swine pathogens (ie, porcine reproductive and respiratory syndrome virus [PRRSV], PEDV, and *M hyopneumoniae*) may or may not be required by the buyer. Although freedom of infectious agents and disease is a desirable attribute in replacement animals, it is hypothesized that in certain circumstances the health conditions of the recipient farm may be more severely affected by the introduction of naïve pigs. This is the case for *M hyopneumoniae* infections, which are considered endemically prevalent in a significant proportion of swine farms.[11] Introduction of naïve gilts into *M hyopneumoniae*-positive farms is hypothesized to be a risk factor for sow herd disequilibrium and results in difficulty to control disease presentation in downstream flows.[12-14]

Various options can be pursued to address the issue of naïve gilt introductions into *M hyopneumoniae* endemically infected farms. Disease elimination is most favorable for any swine production unit, and recently efforts for *M hyopneumoniae* eradication have increased in the United States.[15] One of the most commonly utilized strategies for *M hyopneumoniae* elimination, which is herd closure and medication, implies uniform exposure of the entire herd at the same time prior to the start of closure.[16] A protocol directed at exposure with *M hyopneumoniae* is needed when pursuing disease elimination. To achieve and maintain the elimination of *M hyopneumoniae,* farm geographical location, area prevalence, facility design, production system flow, and constant and continuous supply of negative gilts should be accounted for. However, these factors often cannot be modified to achieve successful elimination. Therefore, disease control is viewed as one of the oldest and most cost-effective strategy to deal with *M hyopneumoniae* on endemically infected farms, keeping in mind the necessity to maintain the health of incoming and resident dam populations.

One common question in the industry is whether control can be achieved with commercial products directed at treating or controlling *M hyopneumoniae* infections. The species-specific vaccines and antimicrobial drugs with activity towards mycoplasmas play an important role in decreasing the negative outcomes of EP. However, it is widely known that partial protection is conferred by *M hyopneumoniae* bacterins[17] and vaccinated pigs can become colonized after contact with shedding pigs.[18,19] In addition, elimination of the bacterium from the respiratory tract of pigs has not been achieved with antimicrobial treatment alone, even during the chronic phase of infection.[20] Therefore, a need exists for a practical protocol for herd exposure to *M hyopneumoniae*. In this practice tip, we describe a procedure to develop a herd-specific lung homogenate for *M hyopneumoniae* exposure under field conditions to potentially stimulate immunity and decrease the proportion of susceptible

animals in the population. This practice tip is intended to be used as a resource for swine veterinarians who are designing gilt acclimatization strategies that involve the procurement of a herd-specific lung homogenate.

Definitions

For the purpose of providing clarity to this practice tip, the following definitions are proposed:

> **Gilt acclimation:** The process of adapting gilts to a new environment or exposure to an infectious agent prior to introduction into a recipient breeding herd.[13,21]
>
> **Lung homogenate**: Lung tissue made uniform through a blending process that is used for exposure.

Animal care

All animals were under veterinary oversight and care with a veterinarian-client-patient relationship and Pork Quality Assurance Plus certification in place. Feed and water were available *ad libitum* in stainless steel feeders and through water nipples, respectively. Pigs and their environment were monitored daily by caretakers. All feed rations were formulated to meet or exceed nutritional recommendations for swine.[22] Gilts were raised in standard indoor production facilities with fully slatted floors, fed a diet to meet or exceed their nutritional needs, and received immunizations against porcine circovirus type 2 (PCV2), PRRSV, and *M hyopneumoniae* as a growing pig, followed by a booster immunization for *M hyopneumoniae*, PCV2, and PRRSV at selection (26 weeks of age). All injections were performed with a needleless device using commercially available products.

Procuring a herd-specific *M hyopneumoniae* lung homogenate

Under experimental conditions, viable culture and tissue homogenate have been administered to stimulate *M hyopneumoniae* exposure.[23-25] However due to the fastidious growth of this microorganism, the procurement of a herd-specific lung homogenate was proposed. To obtain a herd-specific lung homogenate, a procedure focusing on lung homogenate preparation from tissue donor gilts was developed for use in field scenarios (Figure 1). Several factors including farm history and health status, clinical observations, and diagnostic testing were taken into consideration by the herd veterinarian during the selection of donor gilts and lung tissue. With diagnostic aid, the concentration of *M hyopneumoniae* and presence of secondary agents were evaluated to ensure adequate lung homogenate quality. It was up to the herd veterinarian to consider the herd's indigenous organisms when developing parameters for homogenate quality. In addition, the infectivity and genomic stability of the *M hyopneumoniae* lung homogenate were assessed under field conditions.

Donor gilt selection

Initial tissue donor gilt

The initial tissue donor gilt was from a PRRSV, influenza A virus (IAV), PCV2, and *Mycoplasma* species positive farm and was selected at 31 weeks of age when she exhibited clinical signs (ie, dyspnea and loss of body condition) suggestive of *M hyopneumoniae* infection.[26] Alternatively, an initial donor may be chosen through testing of ante-mortem samples (eg, laryngeal swabs)[27] using sterile swabs (BBL CultureSwab, Sparks, Maryland) and tested for *M hyopneumoniae* by species-specific real-time polymerase chain reaction (real-time PCR) to confirm infection.[28] The donor was humanely euthanized and lung tissue harvested if macroscopic lesions (ie, consolidation of apical and cardiac lung lobes) consistent with *M hyopneumoniae* infection were observed[26] and no lesions of secondary bacterial infection (eg, polyserositis) were evident. A bronchial swab was obtained by inserting a sterile swab into bilateral bronchioles of affected lung tissue and submitted to the University of Minnesota Veterinary Diagnostic Laboratory (UMN VDL), along with a portion of the affected lung lobe for diagnostic testing. Remaining lung tissue was stored at -20° C for a minimum of 48 hours and until diagnostic testing was completed to ensure a high recovery of *M hyopneumoniae*.

Diagnostic criteria were established for the initial donor to ensure adequate exposure following *M hyopneumoniae* infection and to minimize the risk of introducing and spreading secondary respiratory pathogens (Figure 1). The criteria for initial lung selection were: 1) observation of macroscopic lesions (ie, consolidation of apical and cardiac lung lobes) suggestive of *M hyopneumoniae* infection; 2) *M hyopneumoniae* real-time PCR cycle threshold (Ct) value ≤ 26; 3) *Mycoplasma hyorhinis* real-time PCR Ct value ≥ 33; 4) PRRSV and IAV negative real-time PCR result; 5) PCV2 real-time PCR Ct value ≥ 30; 6) no *Haemophilus parasuis* growth on culture; and 7) identification of < 1+ bacteria on aerobic culture.

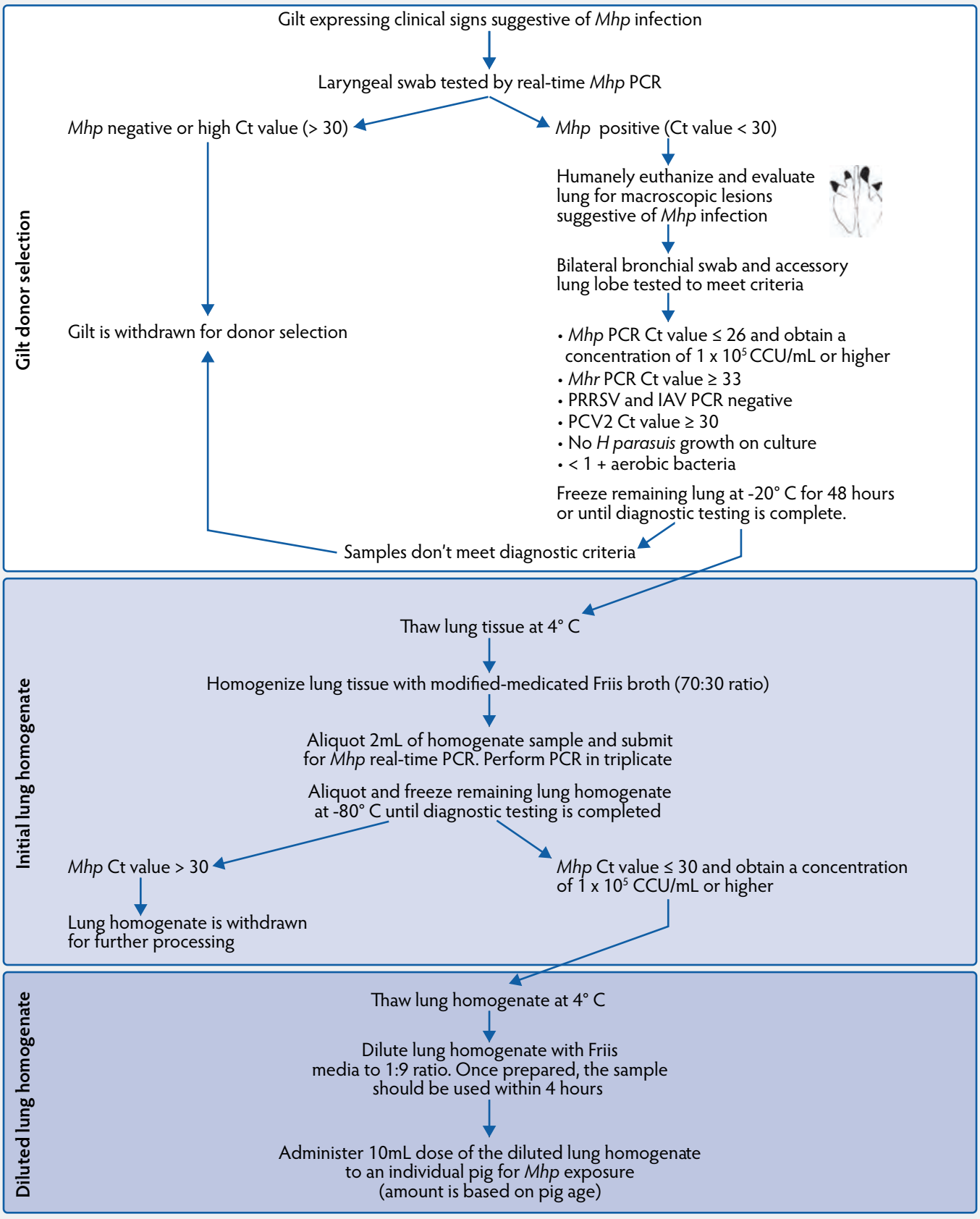
The diagnostic parameters were designed to prevent the introduction, amplification, or spread of secondary respiratory pathogens, including but not limited to PRRSV, IAV, PCV2 and *H parasuis,* which could cause unintended infection and compromise gilt health. *Mycoplasma hyorhinis* is a commensal microorganism in swine; however, clinical disease associated with polyserositis is often evident at high bacterial concentrations.[29] Therefore, an *M hyorhinis* Ct value ≥ 33 was chosen as the cut-off parameter while considering the ubiquitous nature of this microorganism in swine herds and the clinical history of the herd. A PCV2 Ct value ≥ 30 was chosen as the cut-off parameter due to the endemic nature of this microorganism in swine herds.[30] If additional respiratory pathogens were detected, continuation of lung homogenate development protocol was at the discretion of the veterinarian.

