Journal of
SWINE HEALTH & PRODUCTION
January and February 2021 • Volume 29, Number 1

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The Journal of the American Association of Swine Veterinarians

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Journal of Swine Health and Production is indexed in ISI®Focus On: Veterinary Science & Medicine,® and in CAB Abstracts, Euroscience VETLINE on CD-ROM

JOURNAL OF SWINE HEALTH AND PRODUCTION

(ISSN 1537-209X) Volume 29, Number 1; January and February

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JSHAP SPOTLIGHT

Dr Mike Tokach

Kansas State University

Dr Tokach earned his BS from North Dakota State University (86), MS from Kansas State University (88), and PhD from University of Minnesota (91). Currently, Mike is a swine nutritionist and extension specialist at Kansas State University and works to train the next generation of applied swine nutritionists. Mike encourages everyone with unique case studies and prospective research to publish and share their experiences with others. "The peer review process can be daunting, but you always learn from feedback from others. You learn how to improve your investigation skills and how others may interpret your data."

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[1]USDA APHIS. October 2007. "Swine 2006 Part I: Reference of Swine Health and Management in the United States, 2006." National Animal Health Monitoring System. Available at: http://www.aphis.usda.gov/animal_health/nahms/swine/downloads/swine2006/Swine2006_dr_PartI.pdf. Accessed on March 11, 2013.
[2]Merck & Co. 2008. "Respiratory Disease of Pigs: Introduction." The Merck Veterinary Manual. Available at: http://www.merckvetmanual.com/mvm/htm/bc/121400/htm. Accessed on March 31, 2009.

Opportunities in a new year

As we approach the end of this year of perfect vision, I begin contemplating the past and the future. The upcoming holidays will be as different as any time in my memory. Due to a COVID-19 exposure and not wanting to leave my 16-year-old son home alone on Thanksgiving, we will have 7 at our Thanksgiving table. I can remember Thanksgiving dinners with the Harker family with as many as 75 at my parents' home (thankful for the heated 3 car garage!). Hopefully, you are also planning modified gatherings to slow the spread of this virus.

As I finish my 10th year of serving on the AASV Board of Directors, I am very thankful for the opportunities AASV has given me to be a leader in this organization. I want to take this occasion to encourage all members to consider serving as an AASV volunteer.

Many years ago, one of our colleagues just a few years older than me told me "don't expect immediate payback from volunteering." At the time I was on the board of the Indiana Pork Producers Association. A couple of years later one of my fellow board members called me to schedule a second opinion herd visit. Since then, many other contacts from those years of service have given me opportunities that I would not have had otherwise. Similarly, serving on the AASV Board of Directors and the AVMA House of Delegates has exposed me to veterinary experts that I now call friends. These relationships help build the network for all of us in the veterinary community. Also, do not forget to volunteer to promote the pork industry through Operation Main Street as I described in my previous message.[1]

There are many places to volunteer within AASV beginning with committees. Anyone is welcome to volunteer to serve on any of the many AASV committees. Simply communicate your desire to serve to Dr Abbey Canon, or any other staff or board member, and you will be pointed in the right direction. Other opportunities are AASV positions within the AVMA including as a delegate to the house of delegates or a member to AVMA committees or task forces. Finally, volunteer to be nominated to the AASV board of directors as a district director or officer. It is wonderful that we have 2 excellent candidates nominated nearly every year for AASV Vice President. Do not succumb to the notion that you have nothing to offer. Everyone has a unique perspective and experience that benefit the association by sharing our diverse points of view.

In addition to volunteering, attend the AASV Annual Meeting every year. I have attended every year since 1992. This habit was instilled in me by the commitment of Dr Rodibaugh for both of us to always go no matter what our practice circumstances were. The value of the educational sessions and social interactions are immeasurable. That is why it is so disappointing that the upcoming annual meeting will be virtual. I hope you all will join me in the commitment to spend the hours each day online to learn and interact as much as possible. We did our family Easter celebration via Zoom and it was very enjoyable, I am hopeful that we can all enjoy something similar with the 2021 annual meeting.

"Many years ago, one of our colleagues just a few years older than me told me don't expect immediate payback from volunteering."

By the time you read this, 2020 will thankfully be over. We can only hope that 2021 can be a year of healing for our association, our industry, and all individuals affected by this pandemic. I cannot wait to see everyone's smiling faces during the virtual AASV Annual Meeting, so please get your webcams up and running beforehand so we can have some semblance of social interactions. See you in March!

Jeff Harker, DVM
AASV President

Reference

*1. Harker J. Dispelling myths and sharing your passion [Editorial]. *J Swine Health Prod*. 2020;28(6):289.

* Non-refereed reference.

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The virtual experience

As I am sure you will recall, my last message described the uncertainty surrounding the format of the 2021 AASV Annual Meeting. Well, it is now late November (actually the night before Thanksgiving or, as the journal staff so politely calls it, "way past the deadline!"), and the decision to go virtual was confirmed by the AASV Board of Directors during their fall meeting on October 2nd. Since that time, the AASV staff has been hard at work exploring the numerous options for virtual providers.

Virtual meeting facilitation seems to be a rapidly expanding specialty with lots of innovation designed to provide participants with an effective platform to share information and interact with fellow attendees. One thing is for sure, they speak a different language than I do. Who would have thought that the word "session" could mean so many different things? I have to hand it to Sue, Abbey, and Sherrie, they have hung in through what seems like countless demonstrations of the various chat functions, poster session formats, exhibitor opportunities, and the challenges of live versus semi-live presentations. After wading through all that, I am confident that by the time you read this we will have selected a provider and be well on our way to finalizing registrations and organizing speaker videos.

It became evident as we progressed through the stages of understanding virtual meetings that they are different than the in-person experience we all value so much. We came to realize that the more we tried to reproduce the in-person feel, the more contrived it seemed to be. The ability to meet in the hallway and greet someone you have not seen in a year with a fist bump, elbow bump, toe tap, or knowing nod of the head all the while wishing the name tag print was larger so you could remember who this person is cannot be replicated to the same effect virtually. Thus, it is good to go into a virtual meeting with realistic expectations.

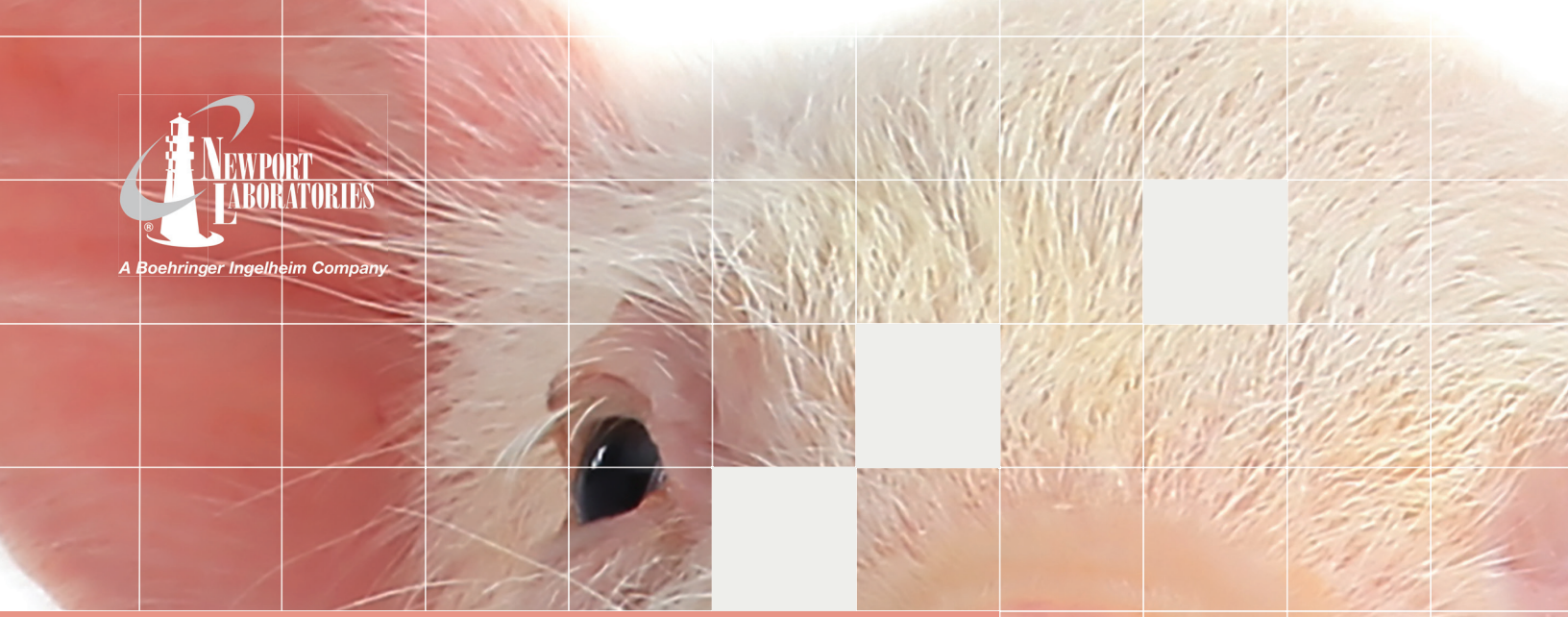
While a virtual meeting will not replace the traditional face-to-face gathering, it can be a very worthwhile platform for the exchange of information and even a little fun in its own right. For example, I have attended several virtual events since the onset of COVID-19 and have noticed that there seems to be much greater attendee question and answer interaction with presenters via the chat function than we normally see during a traditional meeting. In addition, the electronic medium enhances the ability to share information in multiple communication types including proceedings, chat exchanges, slide shows, and video presentations. It also provides the ability to view sessions on demand promoting the opportunity to take in many more presentations than you could during a traditional meeting.

"While a virtual meeting will not replace the traditional face-to-face gathering, it can be a very worthwhile platform for the exchange of information and even a little fun in its own right."

Over the next few months, the AASV staff will be working diligently to ensure the platform provides the needed functionality and ease of use that will make our meeting a technological and organizational success. Likewise, the leadership and session moderators will be focused on providing the educational experience you expect from the Annual Meeting. The ultimate key to a meeting's success, however, does not differ between a virtual and in-person format. That key element is you and your participation, attitude, and willingness to work with the format or the venue to get the most out of the presentations and glean the take home messages that can enhance your professional and personal lives. Ok, so the food can also influence the success of the meeting, but in 2021 you are responsible for the food so there should be no complaints there.

This format will be new to all of us, so it is even more important this year that you watch the e-Letter and email announcements for instructions regarding how to access the virtual meeting. Thanks to the AASV staff, leadership, and planning committee for working so hard to make the 52nd AASV Annual Meeting the best virtual meeting we've ever held! Come join us and do your part.

Harry Snelson, DVM
Executive Director



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Introducing JSHAP Spotlight

Welcome 2021 and so long 2020. Here we are entering a new year of publications, knowledge, information, and likely more change. Despite the pandemic putting a curve ball into the timelines, goals, and plans of many, the time sure seems to have gone by quickly since my last new-year message. This year will continue to bring many new things our way. The continuation of many meetings being offered virtually, including this year's AASV Annual Meeting, has minimized our face-to-face networking opportunities and the ability to really connect with one another. With minimal opportunities for face-to-face meetings forecasted for the time being, the journal staff wanted to bring something fresh to the journal, as well as recognize the contributions of many individuals. We wanted to bring the people to you!

I would like to introduce the JSHAP Spotlight section. You can find JSHAP Spotlight directly below the table of contents and only one page-turn from the front cover. Every issue will feature a member of the JSHAP Editorial Board. Have a look and read about the contributions of your colleagues.

You may also note a few changes with the font and layout of the journal. The journal staff has been working hard to streamline the font type and sizes to optimize both online screen reading as well as print reading. These changes will also help journal staff to index the manuscripts for search engines. Some of the changes are subtle, but I can tell you the behind the scenes work that has gone into this has been substantial. I have learned more than I thought possible about font types, sizes, bullets, hanging indents, etc. The information and learning just keeps on going.

"With minimal opportunities for face-to-face meetings forecasted for the time being, the journal staff wanted to bring something fresh to the journal, as well as recognize the contributions of many individuals. We wanted to bring the people to you!"

I hope you enjoy this issue and that the information it brings keeps you inspired.

Terri O'Sullivan, DVM, PhD
Executive Editor

Increasing creep pellet size improves creep feed disappearance of gilt and sow progeny in lactation and enhances pig production after weaning

Jessica R. Craig, PhD; Jae C. Kim, PhD; Chris J. Brewster, MAgSci; Robert J. Smits, PhD; Casie Braden, BAnimSci; John R. Pluske, PhD

Summary

Objective: To determine if feeding a larger diameter pellet increases creep feed intake and growth rate of piglets during lactation, especially that of gilt progeny (GP) compared to sow progeny (SP), and stimulates feed intake after weaning.

Materials and methods: Over two replicates, GP and SP (n = 2070) were allocated to two creep feed treatments, receiving either a 4 mm diameter × 4 mm length pellet or a 9 mm diameter × 12 mm length pellet, from 3 days of age until weaning. After weaning, pigs were split into male and female pens according to the type of pellet fed in lactation and fed a common diet. Feed disappearance was recorded before and after weaning (up until 10 weeks of age), along with piglet growth performance and all piglet mortalities and removals.

Results: Total creep feed disappearance in lactation was higher ($P < .001$) in litters offered the larger pellet, but litter weaning weight for GP was not improved (interaction, $P > .05$). Gilt progeny were weaned lighter ($P < .001$) than SP. After weaning, pigs offered the larger pellet during lactation showed a tendency to receive less medication ($P = .07$) than pigs offered the smaller pellet. Growth rate and feed intake after weaning were both stimulated ($P = .02$ and $P = .09$, respectively) in pigs offered the larger pellet during lactation irrespective of sex.

Implications: Offering a larger pellet creep feed to piglets in lactation can improve postweaning performance and reduce the postweaning medication rate.

Keywords: swine, gilt progeny, creep feed, weaning, pellet diameter.

Received: May 20, 2020
Accepted: July 20, 2020

Resumen - Aumentar el tamaño de pellet del alimento de la camada mejora la desaparición del alimento de las camadas de primerizas y de la progenie de las multíparas en la lactancia y mejora la producción porcina después del destete

Objetivo: Determinar si la alimentación con un pellet de mayor diámetro aumenta la ingesta de alimento y la tasa de crecimiento de los lechones durante la lactancia, especialmente la progenie de primerizas (GP), en comparación con la de multíparas (SP), y si estimula la ingesta de alimento después del destete.

Materiales y métodos: Durante dos repeticiones, a partir de los 3 días de edad y hasta el destete, la GP y SP (n = 2070) se asignaron a dos tratamientos de alimento de camada, un pellet de 4 mm de diámetro × 4 mm de largo o un pellet de 9 mm de diámetro × 12 mm de largo. Después del destete, los lechones se dividieron en corrales de machos y hembras según el tipo de pellet con los que se alimentaron durante la lactancia, y se les ofreció una dieta común. La desaparición del alimento se registró antes y después del destete (hasta las 10 semanas de edad), junto con el crecimiento, la mortalidad y el desecho de lechones.

Resultados: La desaparición total del alimento en la lactancia fue mayor ($P < .001$) en las camadas a las que se les ofreció el pellet más grande, pero el peso de la camada al destete para la GP no mejoró (interacción, $P > .05$). La progenie de las primerizas se destetó más ligera ($P < .001$) que la de SP. Después del destete, los cerdos a los que se les ofreció el pellet más grande durante la lactancia mostraron una tendencia a recibir menos medicación ($P = .07$) que los cerdos a los que se les ofreció el pellet más pequeño. Independientemente del sexo, tanto la tasa de crecimiento, como el consumo de alimento después del destete fueron estimulados ($P = .02$ y $P = .09$, respectivamente) en los cerdos a los que se les ofreció el pellet más grande durante la lactancia.

Implicación: Ofrecer a los lechones un alimento con pellets más grandes durante la lactancia puede mejorar el rendimiento después del destete y reducir la tasa de medicación posterior al destete.

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

Craig JR, Kim JC, Brewster CJ, Smits RJ, Braden C, Pluske JR. Increasing creep pellet size improves creep feed disappearance of gilt and sow progeny in lactation and enhances pig production after weaning. *J Swine Health Prod*. 2021;29(1):10-18.

Résumé - L'augmentation de la taille des granules lors de l'alimentation à la dérobée améliore la consommation de l'aliment de démarrage par la progéniture des cochettes et des truies en lactation et augmente les performances de production après le sevrage

Objectif: Déterminer si l'alimentation avec des granules de diamètre plus gros augmente la consommation d'aliment de démarrage et le taux de croissance des porcelets durant la lactation, spécialement la progéniture des cochettes (GP) comparativement à la progéniture des truies (SP) et stimule la consommation d'aliment après le sevrage.

Matériels et méthodes: Au cours de deux réplicas, GP et SP (n = 2070) furent répartis en deux traitements de moulée de démarrage, recevant soit des granules mesurant 4 mm de diamètre × 4 mm de long ou 9 mm de diamètre × 12 mm de long, à compter de 3 jours d'âge jusqu'au moment du sevrage. Après le sevrage, les porcelets étaient séparés dans des enclos de mâles et de femelles en fonction du type de granule reçu durant la lactation et nourris avec une moulée commune. La consommation d'aliment fut notée avant et après le sevrage (jusqu'à 10 semaines d'âge), ainsi que les performances de croissance des porcelets et toutes les mortalités de porcelets et les retraits.

Résultats: La consommation totale de la moulée de démarrage durant la lactation était plus grande ($P < .001$) dans les portées recevant les granules plus gros, mais le poids de la portée au moment du sevrage pour les GP n'était pas amélioré (interaction, $P > .05$). La progéniture des cochettes était sevrée à un poids plus faible ($P < .001$) que SP. Après le sevrage, les porcs ayant reçu les granules plus gros durant la lactation avaient tendance à recevoir moins de médicaments ($P = .07$) que les porcs recevant les granules plus petits. Le taux de croissance et la consommation de nourriture après le sevrage étaient tous les deux stimulés ($P = .02$ et $P = .09$, respectivement) chez les porcs recevant les granules plus gros durant la lactation indépendamment du sexe.

Implications: Offrir une moulée de démarrage avec des granules plus gros à des porcelets durant la lactation peut améliorer les performances post sevrage et réduire le taux de médication post-sevrage.

Creep feed consumption before weaning is typically low and variable between and within litters.[1] However, supplying creep feed to piglets during lactation to familiarize them with solid feed before weaning is linked to a greater interest of pigs in their feed after weaning, improving subsequent feed intake and performance.[2,3] Surprisingly, relatively little attention is paid to physical characteristics of creep feed offered to piglets and its impacts on creep feed intake and performance before and after weaning. Commercial creep feed pellet sizes are typically ≤ 4 mm in diameter, and it is generally thought that this size allows for effective chewing and swallowing and therefore maximum intake; however, there is evidence suggesting that young pigs are adaptable to a variety of pellet diameters. For example, van den Brand et al[4] showed that offering a creep pellet with a diameter of 12 mm from day 3 or 4 of lactation encouraged creep feed intake in early lactation (before day 18), stimulated growth, and enhanced feed conversion efficiency after weaning, in comparison to piglets offered a 2 mm diameter pellet. Moreover, Middelkoop et al[5] showed that a novel offering of two different pellet sizes simultaneously improved feed intake shortly before weaning. However, neither of these studies examined the impact of dam parity on these improvements in creep feed intake.

Progeny born to primiparous sows (gilt progeny; GP) show compromised performance both before and after weaning.[6,7] Furthermore, GP exhibit higher mortality rates shortly after weaning in comparison to sow progeny (SP). This may be a result of an inability to physiologically adapt to the additional stressors at this time, which in turn may be a direct result of differences present at birth.[7] Provision of creep feed during lactation may encourage GP to consume more both before and after weaning, resulting in production improvements and greater survival. Furthermore, the sex of the pig has been shown to influence a number of physiological traits after weaning.[8] Female pigs tend to have improved mortality rates and performance compared to their male counterparts, despite generally showing heightened nervous and immune activation, increased intestinal permeability, and increased diarrhea.[8] Therefore, offering a larger pellet might be more beneficial to male pigs after weaning.

We hypothesized first, that feeding the larger creep pellet would improve creep feed disappearance and the performance of GP compared to SP in lactation; and second, that feeding a larger creep pellet would improve feed intake and growth rate after weaning, and be more beneficial to male pigs compared to their female counterparts.