The *M hyopneumoniae* Ct value of ≤ 26 was selected by fitting a standard curve with known concentrations of bacterial infectivity (color changing units/mL [CCU/mL]) to the real-time PCR assay and obtaining a Ct value equivalent to 1×10^3 CCU/mL. A concentration of 1×10^5 CCU/mL of *M hyopneumoniae* has been suggested as the minimum required infectious dose for successful colonization of a pig's lung in experimental conditions.[31] Differences in virulence across *M hyopneumoniae* strains have been observed,[32] therefore, a potentially lower infectious dose equivalent of 1×10^3 CCU/mL was chosen by the veterinarian. In addition, within-sample variation was assumed based on the nature of the sample, therefore, the infectious dose may potentially vary. Lungs fulfilling the diagnostic criteria, with the intent to inoculate *M hyopneumoniae*-negative gilts, were used to make enough homogenate for the herd-specific gilt acclimation program recommended by the veterinarian.

Donor gilts for amplification and lung homogenate procurement

To amplify and procure lung homogenate for *M hyopneumoniae* exposure for replacement gilts to a 65,000-sow herd, 3- to 5-week old PRRSV, IAV, and *M hyopneumoniae*-negative

Figure 1: Procedure to obtain a *Mycoplasma hyopneumoniae* lung homogenate. *Mhp* = *Mycoplasma hyopneumoniae*; PCR = polymerase chain reaction; Ct = cycle threshold; CCU = color changing units; *Mhr* = *Mycoplasma hyorhinis*; PRRSV = porcine reproductive and respiratory syndrome virus; IAV = influenza A virus; PCV2 = porcine circovirus type 2; *H parasuis* = *Haemophilus parasuis*.



gilts (n = 38) were intra-tracheally inoculated with 10mL of the diluted lung homogenate.

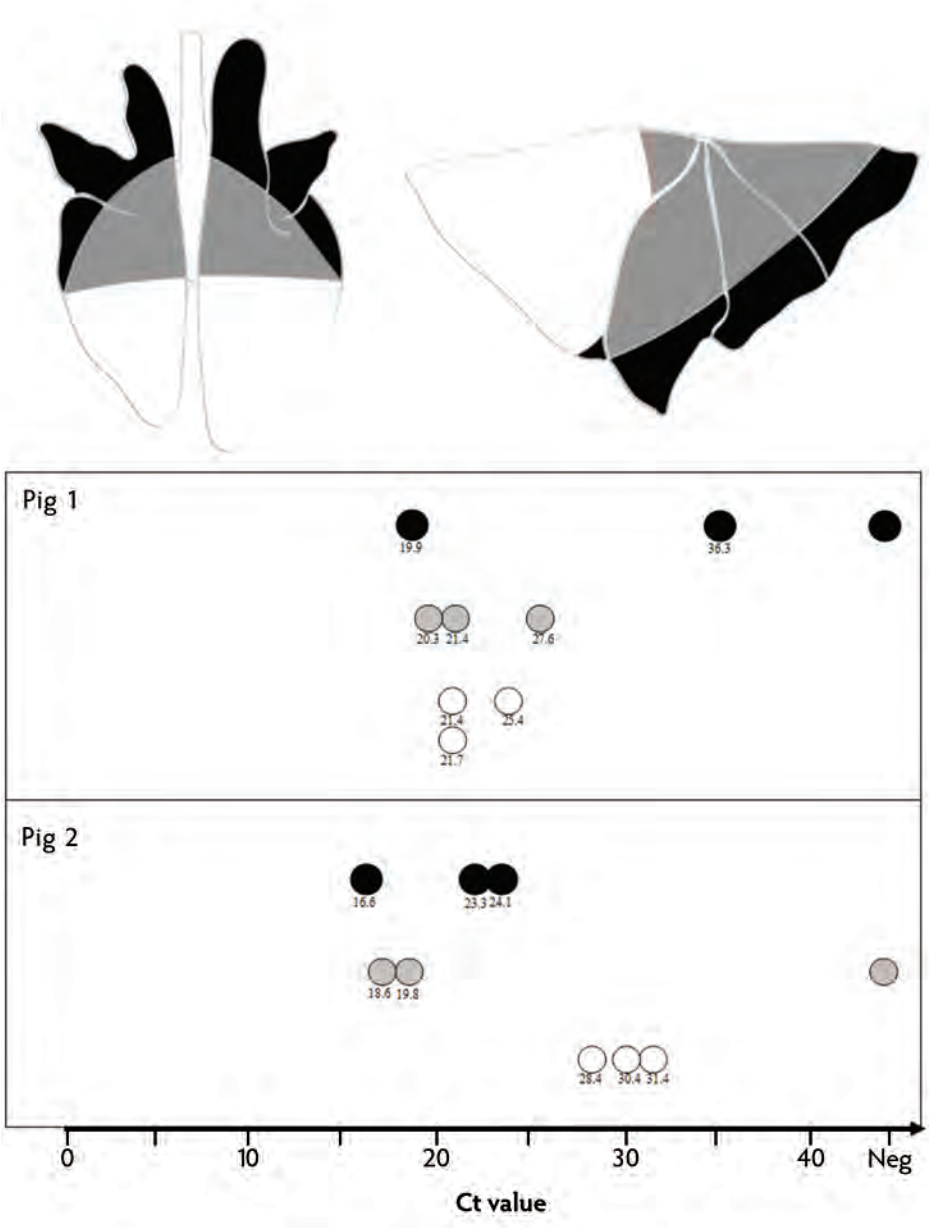
Four weeks post inoculation, laryngeal swabs were collected and tested for *M hyopneumoniae* by species-specific real-time PCR to confirm infection. If swabs were positive, lungs were harvested at 5 weeks post inoculation and diagnostic testing was performed as previously described for the initial donor (Figure 1). The accessory lung lobe was submitted for diagnostic testing to evaluate the presence of viruses and secondary bacteria while preserving the remaining lung sections for subsequent lung homogenate development. Sample collection and tissue harvest took place 5 weeks post inoculation because peak *M hyopneumoniae* shedding has been shown to occur at 4 weeks post inoculation under experimental conditions[33] and to account for the lower *M hyopneumoniae* infectious dose (1×10^3 CCU/mL). Lungs that fulfilled the diagnostic criteria were processed into lung homogenate and used to expose larger gilt populations as part of the herd-specific acclimation program.