Materials and methods

Experimental design

All procedures in this experiment were approved by the Rivalea Australia Animal Care and Ethics Committee (protocol No. 18N042C) and the Murdoch University Animal Ethics Committee (protocol No. R2947/17) under the Australian Code for the Care and Use of Animals for Scientific Purposes.[9]

The study was conducted at a commercial piggery in Corowa, New South Wales, Australia (Rivalea Australia Pty Ltd). Two-hundred forty F1 (Large White × Landrace; Primegro Genetics) primiparous (parity 0; n = 119) and multiparous sows (parities 1-8; mean [SE] parity was 3.1 [0.2]; n = 121) over two replicates were randomly allocated upon entry to the farrowing house according to a 2 × 2 factorial arrangement of treatments. The factors were 1) sow parity (gilts vs sows) and 2) pellet diameter (small [CON; n = 118] vs large [LRG; n = 122]). After weaning, GP and SP were mixed randomly into pens of either male or female pigs according to whether they received the CON or LRG pellet during lactation.

Diets

Piglets were supplied one of two creep diets with different pellet diameters: a 4 mm diameter short-cut (4 mm length) pellet (CON) vs a larger 9 mm diameter pellet of 12 mm length (LRG; Figure 1). The creep diets were produced at a commercial feed mill (Ridley AgriProducts Pty Ltd) and were both identical in nutrient composition (15.0 MJ/kg digestible energy [DE] and 0.87 g standardized ileal digestible lysine [SID Lys]/MJ DE, as-fed basis). Creep diets were supplied from day 3 of lactation until weaning (mean [SE] 26.3 [0.1] days of age) in creep feeders made in-house from polyvinyl chloride pipe (approximately 40 cm high, mouth of feeder 15 cm wide), installed on the wall of the pen adjacent to the creep area. Feeders were checked and filled daily, with all the feed offered being weighed and residual weighed out at weaning. Following weaning, all pigs,

Figure 1: Different creep feed pellets used in the experiment, with A) a larger creep pellet (9 mm diameter × 12 mm length; LRG) and B) a small creep pellet (4 mm diameter × 4 mm length; CON).

regardless of creep pellet diameter treatment, were fed a standard weaned pig diet (14.8 MJ/kg DE and 0.90 g SID Lys/MJ DE, as-fed basis), presented as a 4 mm diameter short-cut pellet, for the first 21 days after weaning.

Animal management

Sows and piglets were housed in individual farrowing crates. Creep areas were fitted with a creep mat and heat lamp. Each farrowing crate had a slatted floor and was fitted with drinker nipples with *ad libitum* access to water for the sow and piglets. Twenty-four hours after farrowing, minimal fostering was conducted to standardize litters, fostering within dam parity and dietary treatments wherever possible. At 3 days of age, all piglets had their tails docked and were given a 200 mg intramuscular (IM) iron injection (Gleptosil; Champion Alstoe Animal Health) and 2 mL of oral toltrazuril for control of coccidiosis (Baycox; Bayer Animal Health). They were then vaccinated against *Mycoplasma hyopneumoniae* and porcine circovirus type 2 (first replicate: Fostera Gold PCV MH; Zoetis Australia Pty Ltd; second replicate: Ingelvac CircoFLEX and MycoFLEX; Boehringer Ingelheim Pty Ltd) approximately 1 week before weaning. All litters were weighed within 24 hours of birth (after fostering) and again at weaning. Creep pellets were offered to litters (F1 × Duroc Synthetic; Primegro Genetics) on an *ad libitum* basis from day 3 of lactation to weaning, and total feed disappearance (feed delivered - residual removed) was recorded.

All piglets within replicate were weaned on the same day at a mean (SE) of 26.3 (0.1) days of age. After weaning, 2070 pigs born to gilts and sows were mixed and divided into pens of entire males and females. Pigs were moved to the commercial weaned pig facility and placed into pens of 18 pigs (n = 115) across two rooms based on the pellet diameter offered in lactation and size, for within-pen uniformity. Each pen was weighed upon entry to the room and again 21 days after weaning, with total feed intake recorded on a per pen basis. The first replicate ran from December 2018 to February 2019, and the second replicate ran from February to April 2019. All piglet mortalities were recorded for the entirety of the experiment. After weaning, all individual injectable medications and all pig removals were recorded.

Each pen in the room was fitted with one feeder with four feeder spaces, and two to three drinker nipples ensuring *ad libitum* access to water. Each pen in the barn had a solid floor area at the front where the feeder was situated, and a slatted floor in the back two thirds of the pen. Pigs that were observed to be suffering from ill thrift, lameness, or meningitis symptoms were medicated with an IM injection of both meloxicam (Recocam; Abbey Animal Health Pty Ltd) and either amoxycillin (first replicate; Moxylan; Jurox Pty Ltd), oxytetracycline, or penicillin (second replicate; Alamycin and Ultrapen, respectively; Norbrook Laboratories Australia, Pty Ltd) as per the product labels. Pigs were removed from the experiment if, in the view of the stockperson, they failed to recover or lost a large amount of body weight, body condition, or both.

Statistical analysis

Continuous variables were analyzed as linear mixed models using the MIXED procedure of SPSS (version 25; IBM). Preweaning data were analyzed as a 2 × 2 factorial comparison with creep diet (CON vs LRG) and parity group (GP vs SP) as fixed factors. Replicate was used as a blocking factor and litter as the experimental unit. Age at weaning was used as a covariate when it had a significant effect ($P < .05$) on the model, ie, for creep disappearance from day 3 to weaning and day 21 to weaning, for average piglet weight at weaning and for average daily gain (ADG) from birth to weaning. This was used to adjust for unforeseen differences in weaning age between dam parity groups in the CON group. Litter preweaning mortality rate was analyzed as a continuous variable. Postweaning data were analyzed as a 2 × 2 factorial comparison with creep diet (CON vs LRG) and sex (female vs male) as fixed factors, and replicate was used as a blocking factor and pen as the experimental unit. Random effects of barn and sow parity (nested within sow parity group) were tested as appropriate and removed from the model if not significant ($P \geq .50$). Chi-squared analysis was conducted for determining the effect of creep pellet diameter on the binomial variables postweaning mortality (died or lived), removal (removed or not), and medication rates (medicated at least once or not medicated). A P value < .05 was considered significant, and a P value < .10 was considered a trend.

Results

Litter performance at birth

Three primiparous and 6 multiparous sows were removed from the analysis due to mortality, or their udders dried up before weaning and their piglets were fostered onto another sow. Five sows (4 primiparous and 1 multiparous) had piglets fostered that were not recorded and therefore their weaning data were

not included in the analysis; however, these piglets were included in the post-weaning stage of the experiment. Litters from 14 sows (6 primiparous and 8 multiparous) were inadvertently mixed when separation boards between farrowing crates dislodged, and therefore their data at weaning was not included in the analysis.

There was no difference ($P \geq .05$) in number of piglets born alive or total piglets born between dietary treatments (Table 1). Multiparous sows had higher ($P = .003$) total piglets born compared to primiparous sows. Primiparous sows had lighter ($P < .001$) litters than multiparous sows after fostering with a lower ($P = .001$) mean piglet weight, but there was no difference ($P \geq .05$) between dietary treatments (Table 1).

Creep feed disappearance

There were a number of instances in the first replicate where creep feed became wet or the feeder tipped over, and therefore this replicate had a highly significant effect on all creep consumption measures ($P < .001$) and was left in the model as a blocking factor. Creep feed disappearance was lower ($P < .001$) in the second replicate, likely due to a lower rate of feed wastage as feeders were not allowed to become wet and soiled as often.

From day 3 (introduction of creep feed) to day 10 of age, piglets provided LRG pellets during lactation had a higher ($P = .004$) creep feed disappearance than piglets provided CON pellets (Figure 2A). However, there was a strong trend ($P = .06$) for an interaction between sow parity and creep feed pellet size, where higher intake was observed in GP than SP (Figure 2A). From day 11 to day 20, creep feed disappearance was again higher ($P < .001$) in the LRG group (Figure 2B); however, there was no significant interaction effect between sow parity and creep pellet size. Sow progeny (SP) had a higher ($P = .03$) creep feed disappearance than GP in this period. Higher ($P = .002$) creep feed disappearance was observed in the LRG group than in the CON group from day 21 to weaning (Figure 2C), and SP and GP had a similar ($P = .98$) creep feed disappearance. There was no creep pellet size by sow parity interaction ($P = .92$).

Over the entire creep-supplemented period (day 3 to weaning; mean [SE] 23.1 [0.1] days), both GP and SP fed the LRG diet had a greater ($P < .001$) creep feed disappearance than those fed the CON diet (2770 g vs 2111 g, respectively). There was no difference ($P = .33$) between GP and SP creep feed disappearance in the entire preweaning period.

Preweaning growth performance

There was a significant interaction ($P = .041$) between dam parity and diet for age at weaning, with GP one day younger than SP in the CON group (25.7 [0.3] vs 26.8 [0.3] days of age, respectively), whereas there was no difference in the LRG group (both 26.4 [0.3] days of age). Hence, age at weaning was used as a covariate for measures where it made a significant contribution to the model ($P < .001$ in all cases). Litter weight at weaning was similar ($P = .67$) between the CON and LRG groups (68.2 [1.4] kg vs 69.1 [1.4] kg, respectively), and multiparous sow litters were heavier ($P < .001$) than litters from primiparous sows. There was no interaction ($P = .57$) between

Table 1: Mean (SE) preweaning performance of experimental litters comprised of gilt (GP) or sow progeny (SP) provided either a small 4 mm diameter × 4 mm length creep pellet (CON) or a larger 9 mm diameter × 12 mm length pellet (LRG) from day 3 of lactation until weaning

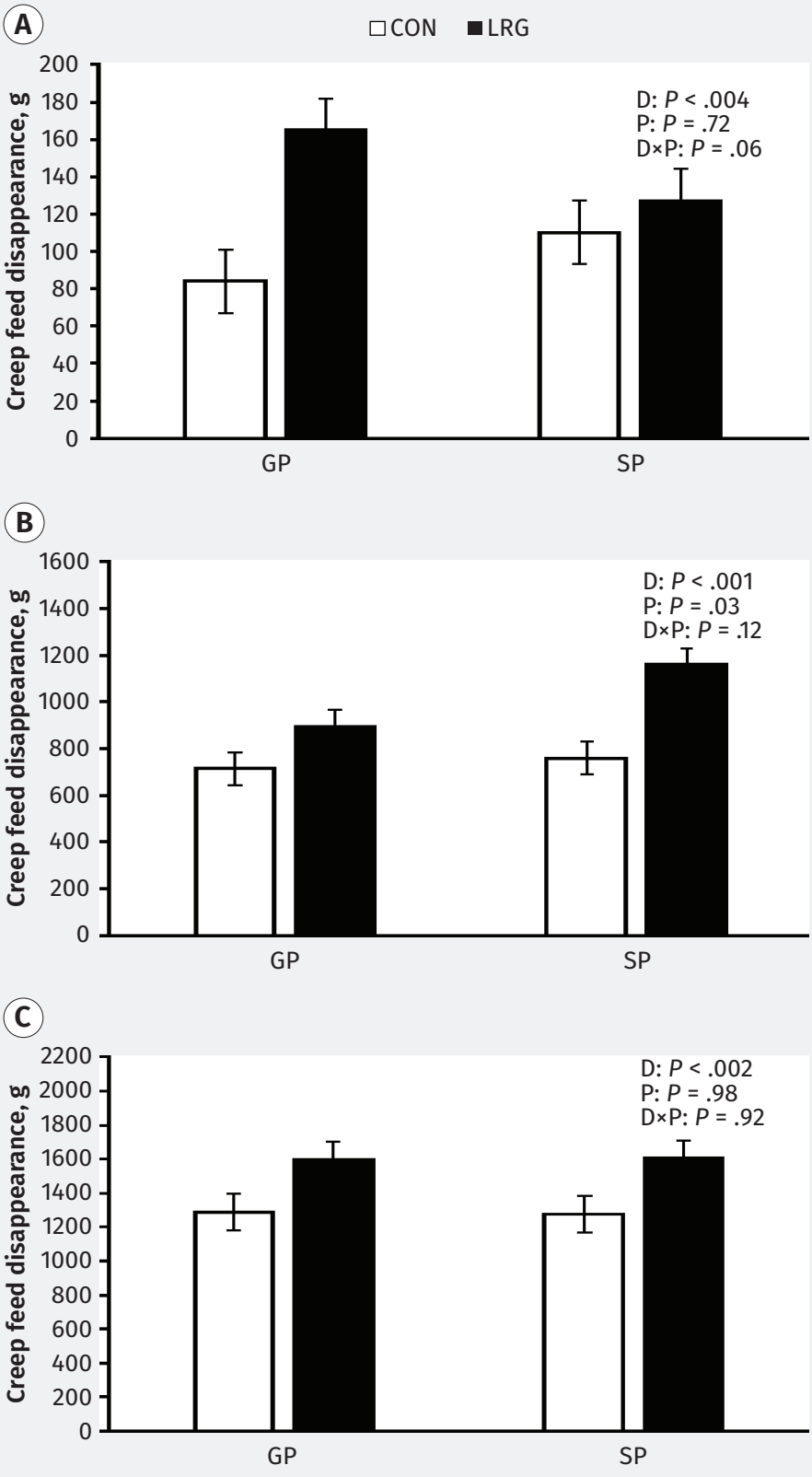
	GP		SP		*P* value		
Parameter	**CON**	**LRG**	**CON**	**LRG**	**Diet**	**Parity**	**Diet × Parity**
BA	11.9 (0.4)	11.8 (0.4)	12.4 (0.4)	12.4 (0.4)	.98	.12	.98
TB	12.5 (0.4)	13.0 (0.4)	13.8 (0.4)	14.1 (0.4)	.84	.003	.32
Day 0 (post foster)							
Litter size, No.	11.7 (0.2)	11.8 (0.2)	11.8 (0.2)	12.0 (0.2)	.88	.45	.28
LW, kg	16.5 (0.3)	16.5 (0.3)	17.8 (0.3)	17.8 (0.4)	.99	< .001	.94
Avg BW, kg*	1.42 (0.03)	1.40 (0.03)	1.52 (0.03)	1.49 (0.03)	.76	.001	.37
Weaning							
Weaning age, d	25.7 (0.3)	26.4 (0.3)	26.4 (0.3)	26.8 (0.3)	.64	.06	.04
Litter size, No.	10.0 (0.3)	10.2 (0.2)	10.3 (0.2)	10.2 (0.03)	.51	.74	.91
LW, kg†	64.7 (2.0)	64.4 (1.9)	73.7 (1.9)	71.7 (2.0)	.67	< .001	.57
Avg BW, kg*†	6.43 (0.15)	6.30 (0.14)	7.19 (0.14)	7.10 (0.15)	.87	< .001	.47
ADG D0-wean, g/d†	190 (5)	187 (5)	215 (5)	212 (5)	.91	< .001	.57
PWM, %	15.9 (2.2)	14.4 (2.2)	14.8 (2.1)	15.7 (2.2)	.58	.95	.90

* Average BW = litter weight ÷ litter size.

† Weaning age used as a covariate.

BA = number of piglets born alive; TB = total piglets born; LW = litter weight; BW = body weight; ADG = average daily gain; PWM = preweaning mortality.

Figure 2: Total creep feed disappearance in gilt progeny (GP) and sow progeny (SP) litters when fed either a small creep pellet (CON) or a larger creep pellet (LRG) from A) days 3 to 10 of lactation, B) days 11 to 20 of lactation, and C) day 21 of lactation to weaning (26.3 [0.1] days of age). The *P* values given are results of the linear mixed model analysis for the main effects of diet (D), parity group (P), and their interaction (D×P).



creep pellet size and dam parity. Litter number at weaning was similar between creep pellet size groups ($P = .51$) and dam parities ($P = .74$; Table 1). Litters in the CON and LRG groups had a similar ADG from fostering to weaning (201 [4] g/d; $P = .91$) and a similar average piglet weight at weaning (6.8 [0.1] vs 6.7 [0.1] kg, respectively; $P = .87$). Sow progeny grew faster (214 [4] vs 189 [4] g/d, respectively; $P < .001$) and were heavier (7.1 [0.1] vs 6.4 [0.1] kg, respectively; $P < .001$) than GP at weaning. There was no difference in litter preweaning mortality between the creep pellet treatments ($P = .58$) or dam parity groups ($P = .95$; data not shown). There were no interactions ($P \geq .05$) for any of these preweaning parameters.

Postweaning performance

The creep pellet size by sex interaction was not significant for any postweaning traits (Table 2). Average weight was similar ($P \geq .05$) at weaning and 21 days after weaning, regardless of creep pellet treatment or sex. Piglets offered the LRG pellets before weaning grew faster ($P = .002$) than those offered the CON pellets and consumed more feed (average daily feed intake [ADFI]; $P = .009$) in the first 21 days after weaning. Replicate significantly ($P < .05$) affected the postweaning ADFI and feed conversion ratio of pigs, with pigs in the first replicate consuming less feed and being more efficient than those in the second replicate (data not shown). Male pigs tended ($P = .07$) to be more efficient in the first 21 days after weaning than female pigs (1.26 [0.01] vs 1.29 [0.02] kg/kg, respectively), and although not a statistical trend, were heavier than females at weaning (by approximately 6%) and 21 days after weaning (by approximately 5%; Table 2).

Mortalities, removals, and medications of pigs are shown in Figure 3. There was a higher numerical proportion ($P = .11$) of removals after weaning in the CON group compared to the LRG group. There was a stronger tendency ($P = .07$) for a higher proportion of pigs in the CON group to be medicated after weaning compared to the LRG group. There was no difference between males and females in terms of mortality ($P = .66$), removals ($P = .58$), or medications ($P = .76$); however, within females there was a significant difference in proportion of

Table 2: Mean (SE) postweaning performance (from weaning, day 0; to 21 days post weaning, day 21) of female (F) and male (M) experimental piglets provided either a small 4 mm diameter × 4 mm length creep pellet (CON) or a larger 9 mm diameter × 12 mm length pellet (LRG) from day 3 of lactation until weaning

	CON		LRG		*P* value		
Parameter	**F**	**M**	**F**	**M**	**Diet**	**Sex**	**Diet × Sex**
BW D0, kg	6.59 (0.26)	7.07 (0.25)	6.75 (0.25)	7.09 (0.25)	.72	.10	.77
BW D21, kg	10.9 (0.4)	11.6 (0.4)	11.6 (0.3)	12.0 (0.4)	.12	.14	.74
ADG (D0-21), g/d	207 (7)	214 (7)	230 (7)	234 (7)	.002	.45	.76
ADFI, g/d	270 (9)	269 (9)	295 (9)	291 (9)	.009	.80	.87
FCR, g:g	1.30 (0.02)	1.27 (0.02)	1.28 (0.02)	1.25 (0.02)	.33	.07	.96

BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; FCR = feed conversion ratio.

pigs medicated between CON and LRG pigs (5.6% vs 3.0%, respectively; $P = .04$), whereas within males there was no difference (4.2% vs 3.6%, respectively; $P = .63$).

Discussion

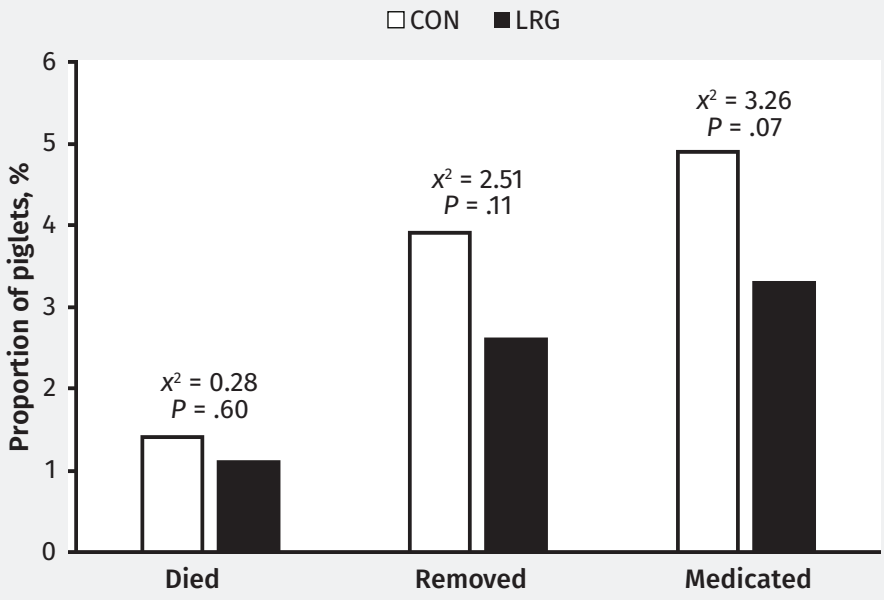
In the current study, providing a larger creep pellet increased creep feed disappearance but there were no production benefits for GP relative to SP during lactation. This partly confirms our hypothesis that providing a creep feed in a larger form would help familiarize piglets with solid feed during lactation, and in turn encourage more feed intake after weaning. The prevailing view commercially is that smaller pellets are easier for small piglets to eat and therefore will encourage more feed intake in early lactation when piglets are not familiar with solid feed.[10] However, larger particle sizes (eg, acorns, nuts, or mushrooms) are often consumed by young wild pigs in nature and in free-range conditions when a more gradual weaning occurs.[11] Providing creep feed early in lactation is thought to encourage exploration behaviors and therefore preweaning feed intake.[12,13] Larger particle sizes may actually be easier to pick up and carry for small piglets, whose teeth and jaw structure are not fully developed before weaning in a commercial production system.[14] The finding that piglets provided the larger pellets had a higher creep feed disappearance in lactation concurs with the study of van den Brand et al,[4] who found that piglets provided a larger diameter pellet (12 mm) explored the creep feeder more often, and had a significantly higher creep feed intake than piglets offered a smaller pellet (2 mm). Clark et al[15] found an increase in creep feed intake of piglets offered larger pellets (13 mm) in comparison to those offered smaller pellets (3 mm) from day 17 of lactation, and a reduction in piglet mortalities from 10 days of age until weaning.