Lung homogenate preparation

Selection of lung tissue

To identify the maximum amount of lung tissue meeting the diagnostic criteria for homogenate production, a pilot study was performed. Localization of *M hyopneumoniae* was evaluated by determining the relative bacterial load within different anatomical lung sections using lung homogenate samples of two gilts, which were evaluated individually (Figure 2). The lung homogenates were obtained 5 weeks post inoculation and tested for *M hyopneumoniae* using real-time PCR. Each lung homogenate was run in triplicate, in which the genetic material from 3 sample subsets was extracted and tested individually to account for possible diagnostic variation due to sample consistency. Of the 2 gilts sampled, *M hyopneumoniae* bacterial loads were numerically higher in the proximal lung sections (median Ct values = 21.4 and 19.8) compared to the distal lung sections (median Ct values = 36.3 and 23.3) and the caudal diaphragmatic lobe (median Ct values = 21.7 and 30.4). However, the amount of viable *M hyopneumoniae* based on anatomical lung sections was not assessed because of the difficulty to obtain an *M hyopneumoniae* culture, especially under field conditions. In addition, the proportion of affected lung within each anatomical lung section was not evaluated.

Figure 2: *Mycoplasma hyopneumoniae* bacterial load (Ct value) based on anatomical lung section. ● =Distal sections of apical, cardiac, and diaphragmatic lobes; ◉ =Proximal sections of apical, cardiac, and diaphragmatic lobes; ○ = Caudal diaphragmatic lobe. Each dot represents one sample tested by real-time PCR. Ct = cycle threshold; PCR = polymerase chain reaction.



For the detection of this microorganism, within-homogenate variance was observed for each anatomical lung region, but to a greater extent in distal and proximal lung sections compared to the caudal diaphragmatic lobe (Figure 2). The degree of within-homogenate variance could have resulted from the anatomic nature of the tissue as the homogenate includes cartilaginous airways, pleura, and lung tissue with the specific localization of the microorganism. In this investigation, a small sample size was evaluated, however, insight regarding the relative bacterial load based on anatomical lung section was gained at the individual pig level. Further research involving a larger sample size and evaluating the impact of different *M hyopneumoniae* infection lengths and lung lesion scores on the relative bacterial load within each anatomical lung section is needed.

Since this microorganism was localized across the three different anatomical lung sections, the relative bacterial load of *M hyopneumoniae* within different lung homogenate preparations was evaluated in 38 gilts at 5 weeks post inoculation. *Mycoplasma hyopneumoniae* Ct values were compared in bronchial swabs and 2 types of lung homogenate samples prepared from either whole lung tissue or from lesioned apical, cardiac, and diaphragmatic lobes that contained adjacent apparently non-affected tissue (Table 1). All bronchial swabs were collected from affected apical and cardiac lung lobes and the lung tissue was homogenized using 70% lung tissue and 30% modified, medicated Friis broth.[34] Samples were submitted for *M hyopneumoniae* testing using real-time PCR, in which the homogenate samples were run in triplicate and the median Ct value was used for data analysis. For statistical analysis, a two-sample *t*-test assuming equal variances was performed using R (v3.5.1; R Core Team, 2018) to compare lung homogenate Ct values based on preparation type. Differences were considered significant at $P < .05$. Based on the conditions of this study, the *M hyopneumoniae* lung homogenate derived from lesioned apical, cardiac, and diaphragmatic lung lobes showed significantly lower Ct values compared to whole lung tissue Ct values ($P = .003$; Table 1). In both lung homogenates, the mean *M hyopneumoniae* Ct values were 20.9 and 24.9, suggesting a high bacterial presence of the microorganism regardless of tissue preparation method (Table 1). In addition, tissue preparation using whole lung provided a larger volume of lung homogenate, resulting in the use of fewer donor gilts. Since the whole lung homogenate preparation met or exceeded the veterinarian's homogenate quality criteria, the lung homogenate was prepared by incorporating the whole lung tissue.

Initial lung homogenate

Frozen whole lung tissue was homogenized using a ratio of 70% tissue and 30% modified medicated Friis broth[34] using a Ninja Professional blender. This ratio was chosen based on the sampling procedure used for viral isolation by the UMN VDL and the feasibility to handle and process the material considering its viscosity. The blending process was repeated until lung tissue reached a slurry consistency. Friis medium was used to support *M hyopneumoniae* viability during the preparation and inoculation of the lung homogenate because this medium is commonly used for the culture and isolation of this microorganism.[34] Lung tissue was processed, aliquoted, and stored at -80° C. Currently, there is minimal information regarding the freeze-thaw effect on *M hyopneumoniae* viability. It is hypothesized that thawing frozen lung tissue aids in the detachment of this microorganism from the targeted tissue leading to a higher bacterial recovery. However, further information on this topic is necessary to assess the viability and storage of frozen *M hyopneumoniae* clinical samples. Previous literature suggests that freezing a *Mycoplasma* organism culture at -70° C and -30° C for up to 2 years may result in up to 1 and 2 $\log_{10}$ reduction in bacterial titers, respectively.[35] Prior to freezing, 2 mL of the lung homogenate was submitted for *M hyopneumoniae* real-time PCR and tested in triplicate, resulting in an average 25.5 Ct value.

Lung homogenate dilution

Thawed lung homogenate was diluted in a 1:9 ratio with Friis base media (Teknova, Hollister, California) in a clean laboratory, while technicians wore personal protective equipment. Since *M hyopneumoniae* adheres to ciliated epithelium within the respiratory airways, the diluted lung homogenate was not filtered to potentially increase the likelihood of infectivity. Ten milliliters of the diluted lung homogenate were delivered intra-tracheally to the 3- to 5-week old donor pigs as previously described.[36] The *M hyopneumoniae* concentration was not evaluated at the time of exposure.

Evaluating lung homogenate infectivity and genomic stability

Lung homogenate infectivity

Diagnostic monitoring post inoculation was performed to evaluate the diluted lung homogenate infectivity. The veterinarian considered the lung homogenate to be infectious if an *M hyopneumoniae* infection was observed or detected post inoculation. Laryngeal swabs were collected 4 weeks post inoculation for *M hyopneumoniae* detection using real-time PCR. All the pigs sampled (n = 38) were *M hyopneumoniae* positive, evidencing sample infectivity. Post inoculation, clinical signs and mortality were closely monitored. If clinical signs suggestive of secondary bacterial infections (eg, unthriftiness, cough, thumping, or increased respiratory effort) were observed, antimicrobials without activity towards mycoplasmas (eg, Ceftiofur) were administered according to label directions.

Genomic stability

Multiple locus variable number tandem repeat analysis (MLVA)[37] was employed to identify *M hyopneumoniae* types in the lung homogenate and to evaluate for potential genomic mutations that could have occurred during the tissue processing and inoculation. The molecular characterization method was performed from *M hyopneumoniae*-positive bronchial swabs that were collected from the initial and subsequent donor gilts' lung tissue. All samples showed an MLVA type 11-15. This suggests a lack of detectable genomic change in the targeted amplicon during the initial lung homogenate preparation and throughout the subsequent exposure-harvest processes. This finding is supportive of other research that describes *M hyopneumoniae in vitro* and *in vivo* genomic stability.[37,38]

Table 1: Detection of *Mycoplasma hyopneumoniae* (Ct values) in bronchial swabs and lung homogenate samples based on tissue preparation. Different superscript letters represent significant difference ($P < .05$) based on a two-sample *t*-test. Ct = cycle threshold.

Lung section	No. of samples	Bronchial swabs, Ct value (SD)	Lung homogenate, Ct value (SD)
Lesioned apical, cardiac, and diaphragmatic lobes	14	22.6 (4.5)	20.9 (3.6)[a]
Whole lung	24	22.9 (2.7)	24.9 (3.9)[b]

Conclusion

In this practice tip, a procedure for the development of a herd-specific lung homogenate for *M hyopneumoniae* exposure under field conditions is described. This practice tip details a step-by-step process focusing on lung homogenate preparation. In doing so, gilt acclimatization practices that encompass herd-specific pathogen exposure methods may be achieved to provide adequate *M hyopneumoniae* exposure and immunization.

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Conflict of interest

None reported.

Disclaimer

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References

1. Alexander TJL, Thornton K, Boon G, Lysons RJ, Gush AF. Medicated early weaning to obtain pigs free from pathogens endemic in the herd of origin. *Vet Rec.* 1980;106:114-119.

2. Clark LK, Scheidt AB, Armstrong CH, Knox KE, Mayrose VB. The effect of all-in/all-out management on pigs from a herd with enzootic pneumonia. *Vet Med.* 1991;86(9):948-951.

3. Truyen U, Streck AE. Porcine Parvovirus. In: Zimmerman JJ, Karriker L, Ramirez A, Schwartz K, Stevenson G, eds. *Diseases of Swine*. 10th ed. Ames, IA: Wiley-Blackwell; 2012:447-455.

4. Alexandersen S, Knowles NJ, Dekker A, Belsham GJ, Zhang Z, Koenen F. Picornaviruses. In: Zimmerman JJ, Karriker L, Ramirez A, Schwartz K, Stevenson G, eds. *Diseases of Swine*. 10th ed. Ames, IA: Wiley-Blackwell; 2012:587-620.

*5. Schwartz KJ. The science of feedback. *Proc Annu Iowa State University Swine Dis Conf Swine Pract.* Ames, IA. 2014;51-66.