It is unclear from previous experiments whether creep feed intake is increased with the larger pellets due to more time being spent at the feeder,[10,16] or to more feed being consumed in the same time frame (ie, in the same feeding event).[4] Edge et al[10] reported that "feed trough directed behavior" of piglets, as measured via video camera, was not highly correlated with actual feed intake. In that study, trough directed behavior was defined as any chewing, manipulating feed, placing head in the feeder, or manipulation of the feeder itself. It is probable that the larger pellets encourage 'playing' with the larger feed items away from the creep area, allowing other littermates more time at the feeder and hence encouraging higher creep feed intakes from the litter as a whole. The presentation of the smaller pellets may have allowed one dominant piglet in the litter to occupy the space at the feeder for a longer period of time, limiting the amount of interaction with the feeder for their fellow littermates.[10] Clark et al[15] found that while improving overall creep feed intake, providing larger pellets did not increase the proportion of piglets in the litter consuming creep feed. The proportion of piglets eating CON or LRG pellets was not ascertained in the current study, therefore it is not known whether offering larger pellets encouraged more creep feed to be eaten over the whole litter or by a greater proportion of piglets.

Farm staff in the current study observed that less of the larger diameter creep pellets fell between the farrowing crate slats than the smaller pellets and hence less were wasted, similar to what was observed by van den Brand et al[4] and Middelkoop et al.[5] Therefore, it may be assumed that the difference in creep disappearance between CON and LRG piglets that was attributable to actual feed intake was larger than is reported here, given that more of the CON diet may have been wasted. Furthermore, a significant effect of replicate on some of the results was observed. In the first replicate it was observed in some instances that the creep feed became wet and had to be replaced (ie, creep feed disappearance was higher overall in this replicate than in the second). Results were analyzed both as a whole, as reported above, but also separately between each replicate to account for this. Regardless of replicate, the results and conclusions were similar and hence the decision was made to analyze the data as the whole cohort, with replicate included in the model.

Daily replacement of creep feed may encourage further preweaning feed intake, as observed by Appleby et al[16] and Wattanakul et al,[1] and could be another reason for significantly higher creep feed disappearance in the large pellet group in our study, as higher feed intakes in this group would encourage more frequent feed replacement. One disadvantage of a larger pellet is that they may be less durable and produce more fine particles during the production process than smaller pellets.[4,17] While physical presence of fine particles was not recorded in the current experiment, the larger pellets were not observed to be any dustier than the smaller pellets. Furthermore, there is evidence that production of larger pellets at the feed mill may be more energy efficient than the production of smaller pellets.[17]

Figure 3: Proportion of piglets that died, were removed from the experiment, or were medicated at least once in the postweaning period. Piglets were fed either a small creep pellet (CON) or a larger creep pellet (LRG) from day 3 of lactation until weaning (26.3 [0.1] days of age). Parameters were analyzed as binomial traits using Pearson's chi-square (χ^2).



Overall, creep feed disappearance during the entire lactation was similar between GP and SP, in contrast to Edwards et al[18] who found creep feed disappearance was greater in SP (from day 19 of lactation to weaning). Potential reasons for GP and SP displaying a preference for larger sized pellets, or creep feed in general, in different stages of lactation in the current study are not easy to interpret. A possible reason might be associated with interactions between litter characteristics, for example suckling intensity and its impacts on nutrient demand and milk production of the sow,[1,19] with primiparous sow litters having less variation in body weights and lower overall litter weights in comparison to multiparous sow litters.[20] The potential effects of this on creep feed consumption differences of GP and SP warrants further investigation. Furthermore, the literature suggests that parity of the sow (birth or foster sow parity) can influence overall creep feed consumption of piglets,[18,21] but these relationships may be quite complex. In this regard, offering creep feed in lactation as early as possible appears to provide benefits,[4,22] even though significant consumption of creep feed does not occur until later in lactation.[23] Our data showed that creep feed disappearance increases throughout lactation and was much higher in the period from day 21 of lactation until weaning, supporting this proposition. Our observation that preweaning growth rates were similar between small and large pellet groups, despite higher creep feed disappearance in the large pellet group, supports this notion and agrees with the findings of Edge et al[10] and Clark et al.[15] This is not surprising given that the improvements in creep disappearance with the larger pellets would not equate to a substantial increase in individual creep feed intake, and therefore energy and nutrient intake, per piglet throughout the lactation period. It also seems that pigs, like other animal species, prefer variety[5,12] and complexity[24] in their diets. Hence, providing more than one creep feed type at a time may also stimulate feed intake before and after weaning.[5] However, results are conflicting as to whether, if given a choice, piglets will show a preference for smaller or larger pellets,[11] or not show a preference to either.[5]

Feed intake after weaning was improved in pigs offered larger diameter creep pellets before weaning, supporting our hypothesis. This agrees with previous studies[4,10,15] and caused a higher postweaning ADG, in agreement with the findings of van den Brand et al[4] and Clark et al,[15] but in contrast to those of Edge et al.[10] Voluntary feed intake of solid food after weaning is typically low and variable[25] and contributes to the postweaning growth check,[26] especially when pigs are not offered solid feed before weaning.[2,27] Supplying creep feed in lactation improves adaptation to a solid diet thereby increasing feed intake[28,29] and performance of pigs after weaning,[3,30] and concurrently may give a sense of familiarity to the pig at this stage that could reduce neophobic reactions to a new environment and ameliorate weaning stressors.[3,26,31] This is critical because even a small improvement in creep feed intake (approximately 60 g/piglet/day) can increase postweaning growth by 1 kg/piglet in the first 2 weeks after weaning.[5,12] It was also suggested by van den Brand et al[4] that chewing of larger pellets may help to further stimulate teething in young pigs, which has been shown to impact their appetite and feed intake after weaning.[32]

The greater ADFI after weaning in pigs eating larger creep pellets before weaning appeared to improve their overall health, as was reflected in the lower removal rates and reduced number of injectable medications (most of which were for ill thrift) given in these pigs. A higher level of feed intake after weaning is important for maintaining gastrointestinal tract structure and function,[33] and greater feed intake is also linked to reduced postweaning diarrhea.[34] van den Brand et al[4] found no differences in diarrhea scores between pigs receiving small or large pellets before weaning, but different conditions in diets and feeding patterns, genetics, disease status, and environmental conditions can contribute to differences between experiments. There were no significant differences in postweaning mortality rate or removal rate between pigs in the CON and LRG pellet groups; however, mortality and removal rates were lower for pigs offered the larger pellet before weaning. We also hypothesized in this study that male pigs would benefit more than female pigs in the postweaning period when offered the large diameter pellet before weaning, given they generally perform poorer.[9] However, we failed to see any production improvements in male pigs compared to their female counterparts aside from a trend for improved feed conversion efficiency, and in fact, offering larger creep pellets significantly reduced the rate of medications after weaning in females but not in males. The mechanisms of this improvement require further investigation. In this regard, postweaning performance

can be heavily influenced by dam parity,[7] and unfortunately dam parity could not be controlled for in the weaner phase of this experiment due to commercial production constraints. It is likely that GP and SP may have been largely kept in separate pens after weaning, as pigs would have been sized into pens (as is commercial practice at this facility) to allow uniformity of body weight within pens as much as possible. Therefore, GP may have been overrepresented in 'light' pens, and SP in 'heavy' pens and it would be of interest to examine these effects more closely after weaning in future studies.

In conclusion, providing a larger creep pellet from day 3 of lactation until weaning may have encouraged higher creep feed intake of both GP and SP before weaning. This caused an increased feed intake after weaning irrespective of sex, and subsequently a significant improvement in ADG and ADFI in the first 21 days after weaning. Offering a larger diameter pellet also resulted in a lower proportion of pigs being medicated after weaning.

Implications

Under the conditions of this study:

- Providing large creep pellets in lactation increases creep feed disappearance.
- Larger pellets enhance postweaning feed intake, growth, and health status.
- Providing larger pellets improves both gilt and sow progeny performance.

Acknowledgments

The authors would like to acknowledge the technical staff at the Research and Innovation Unit at Rivalea Australia for their assistance with the on-farm data collection. This experiment was conducted as part of a project funded by Australian Pork Limited (project No. 2014/461).

Conflict of interest

None reported.

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* Non-refereed references.

Peer Reviewed **Original Research**

Time and temperature requirements for heat inactivation of pathogens to be applied to swine transport trailers

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Summary

Objective: Biosecurity in swine transport trailers is of concern for spreading pathogens between premises, and as such, they require extensive cleaning and disinfection between loads. Our goal in this study was to find the optimal time and temperature required to heat inactivate swine pathogens of high concern to producers in a laboratory setting to then be extrapolated to transport trailers.

Materials and methods: Using standard microbiological techniques for growth and purification, 5 bacterial and 5 viral pathogens important in swine health were produced and tested. Heat inactivation of these pathogens were tested in the lab using several time and temperature combinations. Fecal matter was added to test the effect of biological material on the time and temperatures required for inactivation.

Results: Inactivation was complete for viruses and bacteria tested when heated to 75°C for 15 minutes. The presence of fecal matter resulted in increased time and temperature needed for pathogen inactivation.

Implications: Heat baking of transport trailers is now being applied as a useful tool to reduce the transmission of pathogens commonly associated with swine disease. However, operators must ensure consistent heating to 75°C for a minimum of 15 minutes in all areas of the trailer for reliable inactivation. Cleaning trailers plays an important role prior to heat treatment, as the presence of fecal contamination will insulate the pathogens and inactivation may not be complete even at 75°C for 15 minutes.

Keywords: swine, biosecurity, pathogen inactivation, heating

Received: January 22, 2020
Accepted: July 7, 2020

Resumen - Requisitos de tiempo y temperatura que se deben aplicar para la inactivación por calor de patógenos

Objetivo: La bioseguridad en los remolques de transporte de cerdos es motivo de preocupación para la propagación de patógenos entre las instalaciones y, como tales, requieren una limpieza y desinfección exhaustivas entre cargas. Nuestro objetivo en este estudio fue encontrar el tiempo y la temperatura óptimos necesarios para inactivar por calor a los patógenos porcinos de gran preocupación para los productores en un entorno de laboratorio, para luego extrapolarlos a los remolques de transporte.

Materiales y métodos: Utilizando técnicas microbiológicas estándar para el crecimiento y la purificación, se produjeron y probaron 5 patógenos bacterianos y 5 virales importantes para la salud porcina. La inactivación por calor de estos patógenos se probó en el laboratorio utilizando varias combinaciones de tiempo y temperatura. Se agregó materia fecal para probar el efecto del material biológico en el tiempo y las temperaturas requeridas para la inactivación.

Resultados: La inactivación fue completa para los virus y bacterias probados cuando se calentó a 75°C durante 15 minutos. La presencia de materia fecal resultó en un aumento del tiempo y la temperatura necesarios para la inactivación de patógenos.

Implicaciones: El horneado térmico de los remolques de transporte se está aplicando ahora como una herramienta útil para reducir la transmisión de patógenos comúnmente asociados a enfermedades porcinas. Sin embargo, los operadores deben garantizar un calentamiento constante a 75°C durante un mínimo de 15 minutos en todas las áreas del remolque para una inactivación confiable. La limpieza de los remolques juega un papel importante antes del tratamiento térmico ya que la presencia de contaminación fecal aislará a los patógenos y la inactivación puede ser incompleta incluso a 75°C durante 15 minutos.

Résumé - Exigences de temps et de température pour l'inactivation d'agents pathogènes par la chaleur

Objectif: La biosécurité dans les remorques de transport des porcs est

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

van Kessel J, Strom S, Deason H, Van Moorlehem E, Berube N, Hauta S, Fernando C, Hill J, Fonstad T, Gerdts V. Time and temperature requirements for heat inactivation of pathogens to be applied to swine transport trailers. *J Swine Health Prod*. 2021;29(1):19-28.

une préoccupation pour la transmission d'agents pathogènes entre les sites, et comme tel, elles nécessitent un nettoyage et une désinfection minutieuse entre les chargements. Notre but dans la présente étude était de trouver le temps et la température optimums requis pour inactiver par la chaleur des agents pathogènes porcins hautement préoccupants pour les producteurs dans un environnement de laboratoire et par la suite l'extrapoler aux remorques de transport.

Matériels et méthodes: En utilisant des techniques microbiologiques standards pour la croissance et la purification, cinq bactéries pathogènes et cinq virus pathogènes d'importance en santé porcine furent produits et testés. L'inactivation par la chaleur de ces agents pathogènes fut testée en laboratoire en utilisant plusieurs combinaisons de temps et température. Des matières fécales furent ajoutées pour tester l'effet de matériel biologiques sur le temps et la température requis pour l'inactivation.

Résultats: L'inactivation fut complète pour les virus et bactéries testés lorsque chauffés à 75°C pendant 15 minutes. La présence de matière fécale a résulté en une augmentation du temps et de la température requis pour l'inactivation des agents pathogènes.

Implications: L'exposition à la chaleur des remorques de transport est présentement appliquée comme un outil utile pour réduire la transmission d'agents pathogènes fréquemment associés avec des maladies porcines. Toutefois, les opérateurs doivent s'assurer un chauffage constant à 75°C pour un minimum de 15 minutes dans toutes les parties de la remorque pour une inactivation fiable. Le nettoyage des remorques joue un rôle important avant le traitement à la chaleur, étant donné que la présence de contamination fécale isolera les agents pathogènes et l'inactivation pourrait ne pas être complète même à 75°C pour 15 minutes.

Biosecurity has become an essential part of modern farm management with the aim of reducing exposure of animals to disease both within and between farms.[1] Heat treatment of transport trailers is a relatively new method being incorporated by transport companies to minimize spread of pathogens in the wake of several porcine epidemic diarrhea virus (PEDV) outbreaks in eastern Canada. Heat treatment also results in faster turnaround time as well as less risk to sanitation personnel as compared to fumigation of trailers with formaldehyde or quaternary glutaraldehyde. Thermo-assisted drying and decontamination (TADD) is one way of controlling the spread of PEDV and other porcine pathogens in transport trailers.[2-12]

The question remains for TADD, however, how long should the trailer be heated and to what temperature? Our objective was to determine a reasonable time and temperature that would effectively kill both bacterial and viral pathogens relevant to the swine industry.

Animal transportation is especially a risk for introducing disease to naïve animals and as such, transport trailers require extensive cleaning, washing, and disinfection after each load. In addition, the industry has incorporated the use of heating bays which expose trailers to hot air for varying amounts of time. Current protocols involve heating trailers to 70°C for 10 to 15 minutes. This regimen is based on an extensive amount of studies that have determined the necessary temperatures to inactivate both porcine viruses and bacteria.[2-10,12-14]

Several pathogens have been identified as high risk to swine producers in the last few years, including but not limited to those listed in Table 1. As an added complication, current protocols for disinfection have proven time consuming and costly. This, along with regional trailer shortages, have resulted in increased noncompliance with biosecurity practices.[3,27] Several recent outbreaks of porcine reproductive and respiratory syndrome virus (PRRSV) and PEDV have prompted calls for new methods to sanitize livestock trailers that are robust and cost effective in inactivating targeted porcine pathogens.[2,4-6,8,9] The objective of this study was to find the optimal time and temperature required to inactivate important swine pathogens. For this purpose, we selected a number of common swine pathogens including PEDV, PRRSV, swine influenza virus (SIV), transmissible gastroenteritis virus (TGEV), porcine rotavirus, *Streptococcus suis*, *Salmonella* Typhimurium, *Escherichia coli*, *Actinobacillus pleuropneumoniae* (APP), and *Brachyspira hampsonii*. We chose three different experimental settings for our analysis. First, purified pathogens were inactivated in cell culture. Second, fecal matter was examined for insulating capacity of biological material. Thirdly, the pathogens were incubated inside fecal matter to be more representative of field conditions. Bacteria are differently susceptible to environmental conditions, but generally most bacteria are heat labile (apart from thermophiles which are not of concern in this study).[10,12,14,22,28-30] Although they exist ubiquitously in the environment, pathogenic bacteria usually require certain conditions to make humans and animals sick. Many species of bacteria can survive for years in soil and on fomites unlike viruses. For example, *Salmonella* are normally killed by heat and disinfectants[14,22,28-30] except when they form biofilms on biotic and abiotic surfaces.[28,29] Similarly, *E coli* has been reported to be viable on fomites for more than 14 months, but are also able to form biofilms, and thus pose a risk to naïve animals through direct fomite contact.[10,23,30,31] *Streptococcus sui*s is part of the normal pig microflora but occasionally can mutate into a more pathogenic form or can make animals sick if it gets into an unusual site within the body or as a confounding coinfection with viruses.[22,26,31] Pathogenic species of *S suis* are of particular concern as they can make both humans and animals sick.[31]

Materials and methods

Propagation of viruses

PEDV. Porcine epidemic diarrhea virus is an emerging animal disease in Saskatchewan and as such, all PEDV experiments were performed at VIDO-Intervac in containment level 3 as required by Canadian Food Inspection Agency. The PEDV virus was the US/Colorado/2013 isolate and was obtained from the US Department of Agriculture's National Veterinary Services Laboratories (Lot 025 PDV 1303). Vero76 cells (ATCC) were subcultured 24 hours prior to infection in Dulbecco's Modified Eagles Essential Medium (DMEM; Sigma Aldrich; D5796) supplemented with complete 10% fetal bovine serum (FBS; gamma-irradiated and Australian sourced; PAA Laboratories; A15-503), 0.1M Hepes (Gibco; 15630-080), and 0.05 mg/mL gentamicin (Gibco; 15750078). Cells were 99% confluent at the time of infection. The PEDV inoculum containing tosyl phenylalanyl chloromethyl ketone (TPCK)-treated

Table 1: Important disease pathogens in swine herds and previously reported inactivation conditions in vitro and in environmental studies

Pathogen	Temperature, °C	Time	References
PEDV	60	30 min	2, 9, 15
	71	10 min	
	21	7 d	
TGEV	56	30 min	16
	60	10 min	
PRRSV	56	20 min	17, 18
	37	24 h	
SIV	56	30 min	13, 19
	60	10 min	
	70	<1 min	
Rotavirus	50	5 min	20
	20	7-9 mo	
PCV	70	6 h	21
Salmonella Enteritidis H2292, Heidelberg 21380 Typhimurium avirulent strain 8243	71	< 1 min	14, 22
	70	80-100 min (feces)	
Escherichia coli	70	<1 min	10
Escherichia coli 0157:H7 biofilm forming	22	6 h	23
Swine Brachyspiral colitis	56	10 min	11, 24
	37	60 min	
Mycoplasma hypopneumoniae	60	10 min	25
	45	30 min	
Actinobacillus pleuropneumoniae	42	< 4 h	12
	37	8 h	
Streptococcus suis	60	10 min	26
	25	24 h (dust)	
	20	8 d (feces)	

PEDV = porcine epidemic diarrhea virus; TGEV = transmissible gastroenteritis virus; PRRSV = porcine reproductive and respiratory syndrome virus; SIV = swine influenza virus; PCV = porcine circovirus.

Trypsin-(MJS BioLynx; UB22725S2), with a final concentration of 2 µg/mL, was added to Vero76 cells. Flasks were incubated for 72 hours, harvested by scraping, supernatants frozen at -80°C, thawed and concentrated by ultracentrifugation. The PEDV virus stock was titered and found to be 2.4×10^8 viral copies/mL as determined by quantitative polymerase chain reaction standard curve. Pelleted virus was aliquoted and stored at -80°C.

PRRSV. The PRRSV virus was purchased from ATCC (LN 14832 MARC145 cell line [monkey kidney]) and was subcultured in Eagle's Minimal Essential Medium (MEM; Sigma Aldrich M4655) with complete 10% FBS, 1× non-essential amino acids, and 1× antibiotic/antimycotic for 24 hours prior to infection. Cells were 80% confluent at the time of infection. Monolayers were washed once with phosphate-buffered saline (PBS; Mg^{2+} and Ca^{2+} free) and PRRSV inoculum strain VR2385 was added at a multiplicity of infection (MOI) of 0.1 for 2 hours at 37°C. Inoculum was removed and replaced with MEM with complete 10% FBS. Flasks were incubated for 72 hours until complete cytopathic effect (CPE) was observed. Virus was harvested and subsequently titered. The final titer of viral lysates was 5.0×10^6 median tissue culture infectious dose ($TCID_{50}$)/mL. Virus was aliquoted and stored at -80°C.

SIV. At 24 hours prior to infection, MDCK cells were subcultured in DMEM supplemented with 10% FBS. Cells were 99% confluent at the time of infection. Monolayers were rinsed with PBS prior to infection. An A/SW/SK/02 (H1N1) strain of SIV was isolated from an infected animal in Saskatchewan, was added at an MOI of 0.1 in the presence of TPCK-trypsin and incubated for 48 hours until complete CPE was observed. Flasks were harvested by scraping, supernatants frozen at -80°C, and then thawed before virus was concentrated by ultracentrifugation on a sucrose cushion. Titer of the SIV stock was 4×10^7 plaque-forming units (PFU)/mL. Virus aliquots were stored at -80°C.

TGEV. The TGEV virus was purchased from ATCC. Swine testicular cells (ST cells) were subcultured in MEM with 10% FBS and antibiotic/antimycotic 24 hours prior to infection. Virus was adsorbed onto the cells for 1 hour in low volume MEM (no FBS) and then additional MEM (no FBS) was added. Cytopathic effect was complete in 24 hours and flasks were scraped into the surrounding medium. The cell/virus/medium mixture was frozen at -80°C and then thawed to lyse cells. Cellular debris was spun out of the supernatant by centrifugation and supernatant containing virus was stored at -80°C. The virus titer was 1×10^7 PFU/mL.

Porcine rotavirus. For 24 hours prior to infection, MA-104 cells (ATCC; CRL-2378) were subcultured in MEM supplemented with 10% FBS. Trypsin was added at 10 µg/mL to stock virus and monolayers were washed with MEM alone prior to infection. Virus was diluted 1:10 and adsorbed onto cells for 1 hour in low volume. Additional MEM containing 5 µg/mL trypsin was added and the virus was allowed to replicate for 3 to 4 days. When CPE was 80% to 90% complete, cells were harvested into the medium and both cells and supernatant were stored at -80°C. The virus titer was 6.8×10^5 $TCID_{50}$/mL.

Heat inactivation of viral pathogens

Using 1.5 mL centrifuge tubes, 100 µL of MEM without serum was preheated in digital heating blocks to individual temperatures being tested. For each tube, 20 µL of supernatants containing specified amounts of infectious virus was added and incubated for specific times (Table 2). After the time, 900 µL of ice-cold MEM was immediately added to the tube to rapidly cool the sample and stop any further heat inactivation. Samples were then kept on ice until titrating on fresh tissue culture cells to quantify viable virus. A non-heat-treated sample was used as a positive control. Cells were kept for 3 to 5 days after infection to monitor for evidence of CPE.

Growth and heat inactivation of bacteria

The bacteria of interest were *S* Typhimurium SL1344, *E coli* 0157:H7 (EC1647), *S suis* strain 89-1591, APP AP37 (serotype 1), and *B hampsonii* clade 2 clinical isolate. Each bacterial strain was grown according to established protocols. Briefly, an agar plate (Luria broth agar for *S* Typhimurium and *E coli*; tryptone soya agar with 5% sheep blood for *S suis*; and PPLO agar + 1% IsoVitaleX for APP) was streaked out with bacteria of interest from a -80°C glycerol stock and incubated overnight at 37°C. A single colony was picked from the agar plate and grown in 10 mL of selective growth media (Luria broth for *S* Typhimurium and *E coli*; Todd Hewitt broth for *S suis*; and PPLO broth for APP) overnight (approximately 16 hours) at 37°C and shaken at 200 rpm. The bacteria were subcultured at 1:100 or 1:50 into 10 mL fresh media and grown at 37°C and shaken at 200 rpm for 1.5 to 3 hours until the desired optical density of 600 nm (OD600) was achieved. *Brachyspira hampsonii* bacteria were propagated by streaking out approximately 10 µg of feces or intestinal contents onto BJ and CVS agar plates. Plates were then incubated anaerobically using a commercial system (Anaerogen, Oxoid Limited) at 42°C for 48 hours.

After the desired OD600 was achieved, the bacteria of interest were exposed to 5 temperatures (5° increments from 50°C to 70°C). For each temperature point, 11 tubes (one for each time point), each containing 90 µL of media, were preheated in a heating block set at the desired temperature. After 10 µL of bacteria was added to each tube, the set temperature point was applied for 1, 2, 3, 4, 5, 10, 15,

Table 2: Summary of pathogen inactivation in vitro using known concentrations of virus and bacterial stocks

	Time to inactivate, min						
Pathogen	**80°C**	**75°C**	**70°C**	**65°C**	**60°C**	**55°C**	**50°C**
PEDV	< 1	15	15	45	> 60	> 60	> 60
PRRSV	< 1	< 1	< 1	5	10	60	> 60
SIV	< 1	< 1	< 1	< 1	< 1	3	60
TGEV	< 1	< 1	< 1	< 1	10	30	30
Rotavirus	< 1	< 1	< 1	< 1	< 1	15	15
Streptococcus suis	--	--	< 1	3	3	10	45
Actinobacillus pleuropneumoniae	--	--	< 1	< 1	< 1	< 1	4
Escherichia coli	--	--	< 1	2	3	10	60
Salmonella Typhimurium	--	--	< 1	10	NA*	15	60
Brachyspira hampsonii	--	--	< 1	< 1	< 1	2	15

* No colonies were seen at this temperature.

PEDV = porcine epidemic diarrhea virus; PRRSV = porcine reproductive and respiratory syndrome virus; SIV = swine influenza virus; TGEV = transmissible gastroenteritis virus.

20, 30, 45, or 60 minutes, respectively. At completion, 900 µL of ice-cold medium was added to each tube to rapidly cool the contents. The tubes were incubated on ice until plating. Each suspension was serially diluted 1/10 in medium and then 100 µL of each dilution was spread onto agar. Plates were incubated at 37°C overnight and colonies were counted and recorded. For *B hampsonii*, positive cultures were indicated by zones of strong β-hemolysis. A non-heat-treated sample was also included for each temperature point to serve as a reference sample.

Heat treatment of PEDV samples from clinically infected piglets

In a previous study performed at VIDO-Intervac containment level 3 laboratory, fecal samples from PEDV-infected piglets were collected post mortem, so no additional animal ethics protocol was required for this study. The samples were diluted 1:1 with sterile PBS to make them pipettable. Clinical samples were then diluted 1:5 in cell culture medium and incubated in the heating block for each time point as previously described. Temperatures tested were in 5° increments from 55°C to 80°C for a total of 6 temperatures. Samples were incubated with Vero76 cells in the presence of TPCK-Trypsin as previously outlined and virus was allowed to attach for 1 hour at 37°C before inoculum was removed and replaced with fresh culture medium. Cells were incubated for 2 days and then scraped and frozen. Viral lysate was then passaged once more on fresh Vero76 cells to monitor for CPE. The clinical samples were pooled and diluted as previously described for use as positive control without heat treatment.

Assessing insulating capacity of fecal matter

Fecal material was collected from healthy animals. Thirty grams were formed into a large mass and packed into a fabricated aluminum corner, similar to a normal corner in a transport trailer. This was termed an "exposed environment." The aluminum corner (30 × 30 × 30 cm) was fabricated by the Department of Engineering at the University of Saskatchewan. An OM-EL-USB-TC thermocouple data logger (Omega Engineering Inc) was inserted into the center of the fecal mass and a second logger was mounted on the exterior of the corner to monitor ambient air temperature. The aluminum corner was added to the prewarmed 80°C oven and the temperature rise was monitored overnight for 14 hours.

To account for the thermodynamic effect of water in the feces, a more closed system was used to better monitor the insulating capacity. This was termed a "covered environment." For this, 30 g of feces was packed into a 50 mL conical tube and the thermocouple was inserted into the center of the mass. The 50 mL conical tubes were tightly wrapped in aluminum foil to minimize evaporation. The oven was again set to 80°C and the ambient and internal fecal temperature was monitored for 14 hours.

Assessment of survival of pathogens inside fecal matter

Fecal matter was serially diluted alone and in the presence of 100 ug/mL of Baytril for testing in tissue culture, but even at very low concentration, it was found to be toxic to the Marc145 cells (data not shown). Thus, in order to assess survival of viral pathogens inside fecal matter, 10 µL of PRRSV at 5.0×10^6 $TCID_{50}$/mL was added to MEM for a total of 100 µL volume inside a thin walled 0.2 mL polymerase chain reaction (PCR) tube. The tube was inserted into the center of a 10-g fecal mass. A thermocouple was inserted beside the tube and the feces packed down to minimize air pockets. The conical outer tube was sealed to prevent evaporation. The oven was heated to 80°C from room temperature and samples were removed at 15, 30, 60, and 120 minutes. Following heating, the PCR tube was removed from the fecal mass and kept on ice until serially diluted on tissue culture cells. Tissue culture cells were monitored for 5 days post infection for CPE. This experiment was repeated three times.

To assess survival of bacterial pathogens inside fecal matter, 10 µL of an *S suis* suspension concentrated at approximately 1×10^8 colony forming units/mL was added to 90 µL 0.1M PBS (Mg^{2+} and Ca^{2+} free) for a total of 100 µL volume inside a thin walled 0.2 mL PCR tube. A thermocouple was inserted in the center of the 10-g fecal clump, as well as the PCR tube containing the bacteria, and was covered and packed down to minimize air pockets. The conical outer tube was covered tightly with aluminum foil to prevent evaporation. The oven was heated to 65°C, 70°C, 75°C, or 80°C before the addition of the samples and the rise in temperature within the sample was tracked for 15 minutes. Following heating, the PCR tube was removed from the fecal mass and kept on ice until serial dilution on selective agar growth plates. Bacterial colonies were counted the next day. Triplicate samples were used for each temperature point so that the bacterial counts could be monitored after 5, 10, and 15 minutes of heat treatment (the 65°C temperature point was not in triplicate and only had a 15-minute sample). The experiment was later repeated using a 45-minute heat treatment with bacterial counts being examined after 5, 10, 15, 30, and 45 minutes of heat treatment. A non-heat-treated sample was also included for each temperature point to serve as a reference sample.

Results

Time and temperature required for pathogen inactivation in cell culture

The results from these experiments are summarized in Table 2. When tested, certain viruses proved more heat labile than others. For example, SIV was inactivated in culture after only 3 minutes at 55°C, while TGEV and PRRSV were inactivated after 5 minutes at 65°C. The hardiest virus proved to be PEDV, surviving 15 minutes at 75°C in cell culture (Table 2). All tested viruses were completely inactivated after 2 minutes at 80°C. As bacteria are more sensitive to heat inactivation in general, all bacteria were inactivated after 1 minute at 70°C (Table 2). Lower temperatures could inactivate the bacteria if the heating time was increased. For example, all bacteria were inactivated after 15 minutes at 55°C. Under laboratory conditions and using purified pathogen stocks, 15 minutes at 75°C or 2 minutes at 80°C were sufficient to inactivate all selected pathogens in cell culture.

Heat treatment of PEDV samples from clinically infected piglets

In order to look at more field-relevant specimens, we used frozen fecal samples from PEDV clinically infected piglets. These samples were not separated before freezing and contained typical intestinal content including fecal matter, dead enterocytes, microbiota, gut enzymes, etc. The PEDV titers in these piglets was unknown and samples were obtained after all piglets succumbed to challenge. Using the same temperatures and heating times as described above, all viral particles in these samples were completely inactivated after 15 minutes at 75°C. Positive-control (non-heated) samples were positive for CPE.

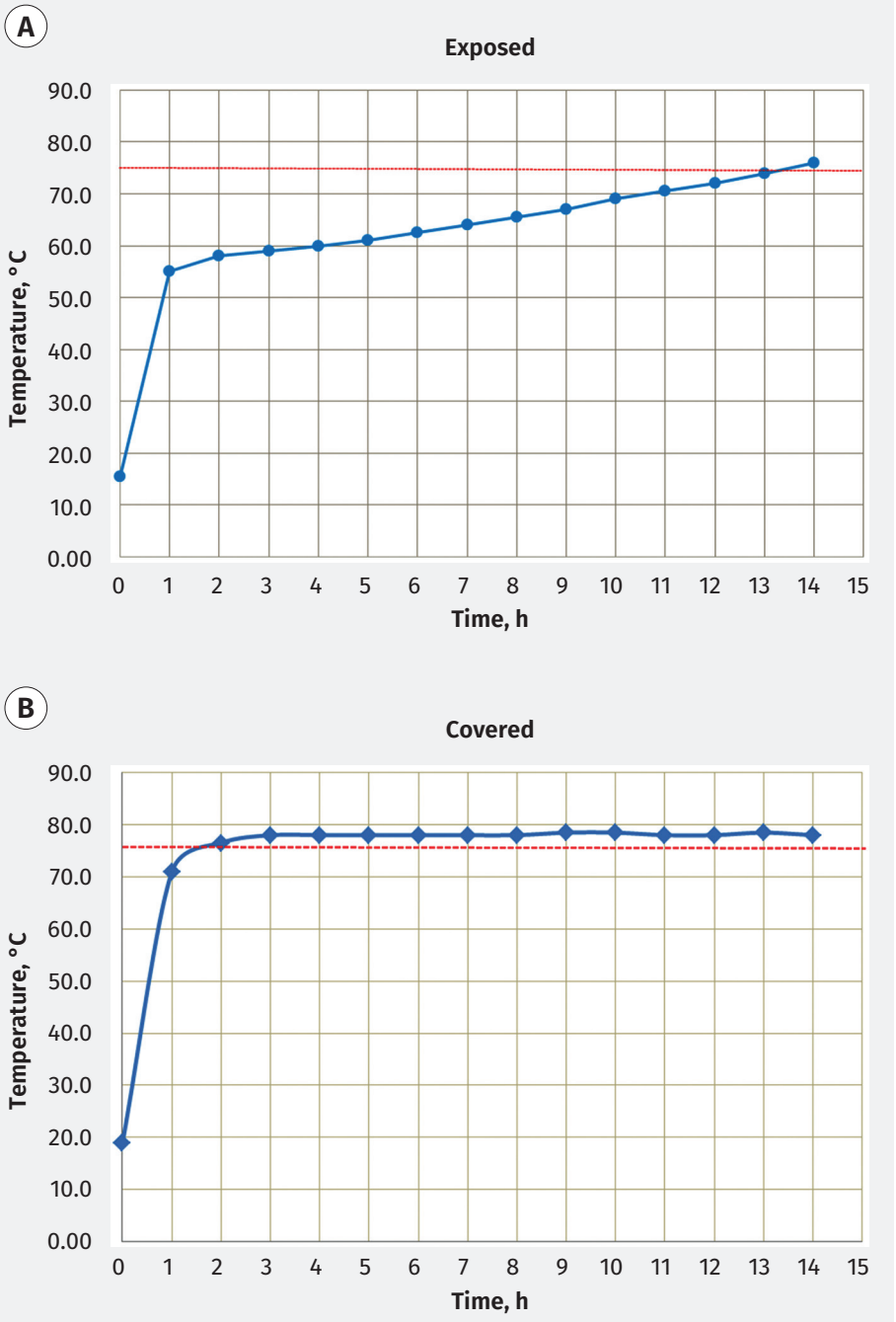
Insulating capacity of fecal matter

To simulate field conditions as much as possible, we added biological material to our experiments. Such material is usually present on commercial transport trailers in the form of feces and animal bedding. If trailers are not properly cleaned, biological material remains hidden behind lights, gates, or in corners. To determine the insulating effect of such material, a 30-g fecal mass was exposed to 80°C temperatures, in open air, for 14 hours with thermocouple data loggers monitoring temperature both inside the mass as well as ambiently. The internal temperature in this exposed sample rose very slowly and reached the desired temperature of 75°C after 14 hours (Figure 1A). The dried outer layer acted as very effective insulation and prevented a rise in the internal temperature. In contrast, the ambient temperature in the oven was able to reach 80°C within approximately 22 minutes from a cold start.

To further investigate the effect of fecal drying, we tested the same amount of fecal matter, 30 g, in a more closed system. The fecal matter was placed in a sealed tube to prevent water evaporation. As expected, the temperature rose internally much faster than in the exposed system. Beginning with a cold oven, internal temperature reached 78°C within 2.33 hours and was maintained until the oven was turned off (Figure 1B). We then reduced the amount of fecal matter to 10 g, as this may represent a more feasible amount of manure left behind post trailer cleaning. It was found that after 15 minutes at 80°C, the internal fecal mass temperature was not above 67.5°C. This temperature would not be high enough to inactivate some of the viruses tested in this study. Next, we placed a small thin-walled PCR tube containing a viral PRRSV suspension inside the fecal matter. When heated from room temperature up to 80°C, total virus inactivation only happened after 30 minutes, which corresponded to an internal temperature > 70°C (Table 3).

In contrast, inactivation of bacteria in these samples occurred faster and at lower temperatures. Heating the samples for 15 minutes (blue line, Figure 2) in an oven set to 75°C or 80°C was sufficient for inactivation of the bacteria, as the internal temperature within the fecal matter was able to briefly reach temperatures above 60°C. This matches our previous data where *S suis* needed 3 minutes at 60°C for inactivation (Table 2). However, when samples were heated for 15 minutes in the oven set to 65°C or 70°C, the bacteria were not inactivated. The internal temperature within the fecal matter never reached 60°C. The fecal matter temperature reached the required minimum threshold of 55°C, but not for the necessary 15 minutes for inactivation to occur (lower red line, Figure 2).

Figure 1: A) Time for internal temperature of 30 g of porcine feces to reach 75°C (red dash line) in an exposed environment. B) Time for internal temperature of 30 g of porcine feces to reach 75°C (red dash line) in a covered environment.



Since we did not expect that the addition of fecal matter would provide so much heat protection, we repeated the experiments with *S suis*, the most heat stable bacterium. This time, we included a 45-minute heat treatment. After 45 minutes (grey lines, Figure 2), all bacteria were inactivated regardless of ambient temperature studied. It is interesting to note that temperatures within the fecal matter only reached 75°C when the ambient temperature was at 80°C for

Table 3: Heat inactivation of PRRSV at 15, 30, 60, and 120 minutes inside 10 g fecal matter*

		Internal fecal matter temperature, °C			Positive CPE,† %		
Time, min	**Ambient air temperature, °C**	**Exp 1**	**Exp 2**	**Exp 3**	**Exp 1**	**Exp 2**	**Exp 3**
15	75	45	45.5	47	100	100	100
30	80	64	69.5	63.5	0	100	0
60	80	75	77	75.5	0	0	0
120	80	76.5	77.5	77.5	0	0	0

* The PRRSV titer was 5.0×10^6 $TCID_{50}$/mL.

† Positive CPE is reported as the percent of positive wells out of 8. The experiment was repeated 3 times.

PRRSV = porcine reproductive and respiratory syndrome virus; CPE = cytopathic effect; Exp = experiment.

Figure 2: Temperature logs tracking the rise in temperature within the 10 g fecal sample when it is placed in an oven and heated at A) 65°C, B) 70°C, C) 75°C, or D) 80°C respectively for 15 (blue line) or 45 (grey line) minutes. The red lines at 55°C and 75°C represent the threshold temperatures that the sample would need to reach for bacterial and viral inactivation, respectively.