*6. Pittman JS. Field experiences with interventions for Rotavirus control. *Proc Annu Iowa State University Swine Dis Conf Swine Pract.* Ames, IA. 2016;30-36.

7. Goodwin RFW, Pomeroy AP, Whittlestone P. Production of enzootic pneumonia in pigs with a mycoplasma. *Vet Rec*. 1965;77:1247-1249.

8. Mare C, Switzer WP. New species: *Mycoplasma hyopneumoniae*; a causative agent of virus pig pneumonia. *Vet Med Small Anim Clin.* 1965;60,841-846.

9. Maes D, Sibila M, Kuhnert P, Segalés J, Haesebrouck F, Pieters M. Update on *Mycoplasma hyopneumoniae* infections in pigs: Knowledge gaps for improved disease control. *Transbound Emerg Dis.* 2017;65:110-124.

10. Stalder K, D'Allaire S, Drolet R, Abell C. Longevity in breeding animals. In: Zimmerman JJ, Karriker L, Ramirez A, Schwartz K, Stevenson G, eds. *Diseases of Swine*. 10th ed. Ames, IA: Wiley-Blackwell; 2012:50-59.

*11. United States Department of Agriculture. Part II: Reference of Swine Health and Health Management in the United States, 2012. In: *National Animal Health Monitoring System Swine 2012*. Fort Collins, Colorado: Center for Epidemiology and Animal Health, Animal and Plant Health Inspection Service, US Dept of Agriculture; 2015. Publication #676.0216.

*12. Lowe L. *Mycoplasma hyopneumoniae*: Gilts, are they the problem? *Proc Allen D. Leman Conf.* Saint Paul, MN. 2012;(39):83-85.

13. Nathues H, Chang YM, Wieland B, Rechter G, Spergser J, Rosengarten R, Kreinebrock L, Grosse Beilage E. Herd-level risk factors for the seropositivity to *Mycoplasma hyopneumoniae* and the occurrence of enzootic pneumonia among fattening pigs in areas of endemic infection and high pig density. *Transbound Emerg Dis.* 2014;61(4):316-328.

14. Pieters M, Fano E. *Mycoplasma hyopneumoniae* management in gilts. *Vet Rec*. 2016;178(5):122-123.

*15. Yeske PE. Economic impact of *M. hyopneumoniae* eliminations. *Proc IPVS.* Cancun, Mexico. 2012;246.

16. Holst S, Yeske P, Pieters M. Elimination of *Mycoplasma hyopneumoniae* from breed-to-wean farms: A review of current protocols with emphasis on herd closure and medication. *J Swine Health Prod.* 2015;23:321-330.

17. Haesebrouck F, Pasmans F, Chiers K, Maes D, Ducatelle R, Decostere A. Efficacy of vaccines against bacterial disease in swine: what can we expect? *Vet Microbiol.* 2004; 100(3-4):255-268.

18. Villarreal I, Meyns T, Dewulf J, Vranchx K, Calus D, Pasmans F, Haesebrouck F, Maes D. The effect of vaccination on the transmission of *Mycoplasma hyopneumoniae* in pigs under field conditions. *Vet J*. 2011;188(1):48-52.

19. Pieters M, Fano E, Pijoan C, Dee S. An experimental model to evaluate *Mycoplasma hyopneumoniae* transmission from asymptomatic carriers to unvaccinated and vaccinated sentinel pigs. *Can J Vet Res.* 2010;74(2):157-160.

*20. Painter T, Kuhn M, Wolff T, Pieters M. Efficacy and duration of infection study for Respisure® and Draxxin® against a *Mycoplasma hyopneumoniae* challenge in swine. *Proc Allen D. Leman Swine Conf*. Saint Paul, MN. 2012;39(12):225.

21. Kraeling RR, Webel SK. Current strategies for reproductive management of gilts and sows in North America. *J Anim Sci Biotechnol*. 2015;6:3. doi:10.1186/2049-1891-6-3

22. National Research Council. *Nutrient Requirements of Swine*. 11th ed. Washington, DC: National Academy Press. 2012.

23. Kobisch M, Ross RF. Experimental infections of swine. In: Tully JG, Razin S, eds. *Molecular and Diagnostic Procedures in Mycoplasmology.* Vol II. San Diego, CA: Academic Press. 1996;371-376.

24. Ross RF, Zimmermann-Erickson BJ, Young TF. Characteristics of protective activity of *Mycoplasma hyopneumoniae* vaccine. *Am J Vet Res.* 1984;45(10):1899-1905.

25. Thacker EL, Halbur PG, Ross RF, Thanawongnuwech R, Thacker BJ. *Mycoplasma hyopneumoniae* potentiation of porcine reproductive and respiratory syndrome virus-induced pneumonia. *J Clin Microbiol.*1999;37(3):620-627.

26. Thacker E, Minion FC. Mycoplasmosis. In: Zimmerman JJ, Karriker L, Ramirez A, Schwartz K, Stevenson G, eds. *Diseases of Swine*. 10th ed. Ames, IA: Wiley-Blackwell; 2012:779-797.

27. Pieters M, Daniels J, Rovira A. Comparison of sample types and diagnostic methods for in vivo detection of *Mycoplasma hyopneumoniae* during early stages of infection. *Vet Microbiol*. 2017;203,103-109.

28. Strait EL, Madsen ML, Minion FC, Christopher-Hennings J, Dammen M, Jones KR, Thacker EL. Real-time PCR assays to address genetic diversity among strains of *Mycoplasma hyopneumoniae*. *J Clin Microbiol*. 2008;46(8):2491-2498.

29. Switzer WP. Studies on infectious atrophic rhinitis. IV. Characterization of a pleuropneumonia-like organism isolated from the nasal cavities of swine. *Am J Vet Res*. 1955;16:540-544.

30. Zhao K, Han F, Zou Y, Zhu L, Li C, Xu Y, Zhang C, Tan F, Wang J, Tao S, He X, Zhou Z, Tang X. Rapid detection of porcine circovirus type 2 using a TaqMan-based real-time PCR. *Virol J.* 2010;7:374. doi:10.1186/1743-422X-7-374

31. Marois C, Dory D, Fablet C, Madec F, Kobisch M. Development of a quantitative real-time TaqMan PCR assay for determination of the minimal dose of *Mycoplasma hyopneumoniae* strain 116 required to induce pneumonia in SPF pigs. *J Appl Microbiol.* 2010;108(5):1523-1533.

32. Vicca J, Stakenborg T, Maes D, Butaye P, Peeters J, de Kruif A, Haesebrouck F. Evaluation of virulence of *Mycoplasma hyopneumoniae* field isolates. *Vet Microbiol.* 2003;97:177-190.

33. Roos LR, Fano E, Homwong N, Payne B, Pieters M. A model to investigate the optimal seeder-to-naïve ratio for successful natural *Mycoplasma hyopneumoniae* gilt exposure prior to entering the breeding herd. *Vet Microbiol.* 2016;184:51-58.

34. Friis NF. Some recommendations concerning primary isolation of *Mycoplasma suipneumoniae* and *Mycoplasma flocculare* a survey. *Nord Vet Med.* 1975;27:337-339.

35. Addey JP, Taylor-Robinson D, Dimic M. Viability of mycoplasmas after storage in frozen or lyophilized states. *J Med Microbiol*. 1970;3:137-145.

36. Pieters M, Pijoan C, Fano E, Dee S. An assessment of the duration of *Mycoplasma hyopneumoniae* infection in an experimentally infected population of pigs. *Vet Microbiol.* 2009;134:261-266.

37. Dos Santos LF, Sreevatsan S, Torremorell M, Moreira MA, Sibila M, Pieters M. Genotype distribution of *Mycoplasma hyopneumoniae* in swine herds from different geographical regions. *Vet Microbiol.* 2015;175:374-381.

*38. Betlach A, Fano E, Sponheim A, Dalquist L, Pieters M. Minimal *Mycoplasma hyopneumoniae* genetic variability within production flows. *Proc AASV*. San Diego, CA. 2018; 342-343.

* Non-refereed references.

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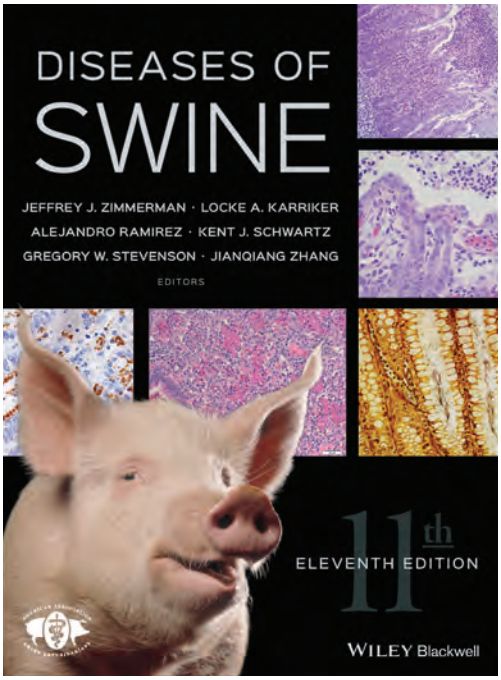

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Checkoff helps create Industry/USDA Feed Risk Task Force

Last spring, the joint industry and US Department of Agriculture (USDA) Feed Risk Task Force was created to look at the risk that imported feed and ingredients pose as a potential source for pathogen introduction. The task force, made up of USDA officials, animal agriculture representatives, and feed industry representatives, will assess the risk and role of feed for the epidemiology of disease transmission and spread, evaluate existing research for feed risks and mitigations, and identify current gaps in knowledge. The first in-person meeting of the group was held on June 11 in Washington, DC.