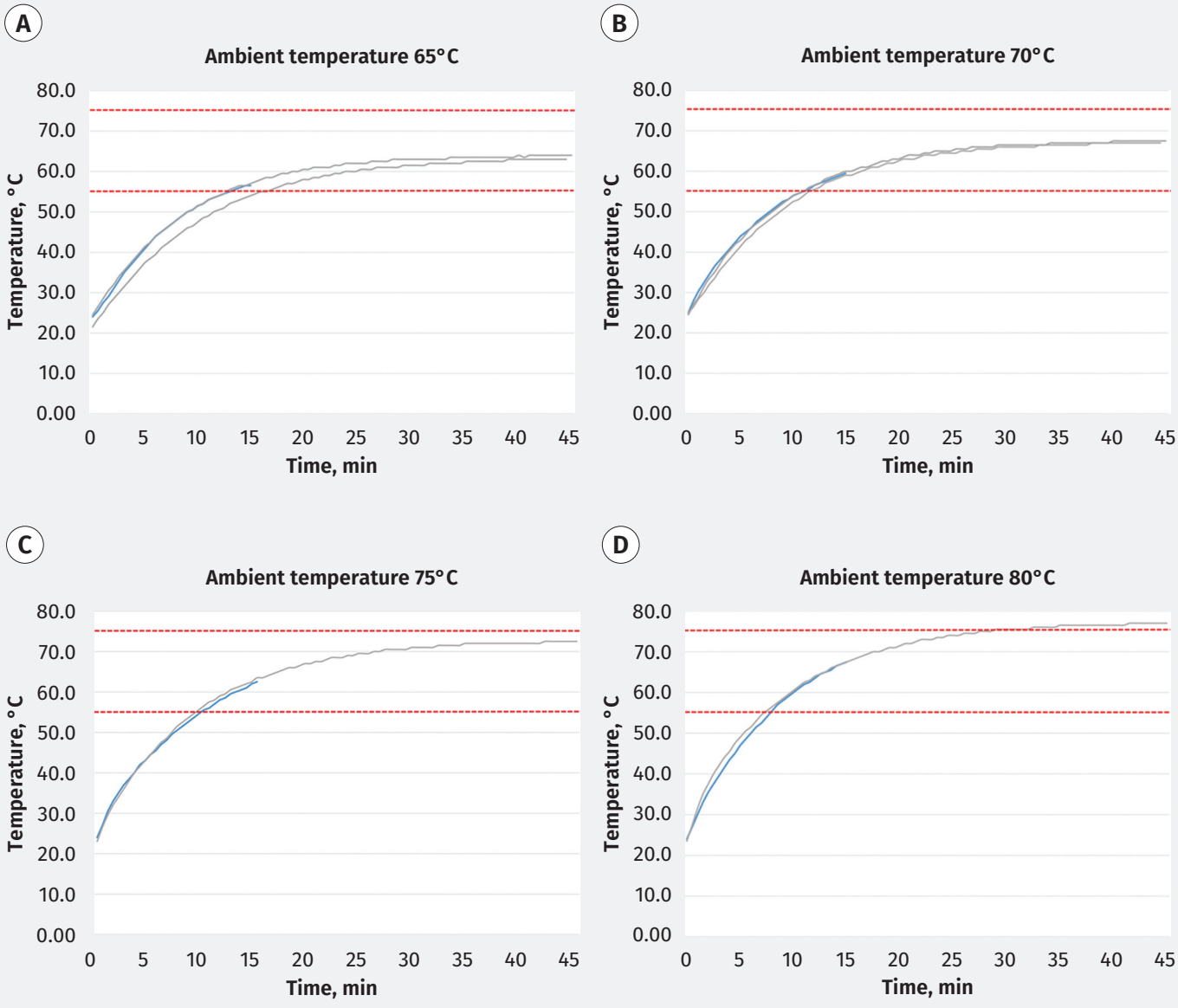


Table 4: Heat inactivation of *Streptococcus suis* at 5, 10, 15, 30, and 45 minutes inside 10 g of fecal matter*

		Fecal matter temperature,† °C			Bacterial growth‡		
Ambient temperature, °C	**Time, min**	**Exp 1**	**Exp 2**	**Exp 3**	**Exp 1**	**Exp 2**	**Exp 3**
80	45	NA	77.0	NA	NA	-	NA
	30	NA	75.5	NA	NA	-	NA
	15	67.5	67.5	NA	-	+	NA
	10	60.0	60.5	NA	+	NA	NA
	5	47.0	49.0	NA	+	NA	NA
75	45	NA	72.5	NA	NA	-	NA
	30	NA	71.0	NA	NA	-	NA
	15	62.5	63.5	NA	-	+	NA
	10	55.5	56.5	NA	+	NA	NA
	5	44.5	44.5	NA	+	NA	NA
70	45	NA	67.0	67.5	NA	-	-
	30	NA	66.0	66.5	NA	-	-
	15	59.5	59.0	60.0	+	+	+
	10	54.0	52.5	54.0	+	NA	NA
	5	44.0	41.5	43.0	+	NA	NA
65	45	NA	63.0	64.0	NA	-	-
	30	NA	61.5	63.0	NA	+	-
	15	56.5	54.0	57.0	+	+	+
	10	51.5	47.5	51.5	NA	NA	NA
	5	41.0	37.5	41.5	NA	NA	NA

* The concentration of *S suis* was 4×10^6 to 2×10^7 colony forming units/mL.

† The experiment was repeated up to 3 times for certain temperature points.

‡ Positive growth is reported as the presence of bacterial colonies on the plate.

NA = this time and temperature combination was not assessed.

30 minutes. This is critical for the complete inactivation of virus (upper red line, Figure 2). Table 4 outlines the fecal matter temperature in comparison to the ambient temperature at the time points of interest. It also shows whether the bacteria were inactivated or not. At the 80°C 15-minute, 75°C 15-minute, and 65°C 30-minute intervals, there is inconsistency in whether the bacteria were completely inactivated. This illustrates that the amount of fecal matter present can significantly affect the inactivation of bacteria. Both adequate time and temperature must be reached, and the introduction of fecal matter greatly impacts this.

Discussion

The aim of this study was to test the most optimal time and temperature for inactivation of a cross section of pathogens (Table 1) to develop improved biosecurity in livestock transport. Current decontamination protocols call for washing for 2 hours followed by overnight (8 hours) drying.[9-12,21,23,32]

It is known that many pathogens, bacterial, viral, and parasitic, can survive outside of a host in various environmental conditions.[11,12,15,17,23,25,26] In general, non-enveloped viruses, as well as most bacterial species, can persist in the environment for longer periods of time.[20,23,27-30] Our study investigated several porcine pathogens to find a time and temperature that would cover most major microbes relevant to swine health. Porcine epidemic diarrhea virus has been of utmost concern to hog producers following recent outbreaks in the United States and Eastern Canada. Being an enveloped RNA virus, PEDV is more fragile in the environment. Under certain conditions, however, the virus can survive on fomites and in organic matter.[2,6-9,15] Current TADD protocols stipulate 10 minutes at 71°C, which may not be enough to fully inactivate PEDV on a transport trailer.[2-6,9] We also investigated TGEV, another enveloped coronavirus and thus does not survive outside the host for long periods of time. It is easily killed by heat and sunlight but is resistant to freezing, thus outbreaks in cold weather are more common.[12] Porcine reproductive and respiratory syndrome virus is also an enveloped virus and does not survive long outside the host unless covered in organic materials.[13,17,18,33] It is quite heat labile but, like TGEV, survives well in temperatures below 20°C.[7] Swine influenza virus is known to be very labile in the environment, as with all enveloped influenza viruses. It

survives longer in cooler temperatures but is rapidly inactivated in sunlight or heat greater than 56°C.[19] Rotavirus, because it is non-enveloped, survives for extended periods in the environment and even longer in manure or manure contaminated environments.[13,20,25] Rotavirus is, however, highly susceptible to heat, and ultraviolet and gamma irradiation.[20,27]

Especially at temperatures below 75°C, PEDV exhibited less consistent inactivation. Because the PEDV stock used in our experiments was a cellular lysate, the variablity in inactivation may be partially explained by the intermittent lysing of cells as the temperature increased, releasing new, previously cell-associated, virus into the medium. It may also be due to heat inactivation of the TPCK-trypsin needed to have an active PEDV infection in vitro. The inactivation of PEDV in our clinical samples appeared to happen at much lower temperatures and less time, ie, 15 minutes at 50°C. Because viral load in these samples was unknown, it may have been significantly lower than that of the previously tested 2.4×10^8 copies/mL. Previous studies have shown 100% PEDV inactivation when surfaces were heated to 71°C for 10 minutes or kept at room temperature (20°C) for 7 days but variable inactivation at all temperatures in between.[2,7-9,15] This would also support our finding that heat inactivation is dose-dependent and as such, trailers contaminated with high amounts of virus from actively shedding animals, would require sufficient heat treatment to ensure all virus is inactivated. Emerging diseases such as African swine fever, Senecavirus A, and foot-and-mouth disease virus have been demonstrated to spread via fomites and infected animal products used as feed. We propose this heating protocol would also be effective against these pathogens in combination with disinfection.[11,34,35] Although heating processes alone in extruding have been demonstrated to kill classical swine fever virus , they may be higher than what is possible in a transport trailer.[11]

Generally, we demonstrated that the bacterial pathogens tested were rendered nonviable at lower temperatures and less time than the viruses. A contributing factor in swine dysentery, *Brachyspira* are anaerobic, but can survive for several days to a few weeks within a permissible environment.[12,25] It is transmitted directly through contaminated feces of both sick and asymptomatic pigs, although indirect contact with contaminated fomites can also be a source of transmission.[25] *Brachyspira* are highly susceptible to high temperatures and drying.[24]

The final bacteria tested in our study was APP. It is a gram-negative bacteria that causes highly contagious respiratory disease with high morbidity and mortality rates making it of particular concern to hog producers.[15,36] It is also a major component of the porcine respiratory disease complex along with *S suis* and PRRSV.[13,17,26,31]

Our data suggest that to effectively kill both bacterial and viral pathogens, the trailers need to reach a consistent temperature of 75°C for 15 minutes in all regions of the trailer. Preliminary data collection using current trailer baking protocols showed that the temperature varied widely across the span of the transport unit leaving "pockets" of potentially infectious material. Swine producers have expressed concerns over the presence of residual biological material (bedding, feces) in the trailers and how that may affect the heat inactivation of pathogens. Indeed, anecdotal reports of fist-sized masses of pig manure falling out of lights after trailers had been washed indicated a need to test the insular capacity of fecal matter. Our findings indicated that presence of large amounts of biological materials, ie, more than 10 g, may reduce the effectiveness of the heat inactivation. As with chemical disinfection, presence of biological material will hamper heat treatment as the insulating properties of feces and bedding are very high. Thorough cleaning of trailers is necessary and must be consistent for the heating process to be effective. Even with intensive cleaning practices, heating to 75°C for 15 minutes should be used to ensure pathogen inactivation. It should be noted that our study did not examine the use of biocides or disinfectants[5,10,19,21,25] routinely used in trailer washing, nor did we examine biofilms as this would have serious implications for cleaning and disinfection practices.[5,22,23,28-30] Our main limitation was not having access to trailers. From a biosafety perspective, we could not take these pathogens out of a laboratory setting to test responses in the field. This work was meant to mimic field conditions as best as possible but may not reflect actual conditions. We also did not examine extreme temperatures found seasonally in Canada. These aspects should be investigated in further studies.

Implications

Under the conditions of this study:

- Heating to 75°C for 15 minutes was sufficient to kill pathogens tested in cell culture.
- All areas of the trailer need to reach 75°C for 15 minutes to be compliant.
- Disinfectant use was not examined, nor was presence of biofilms.

Acknowledgments

This research was supported by Swine Innovation Pork, part of Agriculture and Agri-Food Canada. Published as VIDO-InterVac manuscript series No. 891.

Conflict of Interest

None reported.

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Serologic monitoring of herds with and without bacterin vaccination for *Actinobacillus pleuropneumoniae*

Hugo Dunlop BVMS, PhD, Steven McOrist BVSc PhD

Summary

Objective: Investigate diagnostic serology for *Actinobacillus pleuropneumoniae* (APP) infections in naturally infected and vaccinated pigs.

Materials and methods: The APP status of 12 farms (A-L) was established by lung cultures and isolate serotyping. Screening enzyme-linked immunosorbent assay (ELISA) detected antibodies to ApxIV antigen or multiple APP serotypes. Serotype-specific ELISAs were conducted for serotypes 5 and 7.

Seven groups of farm F pigs (serotype 7) were moved to farm K (serotype 5). Autogenous vaccines (V1/V2) prepared from APP serotype 5 cultures from farm K and a commercial, killed APP vaccine (V3) containing serotypes 1, 7, and 15 were used to vaccinate pigs in each group twice or thrice at 3-week intervals, commencing at 10 weeks of age. Blood samples were analyzed with ELISAs specific for serotype 5 and ApxI and ApxII toxins. Serum titers were compared using an analysis of variance.

Results: Serotypes 5, 7, 12, or 15 were present in lung cultures. The ApxIV screening ELISA and mix-serotype ELISA regularly detected serotypes 5, 7, and 15. Serotype 12 infections were detected in the mix-serotype ELISA, but not in the ApxIV assays. The serotype 5 or 7 specific ELISA regularly detected herd infections with the relevant serotype.

Serotype 5 titers of pigs vaccinated with V1/V2 thrice were higher than those dosed twice with the equivalent volume ($P < .05$). Pigs receiving V3 showed no serotype 5 antibody response. The ApxI and II titers in V1/V2-vaccinated pigs were higher than controls.

Implications: Screening and serotype-specific ELISAs verified APP status. Repeated serotype-specific autogenous APP vaccine doses provided a strong antibody response.

Key words: swine, *Actinobacillus pleuropneumoniae*, ELISA serology, autogenous vaccination

Received: May 4, 2020
Accepted: July 20, 2020

Resumen - Seguimiento serológico de piaras con y sin vacunación con bacterina para *Actinobacillus pleuropneumoniae*

Objetivo: Investigar la serología diagnóstica de las infecciones por *Actinobacillus pleuropneumoniae* (APP) en cerdos infectados naturalmente y vacunados.

Materiales y métodos: El estado de APP de 12 granjas (A-L) se estableció mediante cultivos de pulmón y serotipificación de aislamientos. El monitoreo mediante el ensayo de inmunoadsorción ligado a enzimas (ELISA) detectó anticuerpos contra el antígeno ApxIV o múltiples serotipos de APP. Se realizaron ELISA de serotipos específicos para los serotipos 5 y 7.

Siete grupos de cerdos de la granja F (serotipo 7) se trasladaron a la granja K (serotipo 5). Se utilizaron vacunas autógenas (V1/V2) preparadas a partir de cultivos de APP de serotipo 5 de la granja K y una vacuna comercial de APP muerta (V3) que contenía los serotipos 1, 7, y 15 para vacunar a los cerdos de cada grupo dos o tres veces a intervalos de 3 semanas, comenzando a las 10 semanas de edad. Las muestras de sangre se analizaron con ELISA específicas para el serotipo 5 y las toxinas ApxI y ApxII. Los títulos de suero se compararon mediante un análisis de varianza.

Resultados: Los serotipos 5, 7, 12, o 15 estaban presentes en cultivos pulmonares. La ELISA de monitoreo de ApxIV y la ELISA de serotipo mixto detectaron consistentemente los serotipos 5, 7, y 15. Se detectaron infecciones por el serotipo 12 en la ELISA de serotipo mixto, pero no en las pruebas de ApxIV. La ELISA específica de serotipo 5 o 7 detectó regularmente infecciones en la piara con el serotipo relevante.

Los títulos del serotipo 5 de los cerdos vacunados tres veces con V1/V2 fueron más altos que los que recibieron dos dosis con el volumen equivalente ($P < .05$). Los cerdos que recibieron V3 no mostraron respuesta de anticuerpos del serotipo 5. Los títulos de ApxI y II en cerdos vacunados con V1/V2 fueron más altos que los controles.

Implicaciones: El monitoreo y las ELISA específicas de serotipo confirmaron el estatus de APP. Las dosis repetidas de vacuna de APP autógena específica de serotipo proporcionaron una fuerte respuesta de anticuerpos.

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

Dunlop H, McOrist S. Serologic monitoring of herds with and without bacterin vaccination for *Actinobacillus pleuropneumoniae*. *J Swine Health Prod*. 2021;29(1):29-36.

Résumé - Suivi sérologique pour *Actinobacillus pleuropneumoniae* de troupeaux avec et sans vaccination avec une bactérine

Objectif: Examiner le diagnostic sérologique pour l'infection par *Actinobacillus pleuropneumoniae* (APP) chez des porcs naturellement infectés et des porcs vaccinés.

Matériels et méthodes: Le statut pour APP de 12 fermes (A-L) fut établi à la suite de cultures de poumons et de sérotypage des isolats. Une épreuve immuno-enzymatique (ELISA) de tamisage détecta des anticorps contre l'antigène ApxIV ou de multiples sérotypes d'APP. Une épreuve ELISA spécifique de sérotype fut effectuée pour les sérotypes 5 et 7.

Sept groupes de porcs de la ferme F (sérotype 7) furent déménagés à la ferme K (sérotype 5). Des vaccins autogènes (V1/V2) préparés à partir d'APP sérotype 5 obtenu de la ferme K et un vaccin tué commercial (V3) contenant les sérotypes 1, 7, et 15 furent utilisés pour vacciner les porcs dans chaque groupe deux ou trois fois à 3 semaines d'intervalle, commençant à 10 semaines d'âge. Des échantillons sanguins furent analysés par ELISA spécifique pour le sérotype 5 et les toxines ApxI et ApxII. Les titres sériques furent comparés en utilisant une analyse de variance.

Résultats: Les sérotypes 5, 7, 12, ou 15 étaient présents dans les cultures effectuées à partir des poumons. Le tamisage par ELISA pour ApxIV et ELISA pour sérotypes multiples permit de détecter régulièrement les sérotypes 5, 7, et 15. Les infections par le sérotype 12 furent détectées par ELISA pour sérotypes multiples, mais pas par le test pour ApxIV. L'ELISA spécifique pour les sérotypes 5 ou 7 détecta régulièrement des infections dans les troupeaux avec le sérotype approprié.

Les titres envers le sérotype 5 chez les porcs vaccinés avec V1/V2 trois fois étaient plus élevés que ceux chez les animaux vaccinés deux fois avec un volume équivalent ($P < .05$). Les porcs recevant V3 n'ont présenté aucune réponse en anticorps contre le sérotype 5. Les titres envers ApxI et II chez les porcs vaccinés avec V1/V2 étaient plus élevés que chez les témoins.

Implications: Les épreuves ELISA de tamisage et spécifique de sérotype ont vérifié le statut pour APP. Des doses répétées de vaccin APP autogène spécifique de sérotype ont conféré une forte réponse en anticorps.

Acute porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae* (APP) remains a major clinical problem, particularly in European, Latin American, and Australasian herds; subclinical infections are also common globally. There are 18 recognised APP serotypes, based on their capsule polysaccharide composition.[1,2] These serotype-specific capsule antigens are key factors in the host immune reaction, but without providing significant heterogenous cross-protection.[1] The prevalence of various APP serotypes varies globally, with serotypes 2 and 9 more common in Europe[3] and serotypes 5, 7, and 12 more common in North America and Australia.[4,5] Isolates of serotypes 1, 5, and 9 are considered more pathogenic than others due to their greater expression of the tissue-destroying ApxI and ApxII exotoxins.[6] However, infections with serotype 1 are now less common due to its eradication from many breeding company herds.[4] Infections with APP serotype 5 and 9 are therefore considered of greatest current concern.

Serology is the preferred method for APP surveillance and detection of subclinical infections in pig herds, with established commercial assays available globally. Currently, there are three established antigen formats for APP serology tests. First, a serotype-specific test based on the individual serotype's long-chain lipopolysaccharide (LPS) "O" antigen[7] and second, a test based on the ApxIV exotoxin antigen,[8] which is APP-specific but cannot differentiate between serotypes. The second test is therefore aimed at screening pigs for their APP status. A further screening test has also been established commercially in the first format, by using a pool of long-chain LPS antigens from various serotypes. A third type of test format based on the ApxI or ApxII toxin antigens has also been established,[9] but is considered less specific for APP infection, and therefore less useful for herd screening. Previous studies have used these serologic tests for exploration of APP epidemiology, individual herd status, and response to vaccination.[10-12] Blood samples taken from late-stage finisher pigs (such as 16 to 20 weeks of age) are considered the most sensitive for detection of APP antibody responses representative of herd status.[4,10]

The pathogenesis of APP consists of the separate stages of colonization, resistance to clearance and damage to lungs.[13] Current vaccination programs for APP disease fall into different categories.[14] With knowledge of the APP serotype status of the herd, relevant serotype-specific bacterins can be supplied commercially or prepared as an autogenous vaccination program. These aim to prevent colonization. Subunit vaccines based on the major Apx exotoxin antigens have also been commercially developed and utilised. These aim to prevent tissue damage and can provide protection across APP serotypes. Other vaccine strategies for APP have been developed, such as live attenuated vaccines, but are yet to find wide acceptance.[14] One analysis suggested that 90% of global APP vaccination still occurs via the specific bacterin programs.[15]

In this study, we aimed to further characterize the serologic response for pig herds infected with major serotypes of APP and the response of pigs within a vaccination program for APP serotype 5.

Materials and methods

The Animal Ethics Care Committee of the State Government of Victoria approved the animal use and sampling protocols used in this study.