For more information, contact Dr Lisa Becton at **LBecton@pork.org** or call 515-223-2791.

Checkoff and American Heart Association promote pork

The National Pork Board is working with the American Heart Association to promote the heart health benefits of the pork tenderloin and pork sirloin roast. The American Heart Association has certified the pork sirloin roast and the pork tenderloin as a heart-healthy food. This simple icon delivers results by giving consumers an easy way to cut through the intricate and often conflicting nutrition "noise" and easily identify products as heart-healthy foods.

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

Checkoff to host dietitians on farm tour

In June, the Pork Checkoff showcased all-things pork to 10 highly influential Registered Dietitians (RDs) on a farm tour in Ohio. The 2-day event included touring a sow barn and a nursery/finishing barn. The RDs learned about the latest pork nutrition research and had a hands-on cooking experience. The tour provided a forum to dispel myths about modern pig farming.

Learn more at **library.pork.org** and search for 2017 Pork Promo.

Team pork meets with ARS to build collaboration

An industry group with representatives from the Pork Checkoff, the National Pork Producers Council, and the American Association of Swine Veterinarians recently returned from a meeting with top leaders and scientists at the US Department of Agriculture's Agricultural Research Service in Beltsville, Maryland. Topics covered the research spectrum including animal science, food safety, animal welfare, foreign animal disease, gene editing, public health, and sustainability.

The group will meet regularly to build stronger ties and define mutual research priorities.

For more information, contact Dr Heather Fowler at **HFowler@pork.org** or call 515-223-2633.

Registration Open for Pig Welfare Symposium

The National Pork Board's second biennial Pig Welfare Symposium will take place November 13-15, 2019 in Minneapolis, Minnesota. It will be a forum for sharing ideas, learning from other segments of the industry, and fostering dialog on pig welfare-related issues. Registration is now open at pork.org/pws. For more information, contact Dr Sara Crawford at **SCrawford@pork.org** or 515-223-2790.

NPB continued on page 231

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[2]Trials 6-10 - References available upon request.
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NPB continued from page 229

Blockchain may hold key to We Care metrics

While still murky for many people, the future may hold good things for America's pork producers when it comes to blockchain. That's why Checkoff continues to stay on top of this technology and is collaborating with tech leaders such as Ripe Technologies. Andy Brudtkuhl, Checkoff's director of emerging technology, says blockchain will allow producers to demonstrate how the We Care ethical principles guide pig farming through best practices that benefit people, pigs and the planet.

For more information, contact Andy Brudtkuhl at **ABrudtkuhl@pork.org** or call 515-223-2604.

Biosecurity information targets youth show pig exhibitors

The Checkoff's science and technology, communications, and producer services teams are creating and sharing biosecurity information for youth show pig exhibitors. The cross-departmental team is working with the National Junior Swine Association, Team Purebred, the Agriculture Future of America, and the National FFA. A special page on **pork.org** went live in early June to make getting this critical information easier than ever.

For more information, go to **www.pork.to/showpig**. Or contact Dr Lisa Becton at **LBecton@pork.org** or call 515-223-2791.

Checkoff continues nationwide FAD drills

The Pork Checkoff has been setting up its table top display of a typical small town and rural area for many years to help bring more realism into its ongoing series of foreign animal disease (FAD) drills. Of the roughly two dozen, day-long drills conducted over the last 5 years, most have focused on foot-and-mouth disease. Last August, the emphasis switched to African swine fever.

"We changed gears to meet the changing needs of our industry," said Cindy Cunningham, assistant vice president of communications with the Pork Checkoff. "Most drills have included the state veterinarian, state and federal government officials, packers, state pork associations, and of course, producers."

Cunningham says these drills are designed to help our producers and industry experience what would happen in their particular region of the country, prior to an outbreak. She added, "Now is the time to prepare while we can build a plan, stockpile resources and work to determine the best path forward should we have a confirmation of a foreign animal disease in the United States."

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

AASV NEWS

Call for abstracts – Student Seminar

The American Association of Swine Veterinarians announces an opportunity for veterinary students to deliver a scientific presentation at the AASV Annual Meeting in Atlanta, Georgia, on Sunday, March 8, 2020. Interested students are invited to submit a 1-page abstract of a research paper, clinical case study, or literature review for consideration. The submitting student must be a current (2019-2020) student member of the AASV at the time of submission and must not have graduated from veterinary school prior to March 8, 2020. Submissions are limited to 1 abstract per student.

Abstract submission

Abstracts and supporting information must be submitted online at **aasv2020.exordo.com** (see **www.aasv.org/annmtg/2020/studentseminar** for details). Submissions must be completed before **11:59 PM Central Daylight Time** on **Wednesday, September 18, 2019**. Late submissions will not be considered.

Students will receive an email from Ex Ordo confirming receipt of their submission. If they do not receive this confirmation email, they must contact Dr Andrew Bowman by Friday, September 20, 2019 with supporting evidence that the submission was made in time; otherwise the abstract will not be considered for judging.

The abstracts will be reviewed by an unbiased, professional panel consisting of private practitioners, academicians, and industry veterinarians. Fifteen abstracts will be selected for oral presentation in the Student Seminar at the AASV Annual Meeting. Students will be notified by October 15, 2019, and those selected to participate will be expected to provide the complete paper or abstract, formatted for publication, to AASV by November 15.

Student Seminar and Scholarships

As sponsor of the Student Seminar, **Zoetis** provides a total of $20,000 in support to fund travel stipends and the top student presenter scholarship. The student presenter of each paper selected for oral presentation receives a $750 stipend to help defray the costs of attending the AASV meeting. Veterinary students whose papers are selected for oral presentation also compete for one of several 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 scholarships of $2500 each for 2nd through 5th place, $1500 each for 6th through 10th place, and $500 each for 11th through 15th place.

Student Poster Session

Abstracts that are not selected for oral presentation in the Student Seminar will be considered for presentation in a poster session at the annual meeting. **Zoetis**, sponsor of the Student Poster Session, has joined with AASV to fund a $250 stipend for each student poster presenter who attends the meeting to participate in the session. Those selected for poster presentation will also be expected to supply a formatted paper by November 15 for publication in the conference proceedings.

Veterinary Student Poster Competition

The presenters of the top 15 poster abstracts compete for scholarship 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/2020/studentseminar**. The rules for submission should be followed carefully. For more information, contact the AASV office (Tel: 515-465-5255; Email: **aasv@aasv.org**).

Call for abstracts - Research Topics session

Plans are underway for the 51st annual meeting of the American Association of Swine Veterinarians (AASV), to take place March 7-10, 2020 in Atlanta, Georgia. As part of the meeting, there will be a session highlighting research projects related to swine health and production. Abstracts are now being accepted for potential presentation during the Research Topics session, which will be held Sunday, March 8.

Those interested in making a 15-minute oral presentation should submit a 1-page abstract on applied research related to swine health and production issues (virology, bacteriology, parasitology, environment, food safety, odor, welfare, etc) to **aasv@aasv.org** by **August 15, 2019.** Include the presenting author's name, mailing address, phone number, and email address with each submission.

Abstracts not selected for oral presentation will be considered for poster presentation. All submitting authors will be notified of the selection results in September. Authors of abstracts selected for oral or poster presentation must provide their paper, formatted for publication in the meeting proceedings, by November 15, 2019.

PLEASE NOTE: Participation in the Research Topics oral and poster sessions is at the presenter's expense. The presenting author is required to register for the meeting (nonmember participants may register at the AASV regular member rate). No speaking stipend or travel expense reimbursement is paid by the AASV.

Call for submissions – Industrial Partners

The American Association of Swine Veterinarians invites submissions for the Industrial Partners oral and poster sessions at the 51st AASV Annual Meeting. This is an opportunity for commercial companies to make brief presentations of a technical, educational nature to members of the AASV. The conference will be held March 7-10, 2020 in Atlanta, Georgia.

The oral sessions consist of a series of 15-minute presentations scheduled from 1:00 to 5:00 PM on Sunday afternoon, March 8th. A poster session takes place the same day. Poster authors will be required to be stationed with their poster from noon until 1:00 PM, and the posters will remain on display throughout the afternoon and the following day for viewing.

SUBMISSION PREREQUISITE: All companies submitting topics for presentation during the Industrial Partners sessions must register to participate in the AASV Technical Tables Exhibit before October 1st.

Restricted program space necessitates a limit on the number of presentations per company. Companies that are a member of the *Journal of Swine Health and Production* (JSHAP) Industry Support Council and sponsor the AASV e-Letter may submit up to 3 topics for oral presentation. Companies that are either a member of the JSHAP Industry Support Council or sponsor the AASV e-Letter may submit up to 2 topics. All other companies may submit 1 topic for oral presentation. In addition, every company may submit 1 topic for poster presentation, but the topic must not duplicate the oral presentation. All topics must represent information not previously presented at the AASV annual meeting or published in the meeting proceedings.

To participate, send the following information to aasv@aasv.org by October 1, 2019:

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

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

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

FOUNDATION NEWS

Tee up for the foundation

It's time to recruit your golf team to support the AASV Foundation! Registration is now open for the annual AASV Foundation Golf Outing, slated for **Thursday, August 22** at Veenker Memorial Golf Course in Ames, Iowa. Once again, Dr Josh Ellingson is coordinating the event for the foundation.

Members of AASV, industry stakeholders, and their clients, family, and staff are invited to register a 4-person team for this fun, 18-hole best-ball tournament. Individual golfers and couples are also welcome and will be assigned to a team.

Golfer check-in begins at 11:00 AM on the 22nd, with practice balls available for warming up on the driving range before the contest begins. A shotgun start at noon kicks off the 4-person team, best-ball competition. Golfers compete as a foursome against the challenges of the course (and the other teams) in addition to participating in individual contests along the way.

Boxed lunches, sponsored by **APC**, and beverages, courtesy of **Zoetis**, will be supplied on-course. Sponsored contests, games, and giveaways will add to the fun. When the golfing is completed, team and individual contest winners will be recognized and receive prizes during the pork dinner sponsored by **Boehringer Ingelheim Animal Health**.

The registration fee includes 18 holes of best-ball golf, cart, lunch, beverages, awards dinner, and prizes. Funds raised by the event support AASV Foundation programs, including research grants, travel stipends for students attending the AASV annual meeting, swine externship grants, scholarships for veterinarians pursuing board certification in the American College of Animal Welfare, tuition grants for the Swine Medicine Education Center, and more.

For a sneak peek at the golf course, visit **www.veenkergolf.com**. For more information or to register, contact AASV by phone, 515-465-5255, or email, **aasv@aasv.org**.

Scholarship available to members seeking welfare certification

Two AASV members have already benefited from scholarships supporting their efforts to achieve board certification in the American College of Animal Welfare (ACAW). Will you be the next?

One of the ACAW scholarship recipients, Dr Monique Pairis-Garcia, achieved board certification in July 2018. The other recipient, Dr Madonna Benjamin, has completed her program of study, received approval of her credentials, and will be sitting the board examination this summer.

The AASV Foundation Board of Directors continues to accept applications from AASV members seeking ACAW board certification. Applicants must have a DVM or VMD degree and at least 5 years of continuous membership in the AASV.

To apply, the applicant must submit a curriculum vitae, an ACAW-approved program plan, and 3 letters of reference (one of which must come from the applicant's mentor). There is no submission due date, but there is a limit to the amount of funding available each year. A selection committee reviews applications as they are received.

The scholarship will provide annual reimbursements for actual expenses related to the ACAW program, including travel, course fees, and textbooks, with a maximum reimbursement amount of $20,000. Reimbursement will not cover lost income. An incentive payment of $10,000 will be issued upon successful and timely completion of the ACAW Board Certification.

For more information, contact the AASV office by phone, 515-465-5255, or email, **aasv@aasv.org**.

AASV Foundation Golf Outing

REGISTRATION FORM

Please complete, detach, and return this form with payment to the AASV Foundation by August 5, 2019

☐ **Single registration $125.00**
(per person - includes 18 holes of golf, golf-cart rental, refreshments, box lunch, and closing dinner)

☐ **Team registration $500.00**
(group of four - list names below)

1. _____
2. _____
3. _____
4. _____

☐ **I cannot attend, but will contribute to the AASV Foundation.**

My tax-deductible donation is enclosed: $_____

Name _____

Address_____

Tel _____

Fax _____

Make your check payable to the AASV Foundation
Mail to AASV Foundation,

830 26th Street, Perry, IA 50220-2328

Thursday,
August 22, 2019
11:00 AM – 6:00 PM

VEENKER MEMORIAL GOLF COURSE
2916 Veenker Drive, Ames, Iowa • veenkergolf.com

aasv.org/foundation

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Swine veterinarians and US prioritized zoonoses

As part of a strategic, targeted approach to control and prevent zoonotic diseases, the One Health Office at the Centers for Disease Control and Prevention (CDC) has been hosting One Health Zoonotic Disease Prioritization Workshops in several countries since 2014. A primary outcome of these workshops is to help countries focus limited resources on those zoonoses of greatest concern. Globally, zoonoses commonly prioritized include zoonotic influenza viruses, anthrax, rabies, Rift Valley fever, and viral hemorrhagic fevers, including Ebola.

A critical and first step in these workshops, as well as any One Health activity, is to identify key stakeholders to address the issue from a multisectoral approach, involving human, animal, and environmental health professionals. During December 2017, several departments within the Department of Health and Human Services, the United States Department of Agriculture (USDA), the United States Department of the Interior, the Environmental Protection Agency, the National Oceanic and Atmospheric Association, and state animal and human health agencies collaborated during a workshop focused on identifying the top zoonoses threatening public health in the United States.

Working together to prioritize zoonoses of national concern for the first time, multiple US government agency participants used a One Health approach and the One Health Zoonotic Disease Prioritization tool developed by CDC to identify 8 zoonoses that should be jointly addressed by human, animal, and environmental health sectors.

The workshop report, published earlier this year, describes the process of prioritization, the most concerning zoonoses, and discussions and recommendations on how to develop a coordinated US-specific One Health approach to prevent, detect, and respond to those zoonoses.

Prioritized zoonotic diseases for the United States

1. Zoonotic influenza (zoonotic influenza A viruses)
2. Salmonellosis (*Salmonella* species)
3. West Nile virus (Flaviviridae, *Flavivirus*)
4. Plague (*Yersinia pestis*)
5. Emerging coronaviruses (Coronaviridae; ie, severe acute respiratory syndrome and Middle East respiratory syndrome)
6. Rabies (Rhabdoviridae, *Lyssavirus*)
7. Brucellosis (*Brucella* species)
8. Lyme disease (*Borrelia burgdorferi*)

The disease ranked as the highest priority by all workshop participants was zoonotic influenza A. Swine veterinarians play a significant role in preventing zoonotic influenza.

Influenza A viruses are endemic in the US swine herd. Although rare, some strains of influenza A viruses of avian and swine origin are zoonotic and might infect people. Influenza A viruses of swine (IAV-S) origin are called variant viruses when they infect people and the letter "v" is attached to the influenza strain name (eg, H3N2v).

Of the 462 novel influenza A infections detected in the United States since 2011, 427 were H3N2v. Others of swine origin included H1N1v (9) and H1N2v (25). During late 2016, a veterinarian was infected with avian lineage influenza (H7N2) after prolonged close contact with respiratory secretions of infected cats in a New York City shelter. These novel influenza A cases were all sporadic infections with limited human to human transmission. The people with the highest risk of being infected by a zoonotic influenza virus are those in close contact with infected swine or poultry.

One Health recognizes that the health of people, animals, and the environment are all connected.

The CDC recommends that every person aged 6 months or older receive an influenza vaccine each year. The CDC also recommends that people who work with swine should be trained to recognize the signs of influenza in pigs. If pigs exhibit signs consistent with influenza, even mildly, appropriate veterinary care should be provided, and preventive measures should be implemented by people working with or in close contact with ill pigs. The USDA's Veterinary Services' national IAV-S surveillance program monitors isolates from pigs exhibiting influenza-like illness for any genetic changes.