Case farms and APP status

Twelve separate pig finisher units A to L located across eastern Australia were selected for APP testing. These grower-finisher herds were characterised by their intake of 10-week-old grower pigs per week (Table 1). These herds were each derived from separate breeding/nursery herds either on the same site or under the same management system. The herds A to K had all suffered occasional outbreaks of clinical APP disease, but mortality in these herds was consistently below 2% in the intake to slaughter interval over the study period. All herds incorporated routine vaccination programs for *Mycoplasma hyopneumoniae* infection, but no APP vaccination. The herds had been free of clinical signs associated with porcine reproductive and respiratory syndrome virus and pathogenic porcine circovirus type 2 for 5 years preceding and throughout this study, as

Table 1: Description of study farm *Actinobacillus pleuropneumoniae* (APP) status and APP ELISA results

Farm	APP culture & serotype*	Finisher herd size†	No. pigs examined‡	Pleurisy exam§	ApxIV serology§	APP 5 serology§	APP 7 serology§	APP mix serology§
A	12	800	36	5/36	0/36	0/36	0/36	5/36
B	12	200	27	1/27	0/27	1/27	0/26	5/26
C	15	480	25	0/21	5/25	0/25	0/25	7/25
D	7,15	800	28	2/28	28/28	1/28	8/25	25/28
E	7	240	29	1/29	8/29	0/29	22/29	16/29
F	7	800	20	0/20	12/20	1/20	3/20	19/20
G	7	400	16	3/16	11/14	0/16	16/16	12/15
H	5	400	30	11/30	23/30	28/29	1/29	28/30
I	5	600	16	5/16	11/15	6/14	0/14	2/16
J	5	800	13	6/13	12/13	12/13	0/13	5/9
K	5	180	20	2/20	17/20	16/20	0/20	20/20
L	ND	800	30	0/30	1/30	0/30	1/30	1/30

* APP culture and serotype identification derived from infected lungs harvested immediately prior to study period.

† Herd size expressed as the average number of pigs in each weekly intake batch of 10-week-old pigs.

‡ Pigs examined and blood collection for ELISA at scheduled slaughter at 23 weeks of age.

§ Results expressed as number of positive/number examined.

ND = none detected.

monitored by on-going necropsy, specific serology, and immunohistochemistry studies.

In addition to historical diagnostic results data, five fresh lung samples, some with noticeable lesions of pleuropneumonia, were collected from each farm either at on-farm necropsy or at scheduled slaughter (23 weeks of age). Bacteriologic culture, biochemical identification, and capsule serotyping for APP were performed on each lung sample using established methods [5] to confirm each herd's APP status immediately prior to the study period.

Blood samples were then collected from at least 13 pigs from each farm at scheduled slaughter (Table 1). Lungs from each pig were also examined visually for pleurisy lesions. Serum derived from each blood sample was stored in aliquots at -20°C then thawed and subjected to 4 separate commercial APP serologic assays: 1) indirect ELISA based on recombinant ApxIV antigen (Idexx APP ApxIV ab test; Idexx Laboratories Inc); 2) indirect ELISA based on extract of long-chain LPS antigen for APP serotypes 5 (Swinecheck APP 5a, 5b; Biovet Inc), 4, or 7 (Swinecheck APP 4, 7; Biovet Inc); 3) indirect ELISA based on a mix set of long-chain LPS antigens derived from pools of APP serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 15 (Swinecheck Mix APP 1-9-11, 2, 3-6-8-15, 4-7, 5, and 10-12; Biovet Inc).

The ELISA procedures were performed according to the manufacturer's instructions and were similar to those described previously.[7,8] Briefly, the appropriate dilution of the LPS or ApxIV antigen was determined by checkerboard titration in microtiter plates using 0.5M carbonate buffer. Plates coated with each antigen (50 µL/well) were incubated overnight at 4°C. Plates were then washed with phosphate buffered saline-0.05% Tween 20 (PBST) and blocked with PBST-1% bovine serum albumin (BSA) for 1 hour at 20°C. After washing with PBST, serum samples (diluted 1:100 with PBST-1% BSA) were added; plates were then incubated for 30 minutes at 37°C. After washing with PBST, peroxidase-conjugated rabbit anti-swine IgG (1:10,000; Rockland Immunochemicals Inc) was added and allowed to react for 30 minutes at 37°C. The plates were washed twice with PBST and a chromogenic solution was allowed to react for 30 minutes at 30°C. The optical density at 490 nm was measured with 650 nm as the reference. The ELISA titer = (sample value absorbance − negative reference absorbance) ÷ (positive reference absorbance − negative reference absorbance). Typical absorbances of the negative and positive reference sera were 0.00 to 0.07 and 1.0, respectively. Each ELISA was performed in batches incorporating identical reagents.

Vaccination programs and monitoring

Growing pigs selected for gilt development at 10 weeks of age (n = 164) were assembled and individually tagged on farm F (APP serotype 7 positive; Table 1) for premovement isolation and were later moved to farm K (APP serotype 5 positive; Table 1).

Seven groups, each with 20 to 30 pigs, within the farm F cohort of pigs were enrolled to assess serologic response to two serotype 5 bacterin vaccines and a commercial APP vaccine. The inoculation protocol for groups 1 to 6 (vaccinated) and group 7 (non-vaccinated controls) is outlined in Table 2. All 164 pigs remained in pens in one large barn enclosure, with *ad libitum* feed, water, and bedding. No antibiotics were administered to any pigs during the study period. While pigs remained healthy throughout, occasional pigs were removed during the study for non-study purposes.

Table 2: Inoculation protocol for each *Actinobacillus pleuropneumoniae* (APP) vaccination group*

	No. of pigs				
Vaccine group	**D0**	**D64**	**Dose, mL**	**No. of doses**	**Period between doses, wk**
1) Control	30	27	0	0	-
2) APP serotype 5 V1†	20	16	1	2	3
3) APP serotype 5 V1†	20	18	1	3	3
4) APP serotype 5 V1†	20	19	2	2	3
5) APP serotype 5 V1†	20	17	2	3	3
6) APP serotype 5 V2†	24	19	2	3	3
7) Commercial APP V3‡	30	27	2	3	3

* Seven groups of cohort pigs, each of individually tagged pigs. Test pigs were given vaccine doses on days 0 and 23; and on day 42 for groups 3, 5, 6 and 7.

† Each dose of V1 and V2 had 1×10^9 *A pleuropneumoniae*/mL.

‡ Each dose of V3 had $> 5 \times 10^8$ *A pleuropneumoniae*/mL.

V1, V2 = separate bacterin vaccines derived from APP serotype 5 isolates from farm K; V3 = commercial APP bacterin vaccine containing serotypes 1, 7 and 15 (Porcilis APPvac; Intervet Co).

For autogenous bacterin production, two APP serotype 5 cultures (V1 and V2) derived from farm K had been expanded and finally grown separately for 6 hours in 2 L culture vessels containing Tryptone yeast extract broth, with added nicotinamide adenine dinucleotide (10 µg/mL) and 5% vol/vol inactivated bovine serum. Each batch was then tested for potency (colony forming units/mL) and purity by cultures titrated onto routine aerobic and anaerobic plates. Each batch of pure culture was then inactivated with a final 0.2% vol/vol formalin, blended into the final vaccine strain and a commercial aluminum hydroxide gel adjuvant added at 500 µg/mL. Aliquots from each selected final batch were decanted into 100 mL bottles for use as specific autogenous bacterin vaccines in the assembled pigs.

A commercial, whole-cell, killed APP vaccine (V3; Porcilis APPvac; Intervet Co), stated to contain APP serotypes 1, 7, and 15 and commercial adjuvant, was purchased and used according to the manufacturer's recommendations.

Pigs in each group were dosed intramuscularly behind the ear with 2 mL of V3 vaccine ($> 5 \times 10^8$ APP/mL) or either 1 mL (single dose) or 2 mL (double dose) of V1 or V2 vaccine (approximately 1×10^9 APP/mL). Doses were given either twice or thrice at 3-week intervals, commencing at 10 weeks of age (Day 0 of the study period; Table 2).

Serologic evaluation of the vaccine study was conducted via response to APP serotype 5 LPS, and also to Apx toxins, as APP serotypes 1 and 5 are known to contain ApxI and II, whereas serotype 7 only contains ApxII.[5] Blood samples were collected from each pig at 10, 13, 16, and 19 weeks of age (Days 0, 23, 42, and 64). Serum from each sample was stored in aliquots at -20°C and incorporated into: 1) the indirect ELISA based on extract of long-chain LPS for APP serotype 5, 2) the indirect ELISA based on ApxI toxin antigen, and 3) the indirect ELISA based on ApxII toxin antigen. The procedures for the ApxI and II ELISAs were performed according to methods described previously.[9,16] Recombinant Apx toxin antigens were kindly provided by Dr Han Sang Yoo, of Seoul National University. Briefly, the plates were coated with respective antigen at 4°C overnight. Preliminary checkerboard titration results indicated that the optimal concentration of recombinant ApxI and ApxII antigen was 625 and 100 ng/well, respectively. Plates were washed with PBST after antigen coating and blocked with 10% horse serum for 2 hours at 37°C. After washing with PBST, serum samples (diluted 1:100 with PBST-1% BSA) were added, incubated for 2 hours at 37°C, followed by washing and incubation with peroxidase-conjugated goat anti-pig IgG (Rockland Immunochemicals Inc) for 30 minutes at 37°C. The plates were washed twice with PBST and a chromogenic solution was allowed to react for 30 minutes at 30°C. The optical density at 405 nm was measured with 650 nm as the reference. The largest differences between positive and negative controls were found with anti-pig IgG at a 1:1000 dilution (ApxI) or a 1:2000 dilution (ApxII). Each ELISA titer = (sample value absorbance − negative reference absorbance) ÷ (positive reference absorbance − negative reference absorbance). Typical absorbances of the negative and positive reference sera were 0.05 to 0.1 and 0.1 to 0.15, respectively. Each ELISA was performed in batches incorporating identical reagents.

Differences between serum titers at each blood collection point for each group of vaccinate or control pigs were compared using an analysis of variance.

Results

Serologic analysis of APP herds

The results of lung culture for APP and identification of APP serotypes identified in pigs on farms A to K are shown in Table 1, with the on-farm presence of APP serotypes 5, 7, 12, and 15 identified. These herd designations confirmed historical diagnostic sample results (data not shown). Only one herd (D) had an apparent dual serotype infection with serotypes 7 and 15. One herd (L) was apparently free of APP infection during the study period.

The results of testing with four separate APP ELISAs of blood samples collected from slaughter pigs from farms A to L are shown in Table 1. The results of lung examinations for visible lesions of pleurisy in these sampled pigs are also shown in Table 1. A greater proportion of sampled pigs with pleurisy was

noted in herds positive for APP serotype 5 compared to those infected with other serotypes.

Both the ApxIV ELISA and the APP mix-LPS ELISA regularly detected all apparent herd infections with 5, 7, and 15 serotypes, albeit with a variable ratio of 14% to 100% of blood samples analyzed. However, while the 2 farms identified as having APP serotype 12 infections were detected in the mix-LPS ELISA screen, these reactions were not detected in the ApxIV screen assays performed on the same sets of sera (Table 1). The ELISA employing LPS antigen specific to serotypes 5 or 7 regularly detected herd infections with the relevant serotype, albeit with a ratio of 15% to 100% of blood samples analyzed. Occasional single cross-reactions were detected with these assays in samples taken from pigs in uninfected herds or those herds infected with other serotypes (Table 1).

Vaccination monitoring

A summary of the results of ELISA testing for APP serotype 5 status and Apx toxin antibody status of each group of vaccinated pigs and control pigs from day 0 through day 64 is shown in Figures 1 and 2, respectively. The specific LPS-antigen titers of pigs vaccinated three times with autogenous APP serotype 5 were noticeably higher at day 64 than those dosed only twice with the equivalent 1 mL or 2 mL dose sizes (Figure 1). The analysis of variance indicated a significant difference at day 64 between the groups vaccinated three times with either autogenous serotype 5 strain V1 or V2 (groups 3, 5, and 6) and their titers at day 0 and the control group at day 64 ($P < .05$). The analyses of other vaccinated groups at day 64 and of all groups at other blood collection points indicated no significant difference in the ELISA titers detected from those at day 0 or from the control group.

The ApxI toxin antibody analysis indicated a noticeable anamnestic response within each group of the serotype 5 bacterin vaccine program (Figure 2; groups 2-6). The ApxII toxin antibody analysis indicated a limited response to all bacterin vaccines (Figure 2). Following subsequent movement of pigs to farm K, APP was not detected clinically or at post-mortem examinations of exposed vaccinates for 6 months.

Discussion

The current diagnosis of individual and herd status for APP is established via culture of lungs and serotyping of APP cultures and ELISA serology. Our study confirms the generally good diagnostic relationship between pleurisy lesions, lung cultures, and current ELISA serology techniques for a range of on-farm APP serotype infections. The long-chain LPS antigen ELISA methodology allowed accurate identification of herd status in all 12 farms examined. Analysis of sufficient samples is indicated to account for occasional cross-reactions. We established that the ApxIV antigen ELISA methodology also accurately predicted herd status, except for the two herds known to be infected with APP serotype 12. In contrast, a previous herd study[12] found some false positives with the ApxIV assay (case No. 7). Use of both the LPS and ApxIV antigen assays may be required to fully clarify the status of herds with APP serotype 12. While some APP serology studies have failed to accurately determine herd status with limited sample numbers,[10] other studies found that the combined use of LPS and ApxIV assays on sufficient sample sets was associated with accurate investigations of herd status.[4,12]

The pathogenesis of APP consists of the three separate stages of colonization, resistance to clearance, and damage to lungs.[13] Although all serotypes of APP are considered pathogenic, the lung examinations for pleurisy lesions supplemented the diagnostic information from culture and ELISA results confirming the greater extent of lesions likely to be seen with APP serotype 5 infections. This is considered to be due to greater expression of the Apx exotoxins,

Figure 1: Results of ELISA for *Actinobacillus pleuropneumoniae* (APP) serotype 5 antibodies in pig sera collected in APP vaccine study. The average and mid 50 percentile optical density readings are presented for pigs in each group at each time point following vaccination. Results for day 23 showed no significant differences to day 0 and are not presented. Group 1) Control non-vaccinated pigs; Group 2) Pigs received 1 mL doses of V1 twice; Group 3) Pigs receiving 1 mL doses of V1 thrice; Group 4) Pigs received 2mL doses of V1 twice; Group 5) Pigs received 2 mL doses of V1 thrice; Group 6) Pigs received 2 mL doses of V2 thrice; Group 7) Pigs received 2mL doses of V3 thrice. See Table 2 for group size and designation. V1 and V2 = bacterin vaccines derived from APP serotype 5 isolates from farm K; V3 = commercial APP bacterin vaccine (Porcilis APPvac; Intervet Co).

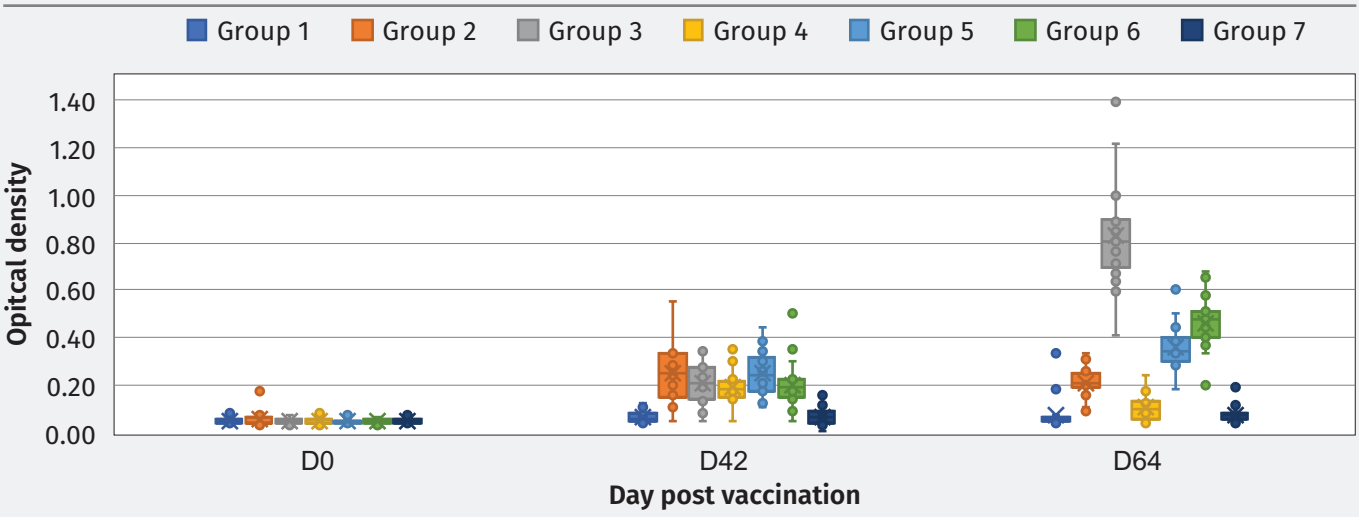
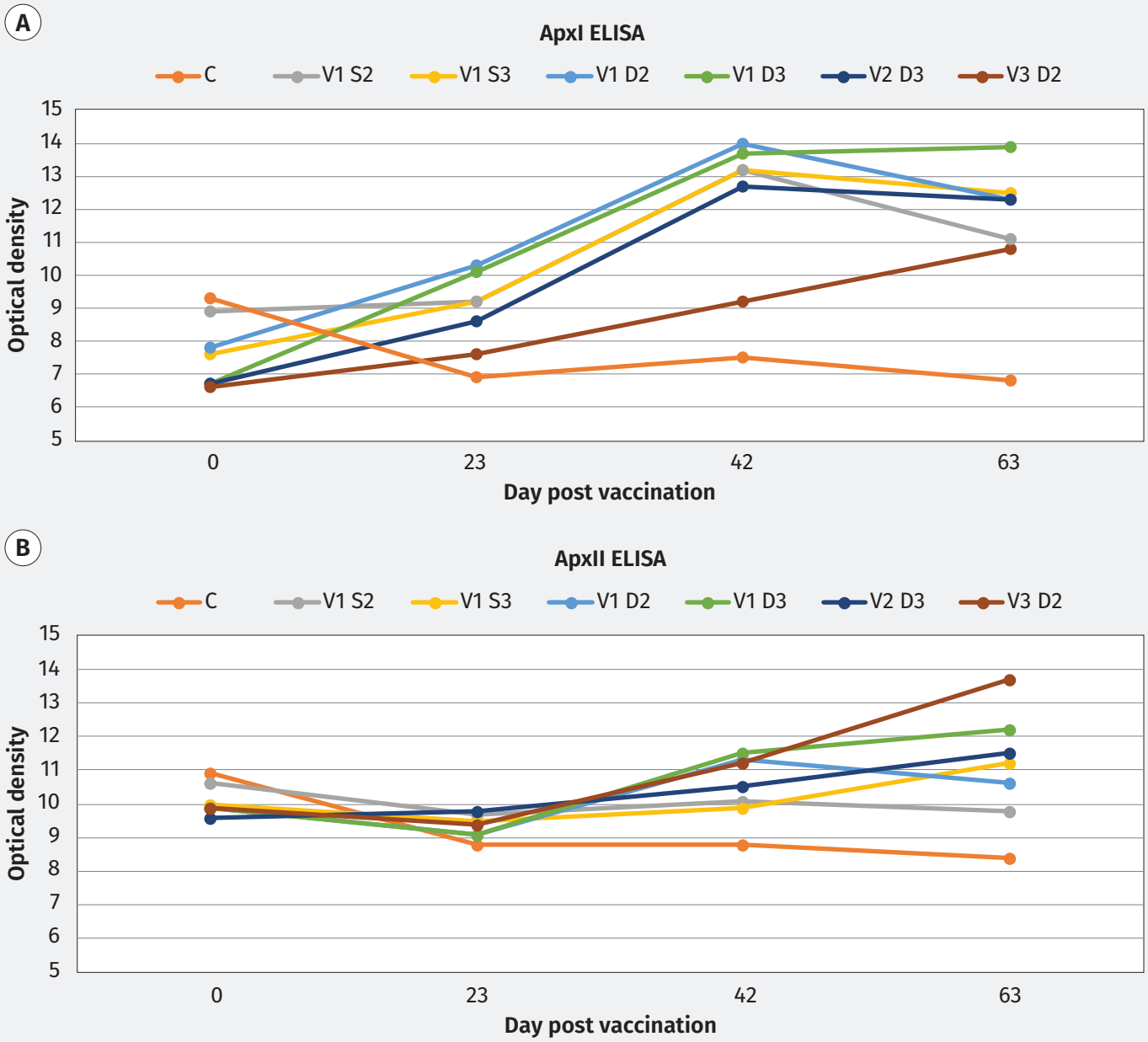


Figure 2: Results of ELISA for A) ApxI and B) ApxII antibodies in pig sera collected in *Actinobacillus pleuropneumoniae* (APP) vaccine study. The average optical density readings are presented for pigs (n = 20-30) in each group at each time point following vaccination. Standard deviations for each assay point were consistently less than 10% of the average. Pigs were vaccinated twice (S2, D2) or three times (S3, D3). See Table 2 for group size and designation. C = control non-vaccinated pigs; V1 and V2 = bacterin vaccines derived from APP serotype 5 isolates from farm K; V3 = commercial APP bacterin vaccine (Porcilis APPvac; Intervet Co); S = 1 mL dose; D = 2 mL dose.



particularly ApxI and ApxII.[6] While the pathogenicity of APP in terms of tissue damage varies according to serotype and Apx content, the number of animals infected within any particular herd may also vary according to serotype. Our study confirms that serologic monitoring of APP herd status is best achieved via approximately 30 samples, of which 3 or more clear positives leads to an accurate indication of APP status, as also suggested previously.[4]

Prevention or eradication of APP infection across serotypes has remained elusive, with novel strategies, such as live attenuated vaccines or outer membrane vesicle subunit toxoids, found to be ineffective.[14] Commercial subunit vaccines based on the Apx toxoids are effective at reducing the tissue damage phase, but do not appear to prevent the initial colonization stage. Their use is therefore considered ineffective at controlling initial APP infections leading to the possible presence of carrier pigs among infected herds.[14,17] In our study, serologic evaluation of bacterin vaccines was conducted via response to APP serotype 5 LPS and Apx toxins, as APP serotypes 1 and 5 are known to contain ApxI and II, whereas serotype 7 only contains ApxII.[6] We confirmed that noticeable antibody production to the ApxI and II toxins occurred in vaccinated pigs, particularly those given APP serotype 5 bacterin. In our study and others,[4] the interpretation

of Apx ELISAs was considered more problematic due to less certain cut-off values and its limited availability. Although our results indicated that repeated dosage of APP serotype-specific vaccines can be monitored successfully by LPS ELISA serology, these assays based on APP LPS antigen are aimed at detection of infection status rather than vaccine responses. It is possible that vaccinated pigs may develop reactions to other protective antigens, unrelated to any detectable LPS response.