Opportunities for swine veterinarians

Participants from all agencies identified the following key themes and next steps for collaboration:

- Increase and leverage leadership engagement
- Create a formalized One Health coordination mechanism at the federal level
- Develop a national One Health framework
- Improve knowledge and data sharing for laboratory, surveillance, and response activities
- Improve coordination during an outbreak response

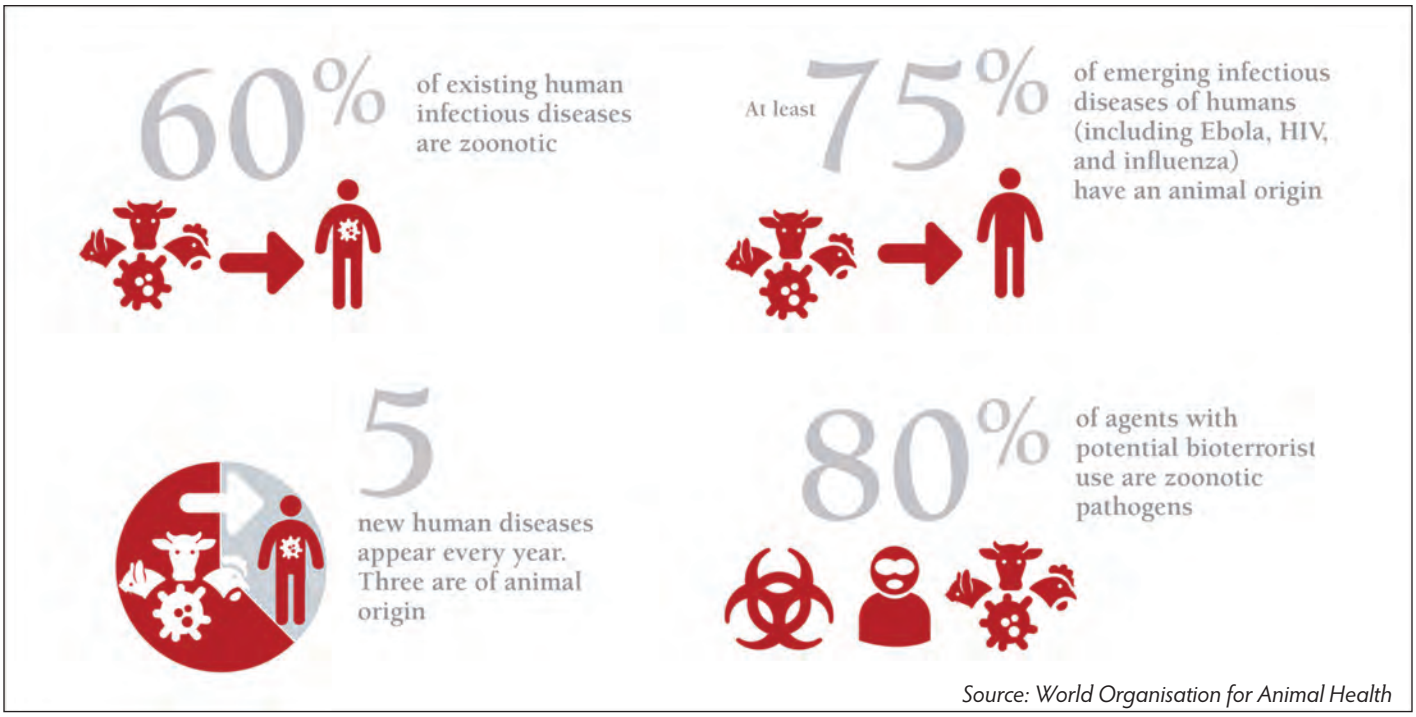
Advocacy continued on page 239

Pigs of #instaham

Share your best pig photos for JSHAP publication.

The *Journal of Swine Health and Production* would like to publish digital photographs submitted by our readers. Images used either on the front cover or in the photo corner on the back page are to represent healthy pigs and modern production facilities. Please ensure that the photos do not include people. Select the largest image size available on your camera (not cell phone) of the quality or compression that allows you to store the fewest images on a given memory card. Do not resize, crop, rotate, or color-correct the image prior to submission to the journal. Please send the images by e-mail attachment to **tina@aasv.org**. Also include your name, affiliation, and the approximate location of the image, or other details that you would like to submit which describe the image.

Advocacy continued from page 237



Source: World Organisation for Animal Health

- Strengthen joint investment for One Health and prioritized zoonoses
- Provide education and awareness
- Discuss One Health research gaps and needs

While we can certainly play a role in each of these next steps (eg, increased laboratory data sharing for surveillance), two of these identified opportunities most directly involve swine veterinarians.

Improved coordination during an outbreak response. The driver of a One Health approach is multisectoral collaboration. Successful One Health responses include veterinarians, physicians, nurses, epidemiologists, diagnosticians, social and behavioral scientists, toxicologists, economists, entomologists, and many others. Even outbreaks of animal diseases that are not zoonotic (eg, African swine fever) require a One Health approach because of the intricate relationship with humans (eg, producer and responder mental health) and the environment (eg, wildlife and disposal).

Standardized multiagency outbreak response plans will improve collaborative response, while combined cross-disciplinary training (eg, tabletop exercises and field epidemiology courses) for all sectors will strengthen responder preparedness. To ensure the best outcome for people, pigs, and the environment, it is essential to have those who understand animal agriculture involved in a One Health response.

Education and awareness. Continuing education in One Health will bolster workforce development, ensuring the right people are participating in zoonotic disease prevention, detection, control, and response.

The American Association of Swine Veterinarians (AASV), Swine Health Information Center, National Pork Board, and National Pork Producers Council have a strong history of coordinated messaging to various audiences, including the general public. We routinely work together to disseminate accurate and timely information. Opportunities exist to more closely collaborate with local, state, and federal colleagues in public health to provide coordinated messaging before, during, and after zoonotic disease outbreaks or other One Health events or issues. The AASV will continue to build and strengthen these relationships.

The swine veterinarian's role

These zoonoses were prioritized because of their pandemic/epidemic potential; the severity of disease in humans, domestic animals, and wildlife; the economic impact to the United States; the potential for introduction or increased transmission in the United States; and the bioterrorism threat to national security. Swine veterinarians in practice, industry, academia, and government can prepare producers, strengthen relationships, and coordinate messaging to prevent, detect, and control zoonotic influenza.

Abbey Canon, DVM, MPH, DACVPM
Director of Communications

Resources

`www.cdc.gov/onehealth/pdfs/us-ohzdp-report-508.pdf`.

`www.cdc.gov/onehealth/domestic-activities/us-ohzdp.html`.

Updated May 2019

Journal of Swine Health and Production Author Guidelines

Journal description

The *Journal of Swine Health and Production* (JSHAP) is published bi-monthly by the American Association of Swine Veterinarians (AASV) and is freely available online. The journal accepts manuscripts for peer review that encompass the many domains of applied swine health and production, ie, the diagnosis, treatment, management, prevention and eradication of swine diseases, swine welfare and behavior, nutrition, public health, epidemiology, food safety, biosecurity, pharmaceuticals, antimicrobial use and resistance, reproduction, growth, systems flow, economics, and facility design.

Types of papers

The *Journal of Swine Health and Production* currently accepts manuscripts that meet the descriptions and formatting requirements defined in Table 1.

Policies and procedures

Animal care and welfare

For animal experiments performed in research facilities or on commercial farms, include a statement indicating that the studies were reviewed and approved by an institutional animal care and use committee or equivalent. For case reports and studies performed under field conditions, in which animals are not manipulated beyond what would be required for diagnostic purposes, it must be clear that housing was adequate and that the animals were humanely cared for. If the study is exempt from animal care and use approval (eg, use of diagnostic records), authors need to clearly state the reasons in the manuscript. Place welfare statements in a paragraph immediately after the "Materials and methods" heading or equivalent position depending on genre.

Authorship

According to the International Committee of Medical Journal Editors, all listed authors must have participated sufficiently to take public responsibility for the work. Individuals should only be listed as authors if contributions have been made in each of the following areas[1]:

1) Conception and design, acquisition of data, or analysis and interpretation of the data,
2) Drafting the manuscript or revising it critically for important intellectual content,
3) Approval of the version of the manuscript to be published, and
4) Agreement to be accountable for all aspects for the work, ensuring questions related to accuracy and integrity are investigated and resolved.

Ethics

Authors are expected to observe high standards with respect to research and publication ethics. Fabrication, falsification, or plagiarism in proposing, performing, or reviewing research, or in reporting research results is considered research misconduct.[2] All cases of research misconduct will be investigated and addressed accordingly.

Conflict of interest

Authors are required to declare the presence of any personal, professional, or financial relationships that could potentially be construed as a conflict of interest for the submitted manuscript, regardless of genre. This declaration is placed just before the reference section, and provides information concerning authors who profit in some way from publication of the paper. For example, one or more of the authors may be employed by a pharmaceutical company that manufactures a drug or vaccine tested in the study reported. Other examples include consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. If there is no conflict of interest to declare, the statement under the "Conflict of interest" heading is "None reported."

Copyright transfer

When a manuscript is submitted to the JSHAP, a pre-review copyright agreement and disclosure statement must be signed by all authors. It is the responsibility of the corresponding author to secure these signatures. This form is available from the publications manager. Scan and email signed copies to Karen Richardson at `jshap@aasv.org`. When the manuscript is accepted for publication, the corresponding author will be required to transfer copyright to the AASV, with the exceptions of US government employees whose work is in the public domain and portions of manuscripts used by permission of another copyright holder. Anyone acknowledged by name in the manuscript will need to sign an acknowledgment permission form.

Prior publication

We do not republish materials previously published in refereed journals. Sections of theses and extension publications that may be of value to our readership will be considered. Prior publication of an abstract only (eg, in a proceedings book) is generally acceptable.

Permissions

If copyrighted material is used, advise the editors of this at the time of manuscript submission. Authors are responsible for securing permission to use copyrighted art or text, including the payment of fees.

Publication fees

There is no fee for publication of manuscripts in the JSHAP.