The long-standing use of serotype-specific bacterin vaccines remains the most popular form of APP vaccination, despite the considerable time and effort required for their autogenous preparation. It is possible that the use of bacterin vaccines may confer both some protection against colonization and some protection against Apx related tissue damage. Our results indicated that repeated dosage of APP serotype-specific vaccines can be monitored successfully by LPS ELISA serology, with an anamnestic response to LPS antigen (note titers at days 0, 42, and 64 presented in Figure 1) similar to vaccinated pigs in previous studies of the administration of APP serotype 5 bacterin vaccines.[18] It is possible that the preparation and use of whole-cell, unwashed bacterial material for production of the autogenous vaccines in our study may have conferred some protective advantage in comparison to other vaccine substrates. These previous studies also confirmed that repeated doses of APP bacterins are required for a measurable response and that there is little difference in the measured LPS antigen titer response to pigs given either 1 mL or 2 mL doses.[18] In our study, we found that a greater response occurred with three doses of APP serotype 5 bacterin compared to only two doses. Whether this may be useful for all APP bacterins, or merely this example of pathogenic APP serotype 5 infection, is not clear.

Implications

Under the conditions of this study:

- Commercial and serotype-specific ELISAs were used to identify herd APP status.
- Repeated autogenous APP serotype 5 vaccine doses provided strong antibody responses.

Acknowledgments

We thank the diligent farm and slaughterhouse managers for assistance with sample collections. We thank the staff of ACE laboratories (Bendigo East, Australia) for assistance in performing some of the culture and ELISAs. We thank the Pork Research Council of Australia for providing financial assistance for the study.

Conflict of interest

None reported.

Disclaimer

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Conversion tables

Weights and measures conversions

Common (US)	Metric	To convert	Multiply by
1 oz	28.35 g	oz to g	28.35
1 lb (16 oz)	0.45 kg	lb to kg	0.45
2.2 lb	1 kg	kg to lb	2.2
1 in	2.54 cm	in to cm	2.54
0.39 in	1 cm	cm to in	0.39
1 ft (12 in)	0.3 m	ft to m	0.3
3.28 ft	1 m	m to ft	3.28
1 mi	1.6 km	mi to km	1.6
0.62 mi	1 km	km to mi	0.62
1 in^2	6.45 cm^2	in^2 to cm^2	6.45
0.16 in^2	1 cm^2	cm^2 to in^2	0.16
1 ft^2	0.09 m^2	ft^2 to m^2	0.09
10.76 ft^2	1 m^2	m^2 to ft^2	10.8
1 ft^3	0.03 m^3	ft^3 to m^3	0.03
35.3 ft^3	1 m^3	m^3 to ft^3	35.3
1 gal (128 fl oz)	3.8 L	gal to L	3.8
0.26 gal	1 L	L to gal	0.26
1 qt (32 fl oz)	0.95 L	qt to L	0.95
1.06 qt	1 L	L to qt	1.06

Temperature equivalents (approx)

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

°F = (°C × 9/5) + 32

°C = (°F - 32) × 5/9

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

Conversion chart, kg to lb (approx)

Pig size	Lb	Kg
Birth	3.3-4.4	1.5-2.0
Weaning	7.7	3.5
	11	5
	22	10
Nursery	33	15
	44	20
	55	25
	66	30
Grower	99	45
	110	50
	132	60
Finisher	198	90
	220	100
	231	105
	242	110
	253	115
Sow	300	136
	661	300
Boar	794	360
	800	363

1 tonne = 1000 kg

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

1 ppm = 1 mg/L

National Pork Board launches AgView, a new tool to help protect the industry from foreign animal disease fallout

Last November, the National Pork Board launched AgView, a technology solution to help the US pork industry respond faster than ever before possible in the event of a foreign animal disease (FAD) outbreak. The web-based tool will allow participating producers to easily share their farm's FAD status updates and pig movement data with state animal health officials. The opt-in, no-fee technology – funded by the Pork Checkoff – will allow for contact-tracing of infected animals to help rapidly contain or regionalize a potential FAD outbreak.

AgView is designed to help the US pork industry coordinate a unified response to FADs across the nation – from grain farmers to producers, to state health officials, and veterinarians. When producer-users grant permission, AgView securely provides state animal health officials with health status and site and pig movement data from registered farms in real-time. This data sharing would go a long way in aiding an effective FAD response and could ultimately help the industry more quickly contain or regionalize an outbreak.

"While local and state reporting protocols already are in place, there is no nationwide repository for this data and no mechanism for real-time sharing," said National Pork Board Chief Veterinarian Dr Dave Pyburn. "Time is money in an FAD response, which is why we're excited to have AgView to help fill that gap and facilitate a quicker return to business for producers, especially in our export markets."

AgView, as a single software platform, allows for the rapid and accurate visualization of relevant pig movement data and diagnostic test results to create visibility, accountability, and trust during an outbreak of African swine fever or another FAD. To make this easier for producers, and ensure data is up to date, AgView can integrate with many existing record-keeping systems for easy synchronization. For those who do manual record-keeping, AgView also accepts imports from an Excel template. For more information, visit **pork.org/agview**. To register for an account, go to **agview.com**.

Surveillance Working Group makes progress, funds multiple African swine fever and classical swine fever projects

Producers on the National Pork Board's Surveillance Working Group voted to fund 10 proposals in the summer and fall of 2020 totaling $615,450. The projects address several areas including evaluating diagnostics to detect African swine fever (ASF) virus antibodies, developing classical swine fever (CSF) diagnostic tests to differentiate vaccinated animals from infected animals, investigating novel sample types to be used for ASF detection, improving oral fluid test performance, and evaluating ASF detection in oral fluid samples collected post outbreak. This research is being performed in the field in Romania, Uganda, China, Vietnam, and Thailand as well as in a laboratory setting in Russia, Canada, and the United States. The goal of this research is to improve ASF and CSF diagnostics and surveillance strategies as well as investigate new potential diagnostic sample types. The National Pork Board will release the results of the studies upon their completion. For more information about the working group or these studies, contact Dr Pam Zaabel at **pzaabel@pork.org** or (515) 223-2764.

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Highlights: AASV Board of Directors and committee leaders

The AASV Board of Directors and committee chairpersons met virtually on October 1, 2020 for a review of AASV committee activities followed by a strategic planning session and update on African swine fever preparedness activities. The AASV Board of Directors met virtually on October 2 to conduct official business. The following are highlights from the meetings:

- Each committee now has a board liaison to represent the AASV Board of Directors in an official capacity at each committee meeting.
- The AASV's representatives to the AVMA are listed at **aasv.org/members/only/AVMAreps.php**.
- At the request of the Early Career Committee, the board approved funding to establish an unmoderated early career veterinarian peer listserv and funding for speakers to participate in an early career veterinarian webinar/podcast series.
- The board approved revisions to the AASV Basic Guidelines of Judicious Therapeutic Use of Antimicrobials in Swine, available at **aasv.org/documents/JUG.php**.
- The AASV COVID-19 webpage, available at **aasv.org/resources/publichealth/covid19**, hosts numerous crisis response and depopulation resources developed for veterinarians in response to the processing disruption due to COVID-19.
- The AASV well-being webpage, available at **aasv.org/resources/wellbeing**, is dedicated to providing members with well-being resources.
- The board approved an increase in annual meeting registration fees by $30. There is no change to AASV membership dues for 2021.
- After receiving updates from Drs Snelson and Canon regarding hotel negotiations and current COVID-19 regulations in San Francisco, the board reviewed options for holding the 2021 AASV Annual Meeting. After considerable discussion, the board voted to hold the 2021 conference virtually, pending successful negotiation with the San Francisco Marriott to rescind the 2021 contract without penalty and meet there in 2025 instead. Those negotiations have since been completed, and the **2021 AASV Annual Meeting will be held virtually**. Future AASV meetings are scheduled for Indianapolis (2022), Denver (2023), Nashville (2024), and San Francisco (2025).
- The board accepted Dr Angela Baysinger and Dr Bill Hollis as nominees for AASV vice president. Ballots will be distributed electronically in January.
- Nominations will be sought for expiring terms of office in districts 1 (northeastern United States), 4 (Indiana and Michigan), and 6 (Iowa). Drs Melissa Billing (district 1) and Darryl Ragland (district 4) have each served one term and are eligible for reelection. Dr Locke Karriker (district 6) has served two terms and is not eligible for reelection. Nominations will open in these districts in late 2020.

Read the complete minutes of the Board meeting on the AASV website at **aasv.org/aasv/board**.

Join us for the first-ever virtual AASV Annual Meeting!

When Program Chair Dr Mary Battrell selected the theme for the 2021 AASV Annual Meeting, little did she know just how appropriate "Navigating the Future … *Together*" would be. With the COVID-19 pandemic looming on the horizon, the AASV Board of Directors voted to change course and steer towards the safe harbor of an online meeting.

While we won't be gathering "together" in the usual, in-person sense of the word, the Program Committee and AASV staff are working hard to ensure plenty of opportunities for conference participants to connect and interact with other attendees, speakers, and exhibitors during the usual exceptional meeting of continuing education for swine veterinarians. As AASV's Executive Director Dr Harry Snelson is fond of saying, "It's going to be the best virtual meeting we've ever had!"

In addition to hearing from Howard Dunne and Alex Hogg Lecturers Drs Jerome Geiger and Jeremy Pittman, conference attendees will be navigating current topics that include pig welfare, the threat of African swine fever, lessons learned from COVID-19, and new tools for that familiar nemesis, porcine reproductive and respiratory syndrome. As in the past, preconference seminars provide opportunities to gain in-depth information on a variety of additional subjects. Check out the full program at **aasv.org/annmtg**, and register before February 5. "See" you soon!

AASV news continued on page 41

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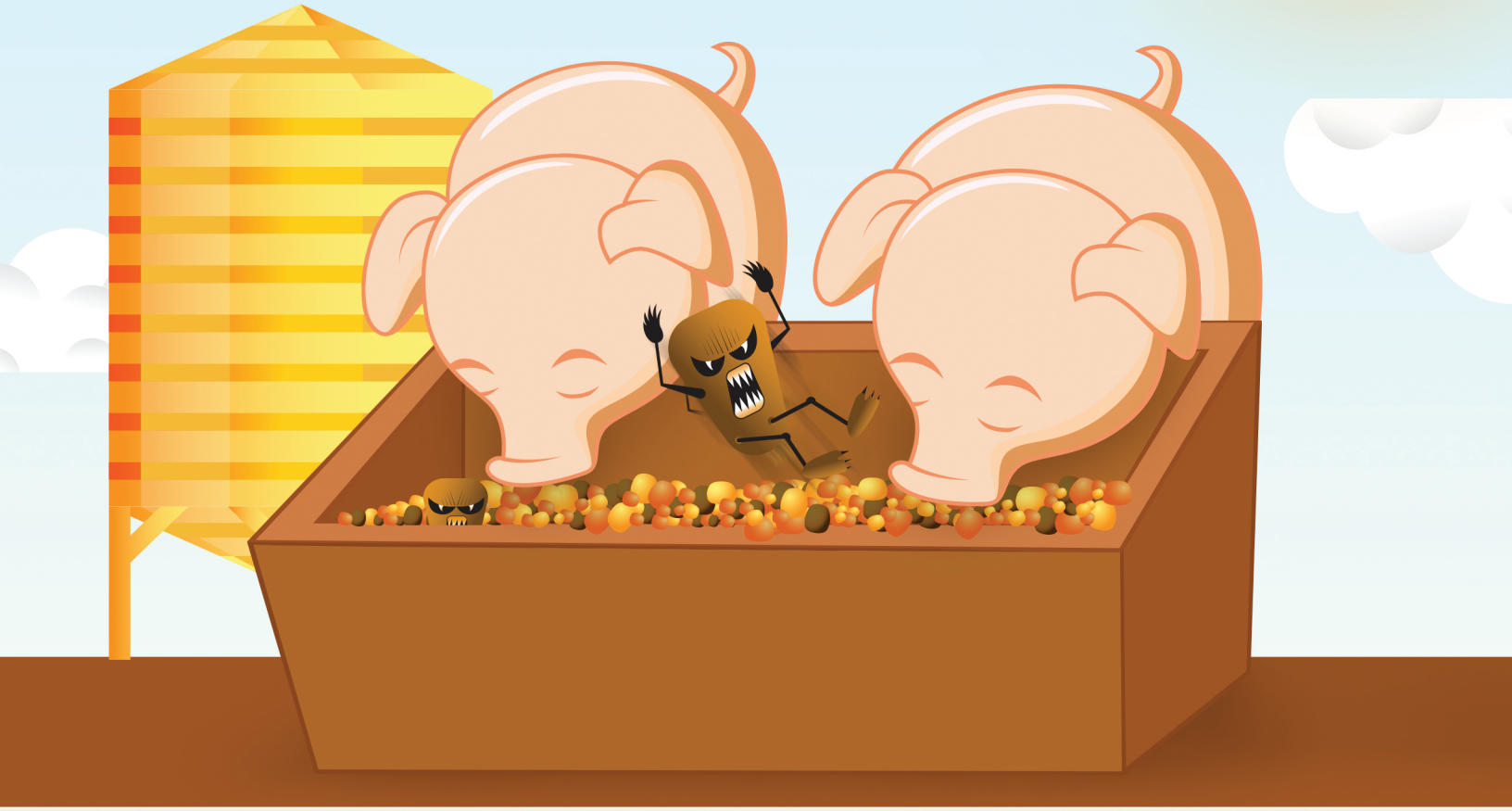

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Find your mycotoxin management strategies at **UnitedAnH.com/MMobilize**

AASV news continued from page 39

AASV committees to meet virtually before annual meeting

The AASV's issue-based committees will meet virtually this year during the winter months before the annual meeting. Meeting times are posted on the AASV committee webpage at **aasv.org/aasv/committee.php**. Agendas will be posted on each committee page as they become available.

Learn about each committee, read their reports and workplans, and review committee guidelines on the AASV committee webpage. All AASV members and student members are welcome to attend any committee meeting, but only committee members are eligible to vote. If you are interested in joining a committee, please contact the committee chair or Dr Abbey Canon. Not sure which to join? The AASV staff can help you fill an open seat!

The AASV Board of Directors relies on the committees as topic experts and seeks their input regarding issues of importance to swine veterinarians. Committees are called upon to examine an issue and advise the board on official positions the association should take or to develop additional resources to educate membership.

AASV publishes 2020 Salary Survey results

The AASV's 7th triennial Salary Survey results are now available for members to view and download at **aasv.org/members/only/SalarySurvey2020.pdf**. The survey requested 2019 salary and employment information from AASV active members in the United States and Canada, and a record-high 49% of the association's 966 eligible members participated. As in past survey efforts, the membership was classified into two categories: practitioners and public/corporate veterinarians, with each category completing a slightly different survey. The published report follows the customary format for presenting information, with the addition of several new tables and figures that present information not shared in previous surveys. A few highlights from the 2020 Salary Survey are:

- Additional information about benefits is provided in this report, including who pays for health insurance (80% of public/corporate veterinarians receive most or all of their health insurance paid by the employer while only 50% of practitioners do), average days of paid vacation and sick leave (higher for public/corporate veterinarians than practitioners), and information about parental leave, a new question on this year's survey.
- New charts provide average salaries based on work activities (clinical medicine, consulting, administration, research, tech/sales support, etc). Salaries for those engaged in management/administration are significantly higher than others.
- Age distribution of respondents shows a peak at 30 to 40 years of age and another slightly lower peak of respondents at around 60 years of age.
- Gender comparisons show that respondents in the older age groups are predominantly male, while the respondents in the younger age groups are more evenly split, with a trend towards more females than males.
- Comparisons show that salaries for females continue to lag behind the salaries of their male counterparts in the same age and employment category, with the sole exception of those less than 30 years of age in the public/corporate category.
- In comparison with previous surveys, the mean and median salaries for public/corporate respondents continued a downward trend since 2013 compared to their price-index adjusted counterparts. The mean and median salaries for practitioner respondents increased over the adjusted values since the 2016 survey but are still less than the adjusted mean and median salaries reported in 2013 (mean) and 2010 (median).

Applications due January 31 for $5000 debt-relief scholarships

The AASV Foundation has increased the number of student debt-relief scholarships to be awarded in 2021. Three $5000 scholarships will be provided to early-career swine practitioners through the "Dr Conrad and Judy Schmidt Family Student Debt Relief Endowment," which was renamed to honor the donors who established the scholarship program.

The scholarships are available to AASV members engaged in private practice who are 2 to 5 years post graduation from veterinary school (2016-2018) and who carry a significant student debt burden.

The scholarship program was initiated two years ago with a $110,000 contribution to the foundation by the Conrad Schmidt and Family Endowment. Dr Schmidt, a charter member of AASV, explained, "Together, Judy and I noticed that many new DVM graduates interested in swine medicine begin their professional life with heavy educational debt obligations. It is our desire to help AASV members who have dedicated their professional skills to swine herd health and production."

Since then, the number of applicants for the scholarship demonstrated a need to expand the program to support more early-career swine veterinarians who are carrying a heavy student debt load.

Applications are being accepted through January 31 for the scholarships to be awarded during the 2021 AASV Annual Meeting. The application form is available at **aasv.org/foundation/debtrelief.php**. The following criteria will be used to select the scholarship recipient:

1. Joined AASV as a student enrolled in an AVMA-recognized college of veterinary medicine
2. Attended the AASV Annual Meeting as a student
3. Maintained continuous membership in AASV since graduation from veterinary school
4. Is at least 2 years and at most 5 years post graduation from veterinary school (2016, 2017, 2018 DVM/VMD graduates)
5. Has been engaged in private veterinary practice, 50% or more devoted to swine, providing on-farm service directly to independent pork producers. Veterinarians who work for production companies, pharmaceutical companies, or universities are not eligible for the scholarship.
6. Has a significant student debt burden

For more information, contact the AASV Foundation: **aasv@aasv.org**, 515-465-5255.

Apply for Hogg Scholarship by January 31

The American Association of Swine Veterinarians Foundation is pleased to offer the Hogg Scholarship, established to honor the memory of longtime AASV member and swine industry leader Dr Alex Hogg.

The intent of the scholarship is to assist a swine veterinarian in his or her efforts to return to school for graduate education (resulting in a master's degree or higher) in an academic field of study related to swine health and production. Twelve swine practitioners, recognized at **aasv.org/foundation/hoggscholars.htm**, have been awarded this prestigious scholarship since it was established in 2008.

Applications for the $10,000 scholarship will be accepted until **January 31, 2021**, and the scholarship recipient will be announced Sunday, February 28 during the 2021 AASV Annual Meeting.

Dr Alex Hogg's career serves as the ideal model for successful applicants. After twenty years in mixed animal practice, Dr Hogg pursued a master's degree in veterinary pathology. He subsequently became Nebraska swine extension veterinarian and professor at the University of Nebraska. Upon "retirement," Dr Hogg capped off his career with his work for MVP Laboratories. Always an enthusiastic learner, at age 75 he graduated from the Executive Veterinary Program offered at the University of Illinois.