Manuscript preparation

File types

All manuscripts must be submitted as a Microsoft Word document using 1-inch margins, Times New Roman 12-point font (unless otherwise specified), and left justification with double-spacing throughout. Include continuous page and line numbers. Do not use numbered or bulleted lists in the summary or the text. Do not include tables or figures in this file, but do include table and figure references, such as (Table 1) or (Figure 1), within the text. Software programs that automatically create endnotes, footnotes, and references should be avoided in the final submitted version of the manuscript as the embedded formatting cannot be read by the publication software.

Table 1: Manuscript genres and formatting requirements currently accepted by the *Journal of Swine Health and Production*.

Genre	Description	Maximum words		Maximum No.		Other requirements*
		Abstract	Manuscript	Figures and Tables	References	
Original Research	Reports the results of original research on topics that are within journal scope.	250	4000	As needed	35	–
Brief Communication	Documents observations made in a narrowly defined research area or a mini-review of a subject area.	50	2000	2	15	–
Case Report	Describes an unusual or interesting case.	100	3000	As needed	As needed	Manuscript should not exceed 20 pages including figures, tables, and references.
Case Study	Describes unusual or interesting cases occurring on two or more farms.	100	3000	As needed	As needed	Manuscript should not exceed 20 pages including figures, tables, and references.
Literature Review	Review of the published scientific literature about a specific topic area in which important advances have been made in the past five years and is of current interest.	200	5000	As needed	As needed but most references should be recent (within 5 yrs) and avoid use of non-refereed references and personal communications.	Manuscript should not exceed 30 pages including figures, tables, and references.
Production Tool	Describes a practical, state-of-the-art technique for improving an individual swine enterprise or the swine industry at large.	100	3000	As needed	As needed	Manuscript should not exceed 20 pages including figures, tables, and references.
Diagnostic Note	Describes methods of diagnosis for swine diseases. A brief literature review may be included and use of non-refereed references and personal communications is not restricted.	100	3000	As needed	As needed	Manuscript should not exceed 20 pages including figures, tables, and references.
Practice Tip	Describes new technological methods likely to be of use to swine practitioners.	100	3000	As needed	As needed	Manuscript should not exceed 20 pages including figures, tables, and references.
Peer-Reviewed Commentary	Commentary on diagnostic, research, or production techniques used in the field of swine health and production.	100	3000	As needed	As needed	Manuscript should not exceed 20 pages including figures, tables, and references.

Table 1: Continued

Genre	Description	Maximum words		Maximum No.		Other requirements*
		Abstract	Manuscript	Figures and Tables	References	
Letter to the Editor (LTE)	Offers comment or useful critique on materials published in the journal.	-	500	0	5	The decision to publish an LTE rests solely with the executive editor. Letters referring to a published article will be forwarded to the author of the article, and both the original letter and the response will be published in the same issue if possible. Letters to the Editor are not peer-reviewed but are subject to editorial changes.

* Page limits are for Microsoft Word documents using 1-inch margins, Times New Roman 12-point font (unless otherwise specified), and left justification with double-spacing throughout.

If the manuscript includes tables, create and submit them in a second Microsoft Word document titled "Art". Multiple tables can be submitted in a single Word document.

If the manuscript includes figures (graphs or images), submit each figure in a separate file titled as the respective figure number. Graphs created in Microsoft Excel should be submitted in the original .xls file(s). A graph created in statistics software can be submitted as a .pdf file. Photographs and images need to be high resolution .jpg files. Figure caption and legend texts should be submitted in a Microsoft Word file titled "Art" (included with Tables if applicable).

Supplementary materials are accepted for online only publication and should be formatted according to these guidelines.

Sample templates have been created for each genre to assist authors in formatting their manuscript and can be accessed at **www.aasv.org/shap/guidelines**.

General style

Manuscripts must be written in English and use American spelling and usage. The JSHAP uses the AMA Manual of Style for guidance on general style and form.[3] Please review the complete author guidelines and author checklist at **www.aasv.org/shap/guidelines** for full details on journal formatting requirements for submitted manuscripts.

Manuscript submission

Submission instructions

All submissions must be accompanied by a cover letter. The cover letter should be on official letterhead, not exceed 1 page, and include the following information:

- a statement acknowledging the manuscript is not currently under consideration for publication elsewhere,
- a statement that all co-authors have reviewed and approve the manuscript submission,
- the intended genre of the submitted manuscript,
- a brief description of how the manuscript relates to the scope of JSHAP (optional),
- suggestions for potential reviewers of the submitted manuscript (optional), and
- signature of the corresponding author.

All manuscript files should be submitted to the JSHAP publications manager via email: **jshap@aasv.org**.

Unless given alternate instructions at the time of submission, we will correspond with the corresponding author.

Questions about manuscript submission or status can be directed to the JSHAP publications manager:

Karen Richardson
Journal of Swine Health and Production
c/o American Association of Swine Veterinarians
830 26th Street
Perry, IA 50220
Tel: 519-856-2089
Email: **jshap@aasv.org**

References

1. International Committee of Medical Journal Editors. Recommendations for the conduct, reporting, editing, and publication of scholarly work in medical journals. **http://www.icmje.org/icmje-recommendations.pdf**. Updated December 2017. Accessed June 20, 2018.

2. Office of Science and Technology Policy. Federal policy on research misconduct. *Fed Regist*. 2000;65(6):76260-76264.

3. Iverson C, Christiansen S, Flanagin A, Fontanarosa PB, Glass RM, Gregoline B, Lurie SJ, Meyer HS, Winker MA, Young RK, eds. AMA Manual of Style: A Guide for Authors and Editors. 10th ed. New York, New York: Oxford University Press. 2007.

Upcoming meetings

LIII National Congress AMVEC 2019

July 23-26, 2019 (Tue-Fri)
Guadalajara, Jalisco, Mexico
Hosted by the Asociación Mexicana de Veterinarios Especialistas en Cerdos A.C.

For more information:
Tel: +52 378 705 0345
Email: administracion@amvec.com
Web: www.amvec.com/blog/amvec-1/post/expo-industrial-amed-2019-1

IXth International Conference on Boar Semen Preservation

August 11-14, 2019 (Sun-Wed)
Hunter Valley, NSW, Australia

For more information:
ASN Events Pty Ltd
Head Office: 9/397 Smith Street
Fitzroy VIC 3065
Australia
Tel: +61 3 8658 9530
Fax: +61 3 8658 9531
Email: rh@asnevents.net.au
Web: www.boarsemen2019.com

Asian Pig Veterinary Society Congress 2019

August 26-28, 2019 (Mon-Wed)
BEXCO, Busan 55, APEC-ro
Haeundae-gu, Busan
Republic of Korea
Tel: +82 51-740-7300

For more information:
Amy Chang (Secretariat of APVS 2019):
802, InnoN, 66, Seongsui-ro
Seongdong-gu, Seoul
Republic of Korea
Tel: +82 2-2190-7331
Email: moon@innon.co.kr

Sue Jo (Secretariat of APVS 2019):
Tel: +82 2-2190-7327
Email: sue@innon.co.kr
Web: www.apvs2019.com

Allen D. Leman Swine Conference

September 14-17, 2019 (Sat-Tue)
Saint Paul RiverCentre
Saint Paul, Minnesota
Hosted by the University of Minnesota

For more information:
Tel: 612-624-4754
Email: vetmedccaps@umn.edu
Web: ccaps.umn.edu/allen-d-leman-swine-conference

2019 ISU James D. McKean Swine Disease Conference

November 7-8, 2019 (Thu-Fri)
Scheman Building
Iowa State University
Ames, Iowa

For registration information:
Registration Services
Iowa State University
1601 Golden Aspen Drive #110
Ames, Iowa 50010
Tel.: 515-294-6222
Fax: 515-294-6223
Email: registrations@iastate.edu

For questions about program content:
Dr Chris Rademacher
Conference Chair
Iowa State University
Email: cjrdvm@iastate.edu

Pig Welfare Symposium

November 13-15, 2019 (Wed-Fri)
Minneapolis Marriott City Center
Minneapolis, Minnesota
Hosted by the National Pork Board

For more information:
Web: www.pork.org/pws

American Association of Swine Veterinarians 51st Annual Meeting

March 7-10, 2020 (Sat-Tue)
Hyatt Regency Atlanta
Atlanta, Georgia

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

26th International Pig Veterinary Society Congress

June 2-5, 2020 (Tue-Fri)
Florianopolis, Brazil

For more information:
Tel: +55 31 3360 3663
Email: ipvs2020@ipvs2020.com
Web: ipvs2020.com

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

AASV Industry Support Council

The JSHAP is made possible by the generous support of these Industry Support Council members.

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Newport Laboratories	Norbrook®	PHARMACOSMOS Committed to Quality
Pharmgate ANIMAL HEALTH	Phibro ANIMAL HEALTH CORPORATION™	provimi®
Tonisity	VIROX™ ANIMAL HEALTH	zoetis™

Photo Corner

Nursery pigs at University of Missouri Swine Teaching Center

Photo courtesy of Barbara Molnar-Smith