The scholarship application requirements are outlined below, and on the AASV website at **aasv.org/foundation/hoggscholarship.htm.**

Hogg Scholarship Application Requirements

An applicant for the Hogg Scholarship shall have:

1. Three or more years of experience as a swine veterinarian, either in a private practice or in an integrated production setting
2. Five or more years of continuous membership in the American Association of Swine Veterinarians

Applicants are required to submit the following:

1. Current curriculum vitae
2. Letter of intent detailing his or her plans for graduate education and future plans for participation and employment within the swine industry
3. Two letters of reference from AASV members attesting to the applicant's qualifications to be a Hogg Scholar

Applications and requests for information may be addressed to: AASV Foundation 830 26th Street, Perry, IA 50220, Tel: 515-465-5255, **aasv@aasv.org**.

Foundation solicits research proposals, due January 15

In recognition of the value and need for research with direct application to the swine veterinary profession, the AASV Foundation has increased the amount of funding available for research proposals in 2021 from $60,000 to $100,000.

Proposals are now being accepted. They are due January 15, 2021 and may request a maximum of $30,000 (US$) per project. The announcement of projects selected for funding will take place during the 2021 AASV Annual Meeting.

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

The instructions for submitting proposals are available on the AASV Foundation Web site at **aasv.org/foundation/2021/research.php**.

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

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

A summary of the research funded by the foundation over the past 14 years is available at **aasv.org/foundation/research.htm**.

For more information, or to submit a proposal:

AASV Foundation
830 26th Street
Perry, IA 50220-2328
515-465-5255
aasv@aasv.org

AASV Foundation Mission

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

- enhancing the image of the swine veterinary profession,
- supporting the development and scholarship of students and veterinarians interested in the swine industry,
- addressing long-range issues of the profession,
- supporting faculty and promoting excellence in the teaching of swine health and production, and
- funding research with direct application to the profession.

AASV Foundation Auction: full speed ahead!

With Dr Chase Stahl at the helm, the AASV Foundation Auction Committee is navigating its way through the choppy waters of conducting a successful fundraising auction during the now-virtual AASV Annual Meeting. Despite the new direction, the crew is making excellent headway, as evidenced by the varied array of items assembled for bids at **aasv.org/foundation/2021/auctionlist.php**.

Their efforts – and your participation – are important, because the foundation depends upon the annual auction proceeds to supplement its investment income in order to fund the many research grants, student travel stipends, swine externship grants, and scholarships it awards every year.

Since the AASV Annual Meeting is being held online, the auction is following in its wake. Some features of the auction will be the same as in the past. For example, you are already accustomed to viewing and bidding on the silent auction items from your phone or other digital device. The bidding site will be available in February; go to **aasv.org/foundation/2021/auctionlist.php** to access the link and sign up for your bidding number to start bidding!

There will be featured Live Auction items as in the past, but there will not be a live (or virtual, for that matter) auctioneer. Instead, you will bid using the bidding app, just like the silent auction. But be sure to pay close attention to the closing time for the "Live Auction" items, as they have different closing times from the Silent Auction items.

Don't miss out – remember, you can use the Max Bid feature on any live or silent item to avoid last-minute disappointment when the bids close. After the auction, the donor of each item will ship it directly to the winning bidder.

The best part: you don't have to be registered for the meeting to participate in the auction. Truly, we can all join together as we navigate the future of the AASV Foundation!

All aboard, mateys!

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Dr Jerome Geiger

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Dr Jeremy Pittman

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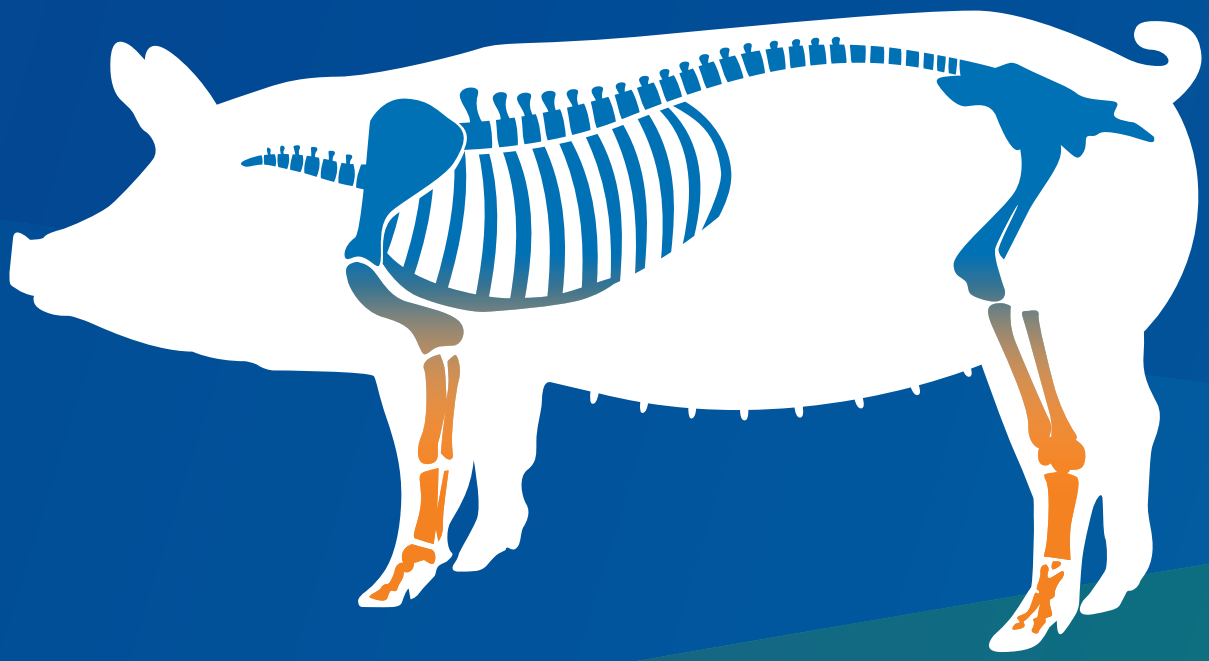

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Assessing pain in pigs

A new $650,000 grant from the US Department of Agriculture's National Institute of Food and Agriculture through their Agriculture and Food Research Initiative will help a consortium of researchers, led by the American Association of Swine Veterinarians, to validate behavioral, physiologic, and biomarker-based endpoints that reliably measure pain associated with surgical castration in piglets. In addition to AASV, the project collaborators include Kansas State University, North Carolina State University, Prairie Swine Centre, Iowa State University, Smithfield Foods, Merck Animal Health, National Pork Board, Beef+Lamb New Zealand, Midwest Veterinary Services, and the US Food and Drug Administration (FDA).

We know that surgical castration of piglets causes acute pain as evidenced by changes in behavior and physiology. The US swine industry is committed to protecting and promoting pig welfare and finding ways to minimize pain and distress to the animals in our care. Immunocastration is a viable alternative to surgical castration, however, there has been limited uptake of its use in the US marketplace to date. Beyond this technology, farmers and veterinarians are currently limited in how to address the pain management challenge.

This is in part due to the lack of analgesic or anesthetic drugs in the United States specifically approved with an indication for the control of pain in swine. There is also a lack of consistent data related to the efficacy of pain mitigation products primarily due to the lack of uniform testing methodology and protocols.[1] This in turn makes evaluating efficacy of pain mitigation interventions difficult and has prevented consensus on best practices for pain relief.[2] Lack of consistent protocols creates difficulty for pharmaceutical companies to submit new product approvals or label claims related to pain, veterinarians to confidently prescribe product for extra-label use, researchers to reliably assess pain and potential mitigation strategies, and pig farmers to make future business decisions regarding animal welfare.

The long-term goal of the funded project is to improve pig welfare on-farm by effectively controlling pain associated with on-farm surgical procedures, such as castration, in a manner that is safe for the animal and the consumer and is compliant with US regulation. The primary goal of the project is to facilitate consistency and rigor through the development of a research protocol utilizing validated endpoints that are well-defined and reliably measure pain in piglets. This research creates a framework that may easily be adapted to address other painful procedures or conditions experienced by swine, such as tail docking, lameness, injury, and parturition.

Establishing these validated endpoints will provide meaningful swine-specific references for FDA as they review future submissions for new drug approvals with a claim for controlling pain associated with castration of swine. The outcomes from this study will also provide a validated tool set for pharmaceutical companies' research and development pursuits in the area of pain control to satisfy substantial evidence of effectiveness requirements for the approval of analgesic drugs in swine. Academic researchers who specialize in pain assessment can use the validated endpoint protocols in their future research and ultimately provide better consistency in the published data.

> *"The US swine industry is committed to protecting and promoting pig welfare and finding ways to minimize pain and distress to the animals in our care."*

Ultimately, this consistency will aid the veterinary practitioner and farmers in evaluating efficacy, developing consensus on best practices for pain management, and making future business decisions regarding animal welfare. As animal welfare has the potential to become more prominent in trade negotiations, having validated endpoints for measuring pain and solutions for controlling pain will help the US swine industry remain competitive in the global marketplace.

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Sherrie Webb, MSc
Director of Swine Welfare

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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

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

		Maximum words		Maximum No.		
Genre	**Description**	**Abstract**	**Manuscript body**	**Figures and Tables**	**References**	**Other requirements***
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.

Table 1: Continued

Genre	Description	Maximum words		Maximum No.		Other requirements*
		Abstract	Manuscript body	Figures and Tables	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.
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.

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.

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).

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

Supplementary materials

Supplementary materials are additional materials that are not essential to the understanding of the manuscript but provide important context to the manuscript and may be submitted for online only publication. Examples of materials accepted include extended descriptions of experimental methods or statistical analysis, extended bibliographies, additional supporting tables and figures, reporting checklists, copies of surveys or questionnaires, handouts, and forms.

For supplementary materials that are too large or in a format not consistent with JSHAP publication (eg, data sheets, presentations, audio, or video), authors are encouraged to upload and publish these files to a repository, such as FigShare, and reference the DOI within the manuscript.

Supplementary materials must be formatted according to the JSHAP Author Guidelines. There is no word or page limit for supplementary materials, but they should be succinctly presented to facilitate peer review. Acceptance of supplementary materials for publication is at the discretion of the editor. All JSHAP published supplementary materials are subject to copyright.

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 **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,

Author guidelines continued on page 53

Author guidelines continued from page 51

- 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**

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3. Christiansen SL, Iverson C, Flanagin A, Livingston EH, Fischer L, Manno C, Gregoline B, Frey T, Fontanarosa PB, Young RK, eds. *AMA Manual of Style: A Guide for Authors and Editors*. 11th ed. New York, New York: Oxford University Press. 2020.

JSHAP Author Guideline Checklist

Title page

- □ My manuscript is a Word document with double spacing, footer page numbers, continuous line numbers, and Times New Roman 12 pt font.
- □ I have provided a short title of 90 characters or less (including spaces).
- □ I have included the genre of publication.
- □ I have created a title that is concise, specific, and informative without using abbreviations.
- □ I have properly formatted the author byline.
  - Alpha B. Charlie, degree, degree; Juliett K. Lima, degree; Mike N. Oscar, degree
  - List only the highest level of degree or professional certification except if additional degree denotes a different field of study or a specialty degree, license, certification or credentials.
- □ I have properly formatted the author affiliations.
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- □ I have included a Summary not exceeding the word limit for the genre:
  - 250 words for original research including these subheadings – Objective(s), Materials and methods, Results, and Implication(s).
  - 200 words for literature review. No subheadings needed.
  - 100 words for case report, case study, production tool, diagnostic note, practice tip, or peer-reviewed commentary. No subheadings needed.
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- □ I have defined abbreviations at the first mention of the term being abbreviated in the summary.
- □ I have only introduced abbreviations if they are used again in the summary and have used the abbreviation whenever the term is mentioned in the summary except at the beginning of a sentence.
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- □ I have included the required sections for the genre of manuscript.
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Implications

- □ I have included up to 3 bulleted implications, each with a maximum of 80 characters or less (including spaces). This section is exempt only for literature review and practice tip manuscripts.

Acknowledgments

- □ I have mentioned any individuals, companies, or funding sources that I would like to acknowledge.
- □ I have disclosed all conflicts of interest for this paper. If none exist, I have included the statement "None reported."
- □ I have included the JSHAP disclaimer.

References

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- □ I have formatted reference numbers in the manuscript as superscripts placed after periods and commas and before colons and semicolons.
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- □ I have provided complete page numbers in all references (eg, 120-128, not 120-8).
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- □ I have identified all non-refereed references with an asterisk (*) to the left of the reference list number and have included the following notation at the end of the reference list.
  - ○ * Non-refereed references.

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- □ I have included the appropriate unit of measure for each row and column.
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- □ I have used parentheses instead of the ± symbol throughout my table (eg, "1 (3.5)" rather than 1 ± 3.5").
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- □ When appropriate, I have provided a footnote to describe the level of significance and the statistical method of analysis used.
- □ When appropriate, I have used lower case letters as superscripts to designate significant differences and have created a footnote to explain the level of significance and the statistical method used.
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Figures

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- □ My figure title is descriptive, brief, and followed by the legend and abbreviations. The legend includes a brief description of treatments, level of significance, *P* values, and the statistical method used. All abbreviations used in the figure are defined.
- □ I have created a separate file for each figure in the acceptable file types (ie, .xls, .pdf, or .jpg).
- □ All axes are labeled with a description followed by the unit of measure, when needed, separated by a comma.

Manuscript submission

- □ I have included my manuscript file and a separate art file with my submission.
- □ I have included a cover letter that does not exceed 1 page and includes the requested information.

Dr Angela Baysinger

I don't know if it was his plan or simply that he needed my small hands, but when Dr Don Hudson guided me through helping a gilt deliver her first litter when I was six years old, he started me on my path to becoming a swine veterinarian. My entire life has been about animal agriculture – in particular, pig production. From growing up on my family's pig farm, studying animal science and veterinary medicine, serving farmers as a practicing veterinarian, supporting the animal health sector of our food system, and volunteering with many organizations; swine health, the well-being of my peers, and welfare of animals has not only been my vocation but my passion. It's my way of life.

Confucius teaches us, "If your plan is for one year, plant rice. If your plan is for ten years, plant trees. If your plan is for one hundred years, educate children." I believe we are all "children" – life-long learners.

Just as Dr Hudson and many others invested in me as a young person interested in veterinary medicine, I desire to ensure the American Association of Swine Veterinarians (AASV) is investing in all of us "children" as we prepare for the future. As a candidate for vice president of AASV, I would be honored to apply my knowledge, skills, and experiences to the future of our organization. As an elected leader of AASV, I will work to ensure the AASV continues to be inclusive and uplifting for all members. And I look forward to working with my fellow board members, committee leaders, and professional staff as, together, we provide visionary leadership for the long-term success of AASV.

I believe my educational, professional, and volunteer experiences will allow me to fulfill this role.

I earned my doctor of veterinary medicine in 1992 from the University of Missouri-Columbia College of Veterinary Medicine. After graduation, I began my professional career as an associate veterinarian at Sutton Veterinary Clinic in Sutton, Nebraska. In 1995, I began my master's degree in epidemiology at the University of Nebraska-Lincoln and served as the interim state swine extension veterinarian. Since completing my master's degree, I have worked as a swine technical services veterinarian with ALPHARMA and Boehringer Ingelheim, a self-employed swine consultant, and a Health Assurance Veterinarian with Pig Improvement Company (PIC).

Today, many within AASV know my calling is animal welfare. My interest in focusing on animal welfare arose during the seven years I served as Vice President of On-farm Food Safety and Animal Welfare for Farmland Foods (Smithfield). I apply the skills and knowledge I have developed in this space to my current role as Animal Welfare Lead, North America, for all species at Merck Animal Health. In this role, I work across the food chain, retail to producer, to foster trust, promote science, and advocate for animal agriculture.

Like many swine veterinarians, I cannot sit still long. In addition to my role at Merck Animal Health, I am pursuing a master's degree in international animal welfare, ethics, and law at the University of Edinburgh and will pursue board certification in the American College of Animal Welfare (ACAW).

Professionally, I am or have served as a member of the animal welfare committees for the American Veterinary Medical Association, North American Meat Institute, National Pork Board, and, of course, the AASV. I also am a 2-time past chair and a current member of the board of directors for the Professional Animal Auditor Certification Organization and a past member of the AASV board.

My husband, Jerry, an agronomist, farmer, and small business owner, and our two sons, Isaac – a computer science major and member of the marching band at the University of Nebraska, and Sam – a high school senior and enlisted member of the Nebraska Army National Guard, call Bruning, Nebraska home. As a family, we are active in the Hebron Nebraska Bible Church, American Legion, Legion Auxiliary, Boy Scouts of America, the National FFA Organization, and many community activities.

I welcome the opportunity to serve AASV. Service is in my heart, and I am here to serve the members of AASV.

To learn more about Angela, scan the QR code.

Dr Bill Hollis

We have a tremendous professional organization for education, advocacy, peer review, technical support, and fellowship. The AASV Annual meeting has been a constant motivator for me professionally and personally. I enjoy regular member connections through our committees, e-Letter, and journal. Hallway talk at the meetings and personal phone calls have led to some of my most meaningful professional growth. The strongest bonds of my professional network have been fostered through connections within the AASV.

It is an honor to be nominated for AASV vice president. I appreciate the calls of support and sincerely ask for your vote. My wife and I have raised two great kids. It has been my desire to see them get started in their own busy lives before I head back to any extended travel or time away from the practice. I also have been blessed with great business partners who are supportive of my desire to run for this office. I am confident the entire veterinary team in Carthage will continue to serve many roles in the AASV.

Mentorship opportunities started for me as an Illinois veterinary student, preparing notebooks for Dr Leroy Biehl as he organized Executive Veterinary Program classes. Introductions to additional AASV members offered me internships and production experiences necessary to begin forming goals and plans to choose a pathway to begin practice. I remember the AASV meeting theme "Standing on The Shoulders of Giants." My early development in AASV was blessed with introductions, experiences, and mentorship from many wonderful AASV leaders, giants in our organization, and in their own communities.

I grew up in a small town in central Illinois with strong local 4-H and FFA programs. My high school ag instructor was the first person to push me to get involved in production agriculture and in leadership education. I served as the Illinois FFA president and later as National FFA vice president. Veterinary medicine was always my goal growing up. Food-animal medicine offered many opportunities for production experience. Even after travelling to several states for FFA and veterinary experiences, I returned to private veterinary practice within 40 miles of home. I have remained at the same private practice for 24 years. The AASV and our swine clients have allowed me to travel internationally and domestically. I appreciate both the need to protect our home base and learn from the needs of the greater global swine herd.

Veterinary practice introduced me to many challenging cases and client health needs. It has also been an excellent opportunity to participate in business ownership in our community. One exciting thing about veterinary medicine is the numerous avenues to put our education to work. The AASV has brought many of us together to share in client service, client education, product use, technical service, and much more. I believe it is up to us as AASV members to serve our clients by bringing together the best minds to solve problems. The AASV provides the framework to safely challenge the status quo while sharing successes and failures in the search for better patient care.

Service in organized veterinary medicine requires a significant amount of volunteer effort year after year. I have always believed in the value of doing your part to benefit the organization and the industry. I also realize a strong dose of patience is needed to struggle through some of the debates and committee processes required to make significant gains for any organization. I have benefitted from watching the good Dr David Madsen challenge those uninterested in the impact of poorly designed federal guidelines. I have also learned from our esteemed Dr Tom Burkgren the need to show up, speak up, and work hard to bring recognition to the needs of our profession. I have served on the AVMA House of Delegates for six years, the National Pork Board's Swine Health Committee for six years, and most recently on the AASV Board of Directors for six years.

Many challenges lie ahead for our organization. The swine industry we serve has been under significant financial pressure for the last two years. The disease pressures from a growing global economy have led to risks we have not considered until recent years. My interest is to engage the membership in critical decisions.

Our organization holds a great deal of public and political respect. Given that advantageous starting position, it is up to us to advocate for our clients and to protect our clients with safe medical products, healthy farms, and a predictable regulatory environment. I welcome the opportunity to participate in the AASV executive team and build on the successful position we have grown to enjoy for our organization.

Please reach out to me if you have questions or concerns about me or the organization. As I mentioned previously, hallway talk and personal phone calls are some of my favorite things about the American Association of Swine Veterinarians.

To learn more about Bill, scan the QR code.

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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.

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Upcoming meetings

American Association of Swine Veterinarians 52nd Annual Meeting - VIRTUAL

February 27 - March 2, 2021 (Sat-Tue)

For more information:
American Association of Swine Veterinarians
830 26th Street
Perry, IA 50220
Tel: 515-465-5255
Email: **aasv@aasv.org**
Web: **aasv.org/annmtg**

International Conference on Pig Survivability

October 27 - 28, 2021 (Wed-Thu)
Hosted by: Iowa State University, Kansas State University, and Purdue University
Omaha, Nebraska

Conference contact:
Dr Joel DeRouchey
Email: **jderouch@ksu.edu**
Web: **piglivability.org/conference**

26th International Pig Veterinary Society Congress

June 2022 - Date to be determined
Rio de Janeiro, Brazil

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

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

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